Basic Concepts in Medicinal Chemistry

3RD EDITION

Marc W. Harrold
Robin M. Zavod

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BASIC CONCEPTS IN MEDICINAL CHEMISTRY

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3rd Edition

MARC W. HARROLD, BS Pharm, PhD

Professor of Medicinal Chemistry
Duquesne University
School of Pharmacy
Pittsburgh, Pennsylvania

ROBIN M. ZAVOD, PhD, FAPhA

Editor-in-Chief, *Currents in Pharmacy Teaching and Learning*
Professor of Pharmaceutical Sciences
Midwestern University College of Pharmacy
Downers Grove, Illinois

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DEDICATION

To our students who blessed us with their joy, presented us with their challenges, and made us better educators.

To our colleagues who provided us with their encouragement and inspiration, and who have served as our role models.

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Marc would like to thank the Duquesne University School of Pharmacy for providing the time and support required for the writing of this third edition; his colleagues who took the time to provide suggestions for various aspects of this text; his wife Barbara for her love and support and for coining the phrase "Structure Analysis Checkpoint" introduced in the second edition; and God for His countless blessings and constant guidance.

Robin would like to acknowledge the two independent pharmacists who shared their world of community pharmacy and who gingerly pointed out that Medicinal Chemistry isn't "spoken" in this setting. Her experience as a pharmacy technician for these two pharmacists reshaped her teaching philosophy and as a result, her approach to drug structure evaluation. LAM and BLC constantly demonstrated the value of questioning students—whether verbally or via practice sets. As a result, the development of scores of practice sets, as well as a highly interactive teaching style became central to her teaching philosophy. As always, the unwavering support of my colleagues and family was truly appreciated, as they watched yet another ball added to an ever-evolving juggling act.

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PREFACE

Welcome to the third edition of *Basic Concepts in Medicinal Chemistry*. We are excited to be able to offer this updated version of our original text. Similar to what we experienced with our second edition, our students, readers, and peers provided us with challenges to enhance this textbook and provide additional explanations and examples. In hindsight, and with a critical review, we identified topic areas that needed additional clarification. While the basic concepts that underlie medicinal chemistry remain the same, the presentation of some of these concepts can always be improved. In this edition, we have sought to provide better examples, better explanations, additional summaries, and additional knowledge links to help those seeking to master these concepts. We are thankful for the feedback that we have received from both students and peers and have worked to address the suggestions and questions provided.

The major revisions provided in this edition include

- A revision of all of the figures and structures to allow for a more consistent "look" throughout the text
- A revision of a number of the examples throughout the text to include a wider range of drugs and drug classes
- A clarification of examples that were potentially confusing
- The creation of additional summary tables in Chapters 3 and 6 to help readers better select the proper drug binding interaction
- The addition of enhanced explanations, discussions, and examples in the following areas:
  - resonance, induction, and electron flow (Chapter 2)
  - discussion of pK_a ranges of acidic and basic functional groups with a specific emphasis on the differences seen among carboxylic acids, amines, and aromatic nitrogen atoms (Chapter 3)
  - specific links that tie together ionization states and possible binding interactions (Chapter 6)
  - the importance of properly identifying a drug binding interaction (Chapter 6)
  - certain metabolic transformations that can cause confusion (Chapter 8)
- The addition of an expanded discussion of pharmacogenomics (or pharmacogenetics) in Chapter 8, including a number of specific examples

Similar to previous editions, this text focuses on the basic, fundamental concepts governing the discipline of medicinal chemistry and emphasizes functional group analysis and the fundamentals of drug structure evaluation. Every drug that is prescribed and dispensed is a chemical structure that contains numerous functional groups oriented in a specific manner. These functional groups determine the interactions of a drug molecule with its biological target, its pharmacological action(s), the route(s) by which it is administered, the extent to which it is metabolized, and the presence or absence of specific adverse drug reactions or drug interactions. It thus seemed appropriate to begin the text with a discussion of the common characteristics and roles of functional groups. Subsequent chapters were then designed to focus upon specific aspects of these functional groups. These include the identification of acidic and basic functional groups, the use of the Henderson-Hasselbalch equation to solve quantitative and qualitative pH and pK_a problems, the formation of inorganic and organic salts of specific functional groups, the roles of water and lipid soluble functional groups and

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the need for a proper balance of solubility, the interaction of functional groups with their biological targets, the stereochemical orientations of functional groups within a drug molecule, and the routes of metabolism that are available for specific functional groups. The final two chapters serve as capstones for the text. Chapter 9 focuses upon structure activity relationships (SARs) and a brief overview of some of the common strategies employed in rational drug design, while Chapter 10 introduces the concept of Whole Molecule Drug Evaluation, an idea that we first introduced and published in our *Medicinal Chemistry Self Assessment* text in 2015.

Several aspects of this text should help students develop a strong foundation in the concepts that govern the discipline of medicinal chemistry. Chapters 2 through 9 contain specific learning objectives that coincide with the key concepts discussed in the chapters. The organization of the subject material was chosen to allow students to incrementally increase their knowledge of the functional groups that comprise drug molecules and their importance to drug therapy. Each chapter contains numerous examples to help illustrate each key concept. In choosing these examples, a conscious effort was made to try to include as many different commercially available drugs as possible. During the many years that the two of us have taught medicinal chemistry, a question that we are commonly asked is, "Why is this important to a pharmacist and the practice of pharmacy?" To address this question, each chapter includes extended discussions that link fundamental medicinal chemistry concepts to their therapeutic relevance.

We firmly belief that these concepts are difficult to learn and master without multiple forms of self-assessment. To better meet this need, we introduced Structure Analysis Checkpoint (SAC) questions in the second edition of our text. These questions "follow" two drugs, venetoclax and elamipretide, throughout the text. As new concepts and skills are introduced in each chapter, these drugs are revisited, and readers are asked to apply their newly acquired knowledge to these two drugs. By the end of the text, readers will have encountered over 30 unique questions for each of these drugs and will have ultimately completed two whole molecule drug evaluations. It is important to note that the SAC questions are based solely on two drugs, whereas the stand-alone end-of-chapter Review Questions purposely use different drugs for each question. Each set of end-of-chapter Review Questions was evaluated to determine if question format and/or question drug example should be retained or changed. Modifications to the review questions (~50%) were made in nearly all chapters. Items were added to reflect the new content, and the total number of questions in each chapter was increased. This provides instructors with an enhanced question bank for every chapter. Additionally, we introduced four additional Whole Molecule Drug Evaluations in Chapter 10, increasing the content in this chapter by 50%. Each Whole Molecule Drug Evaluation is unique and requires a specific level of evaluation. The answers for all questions are provided in an appendix; however, it is strongly suggested that readers attempt to answer the questions prior to consulting the answers.

We are thankful for the opportunity to provide you with what we believe is an updated and improved version of our initial text, for the invaluable contributions provided by our students and peers, and for those who have chosen to use this text to further their knowledge in the area of medicinal chemistry.

Marc W. Harrold

Robin M. Zavod

ABBREVIATIONS USED IN THIS TEXT

Many of these are defined in the chapters in which they appear, but a comprehensive list of all abbreviations used in the text is provided here for your convenience.

ACE	Angiotensin converting enzyme
ADH	Alcohol dehydrogenase
ADME	Absorption, distribution, metabolism, excretion
ADP	Adenosine diphosphate
ALDH	Aldehyde dehydrogenase
ALL	Acute lymphoblastic leukemia
AMP	Adenosine monophosphate
ARB	Angiotensin II receptor blocker (aka angiotensin II receptor antagonist)
ATP	Adenosine triphosphate
APS	Adenosine-5′-phosphosulfate
BID	*bis in die* (Latin for twice daily)
BPH	Benign prostatic hyperplasia
CIP	Cahn-Ingold-Prelog
cLog P	Calculated log P
CoA	Coenzyme A
CNS	Central nervous system
COMT	Catechol-*O*-methyltransferase
COPD	Chronic obstructive pulmonary disease
COX	Cyclooxygenase
CYP450	Cytochrome P450
1,4-DHP	1,4-Dihydropyridine
DNA	Deoxyribonucleic acid
E (isomer)	*Entgegen* (German for opposite)
EDTA	Ethylenediaminetetraacetic acid
FAD	Flavin adenine dinucleotide
FDA	Food and Drug Administration
FMO	Flavin monooxygenase
GERD	Gastroesophageal reflux disease
GI	Gastrointestinal
GMP	Guanosine monophosphate
GTP	Guanosine triphosphate
GSH	Glutathione
HDL	High density lipoprotein
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type 1
HMG-CoA	3-Hydroxy-3-methylglutaryl coenzyme A

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IM	Intramuscular
IMP	Inosine monophosphate
IR	Infrared
IUP	Intrauterine device
IV	Intravenous
LDL	Low density lipoprotein
Log D	Logarithmic expression of the distribution coefficient
Log P	Logarithmic expression of the partition coefficient
LTC_4	Leukotriene C_4
LTD_4	Leukotriene D_4
LTE_4	Leukotriene E_4
MMAE	Monomethylauristatin E
MTT	Methyl-tetrazole-thiomethyl
NADH	Nicotinamide adenine dinucleotide (reduced form)
NAD^+	Nicotinamide adenine dinucleotide (oxidized form)
NAPDH	Nicotinamide adenine dinucleotide phosphate (reduced form)
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidized form)
NAT	*N*-Acetyltransferase
NMR	Nuclear magnetic resonance
NPH insulin	Neutral protamine Hagedorn insulin (aka isophane insulin)
NSAID	Nonsteroidal anti-inflammatory drug
OTC	Over-the-counter
P_i	Phosphate (inorganic)
PABA	*para*-Aminobenzoic acid
PAH	Pulmonary arterial hypertension
2-PAM	Pralidoxime chloride (aka 2-Pyridine aldoxime methyl chloride)
PAP	3′-Phosphoadenosine-5′-phosphate
PAPS	3′-Phosphoadenosine-5′-phosphosulfate
PAR-1	Protease-activated receptor-1
PEG	Polyethylene glycol
Pen VK	Potassium penicillin V
PGE_2	Prostaglandin E_2
PGI_2	Prostaglandin I_2 (aka prostacyclin)
pH	Negative log of the hydrogen ion concentration in a solution
pK_a	Negative log of the K_a, the dissociation constant for an acid in an aqueous environment
PO	*per os* (Latin for once daily)
POMT	Phenol-*O*-methyltransferase
PPARα	Peroxisome proliferator-activated receptor
PP_i	Pyrophosphate (inorganic)
PRPP	5-Phosphoribosyl 1-pyrophosphate
QID	*quater in die* (Latin for four times daily)

R (isomer)	*Rectus* (Latin for right)
RNA	Ribonucleic acid
S (isomer)	*Sinister* (Latin for left)
SAM	*S*-Adenosylmethionine
SAR	Structure activity relationship
SC	Subcutaneous
SULT	Sulfotransferase
T_3	Liothyronine (aka triiodothyronine)
T_4	Levothyroxine
TID	*ter in die* (Latin for three times daily)
T-IMP	Thioinosine monophosphate
tRNA	Transfer ribonucleic acid
TXA_2	Thromboxane A_2
UDP	Uridine diphosphate
UDPGA	UDP-glucuronic acid
UGT	UDP-glucuronyltransferase
VEGF-2	Vascular endothelin growth factor 2
Z (isomer)	*Zusammen* (German for together)

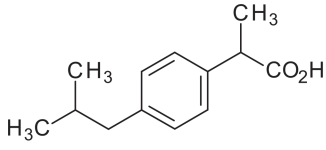
INTRODUCTION

LEARNING OBJECTIVES

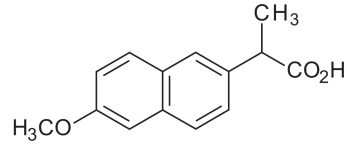
After completing this chapter, students will be able to

- **Discuss the differences among single, double, and triple bonds.**
- **Correctly number alicyclic and heterocyclic rings, sugars, and steroids.**
- **Correctly designate α, β, and ω positions on drugs and biomolecules.**
- **Correctly identify *ortho, meta,* and *para* positions on an aromatic ring.**
- **Correctly explain how peptides are constructed from amino acids.**
- **Correctly identify the components of nucleosides and nucleotides.**

This text focuses on the fundamental concepts that govern the discipline of medicinal chemistry as well as how and why these concepts are essential in therapeutic decision making. In very simplistic terms, *medicinal chemistry* can be defined as the chemistry of how drugs work. In other words, it is the discipline that seeks to identify the specific atoms or functional groups that are responsible for specific biological/biochemical actions. To illustrate this point, let's compare the structures and dosing of two commonly used drugs, ibuprofen and naproxen.



Ibuprofen



Naproxen

Both of these drugs are available without a prescription (i.e., over-the-counter [OTC]) and produce anti-inflammatory, analgesic, and antipyretic actions. Ibuprofen is a shorter-acting drug and must be administered every 4 to 6 hours, whereas naproxen is a longer-acting drug that can be dosed every 12 hours. In evaluating these chemical structures, it is found that there are both similarities (i.e., carboxylic acid and adjacent methyl group) and differences (i.e., bicyclic ring with a methoxy group versus monocyclic ring with an alkyl chain). The discipline of medicinal chemistry seeks to explain how these structural (i.e., chemical) differences result in different durations of

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action. Once this relationship is established, this information can be used to predict the relative durations of action of other agents within this chemical/pharmacological class.

The primary goal of this text is to help the reader develop a solid foundation in medicinal chemistry. Once this foundation has been established, the reader should be able to analyze drug structures and understand how their composite pieces can contribute to the overall properties and/or activity of the drug molecules. Every drug that is prescribed and dispensed is a chemical structure with a specific composition. The atoms and functional groups that comprise these chemical structures dictate the route of administration, the duration of action, the pharmacological actions, and the presence or absence of specific adverse drug reactions or drug interactions.

The organization of topics within this text has been carefully selected to allow the reader to progressively gain knowledge about the chemistry of drug molecules. Each chapter builds on another and, when applicable, relevant examples are cross-referenced. The authors of this text assume that the reader has a basic understanding of inorganic chemistry, organic chemistry, and biochemistry. When applicable, key concepts from these disciplines are reviewed as they apply to medicinal chemistry.

Because every atom within the drug structure is part of a specific functional group, we chose functional group identification and evaluation as the starting point of our discussion. In Chapter 2, we focus on the chemical characteristics of functional groups and the roles they can play in drug action. From there, Chapter 3 examines those functional groups that can be classified as either acidic or basic. We also explore the reasons why it is important to know the acid/base character of a drug molecule. In Chapter 4, we continue our examination of acidic and basic functional groups via introduction of the Henderson-Hasselbalch equation and review of several strategies for solving quantitative and qualitative pH and pK_a problems. Numerous examples are provided throughout that chapter to help the reader become more proficient in solving these types of problems. Similar to Chapter 3, we devote the end of Chapter 4 to selected examples designed to help the reader understand the importance of pH, pK_a, and ionization in drug therapy. In Chapter 5, we discuss how acidic and basic functional groups can form inorganic and organic salts. Additionally, we discuss how these salts influence the water/lipid solubility of a drug molecule and how this relates to various routes of administration. An emphasis is also placed on the need for a balance between water and lipid solubility and the ability to analyze a drug molecule to discern its water and lipid soluble components. The chapter ends with strategies to optimize either the water or lipid solubility of a drug molecule and the associated pharmaceutical and therapeutic advantages.

In some respects, Chapters 2 through 5 share a common thread because they sequentially discuss the roles and properties of functional groups as a whole, identify those that are acidic or basic, review a strategy to calculate the extent to which they are ionized in a given environment, and then examine how all of these characteristics contribute to the overall solubility of a drug molecule. This is extremely important for ensuring that a drug molecule can be administered to a patient via the desired route (e.g., orally, via intravenous [IV] injection, or via nasal inhaler).

In Chapter 6, we examine the types of binding interactions that can occur between a drug molecule and its biological target. Examples of each type of interaction are provided to allow the reader to become more proficient at analyzing drug molecules and identifying the types of interactions that can occur with each of its functional groups. In Chapter 7, we discuss how the stereochemistry of a drug molecule can affect its interaction with biological targets. We review chirality, stereochemical designations, and the differences between enantiomers, diastereomers, geometric isomers, and conformational isomers. A major emphasis is placed on the pharmacological and therapeutic differences that can occur between enantiomers as well as the specific advantages associated with conformational restriction of a drug molecule.

In Chapter 8, we discuss the purpose of drug metabolism and explore the metabolic transformations by which enzymes in the liver and other organs and tissues chemically alter drug molecules. The chapter includes mechanisms and examples for each type of metabolic transformation and identifies the functional groups that are susceptible to each type of transformation. Similar to Chapter 6, the overall objective is to provide sufficient detail to the reader, such that he or she becomes more

proficient at predicting possible metabolic transformations and understanding known metabolic pathways for a given drug molecule.

In Chapter 9, we introduce the concept of structure activity relationships (SARs) and relate this to many examples discussed in previous chapters. Although SARs are an essential component of the discipline of medicinal chemistry, we intentionally reserved the discussion of this topic until after the other concepts were discussed. Taken literally, an SAR defines the relationship between the chemical structure of a drug molecule (or one or more of its component functional groups) and the physicochemical or pharmacological effects it produces. As such, the text introduces the types of relationships (e.g., ionization, solubility, drug binding interactions, stereochemistry, and metabolism) prior to discussing SARs. We close the chapter with an overview of some basic concepts of molecular modification so the reader will understand the common strategies used in the design of new drug molecules as well as analogs of currently approved drugs.

The final chapter focuses on what we call "Whole Molecule Drug Evaluation," a process that requires the reader to use the evaluation skills discussed in the first nine chapters to fully assess specific attributes of known drug molecules. Unlike the end-of-chapter review questions that focus on one or two chapter-specific concepts, this final chapter emphasizes an overall analysis of individual drug molecules.

Each chapter includes a variety of examples and review questions chosen to illustrate and reinforce the concepts discussed. Additionally, Chapters 2 through 9 include Structural Analysis Checkpoint questions. These questions follow specific drug molecules throughout each of these chapters and sequentially probe their chemical nature as new concepts are presented. Answers are provided in the Appendix for all questions so readers can assess their understanding of the concepts that are presented. With perhaps a few exceptions, all examples and review questions are based on currently available drugs. The text is designed to be comprehensive with regard to the fundamental chemical concepts that govern drug action. Through conceptual discussions, examples, and applications, the text is designed to provide readers with the knowledge and skills to predict, discuss, and understand the pertinent chemistry of any drug molecule or class of drug molecules encountered.

Two resources are provided below. They have been placed in this introductory chapter for easy access. The first resource is a review of some selected chemical nomenclature and numbering that are used throughout the text. The second resource is a listing of references used in the writing of this text.

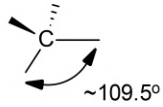
REVIEW OF SELECTED NOMENCLATURE AND NUMBERING

The following topics have been selected due to their relevance in naming and numbering specific atoms and groups in drug molecules. For a full discussion of organic chemistry and/or biochemistry nomenclature, please consult the suggested references listed at the end of this chapter.[1–17]

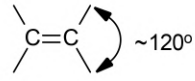
Orbital Hybridization and Bond Formation

Carbon atoms within the structure of a drug molecule are able to form single, double, or triple bonds with one another or with other atoms, such as oxygen, nitrogen, sulfur, and halogens. For this to occur, the 2*s* and 2*p* orbitals must form hybrid orbitals consisting of one *s* orbital and either one, two, or three *p* orbitals. Single bonds are comprised of sp^3 hybrid orbitals and form a tetrahedral shape with bond angles of approximately 109.5°. Double bonds are comprised of sp^2 hybrid orbitals and form a planar shape with bond angles of approximately 120°. There are two components to a double bond: an initial overlap of the two sp^2 orbitals and a side-to-side overlap of the unhybridized *p* orbitals, known as a π bond. Triple bonds are comprised of *sp* orbitals and form a linear shape with bond angles of 180°. Similar to double bonds, there are several components to a triple bond:

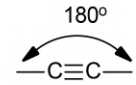
an initial overlap of the two *sp* orbitals and two orthogonal (i.e., at right angles) π bonds formed by the two sets of unhybridized *p* orbitals.



Single Bond
Tetrahedral Shape



Double Bond
Planar Shape

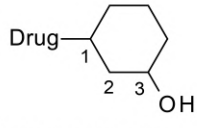


Triple Bond
Linear Shape

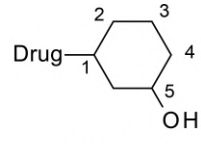
The concept of hybrid orbitals also applies to nitrogen and oxygen atoms; however, due to the presence of additional electrons, a nitrogen atom contains one lone pair of nonbonding electrons while oxygen contains two lone pairs of nonbonding electrons. The hybrid orbitals of these atoms have a similar shape; however, the bond angles are slightly different due to the lone pairs of electrons. Nitrogen is able to form single bonds with carbon, oxygen, nitrogen, and hydrogen; double bonds with carbon, oxygen, and nitrogen; and triple bonds with carbon. Oxygen is able to form single bonds with carbon, nitrogen, and hydrogen or double bonds with carbon and nitrogen.

Numbering of Alicyclic Rings

An alicyclic ring is comprised of hydrocarbon. It may contain double bonds, but it cannot be aromatic. The attachment point of an alicyclic ring such as cyclohexane or cyclopentane to a drug molecule is designated as the C_1 carbon of the ring. When a substituent (i.e., functional group) is attached to the ring, its attachment point is assigned the lowest possible number, as shown below. This also holds true whenever two or more ring substituents are present.



Correct numbering



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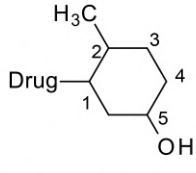

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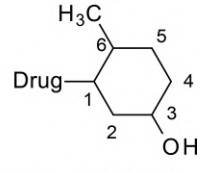
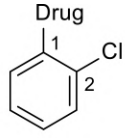


Incorrect numbering

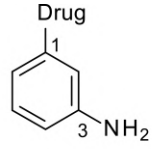
Designation of Aromatic Ring Positions

The designations *ortho*, *meta*, and *para* are commonly used to indicate the positions of substitution on an aromatic ring. These designations are relative to the attachment point of the aromatic ring to the rest of the drug molecule. This attachment point is known as the *ipso* carbon or the C_1 position. As shown below, an *ortho* designation represents a 1,2 substitution pattern on a benzene ring, a *meta* designation represents a 1,3 substitution pattern, and a *para* designation represents a 1,4 substitution pattern. In looking at the 2-methyl, 4-hydroxyl substituted ring, please note that the 2-methyl group is located *ortho* to the rest of the drug molecule, the 4-hydroxyl group is located *para* to the rest of the drug molecule, and the 2-methyl and 4-hydroxyl groups are located *meta* to one another (i.e., relative to one another, they are in a 1,3 substitution pattern or *meta* to one

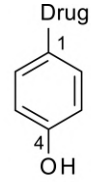
another). As seen with this last example, these designations can get more complicated with multiple substituents and multiple aromatic rings. In some cases there may be more than one *ipso* carbon, and a functional group could be *ortho* to one *ipso* carbon and *meta* to another. Readers who desire a more in-depth discussion of this topic are referred to the texts by either Graham Solomons et al[9] or Dewick[10] cited at the end of this chapter.



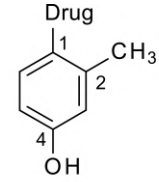
2-chloro
ortho chloro



3-amino
meta amino



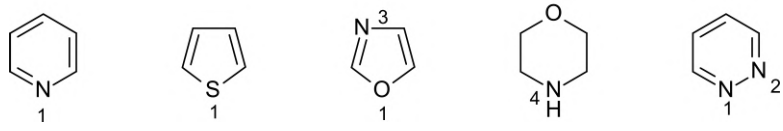
4-hydroxyl
para hydroxyl



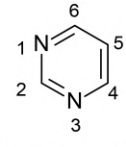
2-methyl, 4-hydroxyl
ortho methyl, *para* hydroxyl

Numbering of Heterocyclic Rings

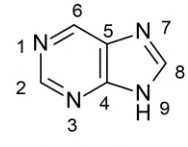
A *heterocyclic ring* contains atoms other than just carbon and hydrogen (i.e., heteroatoms). The three most prominent heteroatoms found in these rings are nitrogen, oxygen, and sulfur. When there is only one heteroatom present within the ring, it is designated as atom "1" in the ring. Similar to alicyclic rings, substituents are assigned the lowest possible number. When there are similar heteroatoms present within the ring (e.g., two nitrogen atoms), one of these is assigned as atom "1" and the other is assigned the next lowest number in sequence around the ring. When there are two different heteroatoms present within the ring, the heteroatom with the highest priority is designated as atom "1," and the other is assigned the next lowest number. Priority is determined by molecular weight; therefore, sulfur has the highest priority, oxygen has the second highest priority, and nitrogen has the lowest priority. Some examples are shown below. For additional examples of heterocyclic rings and their numbering, please consult the text by Lemke et al[1] referenced at the end of this chapter.



Two common heterocyclic rings are the pyrimidine and purine rings seen in DNA and RNA. The numbering of these ring systems is shown below.



Pyrimidine ring

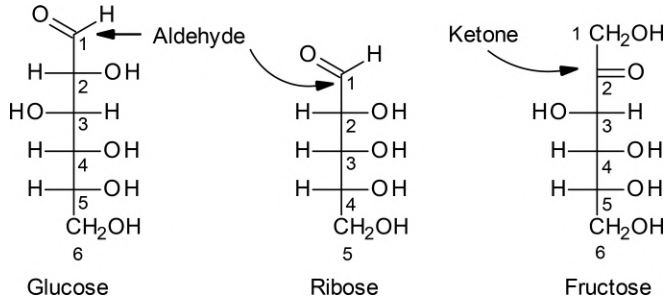


Purine ring

Numbering of Sugars

Sugars are classified as either aldoses or ketoses depending on the presence of an aldehyde or a ketone, respectively. If the sugar is an aldose, the aldehyde carbon is always designated as carbon "1," and the other carbon atoms are sequentially numbered, as shown below with the examples for glucose and ribose. If the sugar is a ketose, it is numbered beginning at the terminal carbon atom

that is closest to the ketone. In most instances, the ketone carbon is at the "2" position, as shown below with fructose.



Sugars can readily assume cyclical structures. The hydroxyl groups within a sugar molecule can react with either the aldehyde or ketone to form a hemiacetal or a hemiketal, respectively, as shown in **Figure 1-1**. Although it is possible for any hydroxyl group within the structure of the sugar to form this cyclical structure, those that form either five or six membered rings are most common. A five-member ring for a sugar is known as a *furanose ring*, while a six-member ring is known as a *pyranose ring*. The numbering does not change; however, the stereochemistry of the carbon atom used to make the hemiacetal or hemiketal can be either α or β, as described in the next section. Shown in Figure 1-1 are the cyclical versions of glucose, ribose, deoxyribose, and fructose.

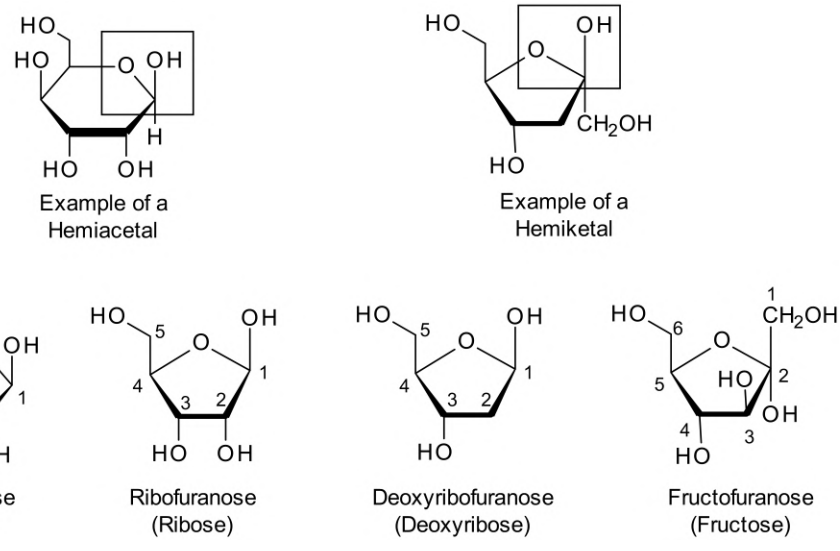


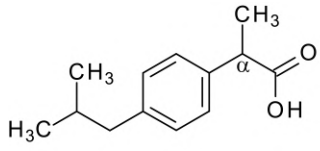
FIGURE 1-1. Examples of a hemiacetal, a hemiketal, and the cyclical structures of some common sugars.

Alpha (α), Beta (β), and Omega (ω) Designations

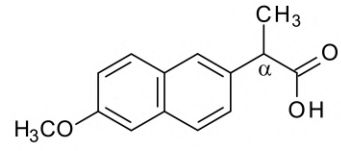
These designations are used to identify specific carbon atoms within the structure of a drug molecule. The α designation is used to indicate a carbon atom that is located directly adjacent to a carbonyl group (C=O) or a heteroatom, whereas the β designation is used to indicate the next carbon atom in the chain. Occasionally, γ and δ are used to indicate the third and fourth carbon atoms in a chain. This represents an alternative way to number carbon atoms. As you progress though different

classes of drug molecules, you will discover that some drug molecules use conventional Arabic numerals (e.g., 1, 2, 3) to number carbon atoms, whereas others use these Greek letter designations.

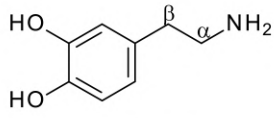
Figure 1-2 provides several examples of drug molecules that use the α/β designation. Both ibuprofen and naproxen contain a methyl group directly adjacent to a carboxylic acid. This methyl group is located at an α position, and these drugs are chemically classified as α-methylarylacetic acids. The carbon atom directly adjacent to the primary amine of dopamine is designated as α, while the next atom in this ethyl chain is designated as β. The addition of a methyl group directly adjacent to the primary amine produces α-methyldopamine. As illustrated with penicillin G and ampicillin, a single molecule can have more than one α designation. Both of these drugs contain a β-lactam ring and are classified as β-lactam antibiotics. This designation comes from the fact the nitrogen atom that is involved in the lactam bond is attached to the carbon atom that is β to the carbonyl.



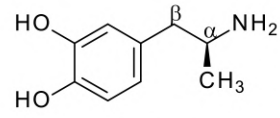
Ibuprofen



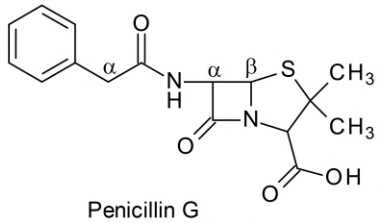
Naproxen



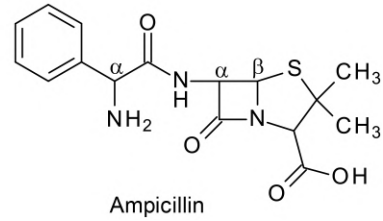
Dopamine



α-Methyldopamine



Penicillin G

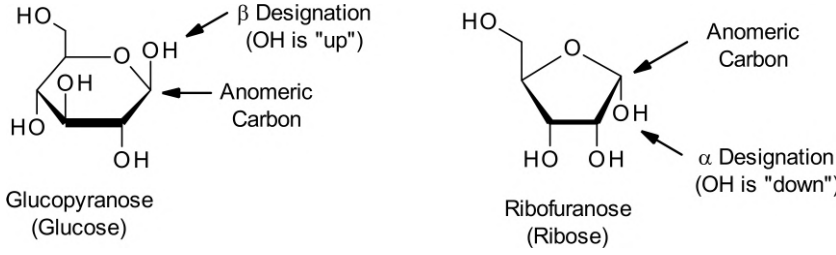


Ampicillin

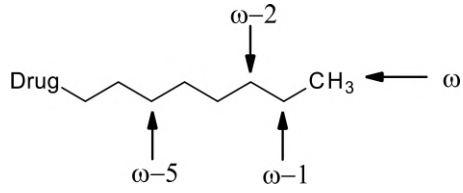
FIGURE 1-2. Examples of α and β designations.

The α/β designations are also used for cyclical sugars. Whenever an aldehyde or a ketone forms a hemiacetal or a hemiketal, a new stereochemical center is also formed. This stereochemical center is unique in that reversible reactions can easily convert linear sugars to cyclical sugars, and vice versa, allowing the chiral center to easily change. This process is known as *mutarotation*, and the chiral carbon atom is known as the *anomeric carbon*. Isomeric forms of sugars that differ only in the stereochemistry of the anomeric carbon of hemiacetals and hemiketals are known as *anomers*. The α and β designations for the stereochemistry of the anomeric carbon are based on a comparison of the stereochemistry of the anomeric carbon to the stereochemistry of the chiral center that is furthest away from the anomeric carbon. While this can get a little complicated, there is an easy way to remember these designations with the most commonly encountered sugars (e.g., glucose, ribose, deoxyribose, fructose, and galactose). Whenever, the hydroxyl group is "down,"

it is designated as α, and whenever the hydroxyl group is "up," it is designated as β. Examples using glucose and ribose are shown below.



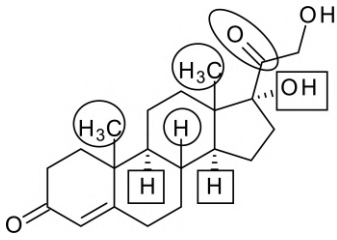
The ω designation is used to identify the carbon atom that is located at the end of an alkyl chain. Additionally, the designations ω-1, ω-2, and so on are used to designate carbon atoms that are sequentially positioned one or two atoms (or more) from the end of an alkyl chain.



Stereochemical Designations

The following designations are used to identify enantiomers and chiral centers. Please note that the term *enantiomer* refers to the drug molecule as a whole, while a chiral center is a single carbon atom. A complete discussion of these designations can be found in Chapter 7.

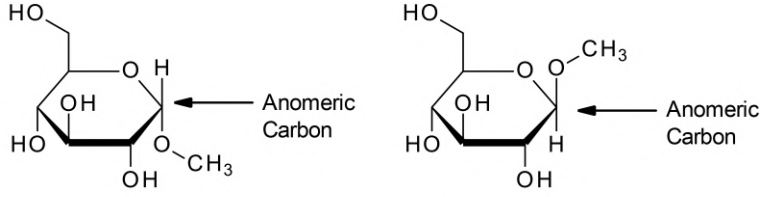
- **(+)/(–):** These designations identify the direction in which an enantiomer rotates plane polarized light. The (+) designation indicates that the enantiomer rotates plane polarized light to the right, or clockwise, while the (–) designation indicates that the enantiomer rotates plane polarized light to the left, or counterclockwise.
- ***d*/*l*:** These designations are similar to the (+)/(–) designations. The *d* designation is an abbreviation for dextrorotatory and indicates that the enantiomer rotates plane polarized light to the right, or clockwise. The *l* designation is an abbreviation for levorotatory and, similar to the (–) designation, indicates that the enantiomer rotates plane polarized light to the left, or counterclockwise.
- **D/L:** These designations refer to the absolute configuration, or steric arrangement, of the atoms about a given chiral carbon atom. The D/L designations are linked to the stereochemistry of D- and L-glyceraldehyde, and their use is primarily limited to stereochemical designations of sugars and amino acids.
- **R/S:** Similar to D/L designations, R/S designations refer to the absolute configuration of atoms about a given chiral carbon atom. These designations are preferred over the D/L designations because they can be assigned via the use of unambiguous sequence rules developed by Cahn, Ingold, and Prelog.
- **α/β:** These designations also refer to the absolute configuration of atoms about a chiral carbon atom; however, their use is primarily limited to steroids and glycosidic bonds. When used with steroids, the α designation is used for functional groups projected away from the viewer (represented as dashed lines), while the β designation is used for functional groups projected toward the viewer (represented as solid lines).



Functional groups highlighted with a square have an α designation

Functional groups highlighted with either a circle or elipse have a β designation

- The α and β designations for glycosidic bonds follow the guidelines discussed above for cyclical sugars. An example is shown in **Figure 1-3** using the α and β isomers of methyl glucopyranoside. A further discussion of the stereochemistry of glycosidic bonds can be found in any of the biochemistry texts referenced at the end of this chapter.



Methyl α-D-glucopyranoside

Methyl β-D-glucopyranoside

FIGURE 1-3. An example of α and β glycosidic bonds.

Steroid Nomenclature

Endogenous steroids are all derived from cholesterol and contain four rings labeled A through D, as shown in estradiol (**Figure 1-4**). The tetracyclic steroid nucleus consists of 17 carbon atoms and is numbered as illustrated with cholesterol. The numbering begins in the A ring and moves counterclockwise around the A and B rings (C_1 through C_{10}), clockwise around the C ring (C_{11} through C_{14}), and then counterclockwise around the D ring (C_{15} through C_{17}). All endogenous, synthetic, and semisynthetic steroids share this 17-atom backbone. The methyl groups attached to the C_{13} and C_{10} atoms are designated as C_{18} and C_{19}, respectively, and the side chain attached to the C_{17} atom begins with the C_{20} designation and is sequentially numbered, as shown in cholesterol.

The stereochemistry at the C_8, C_9, C_{10}, C_{13}, C_{14}, and C_{17} positions for all estrogens, androgens, progestins, glucocorticoids, and mineralocorticoids is the same as that shown in estradiol, cholesterol, and testosterone (Figure 1-4). The only exception here is that the C_{10} position of estrogens (e.g., estradiol) is part of an aromatic A ring and therefore not chiral. Please note that the hydrogen atoms attached to C_8, C_9, and C_{14} are often not shown in steroid structures but are assumed to be present, as shown in estradiol. The hydrogen atom attached to the C_8 position always has β stereochemistry, and the hydrogen atoms attached to the C_9 and C_{14} positions always have α stereochemistry. The naturally occurring functional groups attached to the C_{10}, C_{13}, and C_{17} positions always have β stereochemistry. The C_5 carbon is normally part of an aromatic ring, as seen with estradiol, or a double bond, as seen with cholesterol and testosterone. If the double bond is reduced, the stereochemistry of the C_5 substituent must be indicated, as shown with 5α-dihydrotestosterone.

Peptide Designations

A relatively small number of drugs are peptides or peptide mimics or include a peptide component as part of their structure. Peptides are comprised of amino acids. Each amino acid consists of a basic

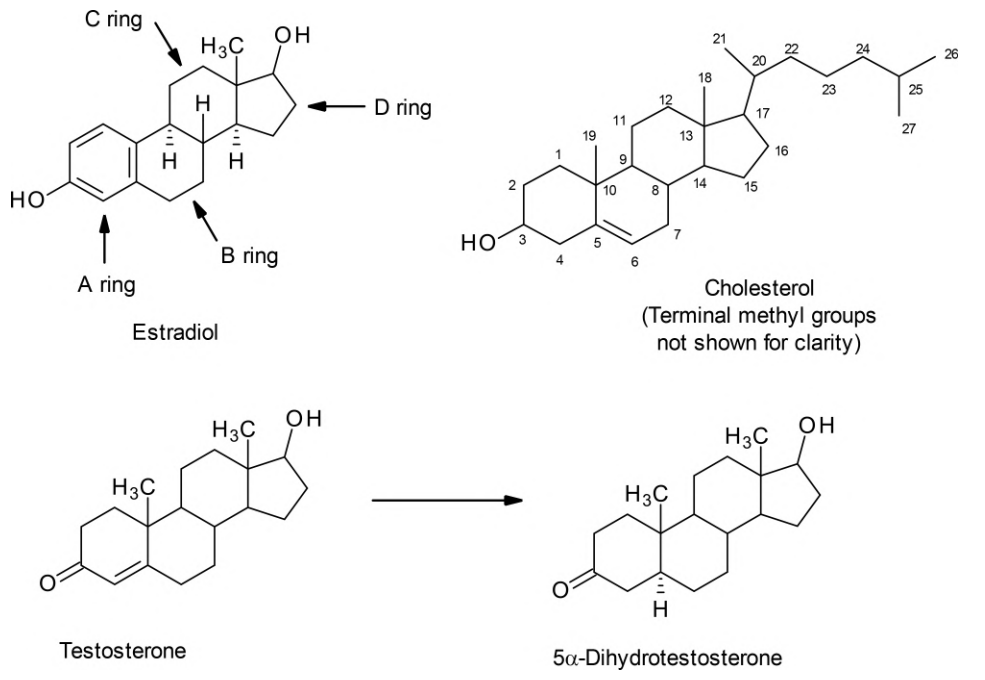
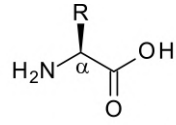


FIGURE 1-4. Standard numbering and designations for steroid based drug molecules.

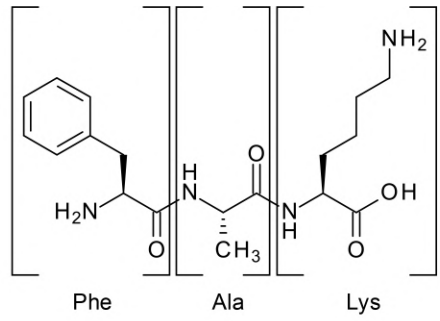
amine, an α carbon that is attached to the side chain of the amino acid, and a carboxylic acid. As previously mentioned, the α designation is assigned because the carbon atom is directly adjacent to a carbonyl atom (i.e., the carboxylic acid). A brief overview of all 20 naturally occurring amino acids is provided in Chapter 2.



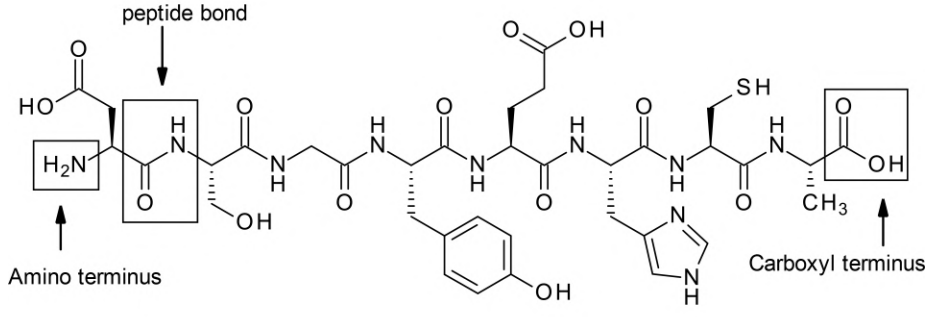
General structure of an amino acid
(R = side chain of amino acid)

A *peptide* is a polymer that consists of two or more amino acids that are linked together by amide bonds (aka peptide bonds). A *dipeptide* consists of two amino acids, a *tripeptide* consists of three amino acids, and so forth. By convention, peptide sequences are normally drawn with the amino end on the left and the carboxyl end on the right, and peptide sequences are normally read left to right, or amino terminus to carboxyl terminus. Exceptions to this occur with cyclical peptides because they don't contain right or left ends. Examples of a tripeptide and an octapeptide are shown in **Figure 1-5**. The individual amino acids have been highlighted in the tripeptide for the reader to better identify the repeating **Nitrogen—α Carbon (with side chain)—Carbonyl** sequence present in peptides and proteins. This triad sequence represents the universal pattern of amino acid building blocks. Recognition of this sequence is essential for the correct reading of a peptide or peptide mimic. The amino terminus, the carboxyl terminus, and a sample peptide bond have been highlighted with boxes in the octapeptide.

There are a large number of enzymes within the human body that catalyze the cleavage of proteins and peptides at specific sites. The designations P1 and P1′ are used to indicate the site of cleavage, with the P1 designation corresponding to the amino acid that contains the carbonyl group and the P1′ designation corresponding to the amino acid that contains the nitrogen atom. The



Tripeptide: Phe-Ala-Lys



Octapeptide: Asp-Ser-Gly-Tyr-Glu-His-Cys-Ala

FIGURE 1-5. Examples of a tripeptide and an octapeptide.

numbering of adjacent amino acids incrementally increases with the P2, P3, P4, etc., designations incrementally moving to the left (or toward the amino end) and the P2′, P3′, P4′, etc., designations incrementally moving to the right (or toward the carboxyl end). As an example, let's examine the octapeptide shown in Figure 1-5. If it is enzymatically cleaved between the valine and tyrosine residue, then the following designations would be assigned.

cleavage site

Asp - Ser - Val - Tyr - Ile - His - Cys - Ala

P3 P2 P1 P1' P2' P3' P4' P5'

These designations are important when evaluating drug molecules that inhibit a specific enzyme by acting as a peptide mimic. As an example, let's look at enalaprilat, a tripeptide mimic that inhibits the enzyme angiotensin converting enzyme (ACE). This enzyme is relatively nonspecific and can cleave dipeptide residues from the carboxyl terminus of a peptide. It is therefore classified as a dipeptidyl carboxypeptidase. Its primary action is to cleave angiotensin I, an inactive decapeptide, to angiotensin II, an octapeptide that, when bound to its receptor, is a potent vasoconstrictor. Inhibition of this enzyme by enalaprilat prevents the biosynthesis of angiotensin II and therefore is useful in the treatment of hypertension and other cardiovascular disorders. As illustrated in **Figure 1-6**, the cleavage site of angiotensin I is located between the phenylalanine and histidine residues. Thus, the P1 designation is assigned to phenylalanine and the P1′ designation is assigned to histidine.

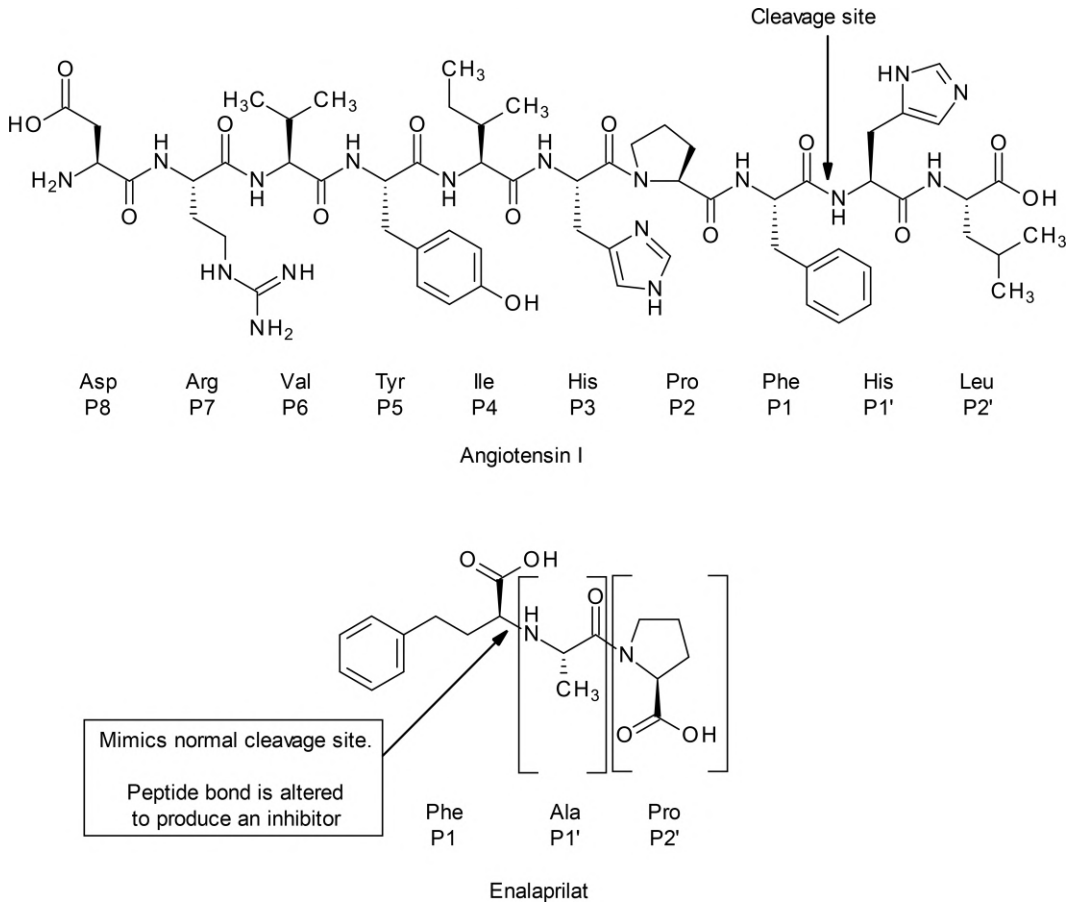


FIGURE 1-6. Angiotensin I and enalaprilat with cleavage site designations.

The other amino acids are designated according to the guidelines described above. Enalaprilat mimics the carboxyl terminal Phe—His—Leu sequence of angiotensin I. Because ACE is a relatively nonselective dipeptidyl carboxypeptidase, it can interact with enalaprilat. The phenylalanine of enalaprilat mimics the P1 amino acid, while alanine and proline mimic the P1′ and P2′ amino acids.

NUCLEIC ACID NOMENCLATURE

A number of drugs that are used to treat cancer, viral infections, and other disease states are structural analogs of naturally occurring nucleosides and nucleotides that comprise the structures of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). A *nucleoside* consists of a nitrogenous base (either a purine or a pyrimidine) and a sugar (either ribose, naturally found in RNA, or deoxyribose, naturally found in DNA). The addition of one or more phosphates to a nucleoside results in a *nucleotide*. The two common purines are adenine and guanine, while the three common pyrimidines are cytosine, thymine, and uracil. The names of these purines and pyrimidines change slightly whenever they are part of a nucleoside or a nucleotide. Since you will encounter these nucleoside and nucleotide analogs, a review of the nucleic acid nomenclature is summarized in **Table 1-1**.

TABLE 1-1. Nucleic Acid Nomenclature

Nitrogenous Base	Nucleoside (Base + Sugar[a])	Nucleotide (Base + Sugar + Phosphate)[b]
Purines		
Adenine	Adenosine	Adenosine monophosphate (Adenylate)
Guanine	Guanosine	Guanosine monophosphate (Guanylate)
Pyrimidines		
Cytosine	Cytidine	Cytidine monophosphate (Cytidylate)
Thymine	Thymidine	Thymidine monophosphate (Thymidylate)
Uracil	Uridine	Uridine monophosphate (Uridylate)

[a] The names assume that ribose is the sugar. If the sugar is deoxyribose, then the prefix "deoxy" is added to the name of the nucleoside (e.g., deoxyadenosine).

[b] The names in parentheses are commonly used to designate the ionized form of the monophosphates. Nucleotides can also be diphosphates and triphosphates.

REFERENCES CONSULTED AND RECOMMENDED

The following references were consulted in the preparation of this text. The text by Lemke et al[1] is recommended to those readers who desire a more in-depth explanation of the organic chemistry of functional groups. *Foye's*[2,3] texts and the *Wilson and Gisvold*[4] text are recommended for those readers who wish to learn more about the medicinal chemistry of specific drug molecules and specific classes of drug molecules. These texts are organized according to major drug classes (e.g., cholinergic agents, sedative/hypnotic agents, diuretics) and therapeutic uses (i.e., indications) and are comprehensive in their discussions of the medicinal chemistry of currently available drugs. The *Essentials of Foye's Principles* provides bullet point explanations of each drug and drug class and is much more "student-friendly" than classic textbooks. The Renslo text discusses some medicinal chemistry concepts; however, it is more focused on organic chemistry than medicinal chemistry.[5] The Silverman and Hollady[6] and *Burger's*[7] texts are a little more advanced in their coverage of medicinal chemistry; however, they are both excellent resources for the topics of drug design and drug development. They are recommended for those readers who desire extended discussions in these areas. The *Goodman & Gilman's*[8] text is also a valuable resource. Although it is primarily a pharmacology text, it nicely integrates pharmacology with both medicinal chemistry and therapeutics. It provides in-depth information regarding the mechanisms of action for all drugs and drug classes and is a nice complement to both the *Foye* and *Wilson and Gisvold* texts. The Graham Solomons et al,[9] Dewick,[10] *Lehninger*,[11] and Berg et al[12] texts are recommended for those readers who desire a review of specific organic chemistry or biochemistry topics. Clinical Pharmacology,[13] Facts and Comparisons,[14] Lexicomp,[15] and Micromedex[16] are excellent online resources for drug information. Each of these references contains comprehensive information for each commercially available drug. DrugBank[17] is a valuable resource for chemical properties of drugs. Along with *Foye's*[2,3] texts, these resources provide the vast majority of individual pK_a values for specific functional groups within the structures of drug molecules.

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FUNCTIONAL GROUP CHARACTERISTICS AND ROLES

LEARNING OBJECTIVES

After completing this chapter, students will be able to

- **Identify the individual functional groups that comprise the structure of a given drug molecule.**
- **Explain the general purpose of functional groups and provide specific examples of how functional groups affect drug activity.**
- **Analyze the electronic, solubility, and steric effects that an individual functional group can impart to a specific drug molecule.**
- **Explain how a specific functional group can serve different purposes on different drug molecules and how the importance of a specific functional group can vary among different drug molecules based on the influence of the adjacent functional groups.**
- **Explain how functional groups can affect therapeutic outcomes.**
- **Identify the key chemical properties of the functional groups present in amino acids, proteins, and nucleic acids.**

This chapter is written with the assumption that the reader has a basic knowledge of organic chemistry and is at least familiar with the terminology used to describe the parts of an organic molecule. The goals of this chapter are to define the term *functional group,* review the major chemical properties or characteristics inherent to any given functional group, relate these chemical properties or characteristics to the discipline of medicinal chemistry, and provide some initial examples of how these properties or characteristics are important for drug action.

Subsequent chapters provide detailed information with regard to the ionization of acidic and basic functional groups, the roles of water- and lipid-soluble functional groups, the types of chemical interactions possible between functional groups and their biological targets, the stereochemical orientation of functional groups, the specific routes of metabolism associated with specific functional groups, and how functional groups can be altered to provide a therapeutic benefit.

WHAT IS A FUNCTIONAL GROUP?

Prior to answering this question, let us begin with two objects with which everyone is familiar: an automobile and a refrigerator. Each of these machines consists of hundreds of interrelated parts

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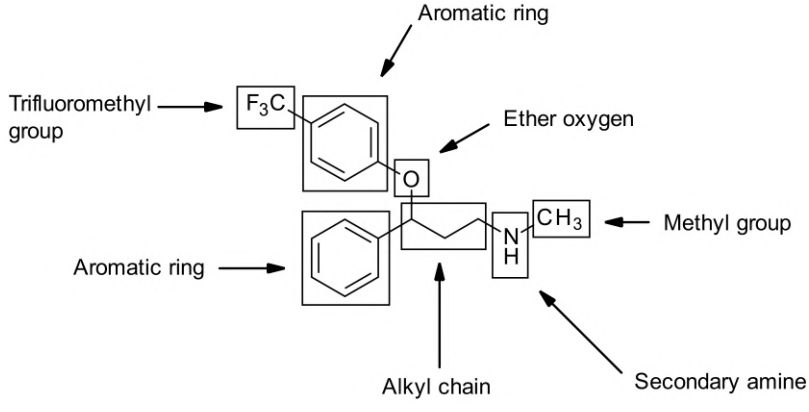
that are essential for specific functions. Some of these functions are absolute requirements, while others are desired but not considered required. As an example, let's consider the wheels. This part of the automobile is essential to allow it to move quickly and smoothly over thousands of miles. In contrast, the wheels found on most modern refrigerators are helpful in moving it a few feet for cleaning purposes or perhaps to remodel a kitchen; however, they are not considered essential. A refrigerator without wheels is still fully functional and it is still possible to move it, either using a dolly or by a couple of people pushing it. On the other hand, an automobile without wheels is no longer functional. **Table 2-1** outlines some additional comparisons between these objects. The key point of this initial example is to emphasize that these familiar objects have different uses and functions, they contain both similar and different parts, and the relative need of a given specific part varies depending on the object. Some parts, such as a power source, are essential for almost all machines, while others, such as a mirror or a horn, are not.

TABLE 2-1. A Comparison of the Specific Requirements for Selected Parts of an Automobile and a Refrigerator

Part	Automobile	Refrigerator
Power source	Essential. Requires gasoline, electricity, or a hybrid.	Essential. Requires electricity.
Doors	Essential. Required to enter and exit the vehicle.	Essential. Required to access food and keep items at proper temperature.
Windows	Essential. Required to see the road, pedestrians, traffic signs, etc. Power windows are somewhat standard but really not required.	Not needed.
Thermostat and coolant system	Essential. Required to ensure the engine does not overheat.	Essential. Required to keep food and drinks at desired temperature.
Lights	Essential. Required for nighttime driving and signaling stops and turns.	Highly desired but not essential.

Similar to automobiles, refrigerators, and other machines, drug molecules consist of various components known as *functional groups*. This is exemplified by fluoxetine, an antidepressant that selectively blocks the reuptake of serotonin. This drug is comprised of seven parts or seven specific functional groups.



From a medicinal chemistry perspective, functional groups provide specific properties and behaviors that allow drug molecules to exert their desired pharmacodynamic and pharmacokinetic effects.

For a given drug molecule, they play a significant role in the following ways:

- overall water/lipid solubility
- route of administration
- ability to interact with specific biological targets
- mechanism of action
- route of metabolism and elimination
- duration of action
- suitability for a specific therapeutic situation
- tendency to cause adverse effects or drug interactions

Definitions

Pharmacokinetic effects have been generally defined as those that explain what the body does to the drug, whereas *pharmacodynamics effects* have been generally defined as those that explain what the drug does to the body. More specifically, pharmacokinetic effects include the absorption, distribution, metabolism, and elimination (ADME) of a drug molecule, while pharmacodynamic effects include the intensity, duration, and mechanism of action of that same drug molecule. Intrinsic within a pharmacodynamic effect is the ability of the drug molecule to interact with its biological target. Biological targets can be organized into four general categories (receptors, enzymes, nucleic acids, and excitable membranes/other biopolymers) and are typically composed of proteins or nucleic acids.

An Exclusive Interview with Some Functional Groups

It is a rare occasion when a functional group will grant an interview; however, the following five functional groups have agreed to talk with Tongue-In-Cheek Productions. Functional groups A through C reside within the structure of enalaprilat, an angiotensin-converting enzyme inhibitor that is used to treat hypertension and other cardiovascular diseases; functional groups D and E reside within the structure of terbutaline, a selective β_2 agonist that is indicated for the treatment of asthma and chronic obstructive pulmonary disease (COPD).

Here are their responses to the question, *"What's your job?"*

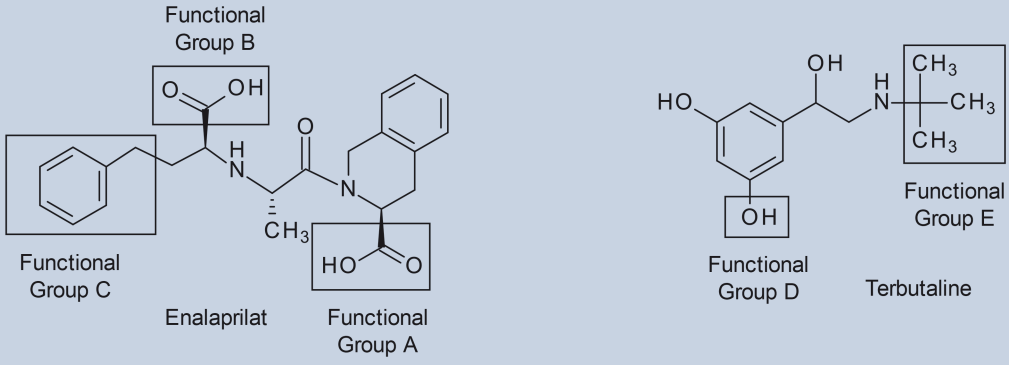
Functional Group A: "I provide the initial ionic bond that allows this drug to interact with its target enzyme."

Functional Group B: "I interact with a zinc atom involved in normal substrate catalysis."

Functional Group C: "I interact with a hydrophobic site and greatly enhance binding."

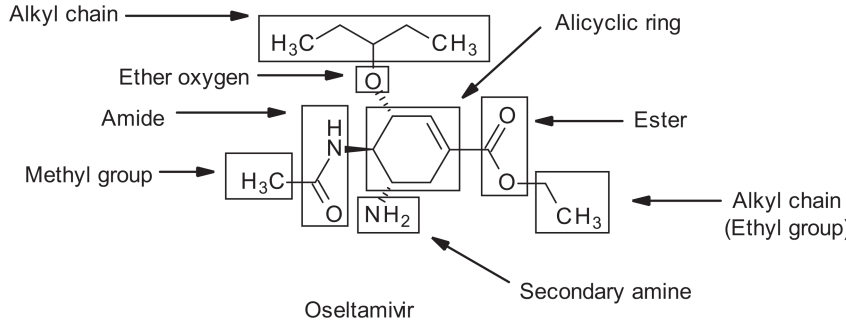
Functional Group D: "I moved one carbon away from the other OH group to decrease the metabolism of our molecule. I also help to provide selective β_2 action."

Functional Group E: "I provide selectivity for receptors located in the pulmonary system that help to decrease unwanted side effects."

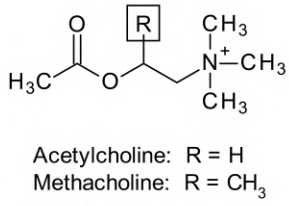


Hopefully, these testimonials help you understand why the term *functional groups* is used to describe the pieces or parts of a drug molecule. The key point here is that each individual group within a drug molecule can serve to provide one or more specific roles, tasks, or functions. As evidenced by functional groups A and B, the same functional group—a carboxylic acid in this case—can serve different roles depending on its location within the structure of the drug molecule.

When examining drug molecules, there are three overriding concepts that you should always consider. First, every atom within the structure of a drug molecule is part of a specific functional group. This was shown above with fluoxetine. An additional example of this concept is shown below using oseltamivir, an antiviral drug used to treat influenza infections.



Second, within any given drug molecule or class of drug molecules, some functional groups are more important than others. This varies among drug molecules and drug classes. As an example, consider the presence or absence of a simple methyl group. As shown in **Figure 2-1**, methacholine and simvastatin have an additional methyl group as compared with acetylcholine and lovastatin, respectively. The methyl group in methacholine offers several advantages for methacholine over acetylcholine as acetylcholine nonselectively interacts with both muscarinic and nicotinic receptors, is rapidly metabolized by acetylcholinesterase, and has an extremely short duration of action. The additional methyl group in methacholine allows it to selectively interact with muscarinic receptors and prevent its degradation by acetylcholinesterase, thus providing a longer duration of action. In comparison, while the additional methyl group in simvastatin does enhance its overall activity as compared with lovastatin, both drugs can produce very similar actions in lowering plasma cholesterol levels. Other pharmacological and pharmaceutical properties of these two drugs are essentially



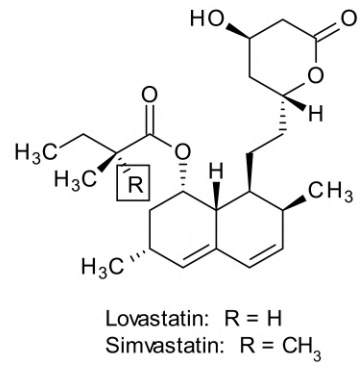
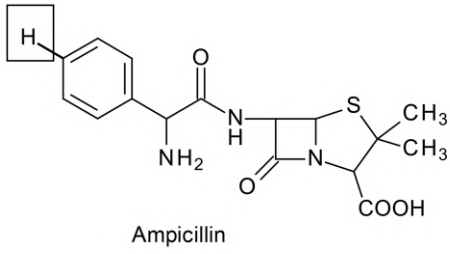


FIGURE 2-1. A comparison of the relative importance of an extra methyl group.

identical. Thus, while the methyl group in methacholine is essential for its therapeutic actions and duration, the methyl group in simvastatin, in comparison, is of much less importance.

Third, it is possible to alter functional groups to enhance activity, increase absorption, decrease adverse effects, or provide other therapeutic benefits. An example of this can be seen with ampicillin and amoxicillin (**Figure 2-2**). Both of these drugs can be used to treat a variety of infections; however, ampicillin has a slower dissolution within the gastrointestinal (GI) tract. This is due to poor water solubility and leads to decreased oral absorption and increased GI adverse effects. Replacement of the highlighted hydrogen atom with a hydroxyl group (present in amoxicillin) increases water solubility. This enhances oral absorption and decreases GI adverse effects. An additional example is seen with terbutaline in Tongue-In-Cheek's "An Exclusive Interview with Some Functional Groups." The movement of an aromatic hydroxyl group (Functional Group D) from one carbon atom to another leads to the enhanced duration and increased selectivity observed with terbutaline.



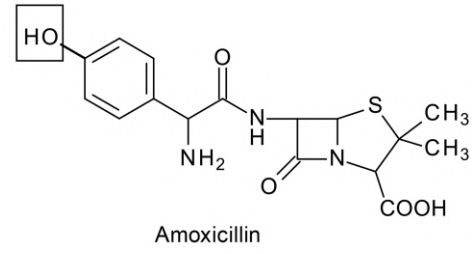
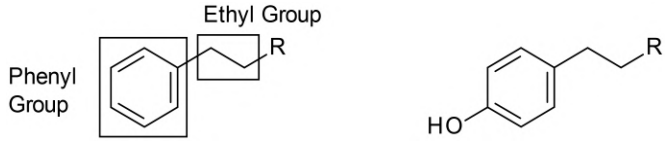


FIGURE 2-2. Ampicillin and amoxicillin.

CHEMICAL PROPERTIES OF FUNCTIONAL GROUPS

There are three major chemical properties that need to be considered for every functional group. Each functional group has an electronic effect, a solubility effect, and a steric effect that needs to be considered when evaluating the overall pharmacodynamic and pharmacokinetic properties of any given drug molecule. Prior to proceeding, there are two key points to keep in mind. First of all, the addition of a single functional group to a given molecule affects the overall electronics, solubility, and steric dimensions of that molecule. *It is impossible for a functional group to alter only one of these properties.* As an example, consider a drug molecule that contains an unsubstituted phenylethyl

group. The addition of a *para* hydroxyl group influences the electron density of the phenyl ring through its ability to interact with the aromatic electrons.

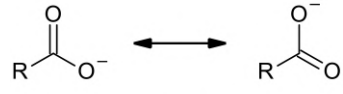


Additionally, the ability of this hydroxyl group to form hydrogen bonds increases the water solubility of the molecule. Finally, since the hydroxyl group is larger than the original hydrogen atom, its addition changes the overall steric dimensions of the molecule. The specifics of these changes are subsequently addressed; however, the key point here is that a single functional group affects the overall electronic, solubility, and steric profile of a drug molecule. The second key point is that the overall effect of a given functional group depends on all other functional groups surrounding it or attached to it. As discussed in the next section, the presence or absence of adjacent functional groups can drastically affect the chemical properties of a specific functional group.

Electronic Effects

The electronic effect of a functional group is measured by its ability to either donate its electrons to adjacent atoms or functional groups or pull or withdraw electrons from adjacent atoms or functional groups. There are two main components that comprise the overall electronic effect of a functional group: its ability to participate in resonance and its intrinsic inductive effects. Let us examine each of these components separately.

Resonance occurs when electrons are shared among a group of atoms that have *adjacent double bonds and lone pairs of electrons*. Because the electrons are equally shared, the overall structure is actually a hybrid of all of the possible resonance structures. As seen with a carboxylic acid, resonance structures can exist within a functional group. In this example, the negative charge is equally shared across the two oxygen atoms. The ability to allow a positive or negative charge to be shared among multiple atoms is extremely important since it enhances the acidity or basicity of specific functional groups. This is discussed in more detail in Chapter 3.



Resonance structures
of a carboxylic acid

Resonance structures can also occur when a functional group donates or withdraws electrons from *adjacent groups*. As shown in **Figure 2-3**, an aromatic hydroxyl group, also known as a phenol, can share its electrons with the adjacent aromatic ring. In this case, the oxygen atom donates its electrons to the aromatic ring. As illustrated by the resonance structures, the negative charge can be equally shared by the three aromatic carbon atoms either *ortho* or *para* to the phenolic group. Similar resonance structures are seen with aromatic amines and adjacent ethers. As mentioned above, the ability of aromatic hydroxyl groups and aromatic amines to donate electrons into an adjacent aromatic ring produces significant effects on their acidity and basicity, respectively.

In contrast to a phenolic group, a nitrile group (**Figure 2-4**) withdraws or removes electrons from the aromatic ring. In this case, the nitrile group acquires a negative charge, and the aromatic ring acquires a positive charge. Similar to the phenolic group, the positive charge can be equally shared among the aromatic carbons *ortho* or *para* to the nitrile. Similar resonance structures are seen when nitro groups and carbonyl groups are directly attached to an aromatic ring. Examples of functional groups that contain a carbonyl group include esters, amides, and aldehydes.

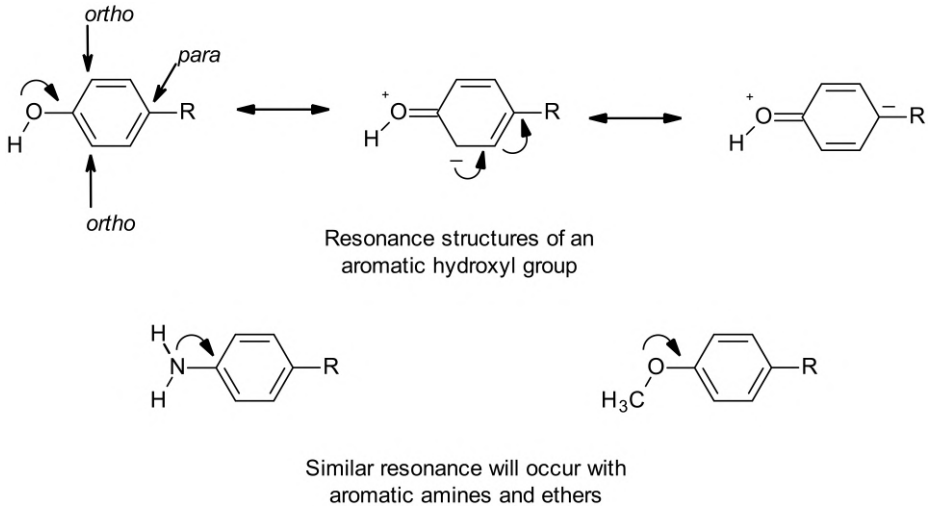


FIGURE 2-3. Examples of functional groups capable of electron donating through resonance.

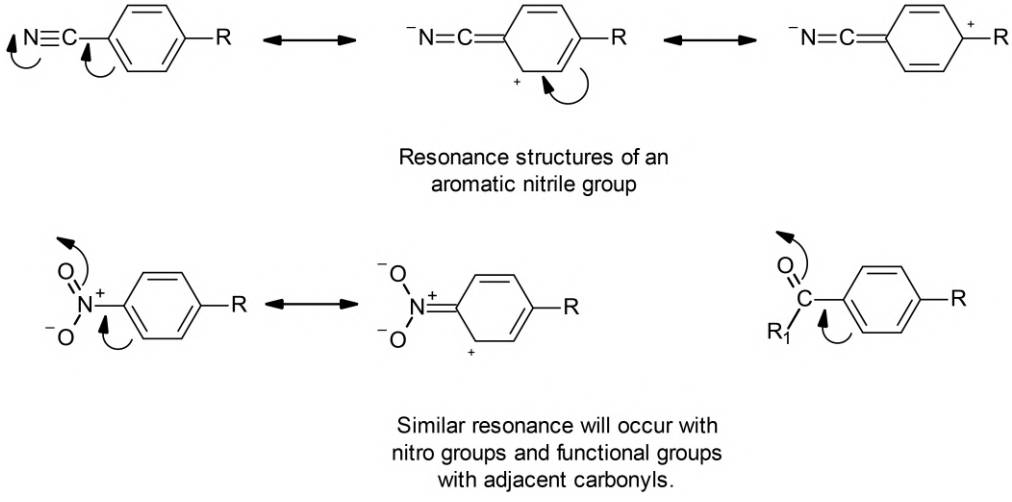


FIGURE 2-4. Examples of functional groups capable of electron withdrawing through resonance.

The *intrinsic inductive* character or nature of an atom or functional group depends on its overall *electronegativity*, a chemical property that defines the ability of an atom or functional group to attract electrons toward itself and away from other atoms or functional groups. The larger the electronegativity, the greater the ability of an atom or functional group to attract electrons. The electronegativity values for atoms commonly seen in drug molecules are shown in **Table 2-2**.

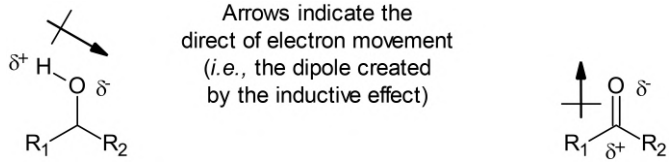
There are a few key points to remember when looking at this table:

- Fluorine, oxygen, chlorine, and nitrogen have the highest electronegativities, respectively, among all of the atoms in the periodic table.

TABLE 2-2. Electronegativity Values for Atoms Commonly Seen in Drug Molecules

Atom	Electronegativity Value
F	3.98
O	3.44
Cl	3.16
N	3.04
Br	2.96
I	2.66
S	2.58
C	2.55
H	2.20
P	2.19

- With the sole exception of fluorine, oxygen inductively attracts electrons from all other atoms.
- Oxygen, nitrogen, and all four halogens (i.e., F, Cl, Br, and I) inductively attract electrons from carbon.
- The above inductive effects (due to differences in electronegativity) create a partial charge separation between the atoms comprising a functional group. This partial charge separation results in a dipole. Two examples of dipoles are shown below. These dipoles are very important in enhancing water solubility (discussed later in this chapter) and allow a drug molecule to interact with its biological target (discussed in depth in Chapter 6).



As previously mentioned, the presence or absence of adjacent functional groups can affect the chemical properties of a given functional group. Let us revisit the phenylethyl group to see how an adjacent phenyl ring can affect the overall electronic effect of a hydroxyl group. **Figure 2-5** shows

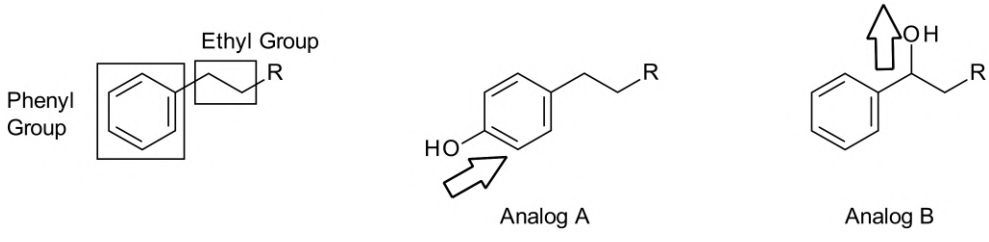


FIGURE 2-5. Varying electronic effects of a hydroxyl group based on adjacent functional groups. The arrows represent the flow of electrons.

the original phenylethyl group along with two analogs, one that contains an aromatic hydroxyl group and one that contains an aliphatic (i.e., nonaromatic) hydroxyl group. As discussed above and shown in Figure 2-3, the aromatic hydroxyl group of Analog A is involved in resonance with the adjacent aromatic ring. This ability to form multiple resonance structures overrides the inductive effect of the oxygen atom and allows this functional group to act as an electron donating group. In contrast, the aliphatic hydroxyl group of Analog B cannot participate in resonance. Thus, its electronic effect is solely the result of its inductive effect. Because oxygen is more electronegative than carbon, an aliphatic hydroxyl group acts as an electron withdrawing group.

Electron Donating Functional Groups

The most commonly seen electron donating groups are shown in **Figure 2-6**. A few key points need to be made here. First, functional groups that contain a lone pair of electrons, such as a hydroxyl group, an aromatic amine, an aromatic thiol, or an ether (e.g., methoxy group), can donate electrons into a phenyl or aromatic ring system as previously discussed. Second, negatively charged functional groups, such as a carboxylic acid, can donate electrons to adjacent atoms through induction. This electron flow allows the carboxylic acid, as well as other acidic functional groups, to enhance its ionization. This is further discussed in Chapter 3. Finally, alkyl groups, such as a methyl group or an ethyl group, can serve as electron donating groups through induction.

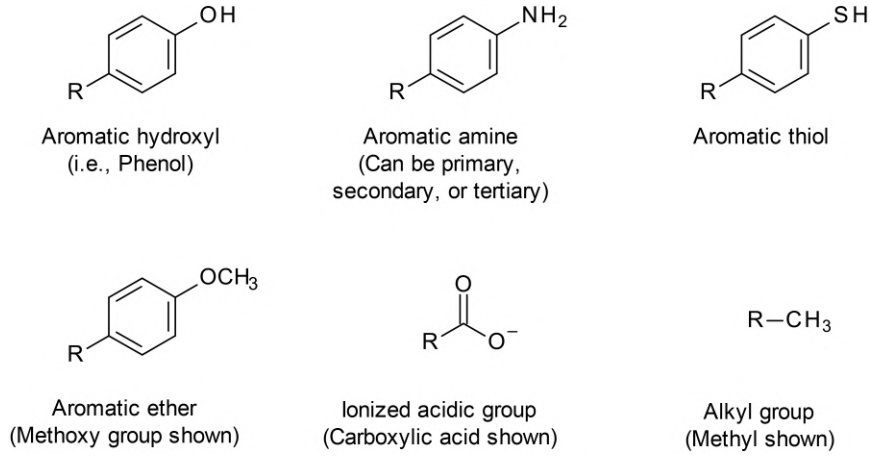


FIGURE 2-6. Common electron donating groups.

Electron Withdrawing Functional Groups

The most commonly seen electron withdrawing groups are shown in **Figure 2-7**. Similar to electron donating groups, there are a few key points to remember. First, halogens, a trifluoromethyl group, as well as positively charged functional groups, such as an ionized amine, pull or withdraw electrons through induction. Resonance is not possible for these functional groups since they cannot move or equally share electrons. Second, when hydroxyl groups, sulfhydryl groups, and ether groups are not adjacent to either an aromatic ring or a conjugated system, they act as electron withdrawing groups as a result of their inductive effects. Finally, all of the other groups shown in Figure 2-7 can withdraw electrons through either ***resonance*** or ***induction***. Adjacent functional groups, as well as the presence or absence of direct attachment to an aromatic ring, determine the relative involvement of these two processes.

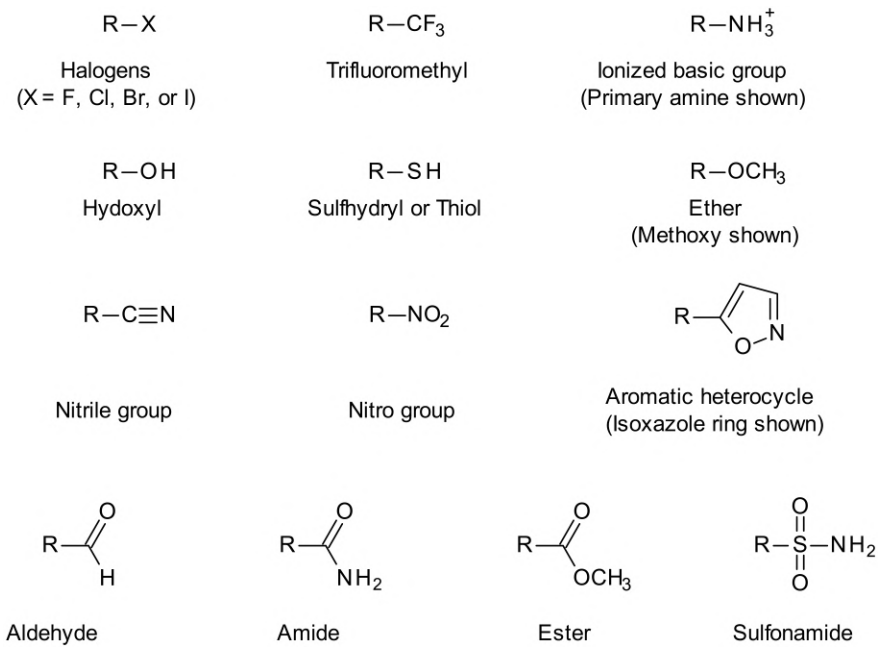
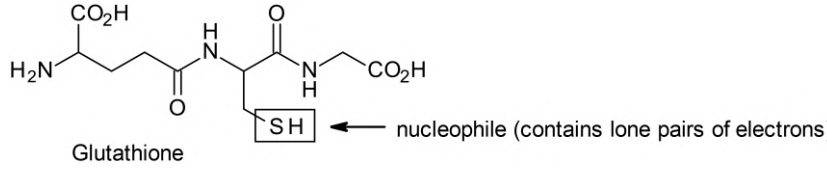


FIGURE 2-7. Common electron withdrawing groups.

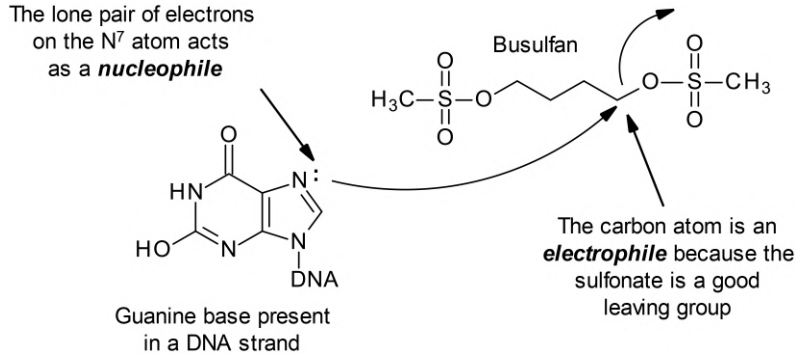
Nucleophilic and Electrophilic Functional Groups

Another aspect of the electronic nature of functional groups is their ability to act as nucleophiles and electrophiles. As the name implies, a *nucleophilic group* (i.e., a nucleophile) is "nucleus loving" and thus attracted to the positive charge present in the nucleus of an atom. Nucleophilic groups contain either a negative charge or a lone pair of electrons that can form a covalent bond with a biological target, drug molecule, or endogenous molecules. It is also important to consider the relative electronegativity of the atom that bears one or more lone pair of electrons. Atoms that have a higher electronegativity have electrons closer to the nucleus and are therefore less nucleophilic. For example, fluorine has three sets of nonbonding electrons and is very electronegative. As a result, it is not nucleophilic. Nitrogen, on the other hand, has one set of nonbonding electrons, is substantially less electronegative, and is a fantastic nucleophile! Some of the abovementioned electron donating groups meet these criteria and can act as nucleophiles. Nucleophilic functional groups are involved in the formation of hydrogen bonds as well as with the irreversible bonds formed in the mechanisms of alkylating agents, a select number of drug molecules, and a few endogenous molecules (e.g., the sulfhydryl group found on glutathione). Three common nucleophiles are hydroxyl groups, sulfhydryl groups, and some nitrogen containing functional groups. As discussed in Chapter 8, the sulfhydryl group of glutathione is very important in the inactivation of highly reactive intermediates.



Opposite of nucleophilic functional groups are electrophilic functional groups. As the name implies, an *electrophilic group* (i.e., an electrophile) is "electron loving." Examples of electrophilic functional groups are those that contain positive charges, a conjugated double bond system, or a good leaving group, such as a halogen or an ester. As alluded to previously, a nucleophilic functional

group can attack an electrophilic functional group to form a covalent bond. An example of this is shown below with busulfan, an antineoplastic drug that exerts its mechanism of action by alkylating DNA. The sulfonate functional group in busulfan is ***electrophilic*** and quickly forms a covalent bond with the ***nucleophilic*** N^7 atom of a guanine residue present within the structure of DNA.



To close out this section, let us examine a specific situation in which an electron withdrawing group bestows a therapeutic benefit. Penicillin G and penicillin V are naturally occurring penicillins; however, the oral bioavailability of penicillin V is superior to that found with penicillin G. As seen in **Figure 2-8**, the only structural difference between penicillin G and penicillin V is the presence of an ether oxygen atom in penicillin V. Before proceeding, let us evaluate this ether oxygen atom. As previously discussed, the oxygen atom of an ether can be either electron donating (via resonance) or electron withdrawing (via induction). In the case of penicillin V, it is both. The ether oxygen can donate electrons to the adjacent aromatic ring via resonance and withdraw electrons via induction from the adjacent methylene carbon and the other atoms attached to this methylene unit. This results in an overall electron flow that withdraws electrons from the right side of the drug molecule and donates them into the phenyl ring as illustrated in Figure 2-8. It is this electron flow, specifically

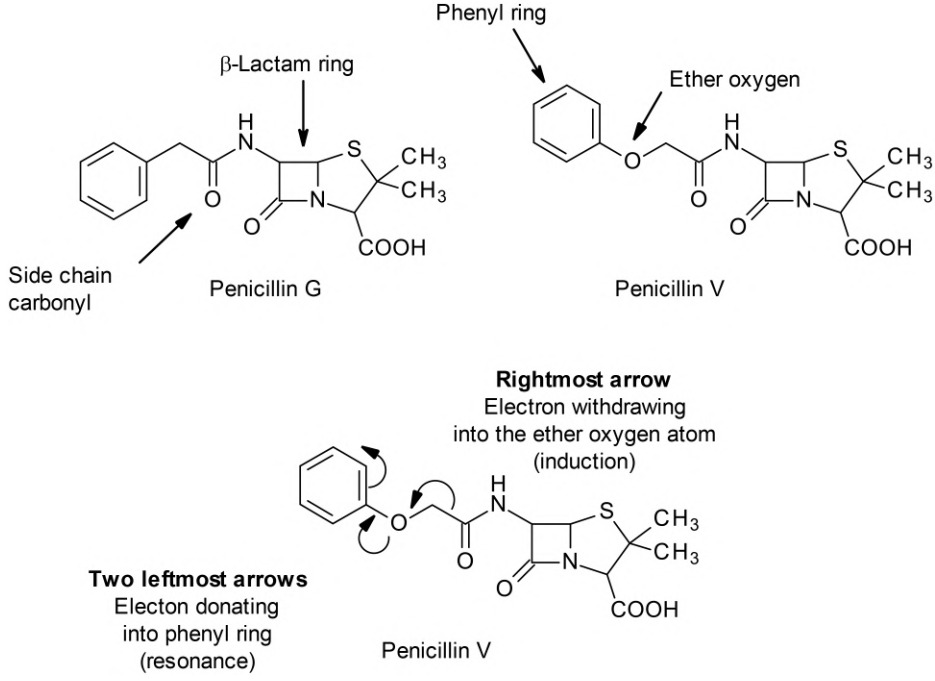


FIGURE 2-8. Comparison of penicillin G and penicillin V.

the electron withdrawing effect, that allows penicillin V to have a better oral bioavailability than penicillin G.

In the acidic environment of the stomach, penicillin G undergoes significant acid-catalyzed degradation. The key mechanistic step involves the lone pair of electrons present on the side chain carbonyl group. In an acidic environment, these electrons attack the carbonyl carbon of the β-lactam ring (**Figure 2-9**). The ultimate effect is destruction of the β-lactam ring in the stomach and inactivation of penicillin G. In contrast, the presence of the ether oxygen withdraws the lone pair of electrons from the side chain carbonyl as discussed above. This decreases the availability of the lone pair of electrons to attack the carbonyl carbon of the β-lactam ring, thus allowing penicillin V to be much more stable in the acid environment of the stomach. From a therapeutic standpoint, both of these drugs have a similar spectrum of antibiotic activity; however, penicillin V can be used orally, while penicillin G must be administered either intravenously (IV) or intramuscularly (IM).

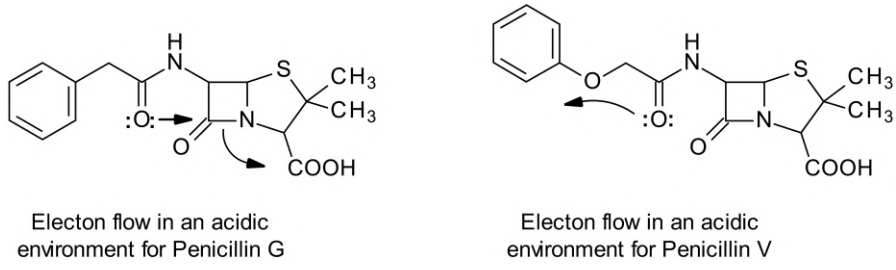


FIGURE 2-9. Overall electron flow of penicillin G and penicillin V.

Solubility Effects

The overall water and/or lipid solubility of a drug molecule affects its route(s) of administration, distribution within the body, metabolism, duration of action, and route(s) of elimination. This overall solubility is a sum of the contributions of each functional group present within the drug structure. The primary purpose of this section is to identify those functional groups that confer water solubility and those that confer lipid solubility. Similar to electronic effects, the overall solubility contribution of a specific functional group can vary depending on adjacent groups. Further explanations with respect to the importance of water and lipid solubility, partition coefficients, the ability to analyze a drug molecule and identify its water-soluble and lipid-soluble components, the need for a balance between water and lipid solubility, the advantages of increasing either water or lipid solubility, and common strategies to alter solubility in a desired direction are discussed in detail in Chapter 5.

Water-Soluble Functional Groups

Functional groups that enhance the water solubility of a drug molecule are often referred to as *hydrophilic functional groups*. There are two major properties that contribute to the water solubility of a functional group:

- ability to ionize
- ability to form hydrogen bonds

Let us examine each of these properties separately. Acidic and basic functional groups are capable of ionization and can become negatively or positively charged, respectively. A permanently charged quaternary ammonium group can also provide a positive charge; however, this functional group is only seen in a small number of drug molecules. The ionization of a functional group imparts an increase in the water solubility of a drug molecule due to the ability of the functional group to form an ion-dipole interaction with water. As such, it is important that you can identify acidic and basic

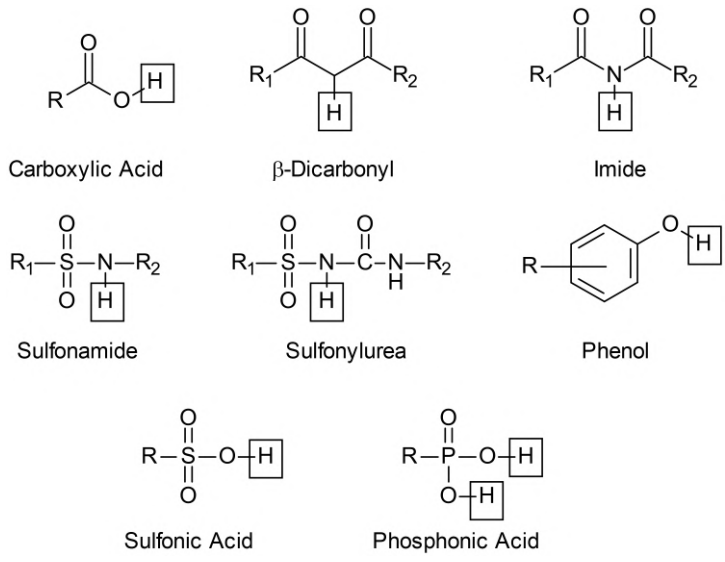


FIGURE 2-10. Common acidic functional groups. Acidic hydrogen atoms have been highlighted with boxes.

functional groups. The most common acidic and basic functional groups are shown in **Figures 2-10 and 2-11**, respectively. Chapter 3 provides a more detailed explanation of the acidic and basic nature of these groups as well as several additional functional groups.

A *hydrogen bond* is a specialized type of interaction between two dipoles (i.e., a dipole–dipole interaction) that occurs whenever a hydrogen atom serves as a bridge between two electronegative atoms. In this type of bond, the hydrogen atom is covalently bound to one atom and noncovalently

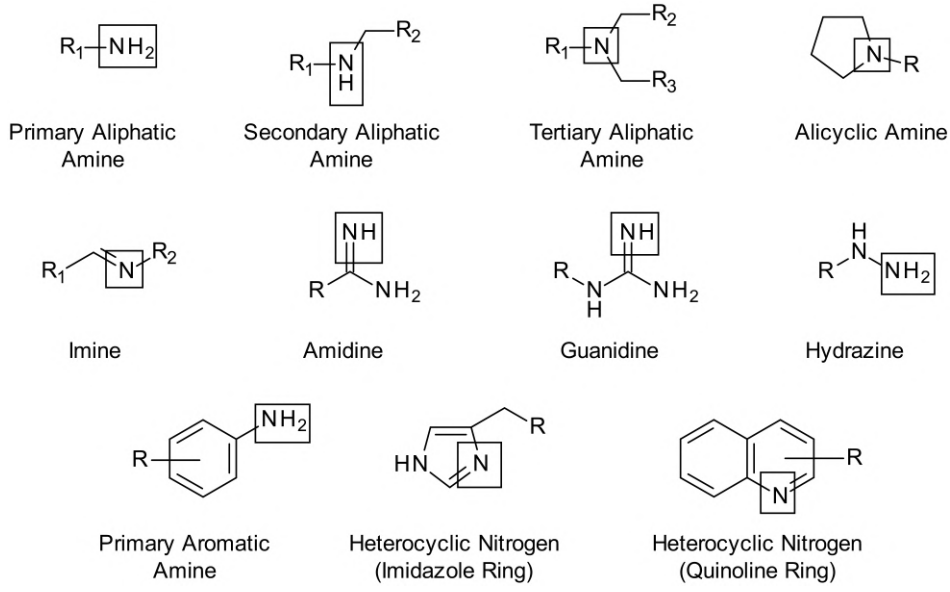
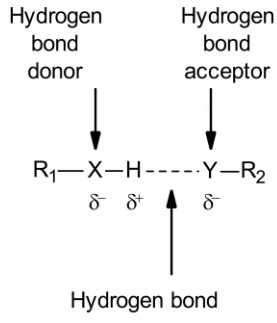
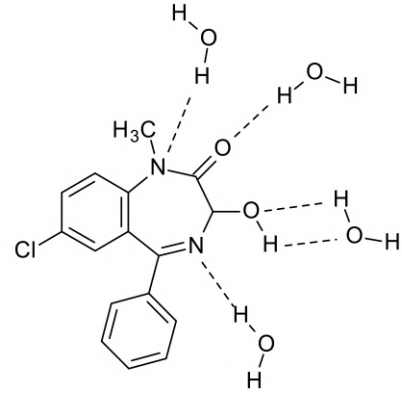


FIGURE 2-11. Common basic functional groups. Basic nitrogen atoms have been highlighted with boxes.

bound to the other. A general representation of a hydrogen bond is shown below. The atom that is covalently bound to the hydrogen atom is known as the *hydrogen bond donor*, and the atom that is noncovalently bound to the hydrogen atom is known as the *hydrogen bond acceptor*. Thus, in the representation below, atom X is the hydrogen bond donor and Y is the hydrogen bond acceptor. In general, oxygen and nitrogen are the most common hydrogen bond donors and acceptors. A water molecule can act as both a hydrogen bond donor and an acceptor, as shown below. Thus, functional groups that are capable of forming hydrogen bonds can form these types of bonds with water and increase water solubility.





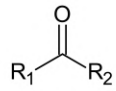
Functional groups capable of forming hydrogen bonds are shown in **Figure 2-12**. They can be divided into three groups. Functional groups, such as ketones, ethers, and esters, lack a hydrogen atom and therefore can be hydrogen bond acceptors but not hydrogen bond donors. Some heterocyclic nitrogen atoms, such as that seen in a pyrrole ring, can only act as hydrogen bond donors, because their lone pair of electrons is part of the aromaticity of the ring. Finally, a number of functional groups contain both a hydrogen atom and a lone pair of electrons and are able to act as either a hydrogen bond donor or a hydrogen bond acceptor. Examples of this latter group are hydroxyl groups, amides, carbamates, and ureas. Hydrogen bonds are discussed further in Chapter 6 since they are also very important for the interactions between a drug and its biological target.

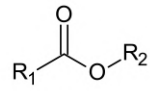
Lipid-Soluble Functional Groups

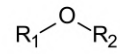
Functional groups that enhance the lipid solubility of a drug molecule are often referred to as *hydrophobic* or *lipophilic functional groups*. Functional groups that lack the ability to either ionize or form hydrogen bonds tend to impart a measure of lipid solubility to a drug molecule. Common lipid-soluble functional groups are shown in **Figure 2-13** and include unsubstituted aromatic rings, alkyl groups (aka aliphatic side chains), unsaturated carbon rings (aka alicyclic rings), and halogens. Fluorine is not included in the list of halogens because its effects on solubility can vary. As shown in Figure 2-12, fluorine can act as a hydrogen bond acceptor and enhance water solubility; however, the substitution of a hydrogen atom with a fluorine atom often slightly enhances the lipid solubility of a drug molecule.

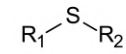
Esters can be viewed as either water-soluble or lipid-soluble functional groups depending on the atoms or groups attached to them. As an example, let us consider the three esters shown below with the assumption that the "R" group is identical for all of the molecules. All three of these functional groups contain an identical ester bond (-CO_2-). The oxygen atoms common in all three of these esters can participate in hydrogen bonds with water and thus contribute to the water solubility of the drug molecule. The difference lies in atoms/groups that are attached to the ester oxygen atom. The ester present within the structure of molecule A contains an ionized phosphate ester and is thus designated as a water-soluble ester. In contrast, the esters present within the structures of molecules B and C contain methyl and dodecenoyl esters, respectively. These alkyl chains do not

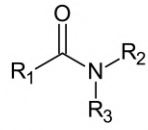
Hydrogen Bond Acceptors

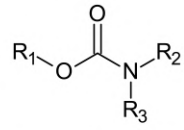

Ketone

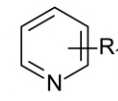

Ester


Ether


Thioether

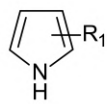

Disubstituted Amides


Disubstituted carbamates

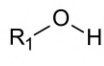

Heterocyclic nitrogens (Pyridine ring shown)

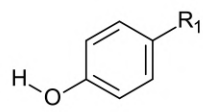

Fluorine

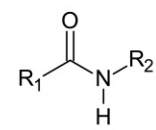
Hydrogen Bond Donors

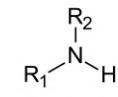

Heterocyclic nitrogens (Pyrrole ring shown)

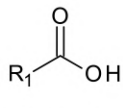
Hydrogen Bond Acceptors and Donors

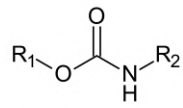

Hydroxyl

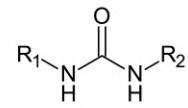

Phenol


Amide


Primary and secondary unionized amines


Unionized carboxylic acid


Carbamate


Urea

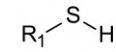
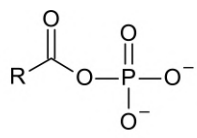
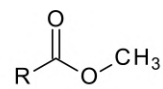
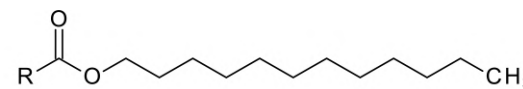

Thiol

FIGURE 2-12. Functional groups capable of forming hydrogen bonds.

interact with water and, thus, are designated as lipid-soluble esters. In general, when viewing an ester and evaluating its overall water/lipid solubility, you need to consider both the CO_2 portion and what is connected to it.


Compound A


Compound B


Compound C

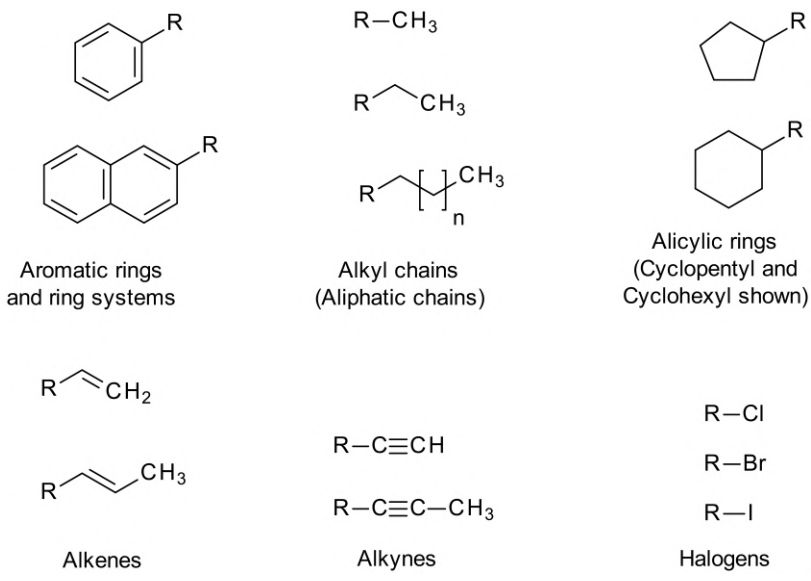


FIGURE 2-13. Lipid-soluble functional groups.

A similar situation exists for ethers, phenols, and aromatic amines. The ether oxygen atom, the phenolic hydroxyl group, and the amine attached to the aromatic ring all contribute to the overall water solubility of the drug molecule, while the alkyl chains and aromatic rings contribute to the overall lipid solubility of the drug molecule. As a suggestion to avoid any confusion here, be as specific as possible when identifying the water and lipid components of these functional groups (**Figure 2-14**).

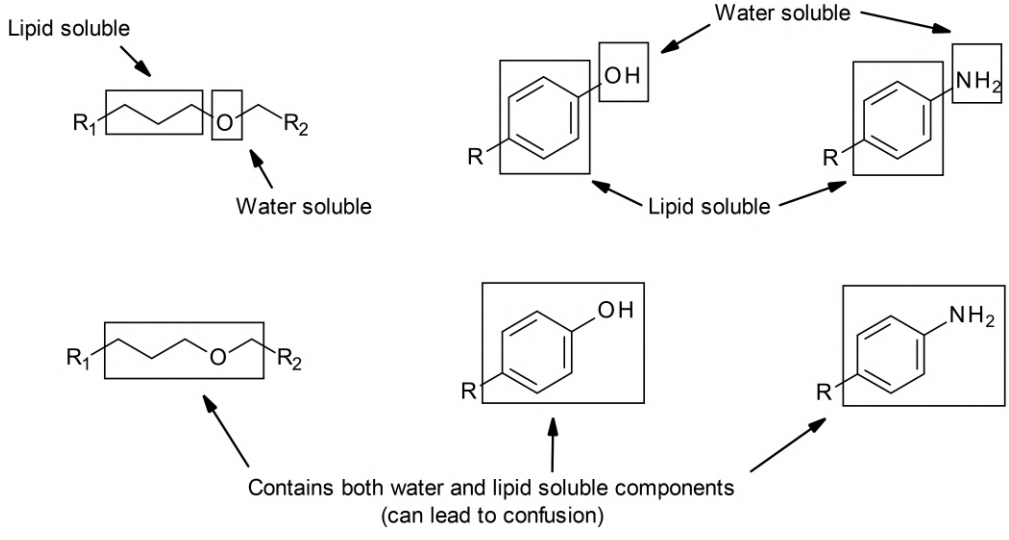


FIGURE 2-14. Suggested designations to avoid confusion or ambiguity.

Steric Effects

Each functional group has a finite size or steric dimension that contributes to the overall conformation or three-dimensional shape of a given drug molecule. Obviously, some functional groups are larger and bulkier than others, and it is impossible for two atoms or functional groups to occupy the

same space. Additionally, the binding sites present at its biological target must be able to accommodate the size and shape of each functional group.

The addition of functional groups to a drug molecule based primarily on their steric effects can provide a number of therapeutic benefits for a drug molecule, including

- increased selectivity for its biological target
- enhanced binding interactions with its biological target
- favorable alteration of its rate of metabolism

Increased Selectivity

We have already seen one example of increased selectivity for a biological target with acetylcholine and methacholine (Figure 2-1). While acetylcholine nonselectively interacts with both muscarinic and nicotinic receptors, the addition of a methyl group to the β carbon of acetylcholine provides methacholine, an analog that is selective for muscarinic receptors. The selectivity results from the fact that the muscarinic receptor has additional space that can accommodate an extra methyl group at this position, whereas the nicotinic receptor does not.

An additional example can be seen with epinephrine and synthetic adrenergic analogs. Epinephrine is a naturally occurring hormone/neurotransmitter that acts as an agonist at all α and β adrenergic receptors. When the size of the alkyl group connected to its secondary amine is increased, resulting drugs tend to be more selective for the β receptor than the α receptor. An example of this is seen in **Figure 2-15** with isoproterenol. Replacement of the methyl group with an isopropyl group results in a drug that provides selective agonist activity at the β_1 and β_2 receptors. Similar to the example with muscarinic and nicotinic receptors, the β receptors can accommodate a larger *N*-group than α receptors. In addition to a number of other physiologic effects, agonist activity at β_1 receptors produces contraction of vascular and cardiac smooth muscle, whereas agonist action at β_2 receptors produces bronchial smooth muscle relaxation. Due to its cardiovascular effects at the β_1 receptor, isoproterenol is useful in treating bradyarrhythmias and AV nodal block. Agonists at the β_2 receptor are useful in the treatment of asthma and chronic obstructive pulmonary disease (COPD); however, nonselective β agonists (such as isoproterenol) can cause unwanted adverse drug reactions due to their ability to constrict vascular and cardiac smooth muscle. Similar to the example with acetylcholine and methacholine, selectivity for the β_2 receptor can be achieved by adding an additional methyl group to take advantage of the different dimensions of these receptors. The *t*-butyl group present within the structure of albuterol (Figure 2-15) allows it to be selective for the β_2 receptor and thus an appropriate drug to treat asthma and COPD.

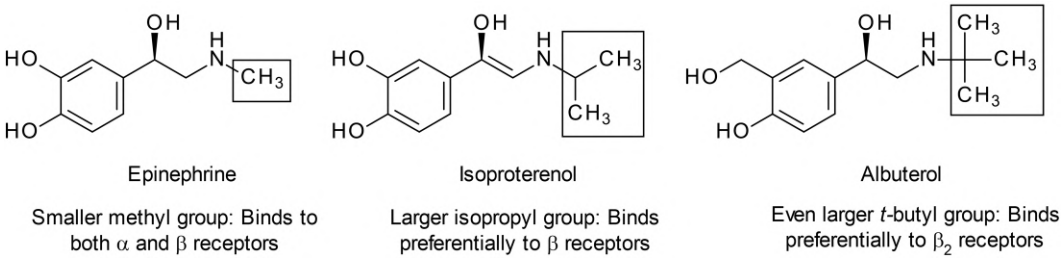


FIGURE 2-15. Comparison of the *N*-alkyl groups of epinephrine, isoproterenol, and albuterol.

Enhanced Binding Interactions

The interaction between a drug molecule and its biological target relies on the ability of the drug molecule to adopt a conformation that is complementary to the three-dimensional shape of the

biological target. Some drugs have a considerable amount of flexibility and can orient their functional groups in different conformations. The energy released when a drug molecule begins to interact with its biological target can be used to allow it to rotate about its single bonds to adopt the specific conformation required by the biological target. However, if the energy required to adopt the proper conformation is similar to or exceeds the energy released through the binding interaction, then the overall binding affinity of the drug for its biological target can be significantly diminished. This can lead to a decrease in the overall potency of the drug and the need to administer higher doses.

One way to enhance the interaction of a drug with its biological target is to decrease the conformational flexibility and essentially lock the drug in its active conformation. This concept is discussed in more detail in Chapter 7; however, one way to accomplish this is by adding adjacent functional groups that sterically hinder the rotation of specific bonds. An example can be seen in the comparison of diclofenac and fenoprofen, two nonsteroidal anti-inflammatory drugs (NSAIDs). Both of these drugs exert their mechanism of action by inhibiting the enzyme cyclooxygenase. This enzyme exists in two isoforms, cyclooxygenase-1 (COX-1) and cyclooxoygenase-2 (COX-2). Diclofenac and fenoprofen, as well as other NSAIDs discussed in this text, nonselectively inhibit both COX-1 and COX-2. As illustrated in **Figure 2-16**, both drugs interact with cyclooxygenase enzymes through three key interactions.

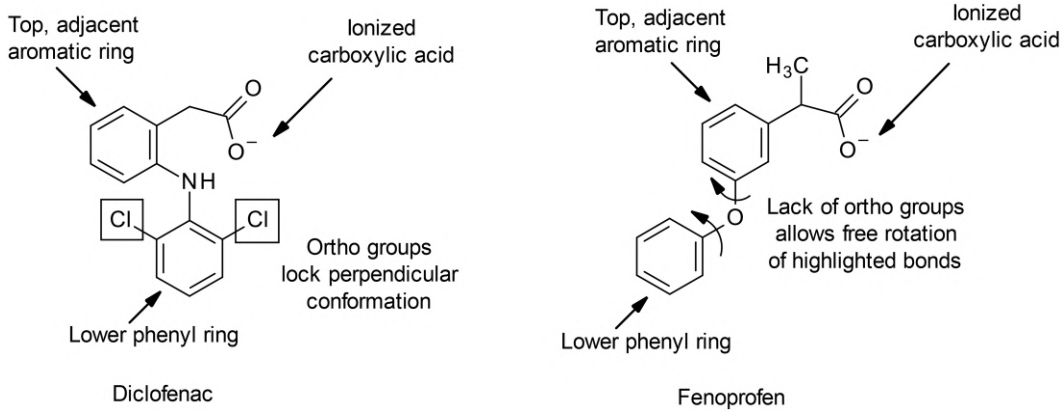
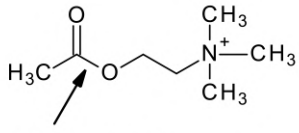


FIGURE 2-16. A comparison of diclofenac and fenoprofen interactions with cyclooxygenase.

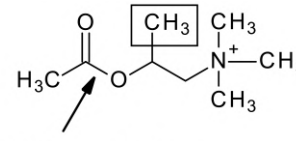
The negatively charged carboxylic acid forms an ionic bond with a positively charged cation within the enzyme. The top or adjacent aromatic ring interacts with a hydrophobic pocket within the enzyme, and the lower phenyl ring interacts with a second hydrophobic pocket within the enzyme. A key component of this binding interaction is that the lower phenyl ring is oriented perpendicular to the top aromatic ring. The key difference between these two drugs is that the structure of diclofenac contains two *ortho* chloro groups. These two functional groups sterically lock diclofenac in its required active conformation. In comparison, the lower ring of fenoprofen is unsubstituted, which allows for free rotation about the indicated bonds. Although fenoprofen can still interact with cyclooxygenase, it requires energy to adopt the required active conformation. As such, its affinity for cyclooxygenase is less than that observed for diclofenac. From a therapeutic perspective, this difference can be seen in the normal doses used for these two agents. The normal dose of diclofenac is 50 mg twice daily (BID) or three times daily (TID), while the normal dose of fenoprofen is 400 to 600 mg TID or four times daily (QID). While there are a number of factors that contribute to the dosing of these two drugs, the steric effect seen in diclofenac definitely plays a key role.

Alteration of Metabolism

Similar to the interaction between a drug and its biological target, the metabolism of a drug molecule requires it to interact with the active site of the enzyme that catalyzes its metabolism. Steric hindrance is a common strategy used to block or slow a specific metabolic pathway. In this approach, additional atoms are added adjacent to the functional group undergoing metabolism in order to block the interaction of the drug molecule with the enzyme carrying out the metabolic transformation. In many cases, these additional atoms need not be very large. Returning to our example of acetylcholine and methacholine (Figure 2-1), the additional methyl group seen on methacholine prevents the enzyme acetylcholinesterase from cleaving the ester bond.

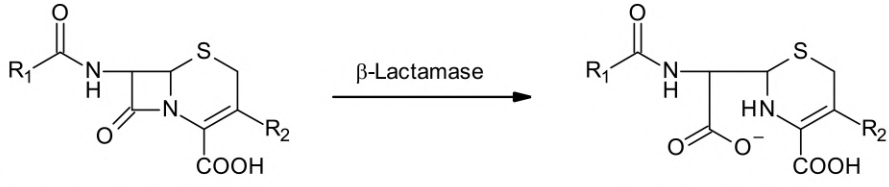


Acetylcholine: Ester hydrolysis occurs very rapidly



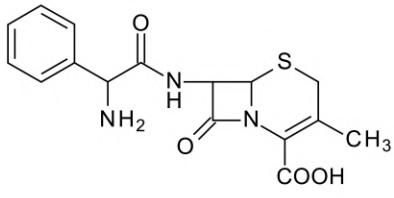
Methacholine: Highlighted adjacent methyl group blocks hydrolysis

Another example of this concept can be seen with the cephalosporin class of antibacterial agents (**Figure 2-17**). This class of drug molecules requires an intact β-lactam ring in order to exert their antibacterial action. Some bacteria can produce β-lactamase, an enzyme that catalyzes the hydrolysis of this ring and, thereby, inactivates the cephalosporin. Cephalosporins, such as cephalexin, that can be inactivated in this manner are known as β-lactamase sensitive. Placing functional groups adjacent to the β-lactam bond can block this inactivation. As seen in Figure 2-17, the methoxy group of cefoxitin produces steric hindrance and permits the drug to be resistant to the actions of β-lactamase.

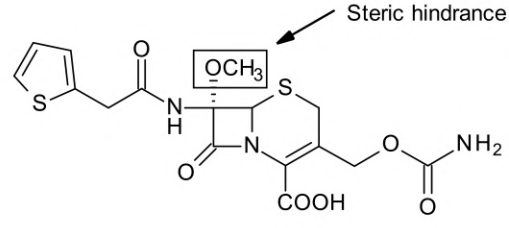


Active cepalosporin with intact β-lactam ring

Inactive cepalosporin with hydrolyzed β-lactam ring



Cephalexin
β-lactamase sensitive



Cefoxitin
β-lactamase resistant

FIGURE 2-17. The role of steric hindrance in inhibiting β-lactam destruction by β-lactamase.

FINAL CONSIDERATIONS FOR FUNCTIONAL GROUPS ON DRUG MOLECULES

There are three key points that need to be emphasized with regard to the concepts and examples discussed previously. First, the same functional group can provide different effects on different drug molecules. We have already seen this in several of the prior examples. The addition of a simple methyl group can result in a number of therapeutic benefits depending on the drug molecule and the location of the methyl group. It can increase the selectivity of a drug for one biological target over another. This was seen in earlier discussions of methacholine compared with acetylcholine (Figure 2-1) and isoproterenol compared with albuterol (Figure 2-15). Addition of a methyl group can also increase the potency of a drug. This was previously discussed in the comparison of lovastatin to simvastatin (Figure 2-1). An additional example can be seen in the comparison of morphine with its *N*-desmethyl metabolite. The *N*-methyl group naturally present on morphine enhances its potency approximately 4-fold as compared with *N*-desmethylmorphine (**Figure 2-18**). Finally, the addition of a methyl group can sterically block metabolism and thus increase the duration of action of a specific drug molecule. This was described previously in the addition of the methyl group to acetylcholine. An additional example of this concept is demonstrated when comparing testosterone with methyltestosterone (Figure 2-18). Testosterone is a naturally occurring androgenic hormone; however, it cannot be taken orally due to rapid oxidation of the C_{17} hydroxyl group to an inactive ketone. Addition of a methyl group at the C_{17} position converts the secondary hydroxyl group into a tertiary hydroxyl group. This blocks oxidative metabolism and allows methyltestosterone to be administered orally.

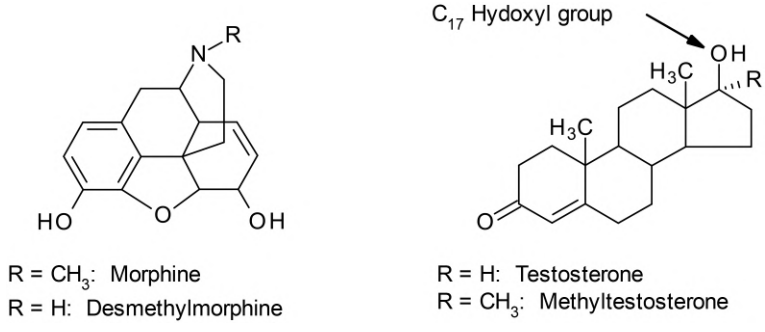
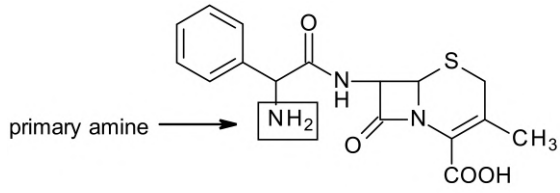


FIGURE 2-18. Morphine and methyltestosterone: the advantages of an additional methyl group.

Second, a single functional group may sometimes serve two distinct purposes. Examples of this can be found in three of the drugs already discussed in this chapter. The methyl group of methacholine serves to both increase selectivity and prevent metabolism. The two *ortho* chloro groups present on the lower phenyl ring of diclofenac (Figure 2-16) provide steric hindrance and lock diclofenac in its active conformation. Additionally, due to their electron withdrawing properties, these halogens deactivate this ring from oxidative metabolism and allow it to have a longer duration of action than fenoprofen. Finally, the primary amine of cephalexin provides acid stability of the β-lactam ring and enhances its ability to enter gram-negative bacteria. In the acidic environment of the stomach, this primary amine is protonated and acts as an electron withdrawing group. Similar to the ether oxygen of penicillin V (Figure 2-8), this electronic effect prevents the acid catalyzed destruction of

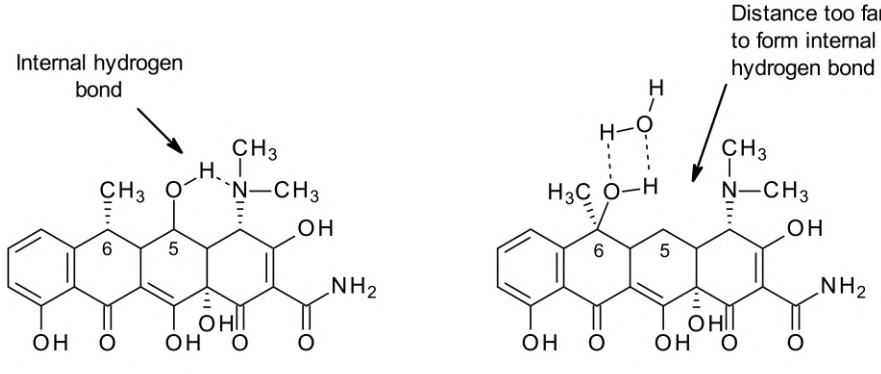
the β-lactam ring. Additionally, this primary amine is primarily ionized at all physiologically relevant pH environments. This enhances the water solubility of cephalexin and better allows it to be transported by porins, the hydrophilic protein channels that allow access of antibiotics to gram-negative bacteria.



Cephalexin

Finally, it is important to reemphasize that adjacent functional groups can alter the properties of a functional group. Examples of this were presented earlier with the discussion of electronic effects, so let us look at one final example that involves solubility. Tetracycline and doxycycline (**Figure 2-19**) have the same molecular formula ($C_{22}H_{24}N_2O_8$) and are chemically identical with the exception that tetracycline contains a C_6 hydroxyl group, whereas doxycycline contains a C_5 hydroxyl group.

Despite their structural similarities, doxycycline is much less water soluble than tetracycline. As shown in Figure 2-19, the C_5 hydroxyl group of doxycycline is able to form an internal hydrogen bond with the adjacent tertiary amine. This decreases the ability of this hydroxyl group to form hydrogen bonds with water and also decreases the ability of the amine to ionize since its lone pair of electrons is involved in this internal hydrogen bond. In tetracycline, the hydroxyl group is no longer adjacent to the tertiary amine and is therefore not able to form hydrogen bonds with it. As such, it is much more available to form hydrogen bonds with water, and the lone pair of electrons of the tertiary amine is much more available to interact with a proton to become ionized. Thus, the position of this hydroxyl group, as well as the presence or absence of an adjacent functional group, plays an important role in its chemical properties. From a therapeutic perspective, this minor change in the position of a single functional group results in a number of benefits. The decrease in water solubility seen in doxycycline allows it to have better oral absorption, enhanced penetration into bacteria, and a longer duration of action.



Doxycycline Tetracycline

FIGURE 2-19. Doxycycline and tetracycline.

Key Summary Points to Consider When Evaluating Functional Groups

- Every atom within the structure of a drug molecule is part of a specific functional group.
- The importance of a given functional group varies among drug molecules and drug classes.
  - A specific functional group can produce different effects on different drug molecules.
  - It is possible for a single functional group to serve more than one distinct purpose on a single drug molecule.
- Each functional group has an electronic effect, a solubility effect, and a steric effect.
  - It is impossible for a functional group to alter only one of these properties without affecting the others.
  - The relative importance of these three properties varies depending on the functional group.
  - The overall electronic effect of a given functional group depends on both its ability to participate in resonance delocalization and its intrinsic inductive effect.
  - Nucleophilic functional groups have either a negative charge or a lone pair of electrons, while electrophilic functional groups have a positive charge, a conjugated double bond system, or a good leaving group.
  - The two key properties that contribute to the water solubility of a functional group are its ability to ionize and its ability to participate in hydrogen bonding interactions.
  - Each functional group has a finite size or steric dimension that contributes to the overall conformation of a given drug molecule.
- The overall effect of a given functional group is dependent on other adjacent or surrounding functional groups.
- Functional groups can be altered to provide specific therapeutic benefits.

A REVIEW OF FUNCTIONAL GROUPS PRESENT ON AMINO ACIDS

As initially mentioned in Chapter 1, amino acids serve as the building blocks for a small number of peptide-based drug molecules. Amino acids also serve a much larger role as the building blocks for proteins and enzymes. Among a plethora of other functions, proteins and enzymes serve as biological targets for drug molecules. As such, it is important to review the functional groups present on the 20 naturally occurring amino acids, as well as the functional groups present on commonly modified amino acids. A more in-depth discussion of drug binding interactions between drug molecules and their biological targets is provided in Chapter 6. The primary goal here is to identify the key chemical properties of the functional groups present in amino acids and, therefore, in proteins. Similar to functional groups present on a drug molecule, the functional groups present on amino acids have electronic, solubility, and steric effects. The primary difference is that unlike drug molecules, the functional groups present on the amino acids of a protein or enzyme cannot be changed. In many examples in this chapter, we discussed the advantages that could be gained by adding, removing, or altering a functional group. Because these types of alterations do not occur with the amino acids present in proteins and enzymes, this section has been intentionally placed at the end of the chapter.

As shown in **Figure 2-20**, each amino acid contains a primary amine, a carboxylic acid, and a side chain at the α-carbon. Although this section focuses primarily on the functional groups present on the side chains, let us briefly examine the amines and carboxylic acids of amino acids. These functional groups are used to form amide bonds or peptide bonds. A protein therefore consists of one or more peptide chains that contain numerous amide bonds, a primary amine at one end, and a carboxylic acid at the other end. These ionizable groups are often masked as amides and esters to avoid degradation by endogenous exopeptidases. The multiple amides that comprise the peptide backbone are polar functional groups and are extremely important for forming internal hydrogen bonds. Once the three-dimensional conformation of the peptide has been established, the functional groups present on the side chains are able to interact with drug molecules or other endogenous moieties.

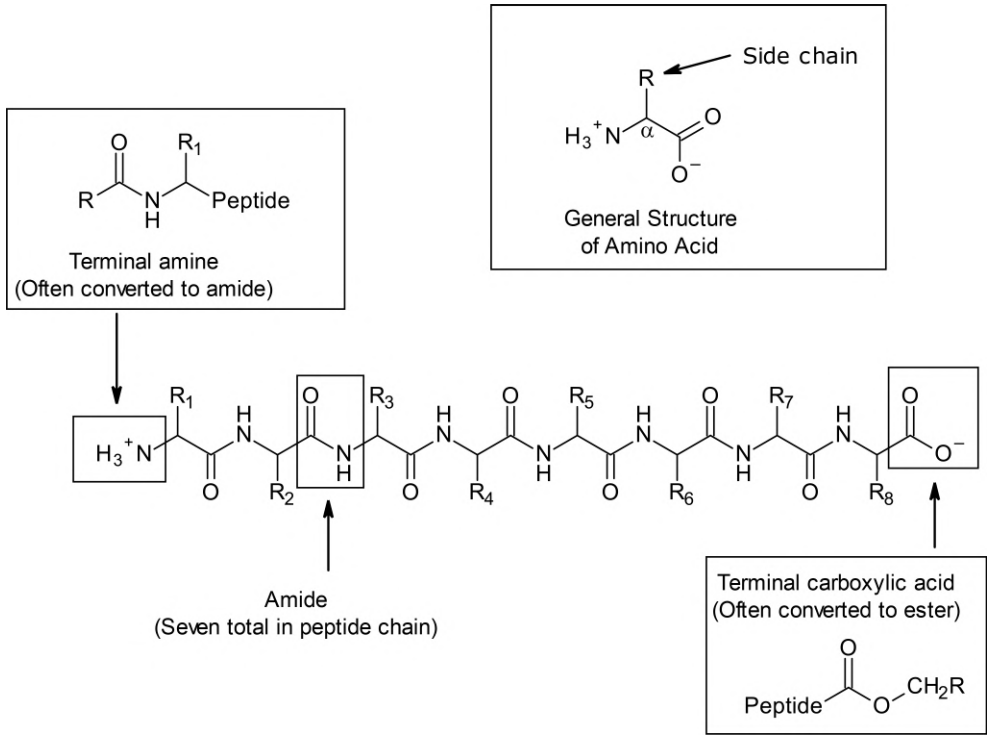
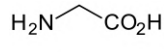


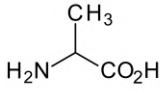
FIGURE 2-20. General structures of amino acids and peptide chains.

Glycine is the smallest and simplest of all of the amino acids. Its side chain is a hydrogen atom; although some texts view glycine has having no side chain. It is essentially neutral in terms of electronic or solubility effects. From a steric perspective, it really has no steric hindrance and conveys flexibility to a peptide chain. Please note that for glycine and all of the other amino acids to follow, the primary amine and the carboxylic acid are intentionally drawn in their unionized forms since they are normally involved in the formation of a peptide bond.

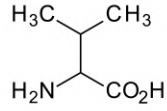


Glycine

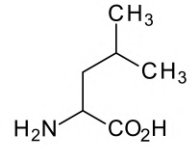
Alanine, valine, leucine, and isoleucine all contain aliphatic, lipid-soluble, hydrocarbon side chains and have varying steric bulk. The side chains on these amino acids are most important for forming lipid-soluble pockets within a protein or enzyme and interacting with other hydrocarbon chains on drug molecules or endogenous molecules. Additionally, the varying steric bulk on these amino acids can either block or allow access to certain portions of a protein depending on which amino acid is present.



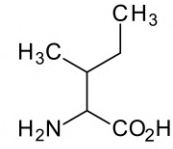
Alanine



Valine

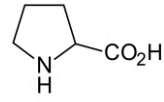


Leucine



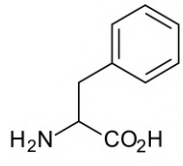
Isoleucine

Proline is unique from all of the other naturally occurring amino acids in that it contains a secondary amine. Similar to the previous four amino acids, proline has a lipid-soluble, hydrocarbon side chain. The key difference is that the side chain forms an alicyclic ring with the nitrogen atom. The most important feature of proline is that the alicyclic ring produces a significant steric affect that causes protein chains to kink or bend. In addition, due to the alicyclic ring, natural human proteases cannot cleave peptide bonds that include a proline amino acid.

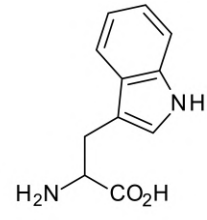


Proline

Phenylalanine and tryptophan contain aromatic rings and similar to the aliphatic and alicyclic amino acids are lipid soluble. Due to the aromaticity of both rings, these functional groups have a greater electronic nature and can interact with aromatic rings present on drug molecules. A key difference between these two amino acids is the presence of the nitrogen atom in the bicyclic indole ring of tryptophan. This nitrogen is not basic due to the fact that its lone pair of nonbonding electrons is required for the aromaticity of the bicyclic ring. This donation of electrons causes the indole ring of tryptophan to be more electron rich than the phenyl ring of phenylalanine. Additionally, the nitrogen atom in the indole ring can act as a hydrogen bond donor and therefore can participate in hydrogen bonds. The phenyl ring of phenylalanine cannot do this. From a steric perspective, both rings are larger than the side chains of the above aliphatic or alicyclic amino acids.



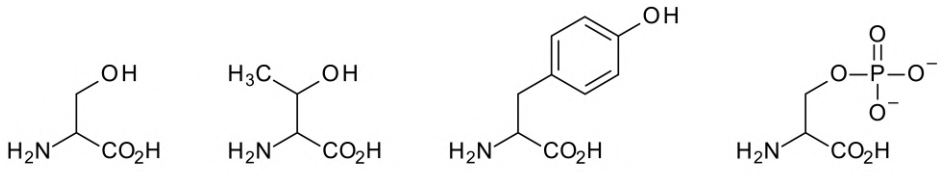
Phenylalanine



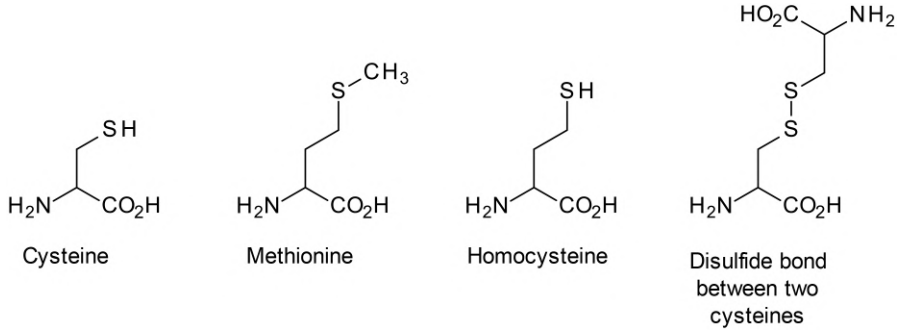
Tryptophan

Serine, threonine, and tyrosine all contain hydroxyl groups within their side chains. These functional groups are very important due to their ability to form hydrogen bonds as both hydrogen bond donors and acceptors with water, as well as with other functional groups present within drug molecules. Both serine and threonine are classified as water-soluble uncharged amino acids. Tyrosine is often classified as an aromatic amino acid along with phenylalanine and tryptophan; however, it is

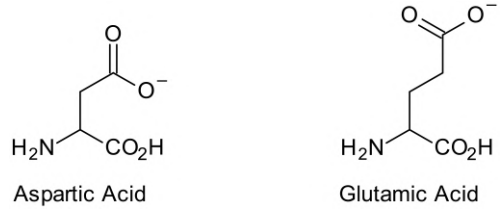
included here due to its phenolic hydroxyl group. It is more water soluble than the phenyl ring of phenylalanine. Additionally, the hydroxyl groups of all three of these amino acids are nucleophiles and are important for specific mechanisms in biochemical pathways and for the mechanisms of action of some drug molecules. Due to their nucleophilic nature, these functional groups are often phosphorylated as part of a regulatory mechanism or are added to increase the number of negative charges associated with the protein or enzyme. An example of this is seen with phosphoserine.



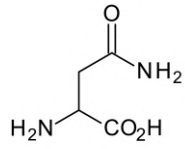
Cysteine and homocysteine are very similar to serine. Cysteine is one of the 20 naturally occurring amino acids, whereas homocysteine is a precursor to methionine and is involved in several biochemical pathways. Both of these are water-soluble uncharged amino acids; however, their ability to form hydrogen bonds is weaker than that observed with serine, threonine, and tyrosine. They are also very nucleophilic. The ability of cysteine to form disulfide bonds is essential for the three-dimensional conformation of a number of peptides and proteins. Methionine, which contains a thioether in its side chain, is much less reactive and is often grouped along with alanine, valine, leucine, and isoleucine as a lipid-soluble amino acid. It can act as a hydrogen bond acceptor and is included here because of its relationship to cysteine and homocysteine.



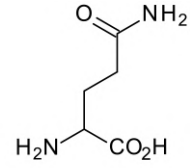
As the names imply, aspartic acid and glutamic acid are the two naturally occurring acidic amino acids. These carboxylic acids are primarily ionized in most physiologic environments, with the stomach being the primary exception. These functional groups are water soluble and provide negative charges for ionic interactions with positively charged functional groups or metal ions. The only difference between these two amino acids is the additional methylene carbon present in glutamic acid. Thus from a steric perspective, glutamic acid has somewhat more steric bulk than does aspartic acid. Similar to the terminal carboxylic acid shown in Figure 2-20, these amino acids can be esterified to remove their ability to be ionized and decrease their overall water solubility.



Asparagine and glutamine are the amide analogs of aspartic acid and glutamic acid, respectively. These amino acids are similar to serine, cysteine, and threonine in that their side chains are water soluble and not able to be ionized. The amide groups can serve as hydrogen bond donors and acceptors. Since the lone pair of nonbonding electrons on the amide nitrogen are involved in resonance with carbonyl group, they are not very nucleophilic.

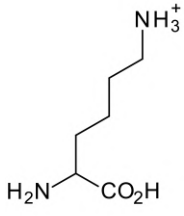


Asparagine

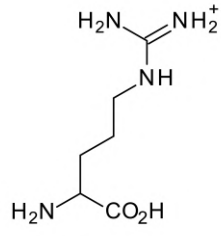


Glutamine

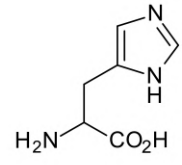
Finally, lysine, arginine, and histidine are the three naturally occurring basic amino acids. The relative basicities of these functional groups is discussed in more detail in the next chapter; however, both lysine and arginine are primarily ionized in all physiologic environments and can provide positive charges for ionic interactions with negatively charged functional groups. Histidine is less basic than either lysine or arginine and is primarily unionized in most physiologically relevant environments. Histidine can act as either a hydrogen bond donor or a hydrogen bond acceptor. The functional groups on all three amino acids enhance water solubility. The primary amine of lysine can be methylated to form a quaternary amine, trimethyllysine. This provides a permanent positive charge within a protein.



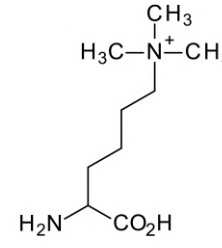
Lysine



Arginine



Histidine

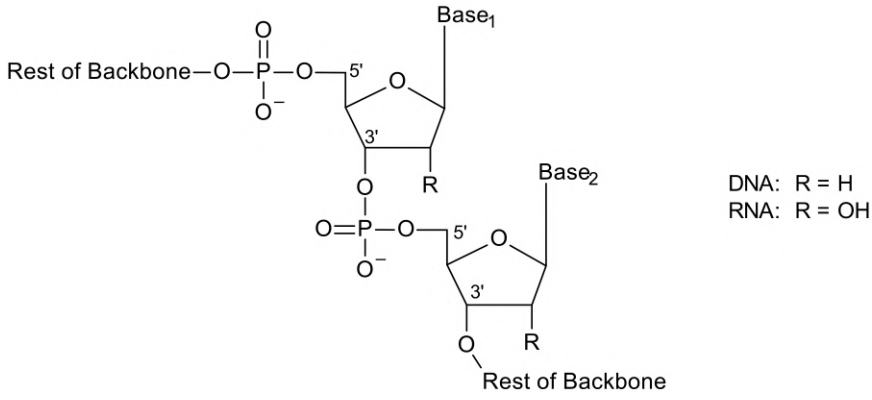


Trimethyllysine

A REVIEW OF FUNCTIONAL GROUPS PRESENT ON DNA AND RNA

While proteins and enzymes comprise the major biological targets for drug molecules, there are a significant number of drugs that bind to DNA or RNA in order to exert their mechanism of action. This last section provides a brief overview of the functional groups present within the backbone of DNA and the purine and pyrimidine bases.

Similar to proteins and enzymes, DNA and RNA are polymers. They consist of bases bound to repeating sugar units, either deoxyribose (DNA) or ribose (RNA), which are linked together by phosphodiester bonds. This backbone consists of numerous water-soluble functional groups that can interact with water, metals, and numerous functional groups present within the structures of drug molecules. The phosphate esters are primarily ionized in almost all physiologic environments. The hydroxyl group of RNA can serve as either a hydrogen bond donor or acceptor, while the oxygen atoms not involved in the resonance of the phosphate can serve as hydrogen bond acceptors.



The bases present in DNA and RNA are structural analogs of either purine or pyrimidine (**Figure 2-21**). All of these bases contain aromatic rings that can form intramolecular π-π stacking interactions with one another as well as intermolecular interactions with aromatic rings present within the structure of drug molecules. The oxygen and nitrogen atoms of these purines and pyrimidines can form hydrogen bonds. This leads to the normal guanosine—cytidine (DNA and RNA), adenosine—thymidine (DNA), and adenosine—uridine (RNA) base pairing present within the structure of DNA and RNA. The hydrogen bonding ability also extends to the interactions with drug molecules. At physiologic pH, these purine and pyrimidine bases are primarily unionized; however, the N^7 atom of guanine, the N^1 atom of adenine, and the N^3 atom of cytosine are weakly basic, while the N^3 atom of thymine and uracil is weakly acidic.

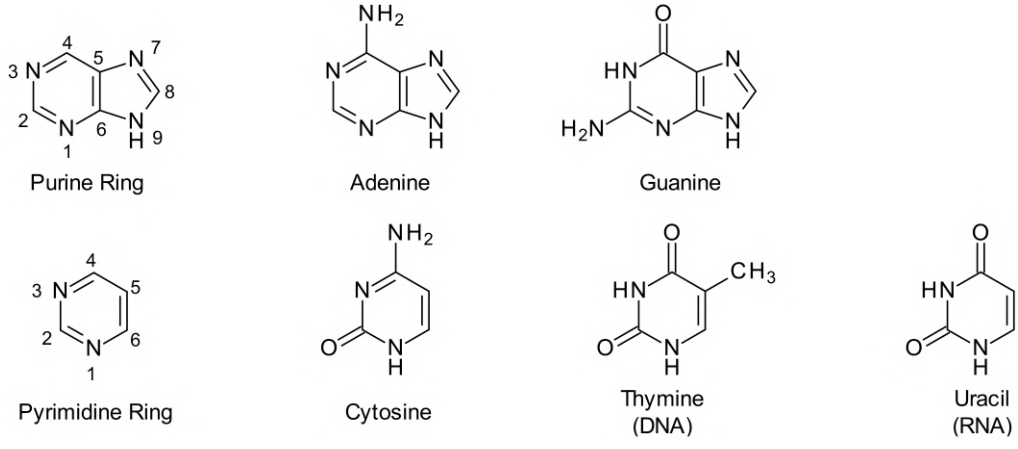


FIGURE 2-21. Purines and pyrimidines present in DNA and RNA.

STRUCTURAL ANALYSIS CHECKPOINT

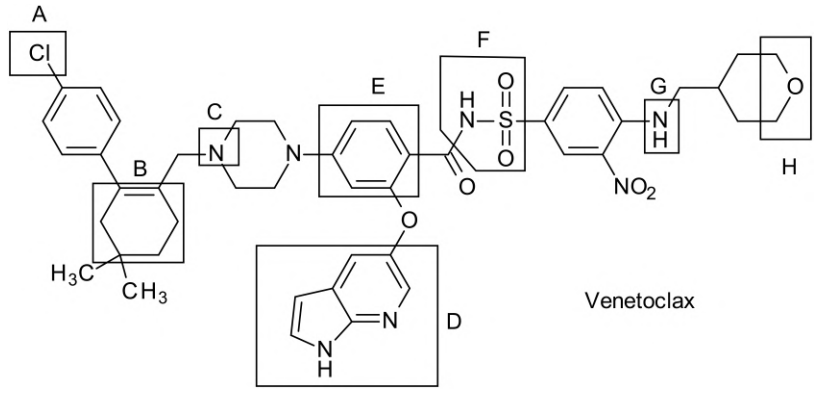
Introduction to This Feature

Review questions are provided for Chapters 2–9. These review questions focus solely on the specific concepts discussed in the respective chapters. A large number of different drug molecules are used so readers can test their ability to apply the concepts with a variety of drugs and situations.

Structural Analysis Checkpoint questions are similar to the review questions with one major difference. Starting in this chapter, we follow two drugs throughout the text. At the end of each

chapter, we check back with these drugs and conduct a structural analysis to determine how the concepts introduced in each chapter are applied to these drugs. By the end of Chapter 9, readers will have conducted a comprehensive evaluation of these two drugs—a whole molecule drug evaluation.

Checkpoint Drug 1: Venetoclax

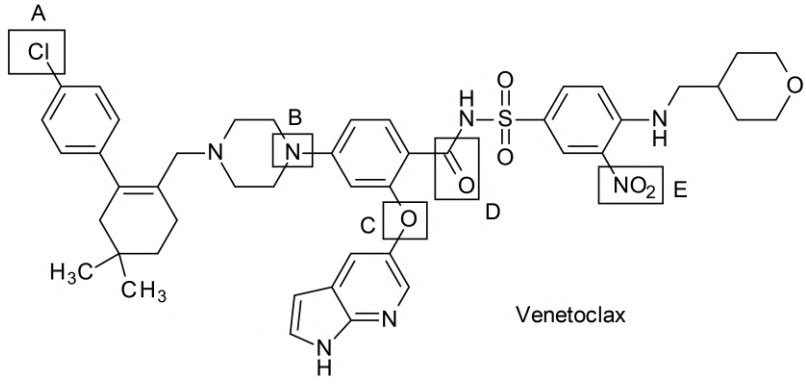


1. Using the table below, provide the name of each boxed functional group and indicate if the functional group contributes to the overall water and/or lipid solubility of venetoclax.

	Functional Group Name	Contribution to Water and/or Lipid Solubility
A		
B		
C		
D		
E		
F		
G		
H		

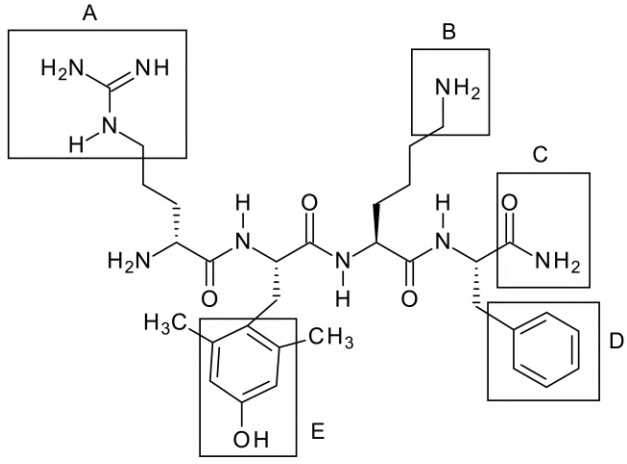
2. Using your answers from the previous question, choose one water-soluble functional group and one lipid-soluble functional group and explain from a physicochemical perspective why they contribute to the overall water or lipid solubility of the drug.

3. Using the structure of venetoclax shown below, identify if the boxed functional groups are electron donating or electron withdrawing with respect to their adjacent rings and if this effect is due to resonance or induction.



	Electron Donating or Withdrawing	Resonance or Induction
A		
B		
C		
D		
E		

Checkpoint Drug 2: Elamipretide



1. Consider the boxed functional groups found in Elamipretide. Complete the grid below for each of the boxed functional groups. When we use the term *solubility*, we think about aqueous solubility, whereas when we use the term *absorption*, we think about the ability of a drug to be absorbed across a lipid bilayer.

	Name of Functional Group	Character: Hydrophobic, Hydrophilic, or both	Function: Contribute to Solubility or Absorption
A			
B			
C			
D			
E			

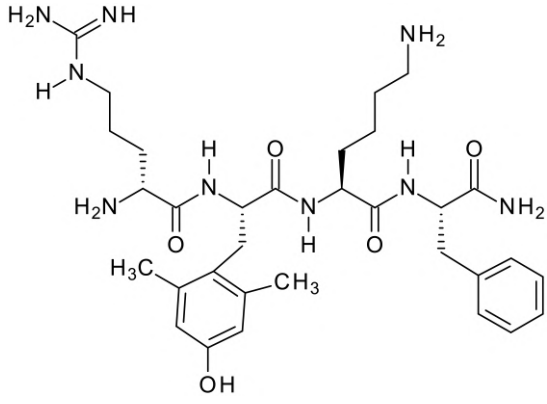
2. Elamipretide is a peptide-based agent being evaluated as a mitochondrial protective agent. This molecule is composed of amino acids and derivative(s) of amino acids. In order to read this molecule, you need to start at the left most primary amine and identify the amino acids found in the peptide chain. Using the molecule below, do the following:

   A. List the three structural components of every amino acid.

   B. Determine how many amino acids or amino acid derivatives are found in this molecule.

   C. Circle and label the portions of the molecule that represent arginine, lysine, and phenylalanine.

D. Amino acid #2 is a derivative of an amino acid. Identify the amino acid.

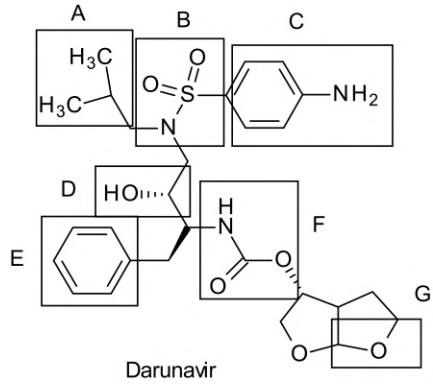


3. For each of the amino acids and derivative(s) identified in the previous questions, complete the grid below:

	Name of Amino Acid or Amino Acid Derivative	Amino Acid Side Chain Evaluation: Hydrophobic, Hydrophilic, or Both	Amino Acid Side Chain Evaluation: Acidic, Basic, Neutral	Amino Acid Side Chain Evaluation: Nucleophilic, Electrophilic, NA
1				
2				
3				
4				

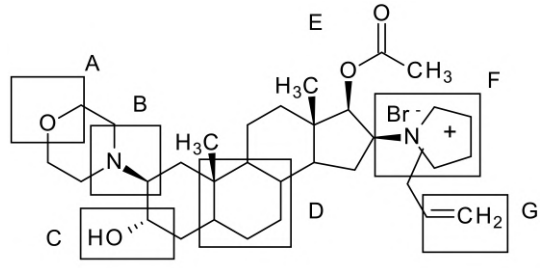
REVIEW QUESTIONS

1. For the structure of darunavir, name all of the boxed functional groups in the grid below.



Box	Functional Group Name
A	
B	
C	
D	
E	
F	
G	

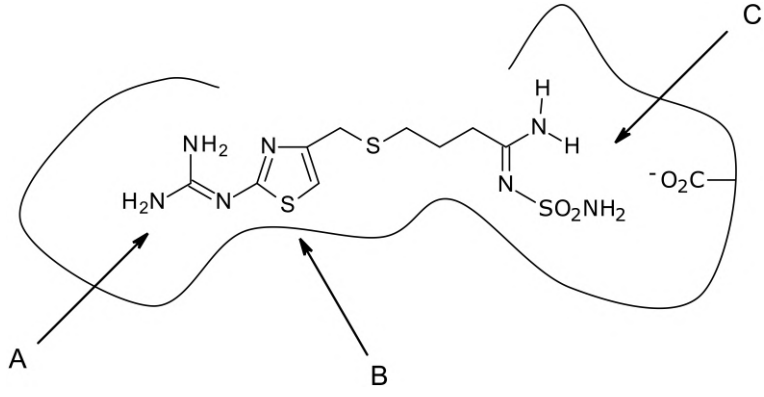
2. For the structure of rocuronium bromide, name all of the boxed functional groups in the grid below.



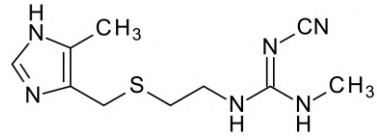
Rocuronium Bromide

Box	Functional Group Name
A	
B	
C	
D	
E	
F	
G	

3. The structural features found within H_2 receptor antagonists include a basic functional group that is protonated at physiologic pH (A), an aromatic ring (B), and a terminal nonbasic polar functional group (C) that is separated from the aromatic ring by the equivalent of a four-carbon chain. The terminal nonbasic polar functional group participates in a key ion–dipole interaction with an ionized carboxylic acid found in the binding region within the H_2 receptor.



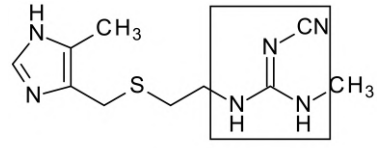
A. Identify all of the functional groups that are present in the structure of cimetidine.



Cimetidine

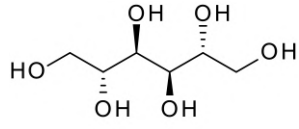
B. Guanidines and amidines that are substituted with electron withdrawing groups have significantly decreased basicity compared with unsubstituted guanidines (pK_a ~12.5) and amidines (pK_a ~9) and are unprotonated (unionized) at physiologic pH. Name the electron withdrawing functional group found within cimetidine.

C. The boxed functional group (show below) participates in a key ion–dipole interaction with an ionized carboxylic acid group found in the H_2 receptor binding region. Provide a brief rationale why this functional group is not protonated (ionized) at physiologic pH.



Cimetidine

4. A 20-year-old male has been rushed to the hospital following a severe head injury that occurred while playing a college football game. Tests have confirmed the presence of cerebral edema. The decision is made to treat the edema with mannitol.



Mannitol

A. Consider the structure of mannitol drawn above when completing the grid below.

Name Two Oxygen Containing Functional Groups	Hydrophilic and/or Hydrophobic	Contribution to Water Solubility and/or Lipid Solubility	Hydrogen Bond Acceptor, Donor, Both, or Neither

B. Mannitol is supplied as an aqueous solution for IV injection and is administered as a bolus of 0.25 to 1 g/kg every 4 to 6 hours as needed. Based on your functional group evaluation, provide a structural rationale for why mannitol can be formulated as an aqueous solution.

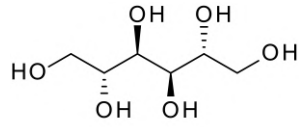
C. Mannitol is classified as an osmotic diuretic and in this case is used to pull fluid across the blood brain barrier to reduce the swelling in the brain. Similarly, at high oral doses it can also be used as an osmotic laxative with which fluid is drawn into the intestine. Based on your functional group evaluation, provide a structural rationale for why mannitol is able to draw water out of the brain to reduce swelling.

D. Using the structure below, show (and label) examples of the following interactions:

   a. Drug–water interaction: drug = hydrogen bond acceptor; water = hydrogen bond donor

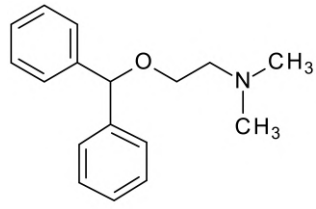
   b. Drug–water interaction: drug = hydrogen bond donor; water = hydrogen bond acceptor

   c. Drug–water interactions: dipole–dipole interaction



Mannitol

5. A 19-year-old male comes into the pharmacy complaining about a skin rash that is berry red and very itchy. He states that he just got back from a camping trip and believes he came into contact with poison ivy. He asks for a recommendation for a cream that will help with the itching. Your recommendation for this patient is Benadryl®, a topical antihistamine cream that is sold over-the-counter.



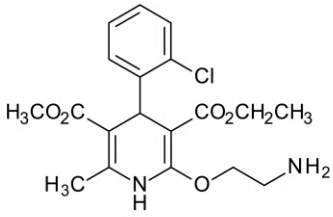
Diphenhydramine

A. Identify all of the structural features present in diphenhydramine and complete the grid below.

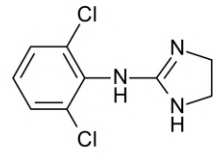
Name of Functional Group	Hydrophilic and/or Hydrophobic	Contribution to Water Solubility and/or Lipid Solubility

B. Using the information that you provided in the grid in the previous question, provide a structural rationale for why diphenhydramine is an agent that can be applied topically. (Hint: Agents that are administered topically must absorb into the hydrophobic components of the skin to be effective.)

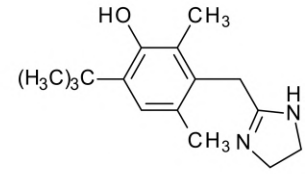
6. Binding interactions with a biological target can be significantly impacted by the presence or absence of functional groups that dictate a molecule's shape. In each of these molecules, functional groups in the *ortho* position of the aromatic rings force the two ring systems to be perpendicular to one another. Circle the functional groups that influence the shape of each of these molecules.



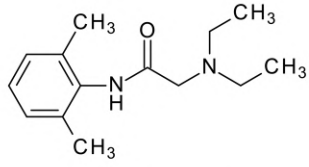
Amlopidine



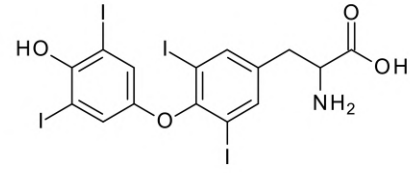
Clonidine



Oxymetazoline



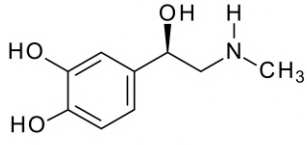
Lidocaine



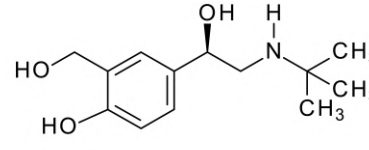
Thyroxine

7. Specificity for a biological target can be significantly impacted by the presence or absence of functional groups that alter the size of a molecule.

   A. Epinephrine is a nonselective agonist at α and β receptors. Determine which functional group in albuterol causes it to have β receptor selectivity and identify if the functional group represents a change in electronic or steric factors.

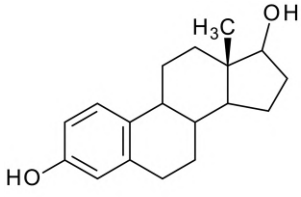


Epinephrine

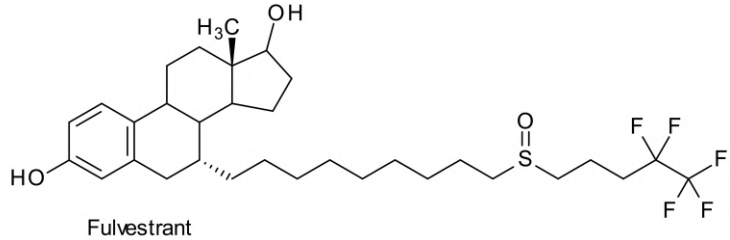


Albuterol

   B. Estrogen is the natural agonist at the estrogen receptor. Determine which functional group in fulvestrant causes it to bind to, but not activate (antagonist), the estrogen receptor. Does this functional group represent a change in electronic or steric factors?

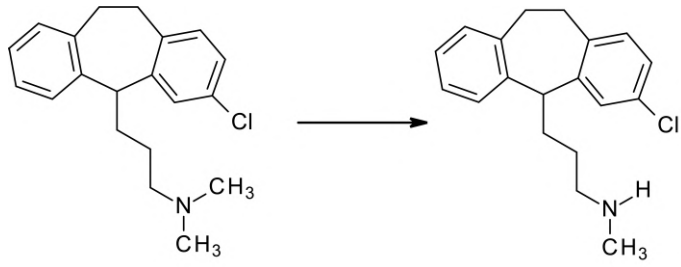


Estrogen



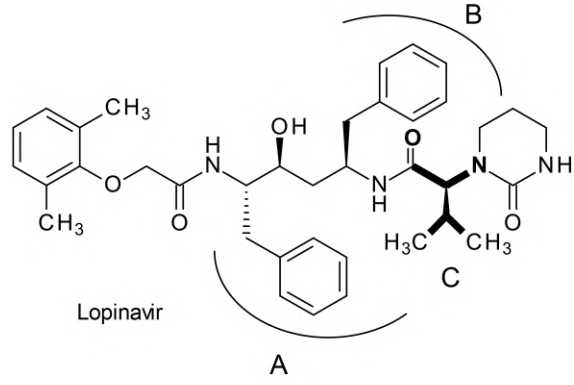
Fulvestrant

8. The addition or loss of one functional group can significantly alter the biological activity that a molecule exhibits. In this case, the loss of a methyl group changes the pharmacological effect of the drug from being a serotonin reuptake inhibitor to a norepinephrine reuptake inhibitor. Determine whether this change is a result of a steric or electronic effect and provide a rationale for your answer.



Clomipramine *N*-Desmethylclomipramine

9. Lopinavir is an HIV protease inhibitor. Structurally, this drug is considered a peptidomimetic, in that it resembles (at least in part), the peptide substrate for the protease. In the presence of this agent, HIV protease is unable to cleave the *gag-pol* polypeptide into its functional proteins during viral assembly and budding.



Lopinavir

   A. Consider the portion of the molecule designated as C. (You will notice that the atoms and bonds of this portion of the molecule have been bolded for you.) Which amino acid is this? How do you anticipate that the side chain of this amino acid will contribute to solubility and absorption of this drug?

   B. Consider the portions of the molecule designated as A and B: while the normal peptide bond sequence has been slightly changed, the side chains are unaltered. Which amino acid do these portions resemble? How do you anticipate that the side chain of this amino acid interacts with the biological target?

10. Aliskiren is an inhibitor of renin, the enzyme that catalyzes the conversion of angiotensinogen to angiotensin I in the renin-angiotensin-aldosterone system. Aliskiren is also a peptidomimetic, in that it mimics the natural peptide substrate (angiotensinogen) for renin. In this peptidomimetic, the hydrolysable peptide (amide) bond has been replaced with a hydroxyethylene functional group.

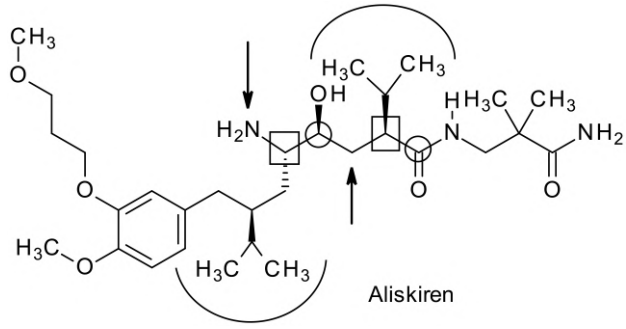
   A. Peptidomimetics often follow the same structural sequence of functional groups as found in a normal peptide—amine, side chain, carbonyl. Find the left most arrow (→) pointing to an amine. If you follow the sequence to the right, you will find a boxed atom followed by a circled atom. What portion of the sequence does the box represent? What portion of the sequence does the circle represent?

   B. Look for the next sequence of →, □, ○. What amino acid does this mimic?

   C. Renin normally catalyzes the cleavage of the peptide bond between Leu and Val. What interactions are possible between renin and the side chains of Leu and Val? If you look

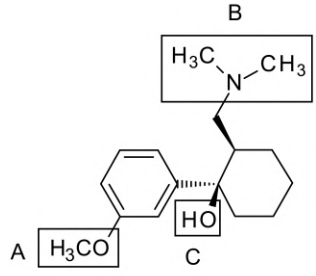
at the structure of aliskiren, you see that two amino acid side chains are docked in enzyme "pockets." What kind of interactions do you expect these amino acid side chains to have with renin?

D. Box the nonhydrolyzable functional group that is replacing the peptide bond that normally links Leu and Val.



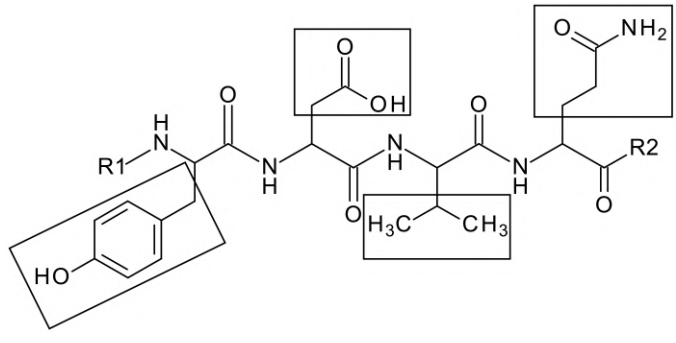
Aliskiren

11. Shown below is the structure of tramadol. Three of its functional groups have been highlighted. Based upon their electronic properties **AND** their relative positions in the molecule, identify if they are electron withdrawing or electron donating. Additionally, identify if this effect is due to resonance or induction.



Tramadol

12. Shown below is the structure of a tetrapeptide that is part of a larger protein receptor. The side chains of the four amino acids have been highlighted.



A. Identify the four amino acids that comprise this tetrapeptide sequence.

B. For each amino acid, identify the key chemical properties of their highlighted side chains.

IDENTIFYING ACIDIC AND BASIC FUNCTIONAL GROUPS

LEARNING OBJECTIVES

After completing this chapter, students will be able to

- **Identify the following acidic functional groups: carboxylic acids, β-dicarbonyls, imides, sulfonamides, sulfonylureas, tetrazoles, phenols, thiols, phosphates, phosphonates, and sulfates.**
- **Identify the following basic functional groups: aliphatic amines, alicyclic amines (aka saturated heterocycles), aromatic amines, imines, hydrazines, amidines, guanidines, and nitrogen containing aromatic heterocycles.**
- **Classify a drug molecule as one of the following: acidic, basic, amphoteric; an electrolyte; or a nonelectrolyte.**
- **Explain how the acid/base nature of a drug molecule can influence its chemical, pharmaceutical, and therapeutic properties.**

Functional groups that are either acidic or basic have the ability to become ionized and as a result become negatively or positively charged, respectively, within the structure of a drug molecule. This ability to ionize increases the overall water solubility of the drug molecule, allows for the formation of specific types of interactions between the drug molecule and its biological target(s), and influences the transport, metabolism, and elimination of the drug molecule. In addition, acidic and basic functional groups can be used to produce water- or lipid-soluble salts for the purpose of providing a specific physicochemical or therapeutic advantage.

This chapter reviews the acidic and basic functional groups that are present within the structures of drug molecules. The discussion of each acidic and basic functional group includes an explanation as to why it is acidic or basic as well as examples of commercially available drugs that contain the functional group within their structures. When applicable, the relative acid/base strengths among the different functional groups are provided along with an explanation. The chapter ends with a discussion regarding the therapeutic significance of the acid/base nature of a drug molecule.

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ACIDIC FUNCTIONAL GROUPS

Acidic functional groups are those that can donate (or lose) a proton (H^+). From a very simplistic view, an acid can be represented as follows:

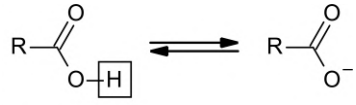
$$HA \rightleftharpoons H^+ + A^-$$

There are two key features of an acidic functional group: the presence of a hydrogen atom that can dissociate from the group (H^+), and the ability of the remaining atoms to delocalize the resulting negative charge via resonance. As a reminder, *resonance delocalization* refers to the ability of the functional group to spread the charge in a manner that allows it to be shared among two or more atoms.

The pK_a values of these functional groups are used to compare their relative acidity. The most important fact here is that the lower the pK_a value, the stronger the acid. As an example, an acidic functional group with a pK_a of 2.4 is more acidic than one with a pK_a of 3.4. A more extensive discussion of pK_a values and their determination can be found in Chapter 4. It is strongly suggested that you focus more on learning the relative acidity and ranges of the groups listed above rather than memorizing specific pK_a values for specific drugs. A summary table of pK_a values and ranges is provided later in this chapter.

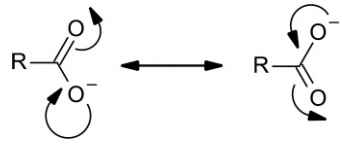
Carboxylic Acids

A carboxylic acid is the most common acidic functional group found in drug molecules. The acidic hydrogen atom has been highlighted with a box below.



Unionized Ionized

The resulting anion (i.e., negatively charged species) is stabilized by resonance delocalization or the sharing of the negative charge between the two oxygen atoms.



The ionized form of a carboxylic acid is known as a *carboxylate*. Four drugs that contain a carboxylic acid as part of their structure are shown in **Figure 3-1**.

Two key points need to be made regarding the drug molecules in Figure 3-1. First, the carboxylic acids present in these drug molecules have purposely been drawn differently to represent the various ways that they are commonly depicted. Gabapentin, alprostadil, and carbidopa are drawn in their unionized, carboxylic acid forms, while cetirizine is drawn in its ionized, carboxylate form. In addition, carboxylic acids can be represented by drawing all of the bonds, as seen in alprostadil, or by using the R-CO_2H or R-COOH abbreviations, as seen in gabapentin and carbidopa, respectively. Carboxylates can be drawn with all of the bonds, as seen in cetirizine, or by using the abbreviated R-COO^- or CO_2^- designations.

The second key point is that carboxylic acids, as well as *all other acidic and basic functional groups*, have a general (or normal) pK_a range. The variation within this range is the result of the electron donating or withdrawing character of *adjacent or surrounding groups*.

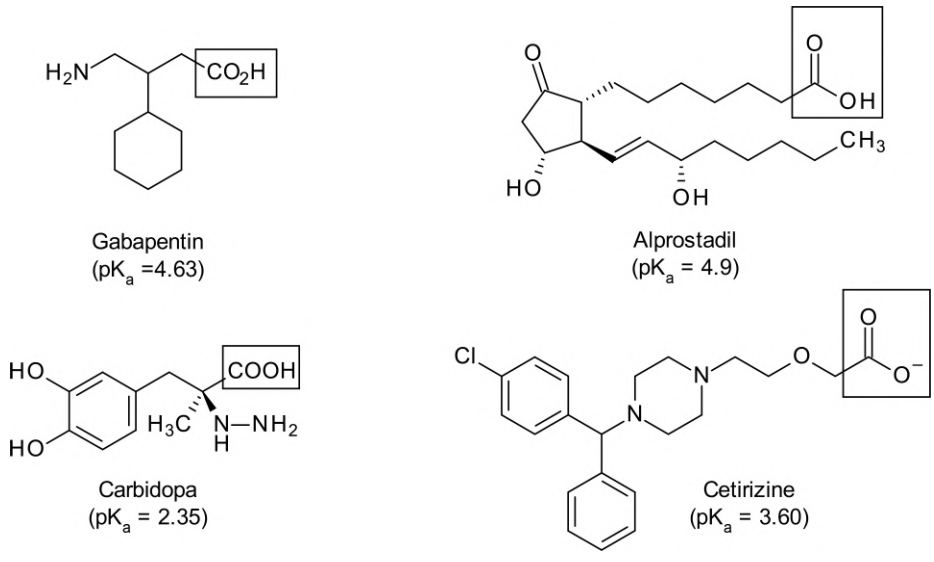


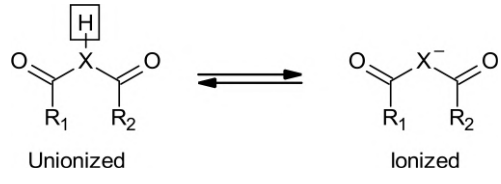
FIGURE 3-1. Examples of drug molecules that contain a carboxylic acid. The various ways this functional group is commonly drawn are represented.

While the normal pK$_a$ range for a carboxylic acid is between 2.5 and 5, there are situations in which the pK$_a$ value of a functional group lies outside of its normal range. To illustrate this concept, the variation in carboxylic acid pK$_a$ value among the four drugs in Figure 3-1 can be explained by their adjacent functional groups. Let's see if we can figure out why the carboxylic acids within the structures of carbidopa and cetirizine have lower pK$_a$ values and hence are more acidic than those within the structures of gabapentin and alprostidil.

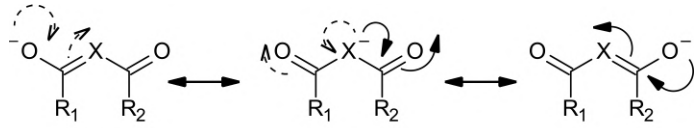
The structure of carbidopa has a basic hydrazine group (NHNH$_2$) that is closely adjacent to its carboxylic acid. This basic functional group acts as an electron withdrawing group and enhances the acidity of the carboxylic acid. A similar situation is seen with cetirizine. The closely adjacent ether oxygen atom is electronegative and withdraws electrons away from the carboxylic acid, thereby decreasing its pK$_a$ value. In contrast, the carboxylic acids within the structures of gabapentin and alprostadil are directly adjacent to alkyl chains. As discussed in Chapter 2, alkyl chains are electron donating in character. The overall electron flow enhances the negative charges of these carboxylic acids and decreases their acidity. It should be noted that the structure of gabapentin does contain a basic amine; however, unlike the basic functional group in carbidopa, this amine is not adjacent to the carboxylic acid and would have minimal effects on its acidity and pK$_a$.

β-Dicarbonyl Groups

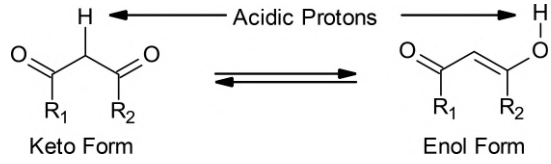
β-Dicarbonyl groups have the following general structure. The acidic hydrogen has been highlighted with a box. The "X" atom can be either carbon or nitrogen. When the "X" atom is nitrogen, the functional group is also known as an imide.



The resulting anion is stabilized by the two carbonyl oxygen atoms via resonance delocalization. Similar to carboxylic acids, the ability to share and stabilize the negative charge among multiple atoms favors ionization of this functional group.



The structures of six drug molecules that contain a β-carbonyl group are shown in **Figure 3-2**. A few key points need to be made as they relate to these structures. When the "X" of a β-dicarbonyl group is a carbon atom, the β-dicarbonyl may exist in either its keto or enol tautomeric form.

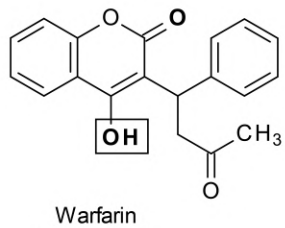


These tautomeric forms are different from the resonance structures shown above and involve the movement of a proton as well as a shift in the bonding electrons. Please note that when this occurs, the acidic hydrogen moves from the carbon atom to an oxygen atom or vice versa. Keto and enol tautomers can also be classified as structural isomers, molecules with the same molecular formula and with a different arrangement of atoms and functional groups. Tautomers, or tautomeric isomers, are distinct from the stereochemical isomers discussed in Chapter 7 and unique from other structural isomers in that they can spontaneously be interconverted. Most drug molecules that contain this type of β-dicarbonyl group normally exist in their enol forms. This is exemplified in Figure 3-2 by warfarin and piroxicam. One drug molecule that primarily exists in its keto form is oxyphenbutazone; however, due to the development of safer agents within this drug class, it is no longer prescribed in the United States.

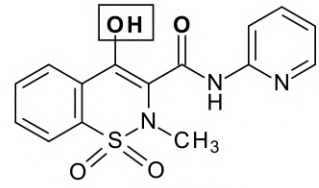
As previously mentioned, when the "X" of a β-dicarbonyl group is a nitrogen atom, the functional group is more commonly known as an imide. Shown in Figure 3-2 are three drug molecules that contain an imide functional group; however, only phenobarbital and phenytoin are acidic. Trimethadione also contains a β-dicarbonyl group that could stabilize a negative charge; however, because the nitrogen atom has been methylated, this functional group does not contain an acidic hydrogen atom. Trimethadione serves as an example to reinforce a key concept: for a functional group to be acidic, *it must contain a hydrogen atom that can dissociate*.

In comparing β-dicarbonyl groups, it should be noted that, in general, imides are much less acidic than their carbon analogs. The primary reason for this is the differences in the relative electronegatives of the atoms involved (see **Table 2-2** in Chapter 2). Oxygen is much more electronegative than carbon and readily accepts the negative charge from the carbon atom once the proton has left. In contrast, while oxygen is also more electronegative than nitrogen, the comparative difference is less, resulting in a decreased electron flow from the nitrogen atom to the oxygen atoms and a decrease in acidity. This fact is exemplified by the drug molecules shown in Figure 3-2. Phenobarbital and phenytoin are imides ("X" = N) and have pK_a values of 7.41 and 8.33, respectively. These pK_a values are two to three log units higher than those for warfarin ($pK_a = 5.05$), piroxicam ($pK_a = 5.10$), and oxyphenbutazone ($pK_a = 4.70$), drugs that have a carbon atom ("X" = C) between the carbonyl groups. The general pK_a range for β-dicarbonyl groups is 4.5 to 8.5, with imides generally having a higher pK_a than when "X" of a β-dicarbonyl group is a carbon atom.

Drug molecules containing a β-dicarbonyl functional group (enol form)

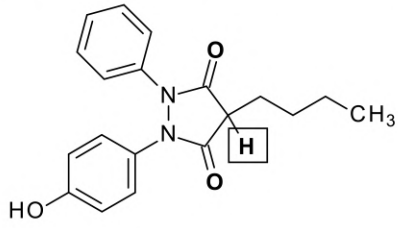


Warfarin
(pK_a = 5.05)



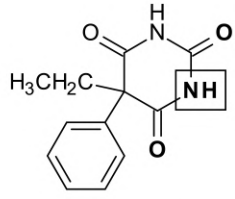
Piroxicam
(pK_a = 5.10)

Drug molecule containing a β-dicarbonyl functional group (keto form)

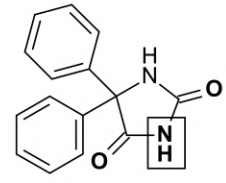


Oxyphenbutazone
(pK_a = 4.70)

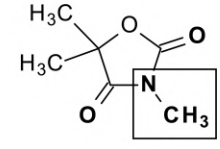
Drug molecules containing an imide functional group



Phenobarbital
(pK_a = 7.41)



Phenytoin
(pK_a = 8.33)

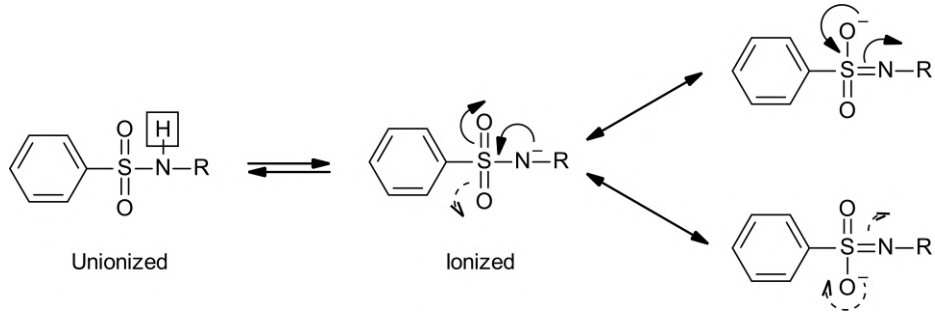


Trimethadione

FIGURE 3-2. Examples of drug molecules that contain a β-dicarbonyl group. The functional groups and acidic hydrogen atoms have been highlighted with boxes.

Sulfonamides and Sulfonylureas

Sulfonamides are similar to β-dicarbonyl groups in that the initial negative charge can be shared by two adjacent oxygen atoms. The only difference here is that both oxygen atoms are attached to a single sulfur atom.



Unionized

Ionized

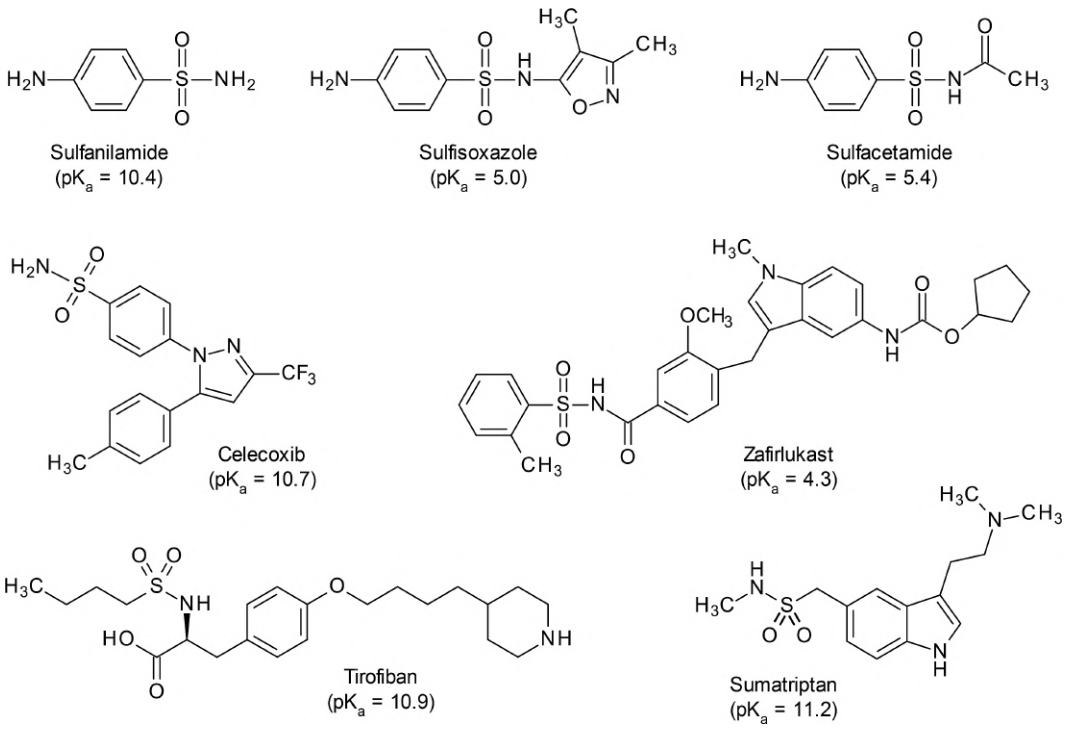
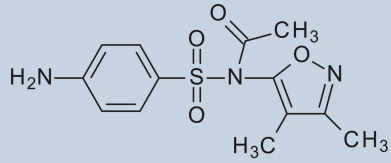


FIGURE 3-3. Examples of drug molecules that contain a sulfonamide and how an adjacent functional group can affect the pK$_a$.

Examples of drug molecules that contain a sulfonamide are shown in **Figure 3-3**. As indicated by these examples, the acidic nature of sulfonamides can vary widely (typical pK$_a$ range is 4.5 to 11) and is strongly influenced by the electronic effects of adjacent groups or resonance-linked groups. Sulfanilamide was one of the first sulfonamide drugs used to treat bacterial infections. The unsubstituted, aromatic sulfonamide present within its structure is weakly acidic. This trend can also be seen with celecoxib, which has a similar functional group. The addition of adjacent electron withdrawing groups such as the aromatic heterocycle present in sulfisoxazole and the carbonyl present in sulfacetamide and zafirlukast increases delocalization of the resulting negative charge and increases the acidity of the proton. For sulfacetamide and zafirlukast, the electron withdrawing ability is due to a direct resonance delocalization of the negative charge into the adjacent carbonyl group. For sulfisoxazole, the heterocyclic ring can withdraw electrons through either resonance or an inductive effect. A key point to remember is that *electron withdrawing groups increase the acidity of adjacent functional groups or resonance-linked functional groups*. The exact opposite is true of electron donating groups. The aliphatic sulfonamide groups seen in tirofiban and sumatriptan emphasize this last point. These sulfonamide groups are adjacent to electron donating alkyl chains. As a result, these functional groups are much less acidic, as indicated by their pK$_a$ values.

Application Question

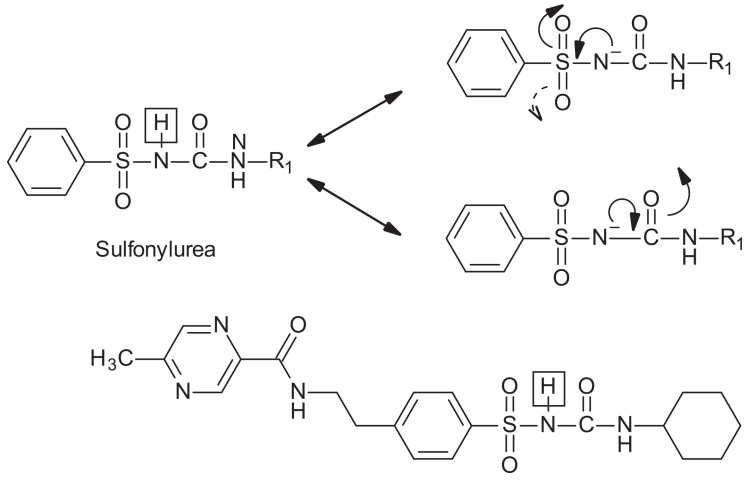
How does the acidity of the following drug molecule compare with those in Figure 3-3?



Sulfisoxazole acetyl

Answer: Similar to what was discussed with drug molecules containing a β-dicarbonyl group, the sulfonamide functional group must contain an ionizable hydrogen atom (proton; H^+). In this case, the nitrogen atom within the sulfonamide functional group has been acetylated. Thus, it no longer contains an acidic proton and therefore is not acidic. It is still a sulfonamide (i.e., it is a "sulfur-containing" amide), but since the nitrogen is substituted, the functional group is neutral. Sulfisoxazole acetyl is actually a prodrug. As discussed in much more detail in Chapters 5 and 8, a *prodrug* is a drug molecule that has been covalently modified to either an inactive or weakly active analog for the purposes of achieving a specific therapeutic benefit. Once the prodrug is administered, it undergoes metabolic activation to release the active drug molecule. In this case, sulfisoxazole acetyl must be converted in vivo to sulfisoxazole for it to exert its activity.

Sulfonylureas are very closely related to sulfonamides. The resonance delocalization of an ionized sulfonylurea can occur across all three adjacent sulfonyl and carbonyl bonds and is similar to the ionizations previously discussed with sulfacetamide and zafirlukast. The pK_a values of sulfonylureas generally range from 5 to 6.

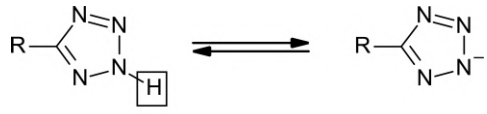


Sulfonylurea

Glipizide (pK_a = 5.34)

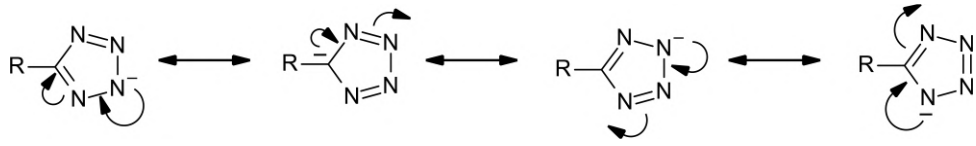
Tetrazoles

Tetrazole rings are isosteres of carboxylic acids. As discussed in more detail in Chapter 9, isosteres are functional groups with a similar distribution and location of electron density and a similar size and shape. The lone hydrogen atom in this ring system is acidic.

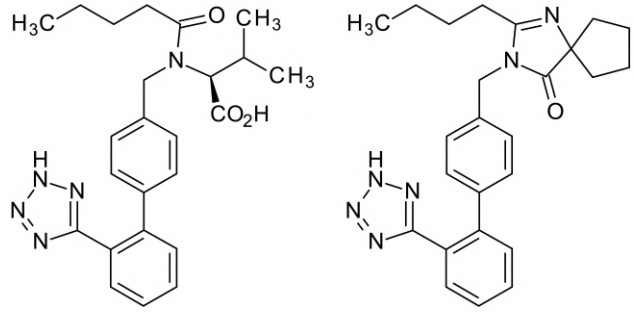


Unionized Ionized

The resulting negative charge can be equally shared among all five atoms of the tetrazole ring by resonance, thus allowing the charge to be distributed over a larger area than that seen for a carboxylic acid.



In comparison with a carboxylic acid, a tetrazole ring is less acidic (typical pK_a range is 4.5 to 6) and more lipophilic. Not many drugs or drug classes contain a tetrazole; however, angiotensin II receptor blockers almost exclusively include this functional group instead of a carboxylic acid (**Figure 3-4**). Advantages gained by using the tetrazole ring instead of a carboxylic acid for this class of drug molecules include better oral bioavailability and enhanced metabolic stability.

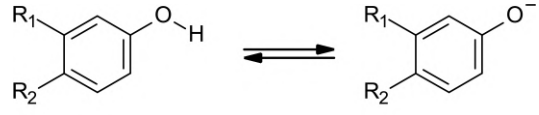


Valsartan (pK_a = 4.9) Irbesartan (pK_a =4.7)

FIGURE 3-4. Examples of drug molecules that contain an acidic tetrazole ring.

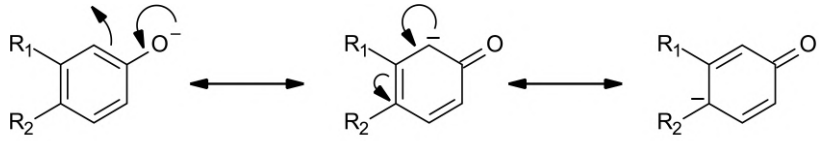
Phenols

Phenols (i.e., hydroxyl groups attached to an aromatic ring) are weakly acidic functional groups.



Unionized Ionized

Similar to previously discussed functional groups, the negative charge can be delocalized. In this case, the negative charge can be shared with the carbon atoms in the aromatic ring.



Although the aromatic ring allows resonance delocalization, it must be noted that carbon atoms are less electronegative than oxygen atoms and thus are less likely to want to share the negative charge. The end result of this effect is that phenols are much weaker acids than those functional groups previously discussed. The pK_a values of phenols normally range from 9 to 10. At any physiologically relevant pH, most phenols are not appreciably ionized. A good example of this guideline is illustrated by estradiol (**Figure 3-5**). Estradiol can be technically and correctly classified as a very weak acid; however, because the phenol is not appreciably ionized at physiologic pH, estradiol is commonly classified as a nonelectrolyte.

Definitions

An *electrolyte* is a molecule that can dissociate into ions in solution and thus can carry an electrical current. Drug molecules that contain one or more of the acidic and/or basic functional groups discussed in this chapter are therefore electrolytes. In contrast, a *nonelectrolyte* is a molecule that does not dissociate into ions in solution and thus does not carry an electric current. Drug molecules that lack acidic or basic functional groups, or only possess weakly acidic or weakly basic functional groups that are not appreciably ionized, are termed nonelectrolytes.

Electron withdrawing groups, such as the iodine atoms seen in liothyronine and levothyroxine in Figure 3-5, increase the acidity of phenols. While estradiol is only 0.1% ionized at a physiologically relevant pH, liothyronine is approximately 10% ionized at a pH of 7.4. Appreciable ionization can occur with very strong electron withdrawing groups or with the presence of multiple electron withdrawing groups. The latter is seen in levothyroxine. The two adjacent iodine atoms increase the acidity almost four log units as compared with estradiol. As a result, levothyroxine is approximately

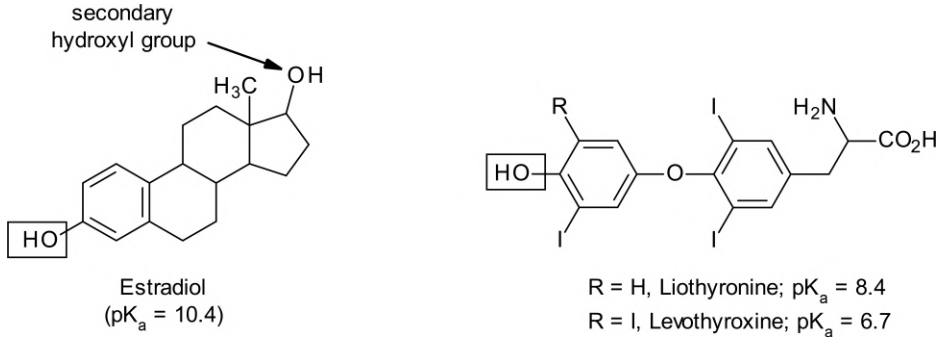
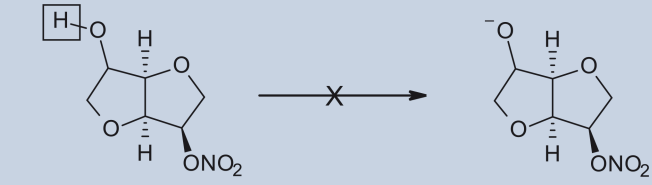


FIGURE 3-5. Examples of drug molecules that contain a phenol (highlighted with a box) and an aliphatic hydroxyl group.

83% ionized at a pH of 7.4. It should be noted that the number of drug molecules with appreciably ionized phenols is extremely small and should be treated as the exception rather than the rule.

What About Aliphatic Hydroxyl Groups?

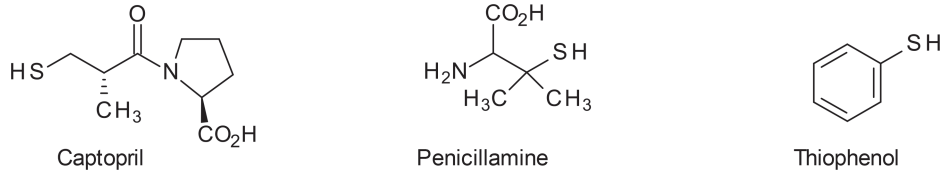
Aliphatic hydroxyl groups, such as the secondary hydroxyl group seen in estradiol (Figure 3-5) and the one seen below in isosorbide mononitrate, are not acidic within physiological environments. The reason for this is the absence of any resonance stabilization of the resulting charge. Unlike the functional groups previously discussed, an aliphatic hydroxyl group is not directly adjacent to a functional group that could share or stabilize a negative charge (e.g., a carbonyl group, a sulfonyl group, or an aromatic ring). As a result, the proton is not ionizable in any physiological environment and the functional group is not acidic.



Isosorbide Mononitrate

Thiols

Thiols (aka sulfhydryl groups) are approximately 4 to 5 log units more acidic than hydroxyl groups. Aliphatic thiols have pK_a values that range from 10 to 11, as illustrated with captopril and penicillamine (**Figure 3-6**). Similar to the phenol in estradiol, aliphatic thiols can be technically and correctly classified as very weak acids; however, since these thiols are not appreciably ionized at any physiologic pH, they are most commonly classified as nonelectrolytes. Aromatic thiols, due to the availability of resonance stabilization, are much more acidic than aliphatic thiols and generally have a pK_a value near 6. Because aromatic thiols are highly nucleophilic and highly reactive, they are not present in drug molecules. In general, the key chemical property of a thiol is its nucleophilicity, not its acidity.



Captopril (pK_a = 9.8)

Penicillamine (pK_a = 10.1)

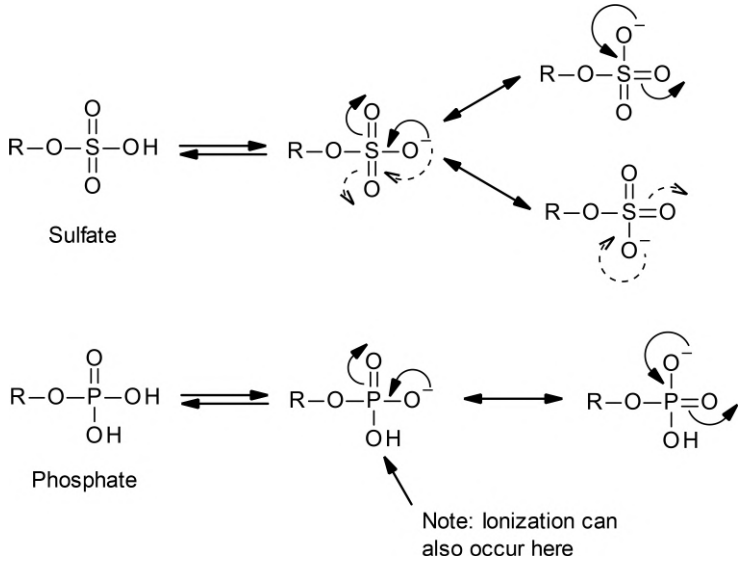
Thiophenol (pK_a = 6)

FIGURE 3-6. Examples of drug molecules that contain a thiol (aka sulfhydryl group).

Sulfates, Phosphates, and Phosphonates

Sulfates, phosphates, and phosphonates are similar to β-dicarbonyls, sulfonamides, and sulfonylureas in that the initial negative charge can be shared with adjacent double-bonded oxygen atoms. Sulfates have one acidic hydrogen atom with a pK_a value that ranges from 1 to 2, while phosphates and phosphonates have two acidic hydrogen atoms. The pK_a value for the ionization of the first

hydrogen atom is approximately 1.5 to 2.5, while the pK_a value for the ionization of the second hydrogen atom is higher, ranging from 6.5 to 7.5.



These acidic functional groups are seen occasionally; however, they are not as prevalent as carboxylic acids and β-dicarbonyl groups. In many cases they are used to create a more water-soluble prodrug to enhance dissolution. Further discussion of this concept is discussed in Chapter 5. As seen in the examples in **Figure 3-7**, drug molecules that contain these functional groups are often marketed as their respective salts.

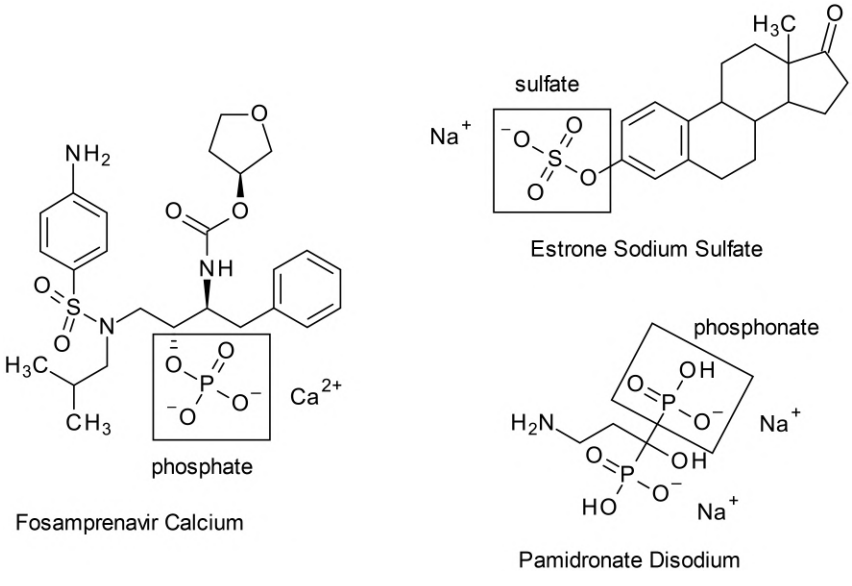
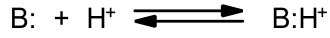


FIGURE 3-7. Examples of drug molecules that contain a phosphate, sulfate, or phosphonate salt.

BASIC FUNCTIONAL GROUPS

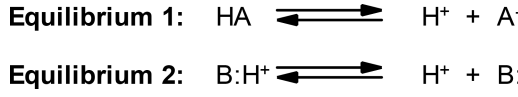
Opposite of acids, basic functional groups are those that can accept (or gain) a proton. In a very simplistic view, a base can be represented in the following way:



The key structural feature of a basic functional group is the presence of an atom with a lone pair of electrons (e.g., nitrogen atom) that can bind to and accept a proton. The availability of these electrons to perform this function determines the basicity of the functional group. Adjacent electron withdrawing groups that ***increase*** the acidity of acidic functional groups ***decrease*** the basicity of basic functional groups. By the same reasoning, adjacent electron donating groups ***increase*** the basicity of the functional group.

Similar to acidic functional groups, pK_a values are used to measure the relative basicity of functional groups. Opposite of acidic functional groups, a higher pK_a value indicates a stronger base. As an example, a basic functional group with a pK_a of 9.8 is more basic than one with a pK_a of 6.7.

Because pK_a values are based on acid equilibrium equations, it is important that you understand why these values can also be used for bases and what the values actually indicate. The pK_a value is based on the dissociation equilibrium of an acid. This can be seen in Equilibrium 1, shown below. To use pK_a values for bases, an analogous equilibrium, Equilibrium 2, must be considered.



In this case, the pK_a of the base in Equilibrium 2 is based on the dissociation of $B:H^+$, its conjugate acid. An important point applicable to both of these equilibrium equations is that a low pK_a value indicates that the equilibrium lies to the right (i.e., favors the loss of a proton and the ionization of HA) and that a high pK_a value indicates that the equilibrium lies to the left (i.e., favors the acceptance of a proton and the ionization of B:). Thus, a low pK_a value is seen with both strong acids and weak bases, whereas a high pK_a value is seen with both strong bases and weak acids.

Back to General Chemistry

Once an acidic functional group loses its proton and becomes ionized (or deprotonated), it becomes what is known as a *conjugate base*. This is because, like a base, it is now able to accept protons. Thus, A^- in Equilibrium 1 is the conjugate base of the acid HA. Likewise, once a basic functional group, such as B: in Equilibrium 2, accepts a proton and becomes ionized (or protonated), it becomes a conjugate acid because it is now able to lose the proton.

Aliphatic and Alicyclic Amines

Shown in **Figure 3-8** are four drug molecules, each of which has an aliphatic or alicyclic amine as part of its structure. These amines have been highlighted and drawn in their unionized form. The lone pairs of electrons available to accept protons are also shown here and in the general structures shown below; however, caution is warranted here. Most drug structures that you will encounter do not show these lone pair of electrons. Thus, it is important that you recognize and understand that these electrons are present and available to accept a proton even if they are not actually shown.

As illustrated in Figure 3-8, aliphatic amines are designated as primary, secondary, or tertiary depending upon the number of alkyl groups attached to the nitrogen atom. Alicyclic amines, or

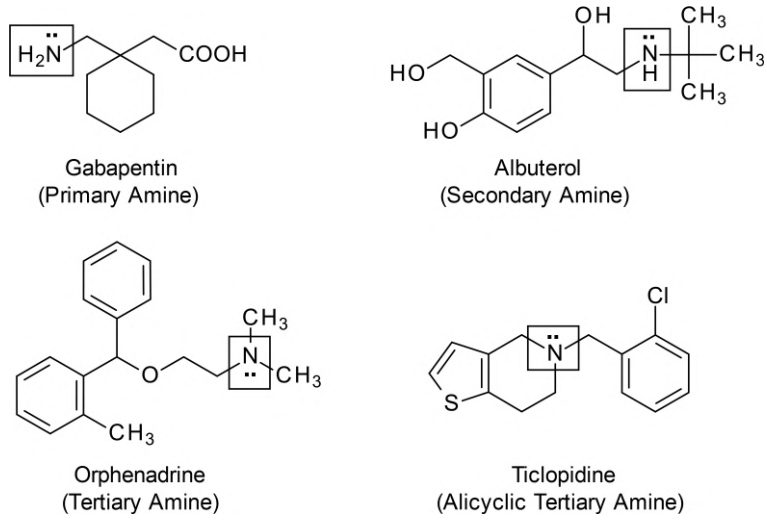
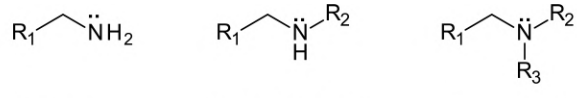


FIGURE 3-8. Examples of drug molecules that contain aliphatic and alicyclic amines. All drug molecules have been drawn in their unionized form.

saturated heterocycles, can be either secondary or tertiary since two carbon atoms are required to form the ring.



The term *aliphatic* denotes that the amine is part of a saturated chain or a nonaromatic ring. The highlighted amine in ticlopidine can also be classified as an alicyclic amine or a saturated heterocycle. Either one of these terms can be used to describe an amine that is part of a nonaromatic ring. Aliphatic amines are the most common basic functional groups present on drug molecules. The ionized forms of each of these drug molecules along with the pK_a values for their respective amines are shown in **Figure 3-9**.

In comparing the relative basicity of primary, secondary, and tertiary amines, two factors must be considered: the presence or absence of adjacent functional groups that are either electron donating or electron withdrawing in character and steric hindrance. Because alkyl groups such as methyl groups, ethyl groups, and methylene carbons are electron donating, secondary and tertiary amines are often more basic than primary amines. Steric effects must also be considered as they can hinder the access of protons to the lone pair of electrons on the nitrogen atom. This issue is more prevalent with tertiary amines; thus, secondary amines are often more basic than tertiary amines. The presence of adjacent electron withdrawing groups decreases the availability of the electrons on the nitrogen atom and decreases basicity and the pK_a. While exceptions arise, the pK_a range generally listed for aliphatic and alicyclic amines is 9 to 11.

The drugs in Figure 3-9 were specifically chosen to illustrate the variations in this range and the concepts listed above. The structure of gabapentin contains a primary amine that is adjacent to an aliphatic chain connected to an alicyclic ring. The lack of steric hindrance, the electron donating effects of the aliphatic chain, and the absence of any electron withdrawing functional group allow the lone pair of electrons to be readily available. Thus, gabapentin contains the strongest basic functional group among these four drugs. Albuterol contains a secondary amine. The *t*-butyl group donates electrons to the basic nitrogen, as does the adjacent carbon atom; however, the hydroxyl

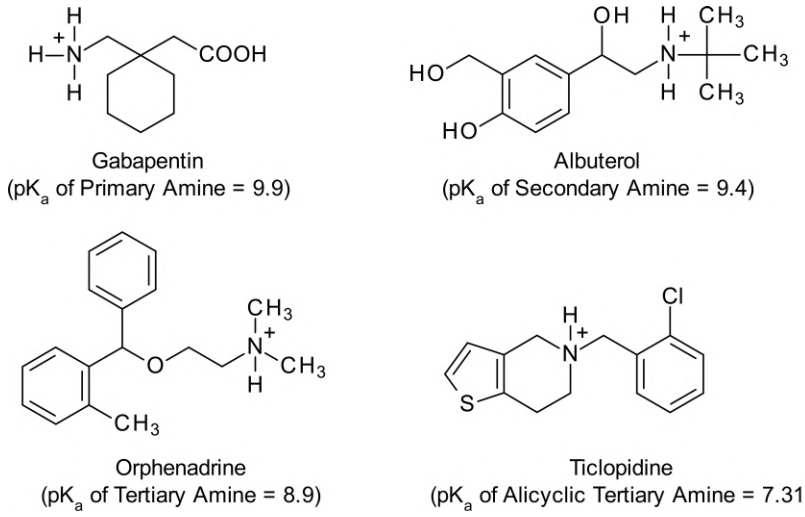
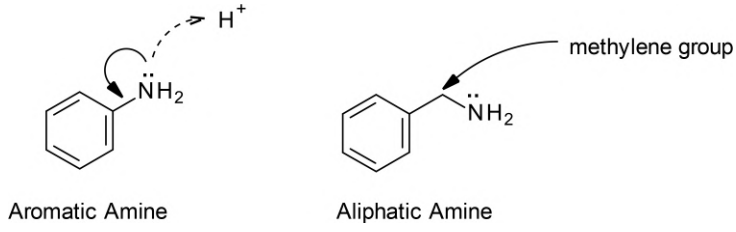


FIGURE 3-9. Examples of drug molecules that contain aliphatic and alicyclic amines with their respective pK$_a$ values. All drug molecules have been drawn in their ionized form.

group, located three atoms away from the amine, has an electronic withdrawing effect leading to a pK$_a$ value less than that seen with gabapentin. The tertiary amine within the structure of orphenadrine has similar electron donating and withdrawing groups, as seen with albuterol. The three directly adjacent methyl and methylene carbon atoms donate electrons, while the ether oxygen is more electronegative and acts as an electron withdrawing group. There is a little more steric hindrance for the tertiary amine of orphenadrine as compared with the secondary amine of albuterol, which is reflected by the slightly lower pK$_a$ value. Finally, the alicyclic tertiary amine seen in the structure of ticlopidine has a significantly lower pK$_a$ value. There are two reasons for this. First, the alicyclic tertiary nature of this amine causes more steric hindrance, as compared with the amines on the other three drugs. Second, the chlorine atom is electron withdrawing in character and decreases the availability of the lone pair of electrons.

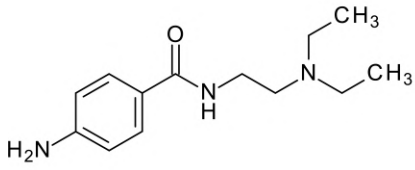
Aromatic Amines

Aromatic amines, also known as anilines, are amines directly attached to an aromatic ring and are much less basic than aliphatic and alicyclic amines. The reason for this decreased basicity lies in the fact that the aromatic ring, through resonance, serves as an electron withdrawing group and significantly decreases the availability of the nitrogen atom's lone pair of electrons. The simple insertion of a methylene group changes the aromatic amine into an aliphatic amine and significantly increases the basicity of the nitrogen atom since it is no longer directly attached to the aromatic ring.

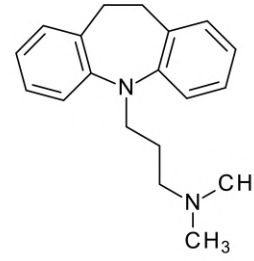


The typical pK$_a$ values for aromatic amines range from 2 to 5. As such, aromatic amines are appreciably ionized in the stomach and perhaps an acidified urine (based on the specific pK$_a$). In all

other physiologic environments, aromatic amines are not appreciably ionized and can be treated as neutral functional groups. Whenever a nitrogen atom is attached to two aromatic rings, it can donate its electrons into either ring. This results in an additional decrease in basicity with pK_a values that are often less than 1. Examples of aromatic amines are shown in **Figure 3-10**.



Procainamide, pKa values:
3.3, aromatic amine
9.2, tertiary amine

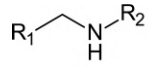


Imipramine, pKa values:
-0.19, aromatic amine
9.5, tertiary amine

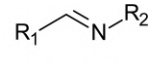
FIGURE 3-10. Examples of drug molecules that contain an aromatic amine.

Imines and Hydrazines

Imines contain an unsaturated bond between the nitrogen atom and an adjacent carbon atom. The term *unsaturated* refers to the presence of the double bond and the fact that the nitrogen atom and the adjacent carbon atom are missing hydrogen atoms (i.e., they are no longer saturated with hydrogen atoms). Imines, also known as Schiff bases, are not commonly seen in drug molecules because they can be easily hydrolyzed unless they are conjugated with an aromatic ring. One example is seen in the benzodiazepine class of drug molecules, exemplified by diazepam (**Figure 3-11**).

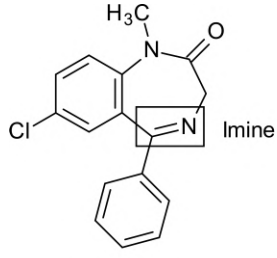


Amine

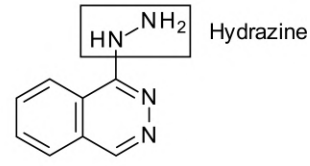


Imine

In general, imines are much less basic than amines, with pK_a values ranging from 3 to 5. This is due to the fact that the nitrogen atoms of amines have sp^3 hybridization, while the nitrogen atoms of imines have sp^2 hybridization. As a result, the lone pair of electrons present on an imine nitrogen atom is closer to the nucleus of the atom than it is in an amine nitrogen atom. Because the electrons are closer to the nucleus, they are less available than those in an amine nitrogen atom. Therefore,



Diazepam

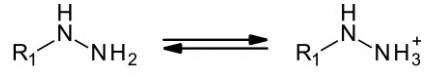


Hydralazine

FIGURE 3-11. Examples of drug molecules that contain basic imine and hydrazine functional groups.

imines are less basic than their respective amines. Additional factors can further influence electron availability and basicity. In the case of diazepam, the imine double bond is in conjugation with the aromatic rings, one of which contains an electron withdrawing chlorine atom. This conjugation causes the imine nitrogen atom of diazepam to have a pK_a value of 3.4. This once again emphasizes that for any given functional group, a variety of factors can influence its overall basicity or acidity.

Hydrazines have the general structure shown below. This functional group is found in only a few drug molecules, with the antihypertensive agent hydralazine being the most notable example (Figure 3-11). The pK_a values for hydrazines range from 7.5 to 8.5.



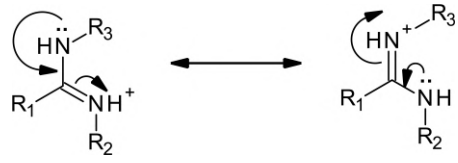
Hydrazine

Amidines and Guanidines

An amidine group can be viewed as a nitrogen-substituted imine. The presence of the extra nitrogen atom allows for resonance delocalization of the positive charge and an increase in the basicity.

Imine Amidine

Similar to a carboxylic acid in which two oxygen atoms share a negative charge, the amidine functional group allows two nitrogen atoms to share a positive charge.



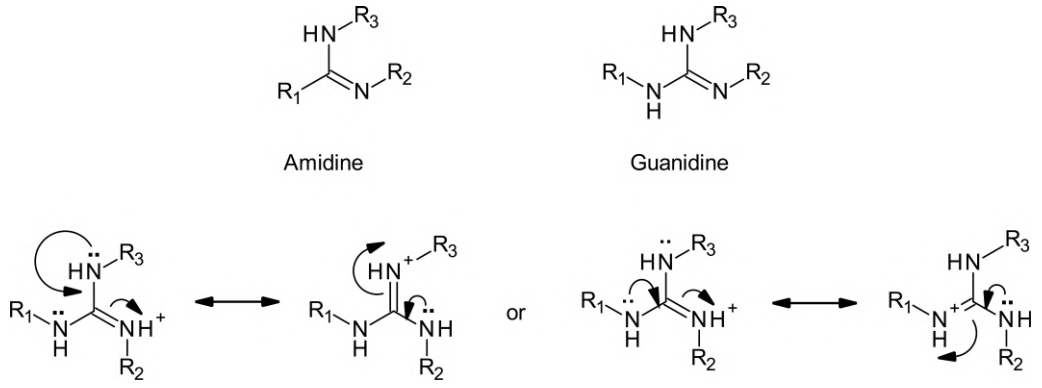
Examples of drug molecules containing an amidine group include naphazoline and tetrahydrozoline (**Figure 3-12**), α adrenergic agonists used as nasal and ophthalmic decongestants. The five-member alicyclic ring containing the amidine is known as an imidazoline ring. Normal pK_a values for amidines range from 10 to 11.

The addition of one more nitrogen atom to the amidine group results in the formation of a guanidine group. The additional nitrogen atom further enhances the basicity of the functional group

Naphazoline Tetrahydrozoline

FIGURE 3-12. Examples of drug molecules that contain a basic amidine functional group.

by allowing the positive charge to be delocalized over all three nitrogen atoms. Due to this additional resonance stabilization, guanidine groups are very strong bases, with pK_a values of 12 to 13.



The most basic nitrogen atom of amidine and guanidine groups is the one that is involved in the unsaturated bond. In reviewing the resonance structures, please note the direction of resonance delocalization and electron flow. If the initial protonation occurred at either of the nitrogen atoms not involved in the unsaturated bond, the same resonance delocalization would not be able to occur. Examples of drug molecules containing a guanidine functional group are shown in **Figure 3-13**.

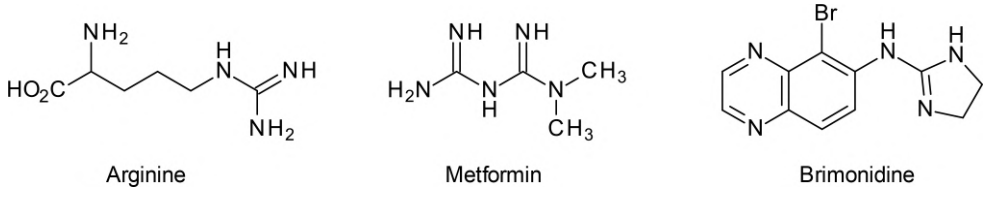


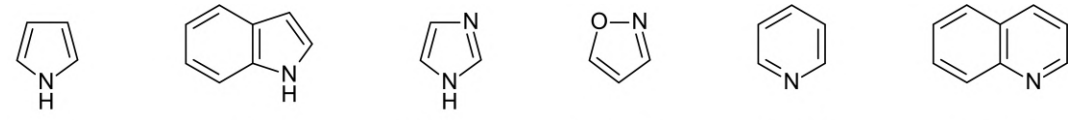
FIGURE 3-13. Examples of drug molecules that contain a basic guanidine functional group.

How Can Resonance Delocalization Both Increase and Decrease Basicity?

Resonance delocalization can either help or hinder the ionization of a nitrogen atom, depending on the direction of delocalization and if the delocalization occurs prior to or after a proton binds to a lone pair of electrons on a nitrogen atom. When delocalization occurs ***after*** a group has been ionized, as shown in the resonance structures for an amidine or a guanidine functional group, the sharing of electrons and the positive charge enhances basicity. This is very similar to the sharing of a negative charge seen with carboxylic acids and other acidic functional groups. When delocalization occurs ***prior to*** ionization, as shown with aromatic amines (aka anilines) and heterocyclic nitrogen atoms, the lone pair of electrons becomes less available to bind with a proton because it is pulled away from the nitrogen atom. This results in a decrease in basicity.

Nitrogen Containing Aromatic Heterocycles

Nitrogen containing aromatic heterocycles vary in their basicity but are generally much less basic than aliphatic and alicyclic amines. The term *heterocycle* refers to a ring system that contains atoms other than carbon. Some examples of heterocyclic rings containing nitrogen atoms are shown below.



The pK_a values for most nitrogen containing aromatic heterocycles range from 1 to 6. These pK_a values are dependent on the size of the ring and the presence, absence, and proximity of other heteroatoms and electron donating and withdrawing functional groups. Five-membered rings and ring systems that contain a single nitrogen atom, such as pyrrole and indole, are not basic because the lone pair of electrons on the nitrogen atom are involved in the aromaticity or resonance delocalization of the ring. As such, they are unavailable for binding to a proton. The presence of a second nitrogen atom, such as that in an imidazole ring, enhances its basicity due to resonance stabilization of the negative charge between the two nitrogen atoms similar to that seen with an amidine. The presence of an oxygen atom decreases the basicity of the isoxazole ring due to the inductive electron-withdrawing property of the oxygen atom. Examples of these effects can be seen in **Figure 3-14** with frovatriptan (indole ring), cimetidine (imidazole ring), and sulfamethoxazole (isoxazole ring).

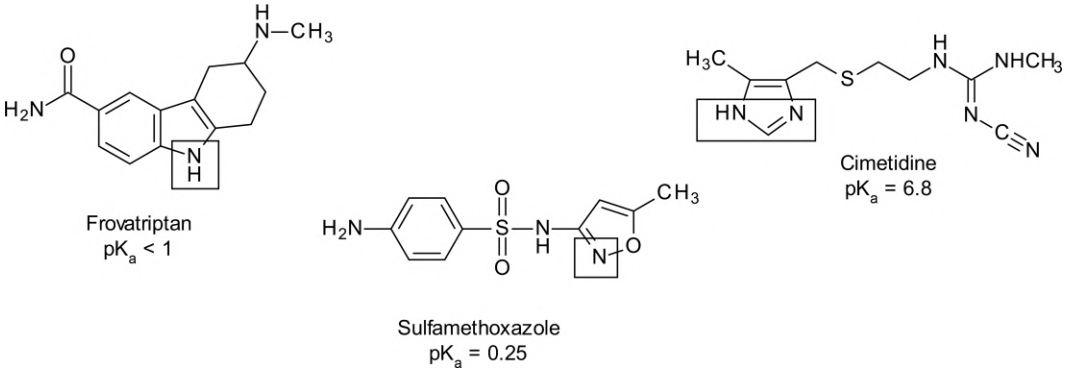


FIGURE 3-14. Examples of drug molecules with five-membered nitrogen containing aromatic heterocycles. Basic functional groups are highlighted with boxes.

Six-membered rings and ring systems that contain a single nitrogen atom, such as pyridine and quinoline, are more basic than pyrrole and indole rings. This is because the lone pair of electrons on the nitrogen atom is not involved in the aromaticity of the ring and is thus available to bind to a proton. Examples of this are seen in **Figure 3-15** with isoniazid and quinidine. The differences in the pK_a values for these two aromatic nitrogen atoms are due to the differences in the functional groups attached to their respective rings. The hydrazide group *para* to the pyridine nitrogen atom is electron withdrawing and decreases the availability of the lone pair of electrons from accepting a proton. In comparison, the quinoline ring within the structure of quinidine contains an electron donating methoxy group that increases the availability of the lone pair of electrons to accept a proton.

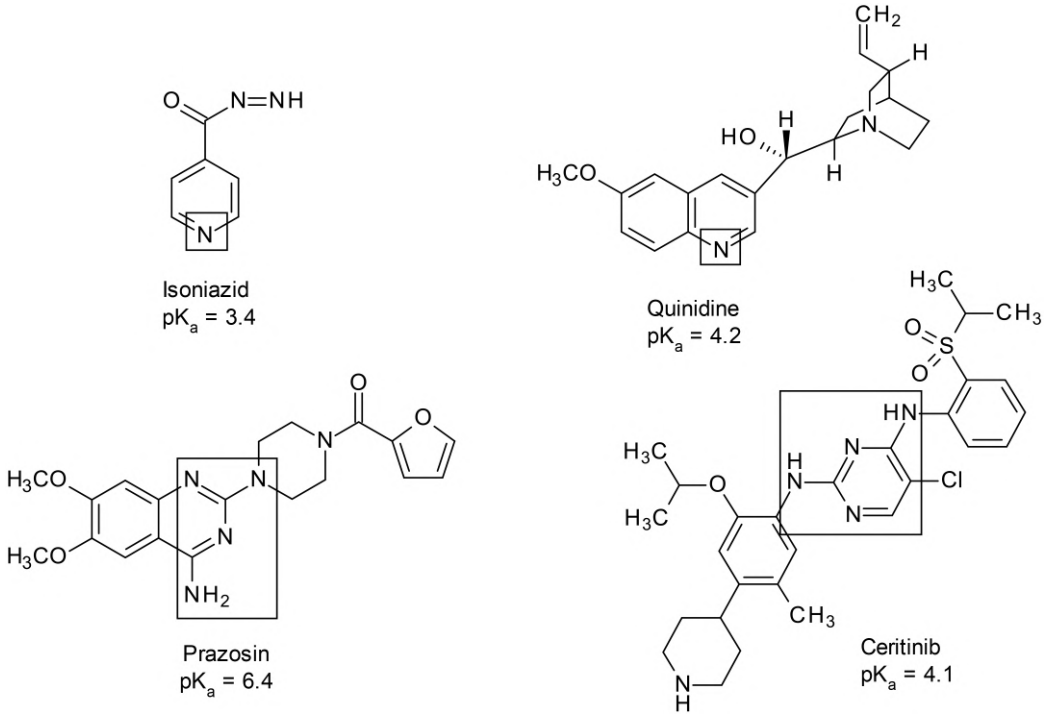


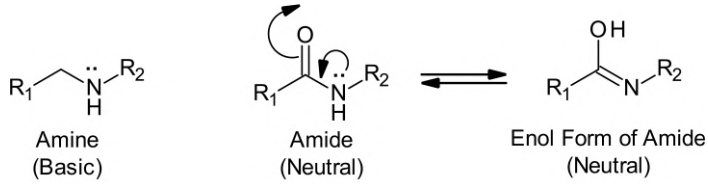
FIGURE 3-15. Examples of drug molecules with six-membered nitrogen containing aromatic heterocycles. Basic functional groups are highlighted with boxes.

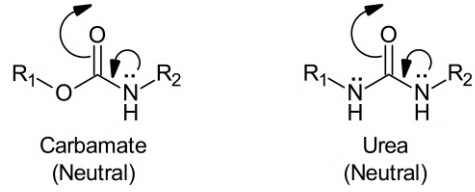
The presence of additional nitrogen atoms within a six-membered ring or ring system can either enhance or detract from the basicity depending on the ability for resonance delocalization of the positive charge or removal of the lone pair of electrons through resonance or induction. Prazosin and ceritinib (Figure 3-15) provide examples of this concept. The basic functional group present within the structure prazosin is very similar to that seen with a guanidine functional group. Because this functional group is part of an aromatic ring system, the overall basicity is substantially lower than an alkyl guanidine. This is similar to the decreased basicity seen in aromatic amines. The structure of ceritinib contains the same basic nitrogen group as prazosin; however, its pK_a is more than 2-fold less. This decreased basicity is due to the electron withdrawing chloro group that is attached to the same aromatic ring. As a final point, there are a large number of structural variations in nitrogen containing aromatic heterocycles. The relative basicities of these rings are due to the cumulative effects of adjacent atoms and functional groups.

Additional Nitrogen Containing Groups

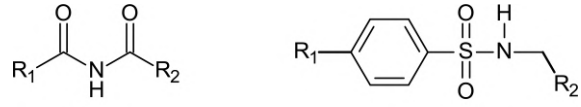
The following functional groups are not basic but are reviewed here to emphasize a key concept. A common mistake made by students is to assume that all nitrogen containing groups are basic when, in fact, these groups can be basic, neutral, acidic, or permanently charged quaternary ammonium salts. When a nitrogen atom is adjacent to a carbonyl group, it is part of an amide group. The lone pair electrons on the nitrogen atom are involved in keto-enol tautomerization with the carbonyl group and are not available for binding to a proton. Similar effects are seen with carbamates and

ureas. *The key point here is that amides, carbamates, and ureas are neutral functional groups, not basic functional groups.*

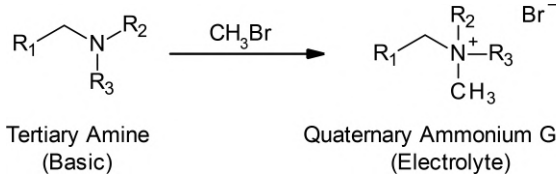




In addition, when a nitrogen atom is adjacent to two carbonyl groups or a sulfonyl group, the functional group is an imide or a sulfonamide, respectively. As previously discussed, these are acidic functional groups.



Alkylation of a tertiary amine results in a quaternary ammonium salt (or group). In the example shown below, please note that the nitrogen atom in this group has four bonds and thus does not have an available lone pair of electrons or a proton. Since this functional group can neither gain nor lose a proton, it is neither basic nor acidic; however, similar to acidic and basic functional groups, it can dissociate into ions in solution and thus can carry an electrical current. As such, drug molecules that contain this functional group are often classified as electrolytes.



Summary of Key Points Regarding Acidic and Basic Functional Groups

- For a functional group to be acidic in character, it must meet two criteria.
  - It must have at least one hydrogen atom that can dissociate from the functional group.
  - The remaining atoms must be able to delocalize the resulting negative charge via resonance.
- Acidic functional groups have general (or normal) pK_a ranges due to the presence of adjacent functional groups.
  - The presence of adjacent electron withdrawing groups enhances the acidity of the functional group and results in a lower pK_a value.

- The presence of adjacent electron donating groups decreases the acidity of the functional group and results in a higher pK_a value.
- For a functional group to be basic in character, it must have a nitrogen atom with a lone pair of electrons that can bind to and accept a proton.
- Basic functional groups have general (or normal) pK_a ranges due to two reasons: (1) the presence of adjacent functional groups and (2) the presence or lack of steric hindrance around the lone pair of electrons.
  - The presence of adjacent electron donating groups enhances the basicity of the functional group and results in a higher pK_a value.
  - The presence of adjacent electron withdrawing groups decreases the basicity of the functional group and results in a lower pK_a value.
  - Steric hindrance around the lone pair of electrons decreases the ability of a proton to bind, resulting in decreased basicity and a lower pK_a value.
- Not all nitrogen containing functional groups are basic.

A summary of the typical pK_a ranges for all of the acidic and basic functional groups discussed in this chapter is shown in **Table 3-1**. Please note that some of the drug molecules that you will encounter have functional groups with pK_a values that are slightly outside of these ranges. This typically occurs whenever adjacent functional groups contribute a significant electronic effect through either resonance or induction; however, there may be situations in which steric effects can hinder ionization. Based on the discussions in this chapter and in Chapter 2, you should be able to offer

TABLE 3-1. Approximate pK_a Values for Common Acidic and Basic Functional Groups

Functional Group	Acidic or Basic	pK_a Range
Carboxylic acids	Acidic	2.5-5
β-Dicarbonyl groups (includes imides)	Acidic	4.5-8.5
Sulfonamides	Acidic	4.5-11
Sulfonylureas	Acidic	5-6
Tetrazoles	Acidic	4.5-6
Phenols	Acidic	9-10
Thiols	Acidic	10-11
Sulfates	Acidic	1-2
Phosphates and phosphonates	Acidic	1.5-2.5 (first phosphate) 6.5-7.5 (second phosphate)
Aliphatic amines and alicyclic amines (aka saturated heterocycles)	Basic	9-11
Aromatic amines (aka anilines)	Basic	2-5
Imines	Basic	3-5
Hydrazines	Basic	7.5-8.5
Amidines	Basic	10-11
Guanidines	Basic	12-13
Nitrogen containing aromatic heterocycles	Basic	1-6

explanations for pK_a values that lie outside these ranges as well as those that lie very close to one end of the range.

Functional Groups Versus Drug Molecules

This chapter has focused primarily on acidic and basic functional groups; however, any given drug molecule may contain one or more of these functional groups within its structure. Each functional group is acidic, basic, amphoteric, a permanently charged quaternary ammonium salt, or neutral (i.e., not ionizable). Examples of each of the possible combinations are shown in **Figure 3-16**. A drug molecule such as gemfibroil that contains one or more acidic functional groups and no basic functional groups within its structure is known as an *acidic drug*. A drug molecule such as clonidine that contains one or more basic functional groups and no acidic functional groups within its structure is known as a *basic drug*. Drug molecules that contain at least one acidic and one basic functional group are known as *amphoteric molecules*. Ciprofloxacin is an example of an amphoteric drug molecule since its structure contains an acidic carboxylic acid and a secondary amine. Drug molecules that contain only neutral functional groups, such as eplerenone, are known as *nonelectrolytes*, whereas drug molecules that contain a quaternary ammonium salt and no other ionizable functional groups (and therefore are unable to accept or donate a proton), such as neostigmine bromide, are known as *electrolytes*.

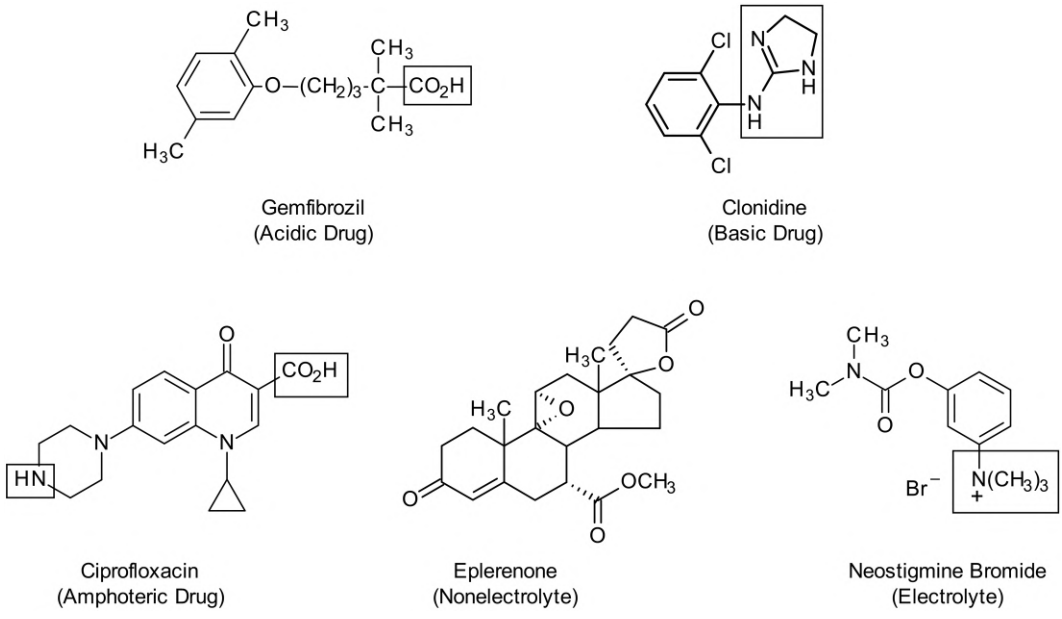


FIGURE 3-16. Examples of drug molecules that are acidic, basic, amphoteric, a nonelectrolyte, and an electrolyte.

THE THERAPEUTIC SIGNIFICANCE OF THE ACID/BASE NATURE OF DRUG MOLECULES

The acid/base nature of a drug molecule influences its chemical, pharmaceutical, and therapeutic properties. The purpose of this section is to identify these key relationships so you gain a better appreciation of the applications of acidic and basic drugs and functional groups. Almost all of these topics are discussed in much more detail in subsequent chapters.

The most important aspect of an acidic or basic functional group is its ability to be ionized (i.e., add a negative or positive charge to the drug). The extent to which a functional group can ionize depends on its pK_a and the environment in which it resides. For example, acids are primarily unionized in an acidic environment, while bases are primarily ionized in an acidic environment. The opposite is true in a basic environment.

Functional group ionization influences the overall water solubility of a drug molecule. An increase in the ionization of a functional group within a drug molecule provides positive or negative charges that substantially increase the drug's water solubility due to enhanced solvation interactions. An increase in water solubility enhances both the rate and extent of dissolution of a drug molecule within the gastrointestinal (GI) tract. This also allows a drug to be concentrated in a small volume for use in intravenous (IV) and ophthalmic solutions. The overall oral absorption of a drug molecule depends first on its ability to dissolve in the GI tract and second on its ability to traverse the lipid bilayer membrane. Although ionized functional groups aid in the dissolution of a drug molecule, the unionized forms of these same functional groups are much more favorable for the passage through lipid membranes. Given that ionization is an equilibrium process, the acid or base strength of a functional group determines the extent to which it is ionized or unionized in any given environment. Thus, the pK_a ranges provided for the various functional groups can help determine the overall effect that a given acidic or basic functional group has on absorption. Ionization of a drug molecule within the urinary tract is also important in terms of passive renal reabsorption. Drug molecules that are highly ionized are much less likely to be passively reabsorbed than those that are unionized. This same concept applies to the passage of any drug molecule across any membrane barrier. A more extensive discussion of pH, pK_a, and ionization is provided in Chapter 4.

Ionization of a functional group allows for the formation of ionic interactions between a drug molecule and its target receptors, transport proteins, enzymes, and/or other endogenous biological targets. As discussed in Chapter 6, ionic interactions are the strongest noncovalent bonds that can be formed between a drug molecule and its biological target(s), and they are often responsible for the initial molecular recognition. Ionic interactions between drug molecules and plasma proteins can extend the duration of action of these drugs by sequestering them from metabolic and elimination pathways. Human serum albumin (aka albumin) is a major transport protein for a number of endogenous substances and drug molecules. Albumin contains numerous binding sites and is somewhat nonspecific in its binding but generally tends to bind acidic drug molecules to a much greater extent than basic drug molecules. In addition, it tends to bind hydrophobic drug molecules to a much greater extent than hydrophilic drug molecules. Although the binding of acidic drugs to albumin is somewhat nonspecific, drug interactions can occur if two different acidic drug molecules are competing for the same binding site. These drug interactions are known as *plasma protein binding interactions* or *plasma protein displacement interactions* and are clinically relevant for those acidic drugs that are more than 90% plasma protein bound. Some examples are shown in **Figure 3-17**.

Basic drugs bind to a different plasma protein, α_1-acid glycoprotein and, similar to acidic drugs, could cause plasma protein displacement interactions with other basic drugs competing for the same binding site on this plasma protein. Some examples are shown in **Figure 3-18**. Since acidic and

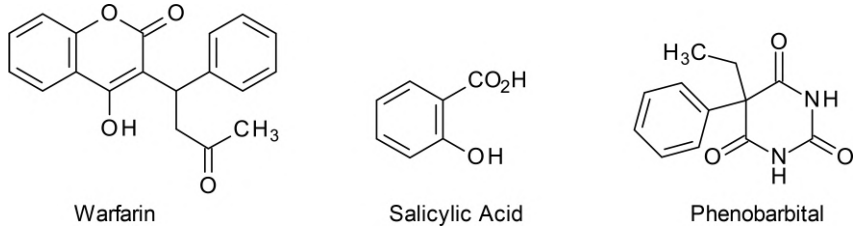


FIGURE 3-17. Examples of acidic drug molecules that are highly bound to the plasma protein albumin.

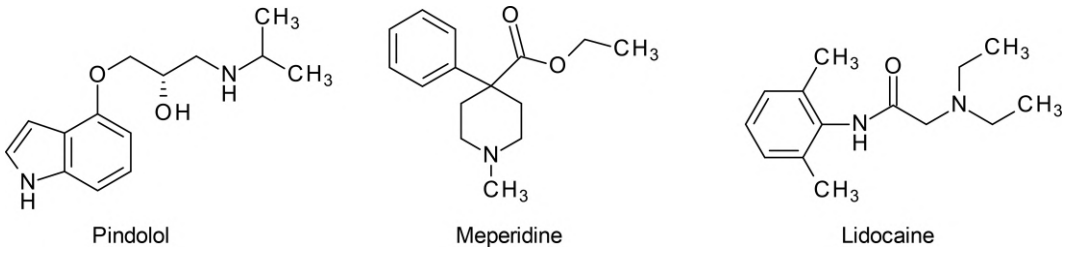


FIGURE 3-18. Examples of basic drug molecules that are highly bound to the plasma protein α_1-acid glycoprotein.

basic drugs bind to two different plasma proteins, acidic drugs generally do not cause displacement interactions with basic drugs and vice versa.

Ionization is also important in the metabolism and elimination of drug molecules. As discussed in more detail in Chapter 8, one of the main purposes of drug metabolism is to ensure that the body can eliminate the drug. In general, this is accomplished by altering existing functional groups or adding more water-soluble groups. Some examples include the oxidation of a hydroxyl group to a carboxylic acid, the hydrolysis of an amide to an amine and a carboxylic acid, the addition of an amino acid, and the addition of glucuronic acid. Drug molecules that inherently contain multiple ionizable acidic and/or basic functional groups already possess significant water solubility and generally do not require extensive metabolism. Examples of this are seen with the aminoglycosides and bisphosphonates (**Figure 3-19**). These drug molecules contain multiple ionizable functional groups, are highly water soluble, do not require metabolism, and are excreted in the urine.

Specific transport proteins for organic acids are present within the renal tubules. These proteins can actively secrete acidic molecules (e.g., penicillins and cephalosporins) from the plasma to the

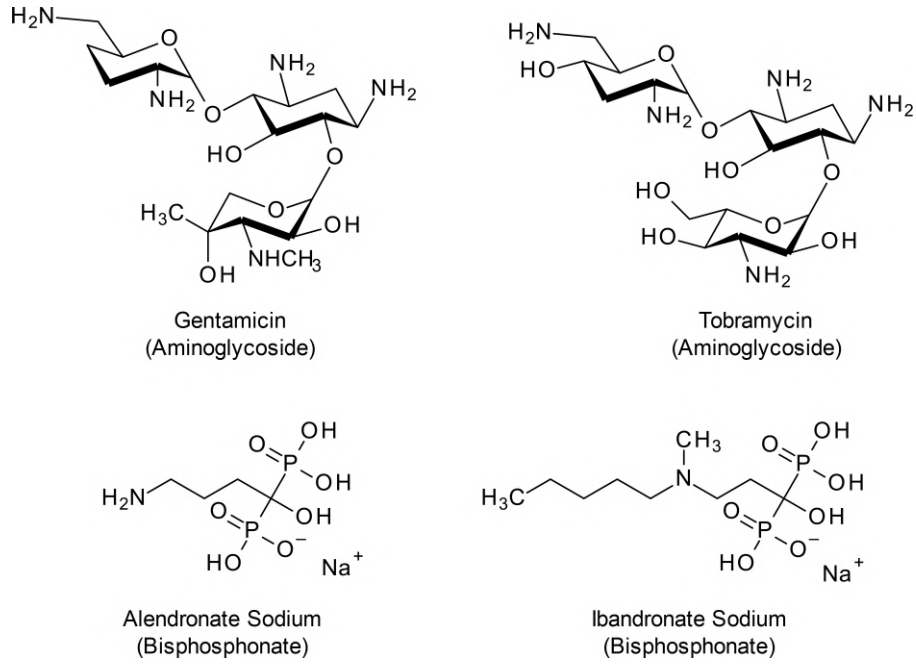


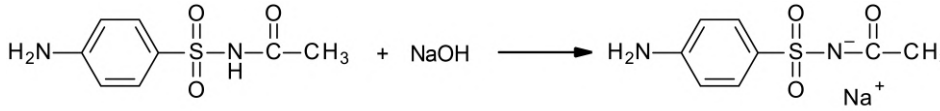
FIGURE 3-19. Examples of drug molecules that contain multiple acidic or basic functional groups.

urine, thus decreasing their half-lives. They can also actively transport other acidic molecules from the urine back into the plasma, thus increasing the half-lives of these molecules. *Two key points need to be made here.*

- First, drug interactions or adverse effects can occur if two acidic drug molecules simultaneously require this transport/secretion pathway since the pathway is saturable.
- Second, basic drug molecules do not use the same transport/secretion proteins as acidic drug molecules and thus would not cause a drug interaction via this mechanism.

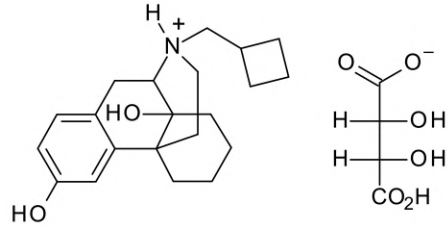
As an example, thiazide diuretics are acidic drug molecules that require this active secretion process to gain access to their site of biological action. Uric acid, a natural breakdown product of purines, also requires active secretion to be eliminated from the body. Thiazides compete with uric acid for the transport sites and block the normal secretion of uric acid. This can lead to hyperuricemia and contribute to the development of gout in some patients.

Acidic and basic functional groups present on a drug molecule can be used to produce commercially available salt formulations. As discussed in more detail in Chapter 5, a *salt* is simply the product that is produced when an acid is combined with a base. As an example, sodium sulfacetamide can be produced by reacting sulfacetamide with sodium hydroxide.

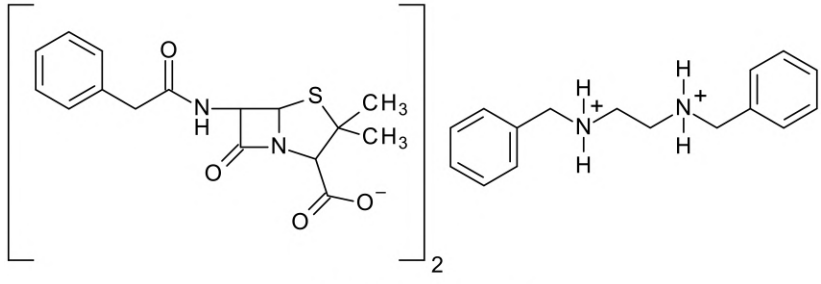


Sulfacetamide Sodium Sulfacetamide

Salts can be formed by using either organic or inorganic acids or bases. Inorganic salts, such as sodium sulfacetamide, are generally used to enhance the water solubility and dissolution of a drug molecule. Water-soluble organic salts, such as butorphanol tartrate (**Figure 3-20**), are similar to inorganic salts and can further enhance water solubility, dissolution, and the ability to form



Butorphanol Tartrate



Penicillin G Benzathine

FIGURE 3-20. Examples of a water-soluble organic salt (butorphanol tartrate) and a lipid-soluble organic salt (penicillin G benzathine).

concentrated IV and ophthalmic solutions. In contrast, lipid-soluble organic salts, such as penicillin G benzathine (Figure 3-20), are used to formulate suspensions for intramuscular (IM) injection (i.e., IM depot injections). After injection, these lipid-soluble salts are slowly released from the injection site and impart a longer duration of action for the drug.

Although salts can provide important therapeutic benefits, it must be noted that the indiscriminate combination of acidic and basic drugs can produce significant interactions. Acidic and basic drugs should never be combined in the same IV infusion bag or infused through the same IV line due to the possibility of organic salt formation and precipitation. Thus, a thorough understanding of the acid/base nature of drug molecules and their functional groups can help prevent these types of drug interactions. In addition, the acid/base nature of any single drug impacts its overall suitability for a given parenteral formulation because its acidity or basicity contributes to the overall pH of the formulation and affects buffers and other components. This is also true in solid formulations. The combination of acidic and basic drugs in solid formulations may lead to the degradation of one or both of the drug molecules.

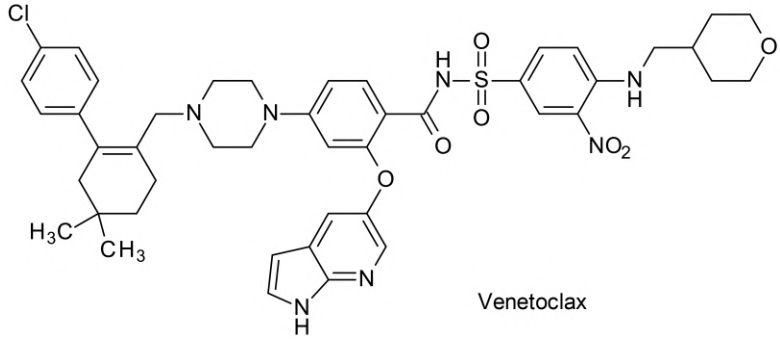
Summary: The Influence of the Acid/Base Nature of a Drug Molecule on Its Chemical, Pharmaceutical, and Therapeutic Properties

The acid/base nature of a drug molecule influences the following conditions:

- the overall water solubility of a drug molecule
- the oral absorption of a drug molecule
- the passive reabsorption of a drug molecule within the urinary tract
- the ability for a drug molecule to interact with its biological target (e.g., target receptor, transport protein, enzyme, or another endogenous biomolecule)
- the metabolism and elimination of a drug molecule
- the ability to form water- or lipid-soluble salts
- the suitability of a drug molecule for a given pharmaceutical formulation and route of administration.

STRUCTURAL ANALYSIS CHECKPOINT

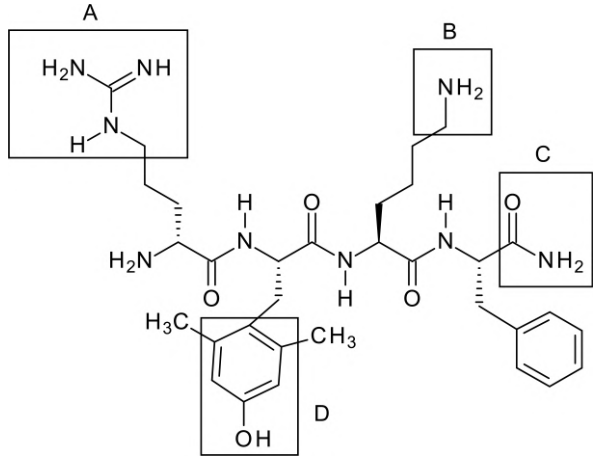
Checkpoint Drug 1: Venetoclax



Venetoclax

1. Identify all acidic and basic functional groups present within the structure of venetoclax and provide the normal pK_a ranges for these functional groups.
2. Using your answer to Question 1, identify if this drug is an acidic drug, a basic drug, an amphoteric drug, an electrolyte, or a nonelectrolyte. Provide a reason for your answer.
3. The sulfonamide functional group in venetoclax has a predicted pK_a value of 4.3. Provide an explanation as to why this functional group has a pK_a value that is slightly lower than the normal range for sulfonamides.
4. The structure of venetoclax contains a pyrrolopyridine, a bicyclic ring system containing two aromatic nitrogen atoms. The nitrogen atom in the six-membered pyridine ring is basic, while the nitrogen atom in the five-membered pyrrole ring is not. Provide an explanation for the differences in these nitrogen atoms.
5. Draw the ionized form of the strongest basic functional group present within the structure of venetoclax.

Checkpoint Drug 2: Elamipretide



1. Consider the structure of elamipretide and evaluate the boxed functional groups in the grid provided.

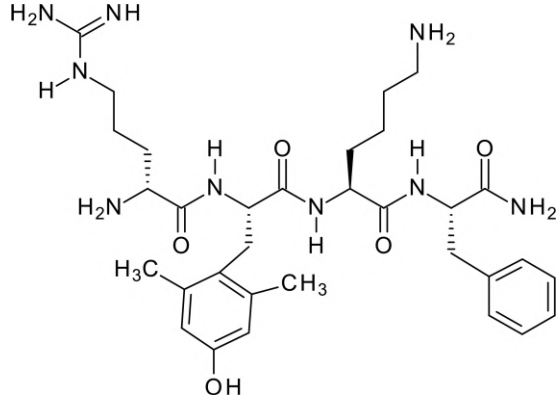
	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range (NA is acceptable)
A			
B			
C			
D			

2. Consider the answer that you provided for the acidic/basic/neutral character of functional group C in the previous question and provide a rationale for this answer.

3. For each of the boxed functional groups, identify its ionization state and acid/base character in each of the indicated physiologic locations.

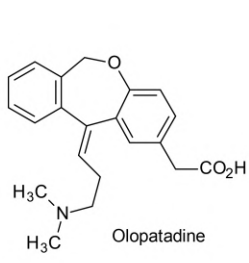
	Stomach (pH = 1) Ionized, Unionized, NA	Acid/Base Character at pH = 1	Intestine (pH = 8) Ionized, Unionized, NA	Acid/Base Character at pH = 8	Urine (pH = 5) Ionized, Unionized, NA	Acid/Base Character at pH = 5
A						
B						
C						
D						

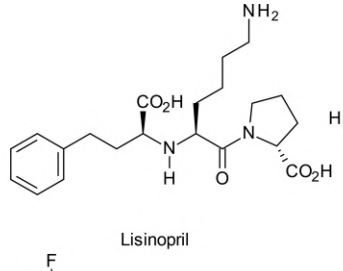
4. Show the ionized form of all of the acidic and basic functional groups.

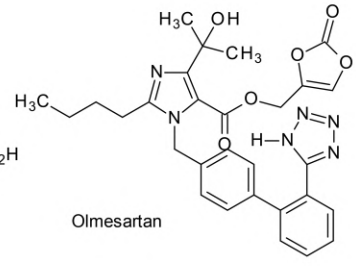


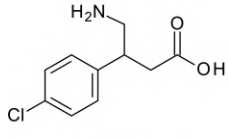
REVIEW QUESTIONS

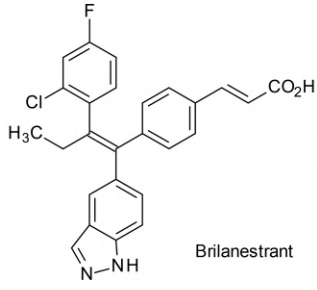
1. For each drug molecule, circle each of the acidic and basic functional groups and identify them by name in the grid provided.

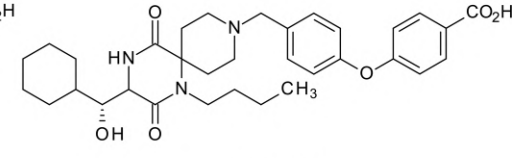

Olopatadine


Lisinopril


Olmesartan

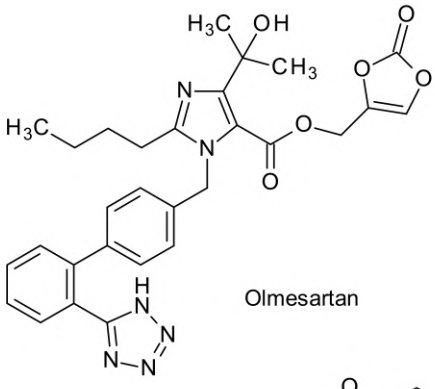

Baclofen


Brilanestrant

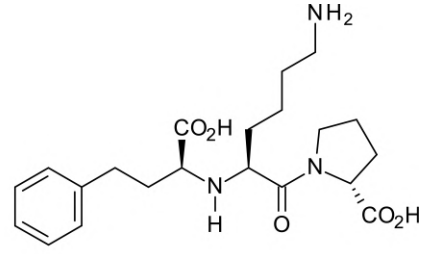

Aplaviroc

Drug Name	Acidic Functional Groups	Basic Functional Groups
Olapatadine		
Lisinopril		
Olmesartan		
Baclofen		
Brilanestrant		
Aplaviroc		

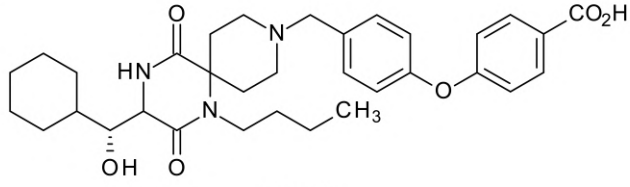
2. Directly modify each acidic functional group to show the ionized form. The ionized form of each of these functional groups represents the conjugate base form of the functional group.



Olmesartan

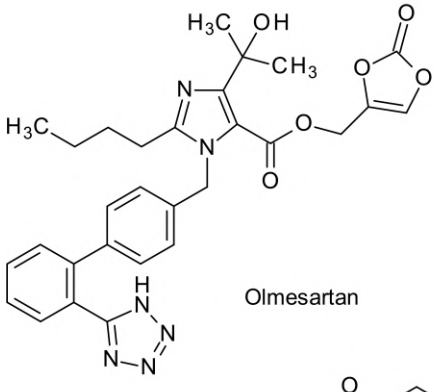


Lisinopril

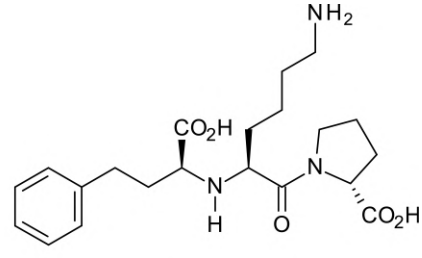


Aplaviroc

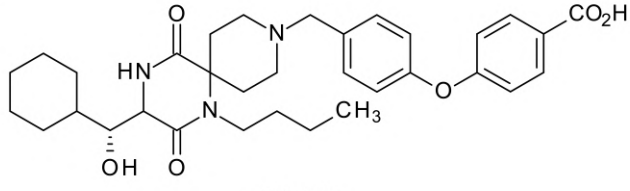
3. Directly modify each basic functional group to show the ionized form. The ionized form of each of these functional groups represents the conjugate acid form of the functional group.



Olmesartan



Lisinopril



Aplaviroc

4. Consider the ionized form of each functional group in Questions 2 and 3. List all of the functional groups that are ***acidic*** when they are in their ionized form. If no functional groups meet this criterion, then leave the cell/column empty.

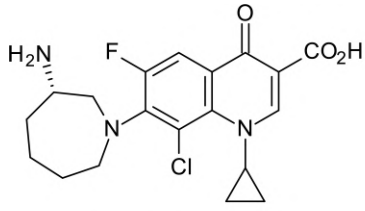
Olmesartan	Lisinopril	Aplaviroc

Consider the ionized form of each functional group in Questions 2 and 3. List all of the functional groups that are ***basic*** when they are in their ionized form. If no functional groups meet this criterion, then leave the cell/column empty.

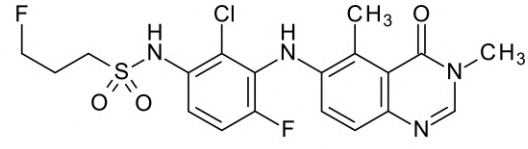
Olmesartan	Lisinopril	Aplaviroc

5. Provide a brief definition for the term *amphoteric*. Determine which structures in Question 1 are amphoteric in nature.

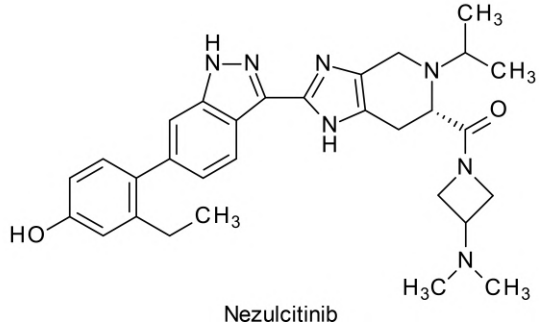
6. For each drug molecule shown below, identify each functional group that is capable of being ionized, the normal pK_a range for the functional group, and whether the drug is acidic, basic, or amphoteric in character.



Besafloxacin



Tinlorafenib

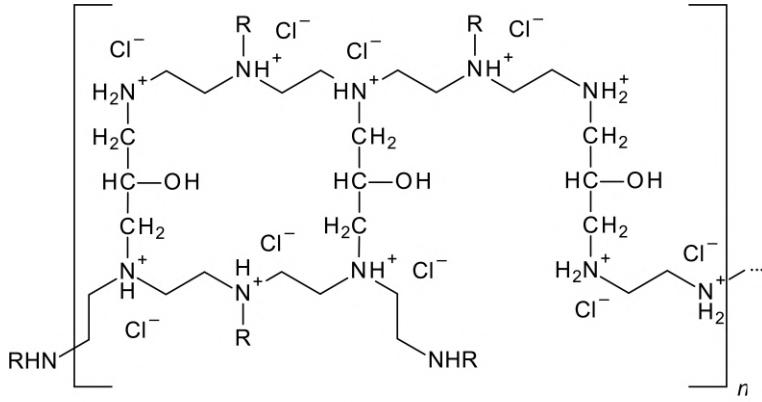


Nezulcitinib

Drug Name	Name of Functional Group(s)	Normal pK_a Range(s)	Acidic, Basic, or Amphoteric
Besafloxacin			
Tinlorafenib			
Nezulcitinib			

7. Bile acids are secreted from the liver into the intestine to help emulsify dietary fat and cholesterol. This enhances the absorption of these dietary substances into the body. In the treatment of high cholesterol, it is important to decrease the intake and absorption of dietary fat and cholesterol.

   Colestipol (Colestid®) is a polymeric drug that can be used in the management of high cholesterol. This polymer acts like an ion exchange resin and exchanges one bile acid (negatively charged) for one chloride ion (negatively charged). Once the bile acid is associated with the polymer, it is readily eliminated. If the bile acids are removed, then they are unable to emulsify dietary fat and cholesterol.



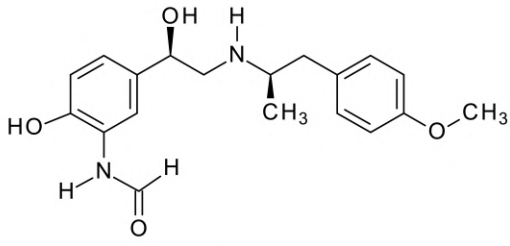
Colestipol

R = H or epichlorhydrin cross link

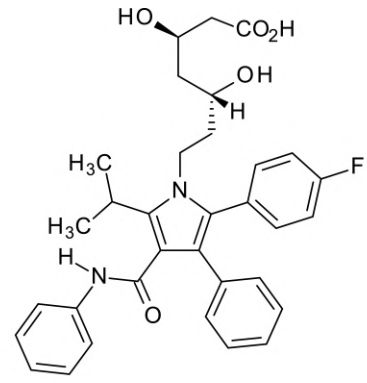
   A. Is colestipol acidic, basic, or amphoteric? Is it an electrolyte or a nonelectrolyte?

   B. Colestipol (and others in the bile acid sequestrant class of agents) is not used frequently because of a significant drug interaction between the polymer and drugs that are anionic in an environment of pH ~8. Describe what you expect to happen when drugs that are anionic are administered at the same time as colestipol.

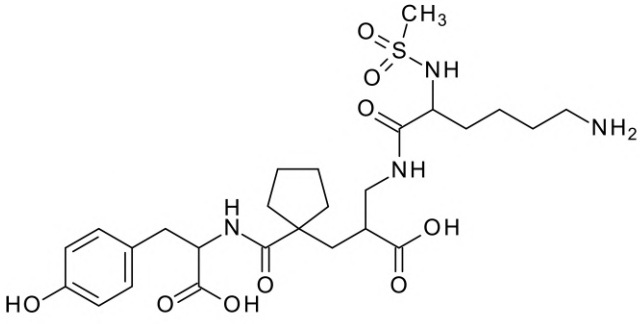
   C. Based on the chemistry associated with this drug interaction, predict which of the following drugs should not be coadministered with colestipol.



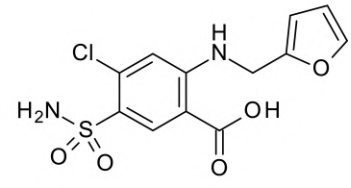
Arformoterol



Atorvastatin



Sampatrilat



Furosemide

8. Consider the structures of arformoterol, atorvastatin, sampatrilat, and furosemide found in Question 7 and do the following:

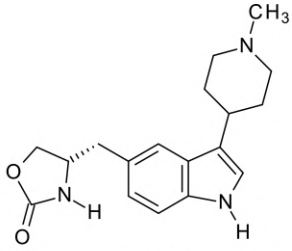
   A. Determine if there are any ionizable functional groups and list each group in the table in the appropriate column.

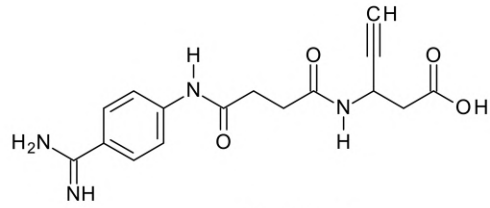
Drug Name	Acidic Functional Groups	Basic Functional Groups
Arformoterol		
Atorvastatin		
Furosemide		
Sampatrilat		

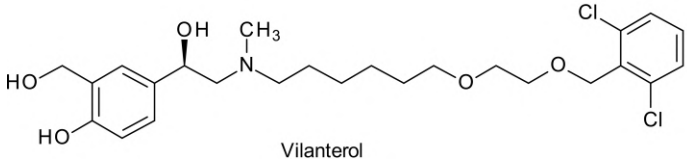
   B. Based on your evaluation in Part A, determine if each drug molecule can be classified as having acidic character, basic character, or both acidic and basic character (amphoteric).

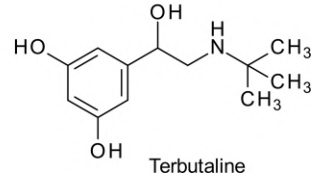
Drug Name	Acidic Character	Basic Character	Both Acidic and Basic Character (Amphoteric)
Arformoterol			
Atorvastatin			
Furosemide			
Sampatrilat			

9. Each of the drug molecules below contains at least one ionizable functional group. Determine whether the drug molecule is acidic, basic, or amphoteric in character.

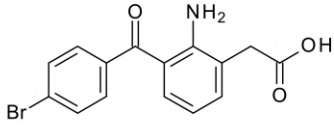

Zolmitriptan

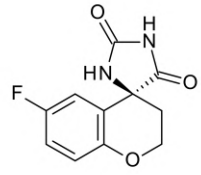

Xemilofiban

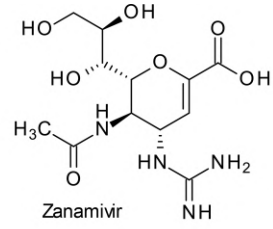

Vilanterol

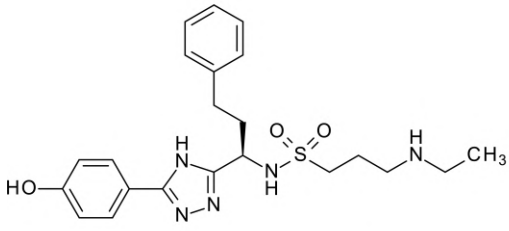

Terbutaline

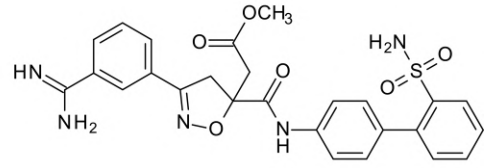
10. For each of the drugs or experimental drugs shown below, identify all of the acidic and basic functional groups and their pK_a ranges.


Bromfenac


Sorbinil

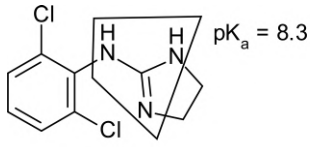

Zanamivir


Experimental antidiabetic agent


Experimental oral anticoagulant

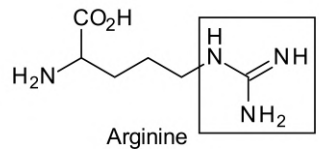
Drug Name	Acidic Functional Groups	Basic Functional Groups
Bromfenac		
Sorbinil		
Zanamivir		
Experimental antidiabetic agent		
Experimental oral coagulant		

11. Shown below is the structure of clonidine, an α_2 adrenergic agonist that can be used to treat hypertension. Clonidine contains a guanidine functional group (highlighted in bold) that has a pK_a of 8.3. Other guanidine functional groups, such as that seen with arginine, are much more basic with a pK_a of 12.5. Provide a chemical explanation for this difference.



pK_a = 8.3

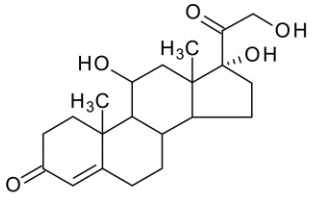
Clonidine



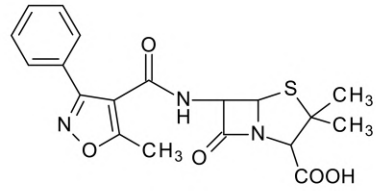
pK_a = 12.5

Arginine

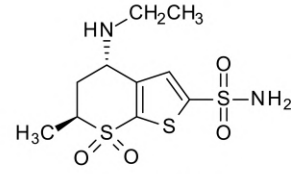
12. For each of the drug molecules shown below, determine if it is an acidic drug molecule, a basic drug molecule, an amphoteric drug molecule, or a nonelectrolyte.



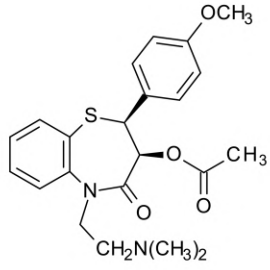
Hydrocortisone



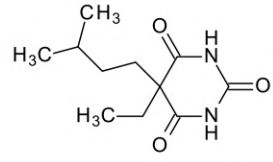
Oxacillin



Dorzolamide



Diltiazem



Amobarbital

Drug Molecule	Acid/Base Character of Drug Molecule
Hydrocortisone	
Oxacillin	
Dorzolamide	
Diltiazem	
Amobarbital	

SOLVING pH AND pK_a PROBLEMS

LEARNING OBJECTIVES

After completing this chapter, students will be able to

- **Explain the similarities, differences, and interrelationships between the pK_a of a functional group and the pH of an environment.**
- **Explain how the Henderson-Hasselbalch equation was constructed and how it can be used to calculate pH values, pK_a values, and the ratio of ionized to unionized functional groups.**
- **Solve both qualitative and quantitative pH/pK_a problems.**
- **Explain how the pH of a given environment and the pK_a of a given functional group can influence the solubility, duration, and binding properties of a drug molecule.**
- **Explain how the pH of a given environment and the pK_a of a given functional group can prevent or contribute to a drug interaction.**

The relative acidity or basicity of the functional groups discussed in Chapter 3 can be measured by comparing their respective pK_a values, while the relative acidity or basicity of the environments in which they reside can be measured by comparing their respective pH values. The *Henderson-Hasselbalch equation* provides a mathematical relationship among the pK_a value of a functional group, the pH of the environment in which it resides, and the ratio that exists between the ionized and the unionized form of the functional group.

This chapter focuses on strategies to solve two basic types of problems: those that determine if a functional group is primarily ionized or primarily unionized at a given pH (i.e., qualitative problems) and those that determine the actual percent that is ionized (i.e., quantitative problems). Within the discipline of medicinal chemistry, the ability to solve qualitative problems and/or estimate the percent to which a functional group is ionized is much more important than the ability to solve quantitative problems. As such, this chapter places a greater emphasis on qualitative problems and the ability to predict or estimate the extent of functional group ionization. This chapter reviews the Henderson-Hasselbalch equation and how to use it to solve both types of problems. This is then followed with descriptions of how to intuitively solve these same types of problem without actually using the Henderson-Hasselbalch equation. For sake of completeness, examples are also provided for the calculation of pH and pK_a values. The chapter ends with a discussion that highlights the importance of pH, pK_a, and functional group ionization in the practice of pharmacy.

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The concepts discussed in this chapter with regard to the Henderson-Hasselbalch equation and the relationship among pH, pK_a, and functional group ionization are also applicable to discussions of drug delivery, permeation, buffering formulations, product stability, and dosage form selection. Most of these topics lie outside the discipline of medicinal chemistry and, therefore, the focus of this text. Additionally, they often require more complicated quantitative calculations than discussed here. Readers interested in any of these topics are referred to either *Martin's Physical Pharmacy and Pharmaceutical Sciences*, *7th edition* (Wolters Kluwer, 2017) or *Aulton's Pharmaceutics: The Design and Manufacture of Medicines*, *4th edition* (Churchill Livingstone/Elsevier, 2013).

DEFINING pH AND pK_a

By definition, pH is equal to the negative log of the hydrogen ion concentration in a solution.

$$pH = -\log[H^+]$$

A very important fact to remember is that a pH value is a property of the environment in which drug molecules reside and is determined by the solutes present in the solution. Thus, aqueous environments such as water, blood, urine, intravenous preparations, and ophthalmic preparations have a pH, while drug molecules and functional groups do not. A second key point in using, evaluating, and comparing pH values is that lower pH values indicate more acidic solutions. As an example, consider two solutions, one with a hydrogen ion concentration of 0.1 M and another with a concentration of 0.00001 M. By simply comparing these concentrations, it is obvious that the solution with the 0.1 M hydrogen ion concentration is the more acidic solution. Using these molar concentrations and the above equation, the respective pH values can be calculated. Please note that the more acidic solution has a lower pH value.

Solution 1: $pH = -\log[0.1] = -(-1) = 1$

Solution 2: $pH = -\log[0.00001] = -(-5) = 5$

By definition, pK_a is equal to the negative log of the K_a, the dissociation constant for an acid in an aqueous environment. While it appears to be a concentration value, K_a is actually a dimensionless term. This is due to a simplification of the actual equilibrium equation to one that excludes the molecule of water.

$$pK_a = -\log K_a, \text{ where } K_a = \frac{[H^+][A^-]}{[HA]}$$

Actual equilibrium equation $\quad HA + H_2O \rightleftharpoons H_3O^+ + A^-$

Simplified equilibrium equation $\quad HA \rightleftharpoons H^+ + A^-$

Similar to the previous discussions regarding pH, it is very important to remember that a pK_a value is a property of a specific functional group and is affected by the steric and electronic factors that surround or are connected to the functional group. Thus, functional groups such as carboxylic acids, sulfonamides, and aliphatic amines all have pK_a values, while the solutions in which they reside do not.

The pK_a values of functional groups can be used to compare their relative acidity and basicity. In examining acidic functional groups, a stronger acidic functional group dissociates more than a weaker one, resulting in a higher hydrogen ion concentration and a higher K_a value. Because the pK_a is equal to the negative log of the K_a, a larger K_a value results in a lower pK_a value. As an example, consider two hypothetical acidic functional groups. Functional group A is a stronger acid and is 10% ionized, or dissociated, in a given environment, while functional group B is a weaker acid and is only

1% ionized, or dissociated, in the same environment. Using 1.0 M concentrations for the drug molecules that contain these functional groups, the pK_a values can be calculated as follows:

Functional Group A: $pK_a = -\log\frac{[H^+][A^-]}{[HA]} = -\log\frac{[0.1][0.1]}{[0.9]}$

Functional Group A: $pK_a = -\log 0.01 = 2$

Functional Group B: $pK_a = -\log\frac{[H^+][A^-]}{[HA]} = -\log\frac{[0.01][0.01]}{[0.99]}$

Functional Group B: $pK_a = -\log 0.0001 = 4$

Math Review

For Functional Group A: Because 10% of a 1.0 M concentration is ionized, 0.9 M exists as HA and 0.1 M exists as H^+ A^-. Therefore, the [HA] = 0.9, while the $[H^+] = [A^-] = 0.1$. The K_a is a dimensionless term (as previously discussed), so the numbers 0.9 and 0.1 are used in the equation without the molar designation.

For Functional Group B: Because 1% of a 1.0 M concentration is ionized, 0.99 M exists as HA and 0.01 M exists as H^+ A^-. Therefore, the [HA] = 0.99, while the $[H^+] = [A^-] = 0.01$.

Similar to acidic functional groups, pK_a values can be used to measure the relative strength of basic functional groups. A strongly basic functional group has a higher pK_a than a weakly basic functional group because the pK_a value of a basic functional group is based on the dissociation of $B{:}H^+$, the conjugate acid of the basic functional group (see Chapter 3 for further discussion of conjugate acids and bases).

$$B{:}H^+ \rightleftharpoons B{:} + H^+$$

In reviewing this equilibrium, it is important to note that a low pK_a value indicates that the equilibrium lies to the right and that the basic functional group is predominantly not bound to the proton. Conversely, a high pK_a value indicates that the equilibrium lies to the left and that the basic functional group is predominantly bound to the proton.

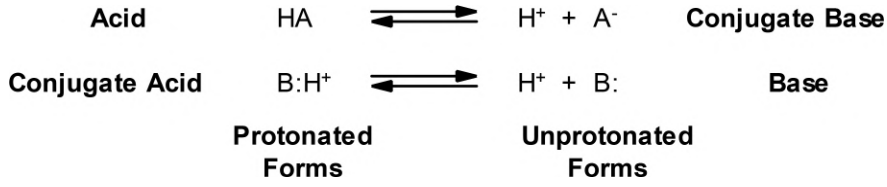
Key Summary Points for pH and pK_a

- A pH value is a property of the environment, or solution, in which drug molecules and functional groups reside. The pH of a solution can change based on what is added or removed from the solution.
- Low pH values indicate acidic environments, while high pH values indicate basic environments. A pH at or about 7.0 indicates a neutral environment.
- A pK_a value is a property of a specific acidic or basic functional group. Although exceptions can occur, pK_a values should generally be treated as constants (i.e., the secondary amine of epinephrine has a pK_a of 10, regardless of whether it is in the stomach fluid, the blood, or the urine).
- Low pK_a values indicate either strongly acidic functional groups or weakly basic functional groups. In comparing functional groups, the one with the lower pK_a value is the stronger acid or the weaker base.
- High pK_a values indicate either weakly acidic functional groups or strongly basic functional groups. In comparing functional groups, the one with the higher pK_a value is the weaker acid or the stronger base.

THE HENDERSON-HASSELBALCH EQUATION

First described by Lawrence Henderson in 1908 and later revised with logarithmic terms in 1916 by Karl Hasselbalch, the Henderson-Hasselbalch equation is very useful for solving a variety of pH, pK_a, and ionization problems. Examples include calculating the pH change of a buffered solution upon the addition of an acid or base, calculating the molar ratio of salt to acid concentrations required to prepare a buffer solution for a particular pH, and calculating the percent to which a functional group is ionized. This chapter primarily focuses on this latter use; however, it provides a few examples of other applications.

The following equations are also discussed in Chapter 3 and are used to review how the equation is derived. To maintain consistency between acidic and basic functional groups, the equations are written such that the acid and base designations lie on the same side of the equations, regardless of the acid/base nature of the functional group.



This same consistency can also be maintained if the groups are designated as protonated (seen on the left) or unprotonated (seen on the right). In contrast, the terms *ionized* and *unionized* would not provide consistency because the ionized forms of acidic and basic functional groups lie on opposite sides of their respective equations.

Why Is Consistency Between Acids and Bases Important?

When using the Henderson-Hasselbalch equation, maintaining consistency between acidic and basic functional groups eliminates confusion and allows the same equation to be used regardless of the acid/base nature of the functional group. Although ionized/unionized designations can be used with the Henderson-Hasselbalch equation, this requires similar but different equations depending on whether the functional group is an acid or a base. Adding an additional step that requires the selection of the appropriate version of the equation is often the root of mistakes on ionization questions.

Derivation of the Henderson-Hasselbalch Equation

To correctly understand and use the Henderson-Hasselbalch equation, it is important to review how it is derived.

Step 1: Starting with K_a, the acid dissociate constant for an acid, substitute the terms *Base Form* for A^- and *Acid Form* for HA.

$$K_a = \frac{[H^+][A^-]}{HA}$$

$$K_a = \frac{[H^+][\text{Base Form}]}{[\text{Acid Form}]} \text{ or } K_a = [H^+]\frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

Step 2: Take the log of each side of the equation.

$$\log K_a = \log[H^+] + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

Step 3: Rearrange the equation.

$$-\log[H^+] = -\log K_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

Step 4: Substituting the terms *pH* for –log $[H^+]$ and pK_a for –log K_a gives the Henderson-Hasselbalch equation.

$$\boxed{pH = pK_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}}$$

Using the descriptors previously identified (i.e., Base Form = unprotonated form and Acid Form = protonated form), the equation can alternatively be designated as shown here. Both representations of the Henderson-Hasselbalch equation are valid for both acidic and basic functional groups.

$$\boxed{pH = pK_a + \log \frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]}}$$

Another form of the equation that compares the concentration of the ionized form to the concentration of the unionized form is often used; however, it often leads to erroneous calculations, and the authors of this text highly discourage its use. The equation is shown below and is valid for monoprotic acidic functional groups such as the carboxylic acid shown in **Figure 4-1**. Because monoprotic

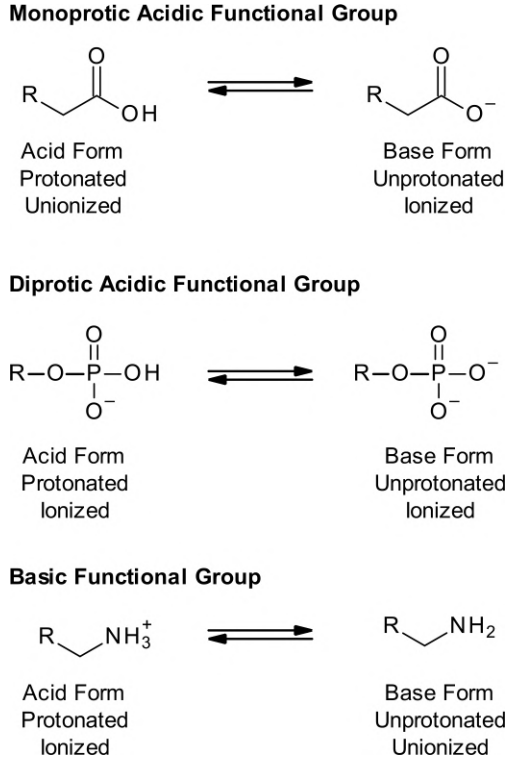


FIGURE 4-1. Equilibria for monoprotic acidic functional groups, diprotic acidic functional groups, and basic functional groups.

acids have only one proton capable of dissociation, the designations base form, unprotonated form, and ionized form are all compatible, and any one of these could be used for the Henderson-Hasselbalch equation.

$$pH = pK_a + \log \frac{[\text{Ionized Form}]}{[\text{Unionized Form}]}$$

In the case of diprotic acids, such as the phosphate group shown in Figure 4-1, the use of this equation can become problematic. This is because diprotic acids have two protons capable of dissociation and two pK_a values. The use of ionized and unionized designations becomes very confusing when evaluating an equilibrium between the mono-ionized and the di-ionized forms of a phosphate group because both forms are ionized. Finally, since the pK_a value of a basic functional group is determined by its conjugate acid (as discussed in Chapter 3), the ionized form of a basic functional group lies on the opposite side of an equilibrium equation as compared with an acidic functional group. As such, the following analogous version of the Henderson-Hasselbalch equation is required for basic functional groups. Please note the presence of the negative log.

Equation for basic functional groups ONLY: $$pH = pK_a - \log \frac{[\text{Ionized Form}]}{[\text{Unionized Form}]}$$

These last two equations using the ionized and unionized designations are presented here solely for sake of completeness and are not used or mentioned in any subsequent sections of this or other chapters. ***Although they are mathematically correct, their use is highly discouraged because they can be confusing and can lead to erroneous calculations***.

SOLVING pH AND pK_a PROBLEMS

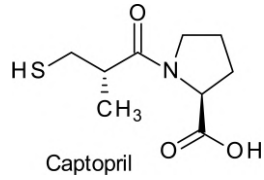
The Henderson-Hasselbalch equation can be used to solve a variety of pH/pK_a problems. For medicinal chemistry, this equation is most commonly used to determine the percent ionization of one or more functional groups present within the structure of a drug molecule. As mentioned previously, there are two types of problems that you are likely to encounter: qualitative problems and quantitative problems. *Qualitative problems* seek to identify the most predominant form of the functional group. In other words, is the functional group primarily ionized or primarily unionized? *Quantitative problems* go one step further and seek to identify what percent of the functional group is ionized and/or unionized.

Regardless of the type of problem, the initial goal is to identify the acidic and basic functional groups for the structure in question and correctly assign any given pK_a values. Questions provided in this text do not require memorization or research of specific pK_a values; however, it is strongly encouraged that you be familiar with the general pK_a ranges provided in Chapter 3.

Solving Qualitative pH and pK_a Problems Using the Henderson-Hasselbalch Equation

The overall goal for these types of problems is to determine whether a specific functional group is primarily ionized or primarily unionized in a given environment. Although the Henderson-Hasselbalch equation can be used to solve these types of problems, it usually is not necessary. For the sake of both comparison and completeness, let us first solve a sample problem using the Henderson-Hasselbalch equation.

Question: Captopril has a functional group with a pK_a of 3.7. Will this functional group be primarily ionized or primarily unionized in the urine at a pH of 5.9?



Captopril

Analysis and Answer: The initial step is to match the given pK_a value with the appropriate functional group. In evaluating the structure, it is easy to identify that the pK_a value belongs to the carboxylic acid. The remainder of the molecule consists of an amide, aliphatic and alicyclic hydrocarbons, and a thiol. While the aliphatic thiol is weakly acidic, its normal pK_a range is between 10 and 11 and does not match a pK_a of 3.7. The remaining functional groups, the hydrocarbons, and the amide are neutral functional groups.

Inserting the given pH and pK_a values into the Henderson-Hasselbalch equation gives the following equation:

$$\textbf{Henderson-Hasselbalch Equation: } pH = pK_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

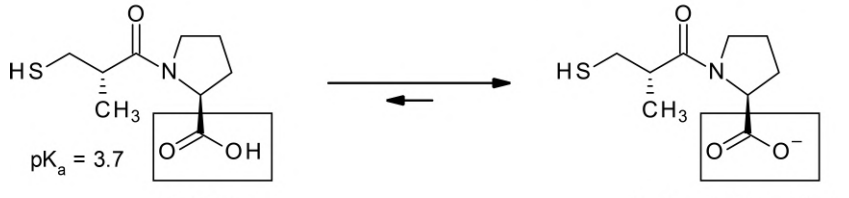
$$5.9 = 3.7 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

Rearranging the equation (i.e., subtracting 3.7 from each side of the equation) gives the following information, which is all that is required to determine if the functional group will be primarily ionized or unionized.

$$2.2 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

The fact that the number on the left has a positive value indicates that the base form (i.e., the conjugate base of the carboxylic acid) is predominant. The question asks if the functional group will be primarily ionized or primarily unionized at a urine pH of 5.9. Since the predominant form is the conjugate base form of a carboxylic acid, the functional group is predominantly ionized.

PREDOMINANT FORM AT pH 5.9

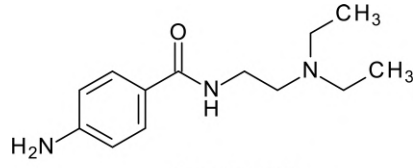


Acid Form
Can Lose Proton

Conjugate Base Form
Can Gain Proton

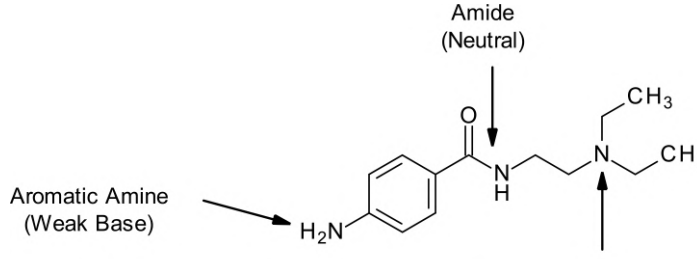
As you work to become proficient in solving these types of problems, assigning the pK_a value to the appropriate functional group is an important initial step in this process. Let's evaluate another sample problem that emphasizes this key point. Shown below is the structure of procainamide.

This drug has a functional group with a pK_a of 9.2. Is this functional group primarily ionized or unionized at physiologic pH?



Procainamide

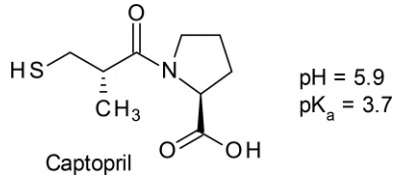
Only one pK_a value is given in this problem; therefore, the first step is to match this pK_a value with the appropriate functional group. In evaluating the structure, you should be able to identify three specific functional groups: an aromatic amine, an amide, and a tertiary amine. Because amides are neither acidic nor basic (i.e., neutral), the pK_a value must belong to either the aromatic amine or the tertiary amine. As discussed in Chapter 3, aliphatic amines have a general pK_a range of 9 to 11 while aromatic amines have a general pK_a range of 2 to 5. Thus, the given pK_a value of 9.2 must be a property of the tertiary amine.



Key Point: To become proficient at solving these types of problems, it is strongly suggested that this initial step be done prior to proceeding with the question. For the question involving procainamide, it is sufficient to know that the drug molecule is basic without identifying the most basic functional group; however, other questions may require you to draw the most predominant form (i.e., ionized or unionized) of the functional group or the drug molecule at a given pH. In this case, it is essential that a thorough structural evaluation be conducted. Assigning the pK_a value to either the amide or the aromatic amine would result in the wrong ionized form being drawn. *It is important to develop good habits and thoroughly evaluate the functional groups within a given structure prior to solving a pH/pK_a problem*.

Solving Qualitative pH and pK_a Problems Without the Henderson-Hasselbalch Equation

In most instances, it is possible to solve qualitative problems without using the Henderson-Hasselbalch equation and without using all of the steps previously outlined. While the principles governing the Henderson-Hasselbalch equation are implicit within the method to be described, the actual equation does not need to be used. To be consistent, let us return to the previous sample problem involving captopril and examine it from a different perspective.



Captopril

For these types of problems, a structure, a pH, and a pK_a are given. As before, it is first necessary to correctly assign the pK_a to the appropriate functional group and determine if the functional group is acidic or basic. This has already been determined for this sample problem, so we can now move to the second step, in which the pH is compared with the pK_a. In doing this, one of three possible scenarios arises. The pH will be less than the pK_a, greater than the pK_a, or equal to the pK_a.

Math Review

The log of the ratio between two values, X and Y, can be greater than, less than, or equal to zero. As examples, let us use the following three equations:

$$\text{Equation 1: } \log \frac{X}{Y} = 0$$

$$\text{Equation 2: } \log \frac{X}{Y} = +0.3$$

$$\text{Equation 3: } \log \frac{X}{Y} = -0.3$$

Calculating the antilog of each side or each equation gives the following:

$$\text{Equation 1: } \frac{X}{Y} = 1$$

$$\text{Equation 2: } \frac{X}{Y} = 2$$

$$\text{Equation 3: } \frac{X}{Y} = 0.5$$

When each side of each equation is then multiplied by "Y," the following relationships are revealed:

$$\text{Equation 1: } X = Y$$

$$\text{Equation 2: } X = 2Y$$

$$\text{Equation 3: } X = 0.5Y$$

Therefore:

- As shown with Equation 1, when the log of a ratio between X and Y is equal to zero, then the values of X and Y are the same.
- As shown with Equation 2, when the log of a ratio between X and Y is greater than zero, then the value of X is greater than the value of Y.
- As shown with Equation 3, when the log of a ratio between X and Y is less than zero, then the value of X is less than the value of Y.

These relationships are directly applicable when using the Henderson-Hasselbalch equation and comparing pH and pK_a values because the value of "pH – pK_a" is greater than, less than, or equal to zero.

$$pH - pK_a = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

Scenario 1: pH Is Equal to the pK_a

This is the easiest of the three scenarios. When the pH = pK_a, then the functional group is 50% ionized and 50% unionized regardless of whether it is an acid or a base. To avoid rote memorization, the Henderson-Hasselbalch equation is used here to verify this fact. If you understand the proof below, it is unnecessary to use the equation in subsequent problems.

$$pH = pK_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

Because the pH is equal to the pK_a, the following is true:

$$0 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

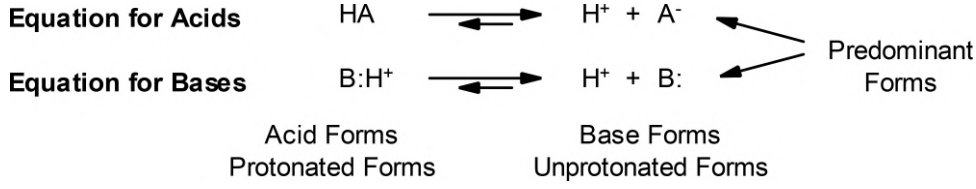
Calculating the antilog of each side of the equation shows that the concentration of the base form is equal to the concentration of the acid form.

$$1 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } [\text{Base Form}] = [\text{Acid Form}]$$

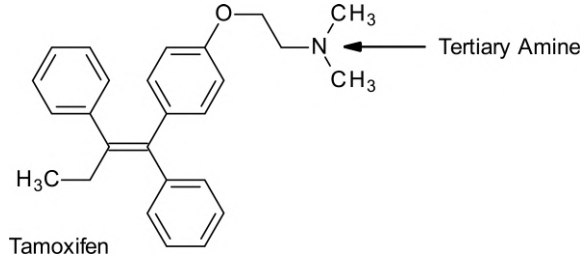
Therefore, regardless of the acid/base character of the functional group, it is 50% ionized and 50% unionized whenever the environmental pH is the same as the pK_a.

Scenario 2: pH Is Greater Than the pK_a

When the pH is greater than the pK_a, then the environment is more basic than the functional group. This scenario is seen with the sample problem involving captopril, in which the pH is 5.9 and the pK_a is 3.7. In a basic environment, protons are less available and are primarily removed from their respective functional groups. As shown below, the equilibria for both acidic and basic functional groups shift to the right, and the basic, or unprotonated, forms predominate.



The key point here is that *in a basic environment, acidic functional groups are primarily ionized and basic functional groups are primarily unionized*. Please note that this is the same conclusion that was reached when the Henderson-Hasselbalch equation was used to solve the sample problem with captopril. Because that sample problem involved an acidic functional group, let us look at an analogous problem involving tamoxifen, a drug molecule with a basic functional group.



Tamoxifen

As shown above, the structure of tamoxifen contains a functional group with a pK_a of 8.9. Since tamoxifen has only one ionizable functional group, the pK_a must be a property of the tertiary amine. For the purpose of this sample problem, assume that this drug molecule resides in a solution with a

pH of 9.5. In comparing the pH and pK_a, the pH is greater than the pK_a; therefore, the basic functional group is in a basic environment and is primarily unionized according to the equilibrium equation for bases. For the sake of completeness, let us use the Henderson-Hasselbalch equation to ensure that both methods provide the same answer.

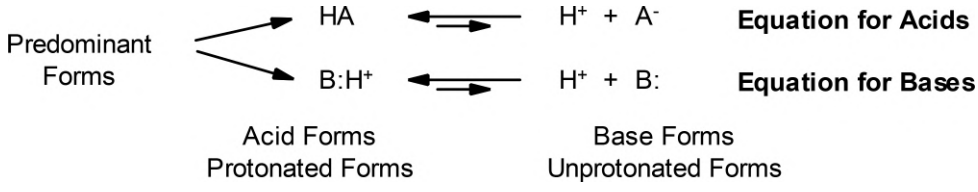
$$9.5 = 8.9 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.6 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

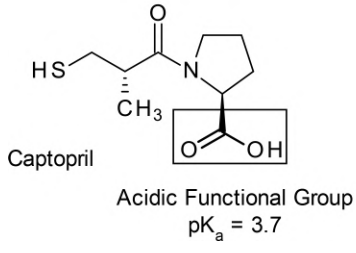
Because this is a qualitative problem, it is not necessary to calculate the antilog of 0.6. The fact that the equation produces a positive value indicates that the base form (i.e., the unprotonated form) is predominant. Since the base form, or unprotonated form, of a basic functional group is the unionized form, this equation verifies and is consistent with the above conclusion.

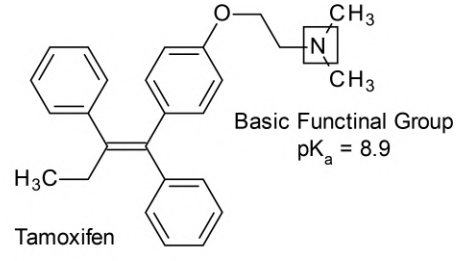
Scenario 3: pH Is Less Than the pK_a

When the pH is less than the pK_a, then the environment is more acidic than the functional group. In an acidic environment, protons are more available and functional groups are primarily protonated. The equilibria for both acidic and basic functional groups shift to the left and the acidic, or protonated, forms predominate.



The key point here is that *in an acidic environment, acidic functional groups are primarily unionized and basic functional groups are primarily ionized*. Similar to what was done in the previous scenario, it is important to verify that these conclusions are consistent with the Henderson-Hasselbalch equation. Let us revisit two previously discussed drug molecules, captopril and tamoxifen, and alter the pH environment in which they reside. For these examples, a gastric pH of 2.1 is used.



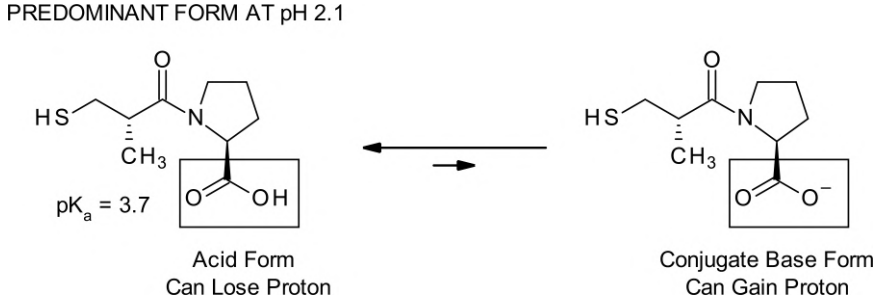


Using the Henderson-Hasselbalch equation for captopril, the drug molecule with the acidic functional group, gives the following initial equation, which can then be rearranged by subtracting 3.7 from both sides of the equation.

$$2.1 = 3.7 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-1.6 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

As before, this type of question simply requires you to identify if the functional group is primarily ionized or unionized. Since the number on the left has a negative value, this indicates that the acid, or protonated, form is predominant. Because the functional group is acidic, this indicates that it is primarily unionized in this environment. This is the same conclusion that was reached without using the Henderson-Hasselbalch equation: *acidic functional groups are primarily unionized in acidic environments*.

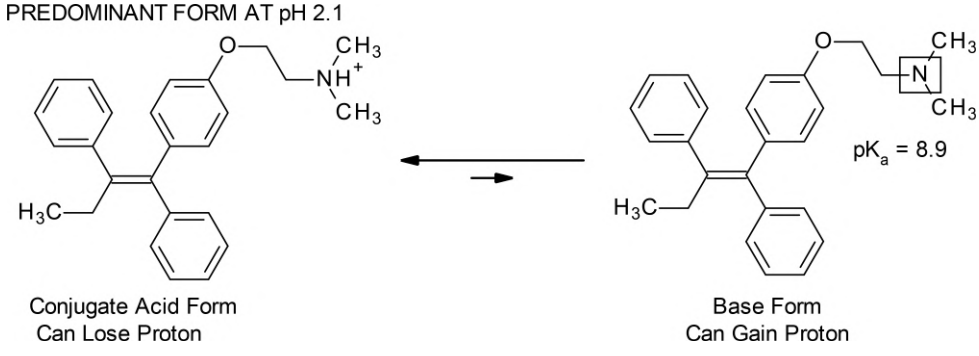


Similarly, the Henderson-Hasselbalch equation can be used for tamoxifen, the drug molecule with the basic functional group.

$$2.1 = 8.9 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-6.8 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

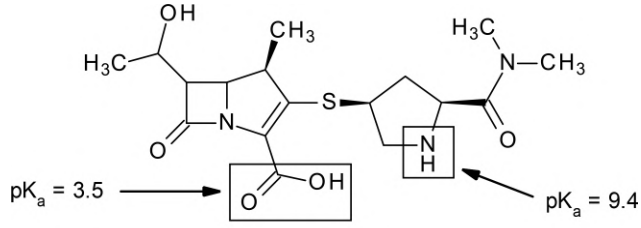
Although the magnitude of the value is larger, the same trend is seen. When the pH is less than the pK_a, the log [Base Form]/[Acid Form] is always a negative value, and thus the acid form always predominates. Again, the exact same conclusion is reached regardless of whether the Henderson-Hasselbalch equation is used: *basic functional groups are primarily ionized in acidic environments*.



Key Points Regarding All Three Scenarios

When solving qualitative pH/pK_a problems, it is essential to understand that the ***relative difference*** between the pH and the pK_a values is more important than the actual pH and pK_a values. This is also applicable to the quantitative problems that are discussed next. ***Failure to recognize the importance of this relative difference commonly leads to incorrect assumptions and answers.*** A common error occurs when a given pH value is compared with a neutral pH value of 7 instead of the given pK_a values. For example, if a pH of 4.5 is immediately considered to be an acidic environment—because it is less than 7—without evaluating the pK_a values of the functional groups, errors are likely to occur. The same is true if a pH of 8.5 is immediately considered to be a basic environment because it is

greater than 7. *To illustrate the potential errors that can occur, let us consider meropenem and these two pH environments.*



Meropenem

First, let us correctly evaluate these two environments using the method previously discussed. Relative to a pH of 4.5, the carboxylic acid ($pK_a = 3.5$) is in an environment that is one log unit more basic than its pK_a. Relative to this same environment, the primary amine ($pK_a = 9.4$) is in an environment that is approximately five log units more acidic than its pK_a. Because the acidic functional group is in a basic environment and the basic functional group is in an acidic environment, both functional groups are primarily ionized if meropenem is in an aqueous environment with a pH of 4.5. A similar situation arises when meropenem is in an environment with a pH of 8.5. Relative to this pH, the carboxylic acid is still in a basic environment, the primary amine is still in an acidic environment, and both groups are still primarily ionized. Please note that in both situations, the exact same environment is viewed differently by these two functional groups. As an analogy, consider that most individuals view the costs of items they purchase as either expensive or inexpensive depending on their perspective (i.e., their income, the prices of similar products, and the need for the product). What is regarded as expensive and unaffordable to one individual may be regarded as inexpensive by another. The same is true of functional groups. The same environment, measured by its pH value, can be acidic to one group and basic to another.

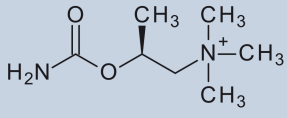
Now let us approach this same problem using *some preconceived ideas and incorrect comparisons*. Because the pH of 4.5 is less than 7, meropenem and its functional groups reside in an acidic environment. The acidic carboxylic acid is primarily unionized in an acidic environment while the basic primary amine is primarily ionized. Similarly, since the pH of 8.5 is greater than 7, meropenem and its functional groups reside in a basic environment. The acidic carboxylic acid is primarily ionized in a basic environment, while the basic primary amine is primarily unionized. These conclusions are obviously not consistent with the previous analysis. The key error here lies in the fact that the given pH was incorrectly compared with a standard pH *and not* the given pK_a values.

It is strongly recommended that you approach these types of problems without any preconceived ideas or comparisons. By using the knowledge of the acidic or basic nature of the functional group and its pK_a, the given environmental pH, and the methods described above, you can correctly answer qualitative pH/pK_a problems.

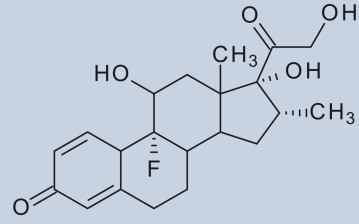
Key Summary Points for Solving Qualitative pH/pK_a Problems

- The initial step in solving these types of problems is to identify the acidic and basic functional group(s) and correctly assign the given pK_a value(s).
- Problems that only require the determination of whether a functional group is primarily ionized or unionized can be solved by simply comparing the pH and pK_a values.
- The Henderson-Hasselbalch equation is not required to solve these types of problems; however, the answers derived are consistent with this equation.
- When the pH equals the pK_a, the functional group is 50% ionized and 50% unionized.

- When the pH is greater than the pK_a, acidic functional groups are primarily ionized and basic functional groups are primarily unionized.
- When the pH is less than the pK_a, acidic functional groups are primarily unionized and basic functional groups are primarily ionized.
- If a drug molecule contains more than one acidic or basic functional group, each functional group and its associated pK_a must be evaluated separately.
- Quaternary ammonium functional groups, such as that found within bethanechol, are always 100% ionized regardless of the environmental pH.
- Nonelectrolytes, such as dexamethasone, are 100% unionized regardless of the physiologic pH.



Bethanechol



Dexamethasone

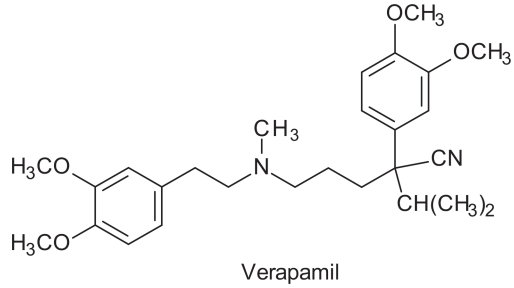
Solving Quantitative pH and pK_a Problems

Quantitative pH/pK_a problems are similar to the qualitative problems previously discussed but require one additional step. Quantitative problems require that you calculate the percent ionization of a functional group, the pH of the environment, or the pK_a of the functional group. Although examples of each are discussed below, problems that involve the percent ionization of a functional group are the most common type of problem that you will encounter.

Calculating the Percent Ionization of a Functional Group

Similar to the discussion of qualitative pH/pK_a problems, initial discussions here involve the use of the Henderson-Hasselbalch equation to solve these types of problems. This is then followed by a discussion of The Rule of Nines, a quick and easy method to solve quantitative problems when the difference between the pH and pK_a is an integer.

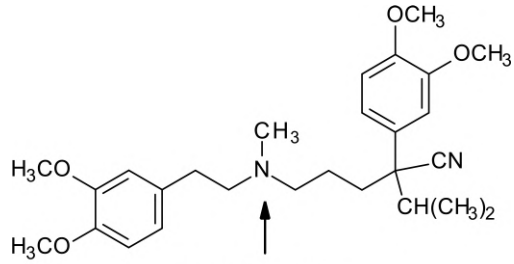
Shown below is the structure of verapamil. Its structure contains one ionizable functional group with a pK_a of 8.9. Using this information, let us calculate the ionization of verapamil at a physiologic pH of 7.4.



Verapamil

Similar to qualitative problems, the initial steps of a quantitative problem involve the identification of acidic and basic functional groups and the assignment of given pK_a values to the appropriate

functional groups. In this problem, only one pK_a has been given, so it can be assumed that only one acidic or basic functional group needs to be evaluated. A structural analysis reveals the presence of a basic tertiary amine, four ether groups, two aromatic rings, numerous aliphatic hydrocarbons, and a nitrile. Since the tertiary amine is the only ionizable functional group, the given pK_a of 8.9 must be a property of this basic functional group.



The next step is to use the principles discussed for qualitative problems to determine if the tertiary amine will be primarily ionized or unionized. Since the given pH of 7.4 is less than the pK_a of the tertiary amine, this basic functional group is in an acidic environment and thus is primarily ionized. These initial steps are very important in that they provide a proof check for the subsequent calculations. Any quantitative answer that is inconsistent with these facts serves as an alert that an error has been made.

Because this type of problem requires a percentage calculation, the Henderson-Hasselbalch equation needs to be used. Inserting the given pH and pK_a values provides the following equation:

$$7.4 = 8.9 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-1.5 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

Taking the antilog of each side of the equation provides the following ratio:

$$0.032 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } \frac{0.032}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that for every one molecule that contains the functional group in the acid form, there are 0.032 molecules that contain the functional group in the base form. To calculate the percent of molecules that contain the functional group in either the acid or base form, it is necessary to first add these numbers together since all molecules must be either in the base or acid form.

$$0.032 \text{ molecules in base form} + 1.0 \text{ molecule in acid form} = 1.032 \text{ total molecules}$$

$$\text{Percent of Molecules in Base Form} = \frac{0.032 \text{ Molecules in Base Form}}{1.032 \text{ Total Molecules}} \times 100\% = 3.1\%$$

$$\text{Percent of Molecules in Acid Form} = \frac{1 \text{ Molecule in Acid Form}}{1.032 \text{ Total Molecules}} \times 100\% = 96.9\%$$

Since it has already been determined that the pK_a is a property of a basic tertiary amine, the base form in these equations is the unionized form and the acid form, or conjugate acid, in these equations is the ionized form. Thus, the tertiary amine is 96.9% ionized. Please note that this answer is consistent with the qualitative analysis that was initially performed prior to the actual calculation.

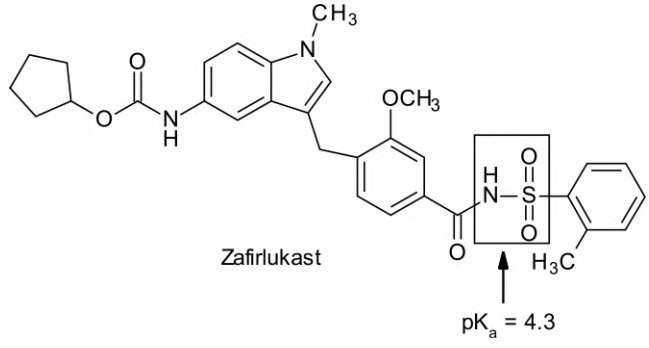
A common error in solving these types of problems is the failure to correctly convert the ratio provided by the Henderson-Hasselbalch equation into a percentage. In the problem above, the ratio between the [Base Form]/[Acid Form] was calculated to be 0.032.

Two incorrect interpretations of this ratio are as follows:

1. The calculated value from the Henderson-Hasselbalch equation is perceived to be the percentage. Thus, for this sample problem, the basic functional group is 99.968% ionized and 0.032% unionized.
2. The calculated value from the Henderson-Hasselbalch equation is multiplied by 100%. Thus, for this sample problem, the basic functional group is 97.8% ionized and 3.2% unionized.

Granted, both of these incorrect calculations are close to the correct answer and are consistent with the initial qualitative analysis; however, this is only because the functional group in this example is highly ionized at the given pH and the acid form is highly predominate. To illustrate the importance of converting the ratio given by the Henderson-Hasselbalch into a percentage and highlight the errors that can result if this step is omitted, let us examine another sample problem.

Shown below is the structure of zafirlukast. Its structure contains a functional group that has a pK_a of 4.3. Using this information, let us calculate the percent to which this functional group is ionized at a urinary pH of 5.



As previously mentioned, the initial step is to match the pK_a value with its appropriate functional group. This has already been done in this example; however, you should be able to recognize that the sulfonamide is the only ionizable functional group present in this structure. In the second step, the pH of 5 is found to be more basic than the pK_a value of 4.3. Since this pK_a value is a property of the carboxylic acid, this acidic functional group is residing in a basic environment and thus is primarily ionized. *Let us now use the Henderson-Hasselbalch equation to calculate the percent ionized.*

$$5.0 = 4.3 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.7 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$5.01 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } \frac{5.01}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that for every one molecule that contains the functional group in the acid form, there are 5.01 molecules that contain the functional group in the base form. Because the functional group is a carboxylic acid, the acid form is the unionized form, and the base form, or conjugate base form, is the ionized form.

The following equations can then be used to correctly calculate the percent of the molecules that are ionized and the percent that are unionized.

$$5.01 \text{ molecules in base form} + 1.0 \text{ molecule in Acid form} = 6.01 \text{ Total Molecules}$$

Base Form = Ionized Form and Acid Form = Unionized Form for This Drug Molecule

$$\text{Percent in Ionized Form} = \frac{5.01 \text{ Molecules in Ionized Form}}{6.01 \text{ Total Molecules}} \times 100\% = 83.4\%$$

$$\text{Percent in Unionized Form} = \frac{1 \text{ Molecule in Unionized Form}}{6.01 \text{ Total Molecules}} \times 100\% = 16.6\%$$

Note that the calculated quantitative answers are consistent with the initial qualitative analysis; the predominant form of the carboxylic acid in a urine pH is the ionized form.

In the above solution to the problem, the value of 5.01 derived from the Henderson-Hasselbalch equation is correctly interpreted as a ratio of 5.01:1.

Let us now revisit the two previously described incorrect interpretations of this ratio and the errors they can cause.

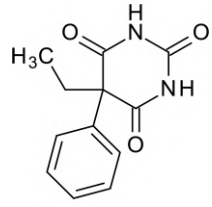
1. This calculated value is actually the percentage. Thus, for this sample problem, 5.01% is in the ionized or conjugate base form, and 94.99% is in the unionized acid form. Please note that this calculation completely contradicts the initial qualitative analysis. Conclusion: An error has occurred somewhere in the analysis or calculation!
2. This calculated value is multiplied by 100%. Thus, for this sample problem, 501% exists in the ionized or conjugate base form. Conclusion: An error has occurred since the calculation indicates that more than 100% is ionized!

In summary, the ratio between the [Base Form]/[Acid Form] that is provided when using the Henderson-Hasselbalch equation is not the same as the percent ionized or unionized. Failure to correctly convert this ratio into a percentage can lead to erroneous calculations.

Calculating the pH of an Environment

In this type of problem, you are given a structure, the pK_a values for ionizable functional groups, and the percent to which each of these groups is ionized. These types of questions are generally not as common as those that require the calculation of the percent ionization but require similar steps and strategies.

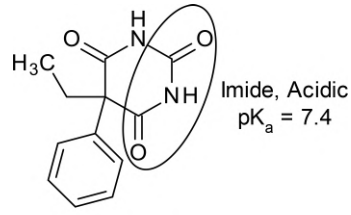
As a sample problem, let us use phenobarbital, shown below, to calculate the pH required for it to be 80% ionized. This drug molecule has a functional group with a pK_a value of 7.4.



Phenobarbital

The initial step in solving this type of problem is to analyze the given structure and to match the given pK_a values with their appropriate functional groups. Please note that this is identical to the initial step for solving percent ionization problems. In analyzing the structure of phenobarbital, you

should be able to identify the acidic imide functional group (highlighted below). Since the heterocyclic ring is symmetrical, it doesn't matter which CO—NH—CO sequence is used.



Phenobarbital

Because this is an acidic functional group, it must reside in a basic environment for it to be primarily ionized, or in this case, 80% ionized. Thus, the pH that is calculated should be greater than 7.4. Similar to the steps used in calculating percent ionization, these initial steps allow us to check our calculations and ensure that they are consistent with the known facts. Let us once again review the Henderson-Hasselbalch equation.

$$pH = pK_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

The pK_a value has been given and can be directly entered into the equation. The functional group is acidic and is 80% ionized. This means that for every 100 molecules of this drug, 80 of the molecules exist in the ionized, conjugate base form and 20 of the molecules exist in the unionized acid form. Thus, the [Base Form]/[Acid Form] ratio is 80:20. Inserting these values provides the following equation:

$$pH = 7.4 + \log \frac{80}{20}$$

Solving this equation gives a pH value of 8.0. As predicted, the calculated pH is greater than the pK_a of the functional group.

$$pH = 7.4 + \log 4$$

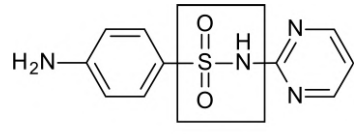
$$pH = 7.4 + 0.6$$

$$pH = 8.0$$

Calculating the pK_a of a Functional Group

This is probably the least common type of problem that you will encounter; however, it is no more difficult than calculating percent ionization of a functional group or the pH of an environment. The steps used here are identical to the previous problems. In this scenario, you are given a structure, a highlighted functional group, a pH, and the percent to which the functional group is ionized in the given pH. Please note that since this type of question requires a pK_a calculation, a specific functional group is highlighted. More than likely, you will still be responsible for identifying if the highlighted functional group is acidic or basic.

As an example, let us use sulfadiazine, shown below. Its highlighted functional group is 10% ionized in a urine pH of 5.5. Given this information and the Henderson-Hasselbalch equation, it is possible to calculate the pK_a for this functional group.



Sulfadiazine

Similar to the previous types of problems, the initial step is to determine if the given functional group is acidic or basic. The highlighted functional group in this example is a sulfonamide, which is an acidic functional group. Because this sulfonamide is acidic and is only 10% ionized at the given pH, this pH of 5.5 must be less than the pK_a of the functional group. *Remember, acids are primarily unionized in acidic environments.* Once again, these initial steps allow us to ensure that our calculated pK_a is consistent with the given facts. The given pH can directly be entered into the Henderson-Hasselbalch equation.

$$5.5 = pK_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

The functional group is acidic and is 10% ionized. Similar to the previous problem, this means that for every 100 molecules of this drug molecule, 10 molecules exist in the ionized, conjugate base form and 90 molecules exist in the unionized acid form. Thus, the [Base Form]/[Acid Form] ratio is 10:90. Inserting these values provides the following equation:

$$5.5 = pK_a + \log \frac{10}{90} \text{ or } pK_a = 5.5 - \log \frac{10}{90}$$

Solving this equation gives a pK_a value of 6.5 for the acidic sulfonamide functional group. As initially predicted, the calculated pK_a is greater than the given pH of the environment.

$$pK_a = 5.5 - \log 0.11$$

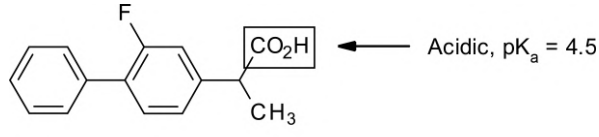
$$pK_a = 5.5 - (-0.96)$$

$$pK_a = 6.46 \text{ (or } 6.5)$$

The Rule of Nines

The Rule of Nines is a quick and easy method to determine the percent ionization of acidic and basic functional groups without directly using the Henderson-Hasselbalch equation. One important criterion must be met before this method can be used: ***the difference between the pH and the pK_a must be an integer*** (i.e., 1, 2, 3).

The Rule of Nines is based on the Henderson-Hasselbalch equation and is best explained by way of a series of examples. *For these examples, we use flurbiprofen, a nonsteroidal anti-inflammatory drug, and ask three new questions. To what extent is this functional group ionized at a urine pH of 5.5, a gastric pH of 2.5, and a solution pH of 7.5?*



Flurbiprofen

Similar to all other pH/pK_a problems, the initial step requires identifying and matching pK_a values to functional groups. Since we have already looked at carboxylic acids in this chapter, this step is omitted here, and this information is shown with the structure. The functional group is acidic, so the acid form of this functional group is the unionized form, and the conjugate base form is the ionized form. In comparing the pK_a of 4.5 with the three environments, this carboxylic acid is primarily unionized at the gastric pH of 2.5 and primarily ionized at the urine pH of 5.5 and solution pH of 7.5. Using this information, let us use the Henderson-Hasselbalch equation to calculate the percent ionization of this functional group in these three environments.

First, we use the urine pH of 5.5. Entering the known values into the Henderson-Hasselbalch equation and solving the equation reveals that this scenario results in a 10:1 ratio of the base form to the acid form.

$$5.5 = 4.5 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$1 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$10 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } \frac{10}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that there are 10 molecules of the ionized conjugate base form for every 1 molecule of the unionized acid form. This is consistent with our initial evaluation that the functional group would be primarily ionized at a pH of 5.5. The following equations can be used to calculate the percentages.

$$\text{Percent in Ionized Form} = \frac{10 \text{ Molecules in Ionized Form}}{11 \text{ Total Molecules}} \times 100\% = 90.9\%$$

$$\text{Percent in Unionized Form} = \frac{1 \text{ Molecule in Unionized Form}}{11 \text{ Total Molecules}} \times 100\% = 9.1\%$$

Thus, the functional group is approximately 90% ionized and 10% unionized when the pH is one log unit greater than the pK_a. At this juncture, there are two key points that need to be emphasized. First, the Rule of Nines requires the use of some approximations, the most noticeable occurring in this particular scenario, in which 90.9% is rounded to 90% and 9.1% is rounded to 10%. Second, the percent ionized to percent unionized can be expressed as a ratio; however, this ratio is different from the ratio calculated using the Henderson-Hasselbalch equation. If a molecule or functional group is 90% ionized, this means that for every 100 molecules, 90 of them are ionized and 10 are unionized. This can then be restated as a 90:10 ratio or a 90% to 10% ratio.

Next, we use the gastric pH of 2.5 and repeat the above process. Using the Henderson-Hasselbalch equation reveals that this scenario results in a 100:1 ratio of the acid form to the base form.

$$2.5 = 4.5 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-2 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.01 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } 100 = \frac{[\text{Acid Form}]}{[\text{Base Form}]} \text{ or } \frac{100}{1} = \frac{[\text{Acid Form}]}{[\text{Base Form}]}$$

This ratio indicates that there are 100 molecules of the unionized acid form for every 1 molecule of the ionized conjugate base form. Once again, this is consistent with our initial evaluation that the functional group would be primarily unionized at a pH of 2.5. As before, the following equations can be used to calculate the percentages.

$$\text{Percent in Ionized Form} = \frac{1 \text{ Molecule in Ionized Form}}{101 \text{ Total Molecules}} \times 100\% = 0.99\%$$

$$\text{Percent in Unionized Form} = \frac{100 \text{ Molecules in Unionized Form}}{101 \text{ Total Molecules}} \times 100\% = 99.01\%$$

Thus, the functional group is approximately 1% ionized and 99% unionized when the pH is two log units less than the pK_a. This relationship can also be expressed as a 99:1 ratio or a 99% to 1% ratio.

Finally, we use the solution pH of 7.5 and follow the same steps. This scenario results in a 1,000:1 ratio of the base form to the acid form.

$$7.5 = 4.5 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$3 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$1000 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } \frac{1000}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that there are 1,000 molecules of the ionized conjugate base form for every 1 molecule of the unionized acid form, which is consistent with our initial evaluation that the functional group would be primarily ionized at a pH of 7.5. As before, the following equations can be used to calculate the percentages:

$$\text{Percent in Ionized Form} = \frac{1{,}000 \text{ Molecules in Ionized Form}}{1{,}001 \text{ Total Molecules}} \times 100\% = 99.9\%$$

$$\text{Percent in Unionized Form} = \frac{1 \text{ Molecules in Unionized Form}}{1{,}001 \text{ Total Molecules}} \times 100\% = 0.1\%$$

Thus, the functional group is approximately 99.9% ionized and 0.1% unionized when the pH is three log units greater than the pK_a. This relationship can also be expressed as a 99.9:0.1 ratio or a 99.9% to 0.1% ratio.

The results from the above three scenarios can be used to establish the Rule of Nines. In the first scenario, the urine pH was one log unit from the pK_a of the carboxylic acid group, and the resulting ratio was approximately 90% to 10%. Note that the number 90 has one nine in it. In the second scenario, the gastric pH was two log units different from the pK_a, and the resulting ratio was 99% to 1%. Note that the number 99 has two nines in it. In the third scenario, the pH of the solution was three log units different from the pK_a, and the resulting ratio was 99.9% to 0.1%. Note that the number 99.9 has three nines in it. At this point, you should easily be able to notice the trend. While the above three scenarios used an acidic functional group, the same ratios would be seen with a basic functional group. The only difference between acidic and basic functional groups would be that the predominant forms would be opposite. As an example, if a basic functional group with a pK_a of 4.5 were used in these scenarios, it would be approximately 90% unionized in a urine pH of 5.5, approximately 99% ionized in a gastric pH of 2.5, and approximately 99.9% unionized in a solution with a pH of 7.5.

By using the absolute value of the difference between the pH of the environment and the pK_a of a functional group, the following relationships, known as the Rule of Nines, can be established.

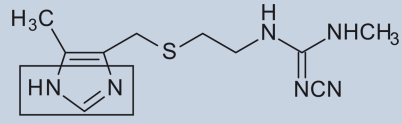
1. If $|\text{pH} - pK_a| = 0$, then there is a 50:50 ratio between ionized and unionized forms.
2. If $|\text{pH} - pK_a| = 1$, then there is a 90:10 ratio between ionized and unionized forms.
3. If $|\text{pH} - pK_a| = 2$, then there is a 99:1 ratio between ionized and unionized forms.
4. If $|\text{pH} - pK_a| = 3$, then there is a 99.9:0.1 ratio between ionized and unionized forms.
5. If $|\text{pH} - pK_a| = 4$, then there is a 99.99:0.01 ratio between ionized and unionized forms.

It is very important to note that the Rule of Nines, by itself, does not provide any information as to whether a functional group is primarily ionized or unionized. It simply provides the ratio between these two forms. To correctly use this method, it is necessary to approach a problem in the same sequential manner that was discussed when the Henderson-Hasselbalch equation was used. Essentially there is a five-step sequence that should be followed when using the Rule of Nines to

solve a percent ionization problem. This is illustrated in the Sample Problem. Please note that steps 1–3 are identical to those that have been used throughout this chapter and that step 4 can be done either before or after steps 1–3.

Sample Problem

Question: Shown below is the structure of cimetidine. The pK_a of the highlighted functional group is 6.8. What percentage is ionized in a solution with a pH of 7.8?



$pK_a = 6.8$ Cimetidine

Step 1 requires the identification of all acidic and/or basic functional groups. In this question, the functional group has already been identified, so all that is required is to determine if the functional group is acidic or basic. In this case, the highlighted functional group is basic. It is part of an imidazole ring and is similar to the basic amidine functional group discussed in Chapter 3.

Step 2 requires the assignment of the given pK_a values to the appropriate functional groups. This has already been done for you in this problem; however, other, more complicated problems may require some discernment among given pK_a values. As previously mentioned, it is very important that you work through these types of problems in a stepwise manner.

Step 3 requires a comparison to determine if the functional group is primarily ionized or primarily unionized. Since the pH is greater than the pK_a, this basic functional group resides in a basic environment and thus is primarily unionized.

Step 4 involves the use of the Rule of Nines to determine the ratio. In this question, the absolute value of pH minus pK_a is equal to 1; therefore, the ratio is 90:10.

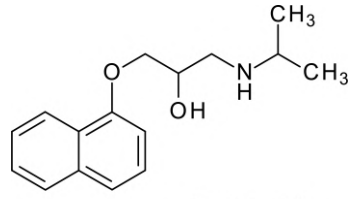
Step 5 involves using the ratio determined by the Rule of Nines, correctly applying it to the information gained in the other steps, and then answering the given question. Step 3 tells us that this functional group is primarily unionized; therefore, 90% is unionized and 10% is ionized. The question asks what percent is ionized; therefore, the answer to this problem is 10%.

If the absolute value between the pH and pK_a is not an integral number, the Rule of Nines can still be a useful tool in approximating the percent to which a functional group is ionized. As an example, consider a basic functional group with a pK_a value of 4.2. If you were asked to calculate the percent to which this functional group would be ionized at a urine pH of 5.4, you would need to use the Henderson-Hasselbalch equation; however, the Rule of Nines can provide an initial estimate that can help you verify your answer. In this example, you have a basic functional group in a basic environment ($pH > pK_a$), so the functional group is primarily unionized. The absolute value between the pH and the pK_a is 1.2. If the absolute value were 1, then 90% would be unionized, and if the absolute value were 2, then 99% would be unionized. Since the absolute value lies between 1 and 2, then your calculated answer should be somewhere between 90% and 99%. Any calculation outside this range would indicate that an error has occurred.

Other Uses for the Rule of Nines

The Rule of Nines can also be used to calculate pH and pK_a values if the percent ionization is 90%, 99%, 99.9%, and so on, or 10%, 1%, 0.1%, and so on. A similar sequential sequence can be used for each of these types of problems.

First, let us use propranolol, shown below, to calculate a pH value. Propranolol has a functional group with a pK_a of 9.5. Given this information, the Rule of Nines can be used to calculate the pH at which this functional group is 99.9% ionized.



Propranolol

The first two steps here are identical to those used for percent ionization problems. They require the identification of all acidic and basic functional groups and the correct assignments of all given pK_a values. Since only one pK_a value is given, it can be assumed that there is only one acidic or basic functional group in this drug molecule. In analyzing the structure, the only ionizable functional group is the secondary amine. Thus, the pK_a is a property of this basic functional group.

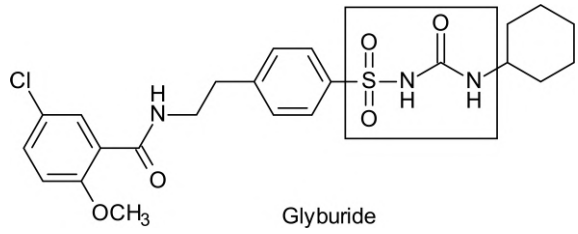
The third step is used to determine if the pH will be greater than or less than the given pK_a. The question here is asking you to calculate a pH in which the functional group is predominantly ionized, specifically 99.9% ionized. Because the functional group is basic, it is primarily ionized in an acidic environment. Hence, the pH must be lower than the pK_a.

The fourth step uses the Rule of Nines. Since the percent ionization is given, this can be used to determine the absolute value of the pH minus the pK_a. There are three nines in 99.9%, so this absolute value must be 3. Thus, since the given pK_a is 9.5, the pH must be either 6.5 or 12.5.

The fifth step once again involves the use of the result from the Rule of Nines and its correct application to the information gained in the other steps. The third step indicates that the pH is lower than the pK_a; therefore, this functional group is 99.9% ionized at a pH of 6.5.

Finally, let us look at an example of how the Rule of Nines can be used to calculate the pK_a value of a specific functional group. In this type of problem, the specific functional group needs to be identified in the question; however, you may be required to discern if the functional group is acidic or basic.

For this final example, let us consider the structure of glyburide shown below. If the highlighted functional group is 10% unionized at a pH of 6.3, what is the pK_a value of this functional group?



Glyburide

The initial step is to determine if the given functional group is acidic or basic. In this example, the highlighted functional group is an acidic sulfonylurea group. As discussed in Chapter 3, the hydrogen atom connected to the nitrogen atom that resides between the carbonyl and sulfonyl groups is acidic.

The second step requires the use of the given information to predict if the pK_a value for the functional group is greater than or less than the given pH. In this example, we are given the fact that the functional group is 10% unionized at a pH of 6.3. This means that it is 90%, or primarily, ionized at this same pH. For an acidic functional group to be primarily ionized, it must be in a basic environment. Thus, the pH of the environment must be greater than the pK_a of the functional group.

The third step uses the Rule of Nines. As stated above, the data provided allow us to determine that the functional group is 90% ionized at a pH of 6.3. Since there is one nine in 90%, the absolute value of the pH minus the pK_a must be equal to 1. The given pH is 6.3; thus, the pK_a for this functional group must be either 5.3 or 7.3.

Once again, the final step requires that the calculation from the Rule of Nines be correctly applied to the information gained in the other steps. The second step indicates that the pH is greater than the pK_a. Because the given pH is 6.3, the pK_a of the highlighted sulfonylurea group is 5.3.

Key Summary Points for Using the Henderson-Hasselbalch Equation for Solving Quantitative Problems

- The Henderson-Hasselbalch equation provides the relationship among the pH of the environment, the pK_a of a given functional group, and the ratio of [Base Form]/[Acid Form] for the functional group in the given environment. If given any two of these three facts, the other one can be calculated.
- Two initial steps are recommended prior to using the equation. Using these initial steps allows a means of verifying that the calculated value is consistent with the given data.
  - Identify the acid/base character of the specific functional group in question.
  - Use the given data to *qualitatively* predict if the functional group should be primarily ionized or primarily unionized, if the calculated pH should be greater than or less than the given pK_a, or if the calculated pK_a should be greater than or less than the given pH.
- The [Base Form]/[Acid Form] value is a ratio. When solving percent ionization problems using the Henderson-Hasselbalch equation, this ratio must be converted to a percentage as explained in the given examples.
- The calculated percentages can be compared and expressed as a ratio; however, this ratio is not the same as that initially calculated by the Henderson-Hasselbalch equation.
- The Rule of Nines can be used in lieu of the Henderson-Hasselbalch equation if the difference between the pH and pK_a is an integer. This method involves some approximations and thus should not be used if very specific and precise values need to be calculated.

THE IMPORTANCE OF pH AND pK_a IN DRUG THERAPY

By now, it should be apparent that the acid/base nature of drug molecules, as well as the pK_a values of their functional groups and the pH of the environment in which they reside, determines whether a drug molecule or a functional group will be primarily ionized or unionized. From a student perspective, there often is one key question that remains regarding this relationship: ***"Why is this so important for me to know as a pharmacist?"***

The purpose of this section is to address this question and provide specific examples that illustrate the importance of acid/base chemistry and ionization in the practice of pharmacy. In doing so, it is hoped that students realize that the pH, pK_a, and ionization relationships discussed in this chapter are very relevant to the practice of pharmacy and, in fact, underscore numerous therapeutic decisions. Granted, most pharmacists never need to ***directly*** solve pH/pK_a/ionization problems in practice; however, the acidic, basic, ionized, and unionized character of drug molecules often determines the appropriateness of one drug over another and can be used in some instances to explain bioavailability, duration of action, drug potency, and drug–drug interactions. The examples that

follow are meant to serve as representative samples and not as a comprehensive list of all important acid/base, pH/pK_a issues. Some of the concepts introduced in these examples, specifically solubility and drug binding interactions, are discussed in more detail in subsequent chapters.

The key concept in all of the following therapeutic examples is that the ***ionization*** of a functional group produces either a positive or a negative charge on that functional group. In terms of ionization, it is often more important to know whether a functional group is primarily ionized or primarily unionized, as opposed to knowing the actual percent of ionization. Additionally, it is often more important to know whether a drug molecule contains acidic (and thus potentially negatively charged) functional groups and/or basic (and thus potentially positively charged) functional groups than it is to know minor differences in acid/base strength.

Outline of Therapeutic Examples

- The influence on the solubility of a drug molecule
  - The effects on absorption and bioavailability
  - The effects on adverse effects and disease states
- The influence on the duration of action for a drug molecule
  - The effects on renal reabsorption
  - The effects on plasma protein binding
- The influence on drug binding interactions
- Ionization and chemical antagonism
- Drug interactions based on pH and/or pK_a changes
  - Changes in gastric pH
  - Changes in urinary pH
  - Alterations in functional group pK_a

The Influence on the Solubility of a Drug Molecule

Ionization plays an important role in the water solubility of a drug molecule. While this concept is discussed in depth in Chapter 5, it is important to introduce a few key concepts here that link pH, pK_a, and functional group ionization to the water solubility of a drug molecule. In general, ionized drug molecules are more water soluble than unionized drug molecules and dissolve faster than unionized drug molecules in an aqueous solution. The presence of ionizable acidic and/or basic functional groups thus aids in the dissolution of drugs in parenteral and ophthalmic solutions as well as within the gastrointestinal tract.

To enhance dissolution, drugs containing acidic and/or basic functional groups can be converted to their inorganic salt forms. An example of this can be seen with verapamil (**Figure 4-2**), a drug molecule that was discussed earlier in this chapter. Verapamil contains a tertiary amine with a pK_a of 8.9.

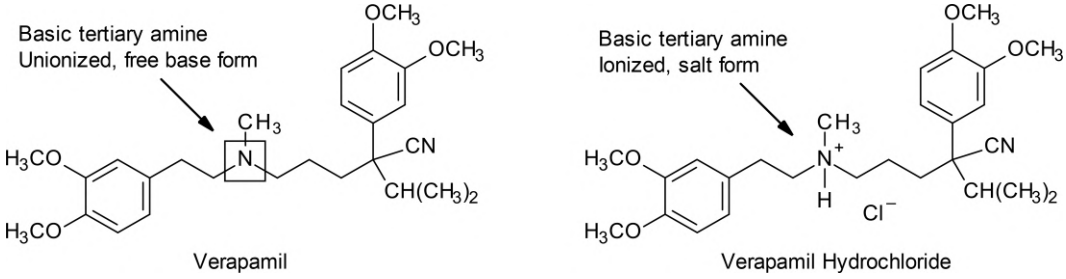


FIGURE 4-2. Verapamil and its hydrochloride salt.

While this drug is predominantly ionized in the stomach, its solid formulation can be administered in either its unionized free base form or as an ionized hydrochloride salt. To ensure that verapamil achieves adequate dissolution and bioavailability, it is marketed as a hydrochloride salt in which the basic functional group is already in its ionized form.

Definition: Free Base

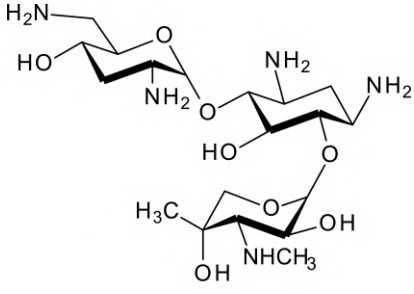
The term *free base* simply means that a basic drug is in its unionized form as opposed to its ionized or salt forms.

The ionization of functional groups also affects the ability of these drug molecules to traverse (be absorbed across) lipid bilayer membranes. In general, ionized drug molecules have difficulty traversing lipid bilayer membranes via passive diffusion; however, it is important to avoid wide sweeping statements such as "all ionized drug molecules are poorly absorbed." These types of statements are incorrect because ionization is an equilibrium process.

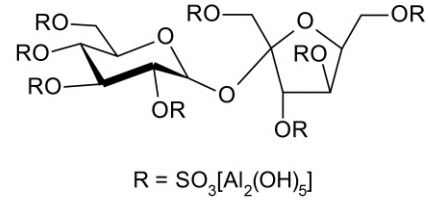
Ionized Drug Molecule (or Functional Group) ⇌ Unionized Drug Molecule (or Functional Group)

While the unionized form of a drug molecule is required for passage across a lipid bilayer, the equilibrium established between the ionized and unionized forms of the drug allows for a constant supply of the unionized form. In other words, if a drug molecule is 99% ionized in the small intestine, the 1% that is unionized can still pass through the intestinal membrane via passive diffusion. The remaining 99% then reestablishes the 99:1 ratio and allows 1% of the remaining drug to be absorbed. This equilibrium dynamic occurs very quickly and can continue until most, if not all, of the drug is absorbed. Another key factor is that the small intestine has a very large surface area that allows for rapid absorption of unionized drug and rapid reestablishment of equilibria.

In some instances, the presence of multiple ionized functional groups prevents oral absorption. Examples include gentamicin, an aminoglycoside antibiotic, and sucralfate, a drug used to treat peptic ulcer disease (**Figure 4-3**).



Gentamicin
Contains 5 Ionizable Amines



Sucralfate
Contains 8 Ionized Sulfates

FIGURE 4-3. Gentamicin and sucralfate.

Because the ionization of acidic and basic functional groups can enhance the dissolution and water solubility of both drug molecules and endogenous moieties, it then follows that changes in the pH of the environment, structural alterations that change the pK_a values, and changes in drug concentration can cause or reduce adverse effects and disease states. The following three examples illustrate this relationship.

Prontosil is a prodrug of sulfanilamide and was the first therapeutically available sulfonamide antibiotic (**Figure 4-4**). Sulfanilamide possesses good antibacterial activity; however, the

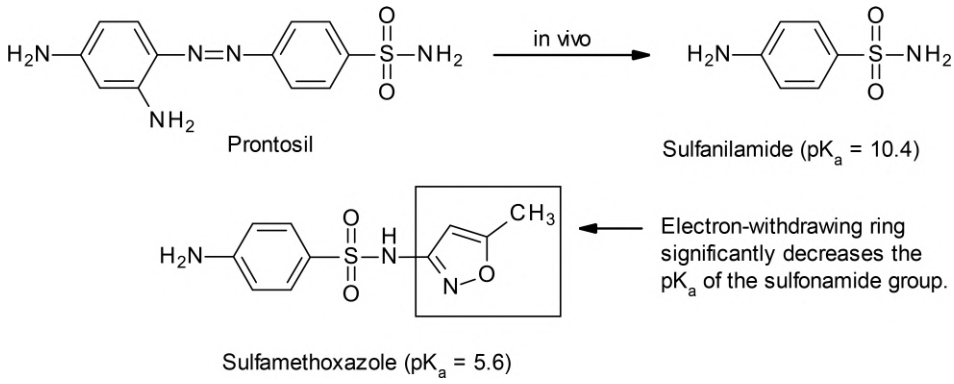
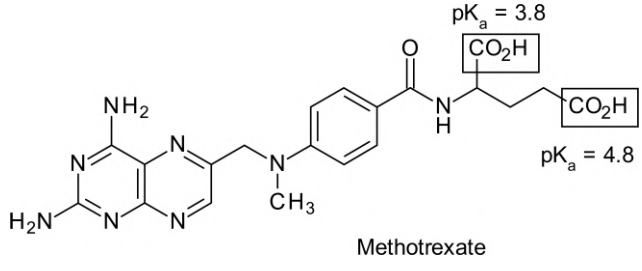


FIGURE 4-4. Prontosil, sulfanilamide, and sulfamethoxazole.

unsubstituted sulfonamide group is only weakly acidic (pK_a = 10.4). Relatively speaking, a normal urine pH of 5 to 6 is much more acidic than this pK_a, and this functional group exists predominantly in its unionized form. As a result, crystalluria—precipitation of the drug molecule in the urinary tract—was a frequent adverse drug event with prontosil. This therapeutic problem was resolved either by altering the pH of the urine or structurally altering sulfanilamide. While neither prontosil nor sulfanilamide is currently used therapeutically, the incidence of crystalluria was initially managed by coadministration of sodium bicarbonate and an increase in fluid intake. Due to its basic nature, sodium bicarbonate increases urinary pH and thus increases the percentage of sulfanilamide in its ionized form sufficiently to alter its aqueous solubility. The increase in fluid intake dilutes the concentration of sulfanilamide and thus decreases its tendency to precipitate. The design of sulfanilamide analogs that contain an electron-withdrawing group provided a better solution to this problem. These drug molecules, exemplified by sulfamethoxazole (Figure 4-4), are significantly more acidic than sulfanilamide, are approximately 50% ionized at a normal urine pH, and are thus much less likely to precipitate in the urine. This is a good example of a concept that was discussed in earlier chapters: electron withdrawing groups can increase the acidity of an acidic functional group.

A similar example is observed with the use of high-dose methotrexate to treat resistant or aggressive tumors. While these higher doses are effective for these indications, they also result in excessive renal concentrations and increase the potential that methotrexate could precipitate in the renal tubules. Similar to sulfanilamide, an increase in fluid intake dilutes the urine, helps prevent drug precipitation, and is recommended for patients taking high-dose methotrexate. Additionally, the structure of methotrexate contains two carboxylic acids with pK_a values of 3.8 and 4.8. To ensure that these two acidic functional groups are highly ionized and thus highly water soluble, it is necessary to alkalinize the urine.



A final example can be seen in patients with gout, a disease characterized by an abnormally high level of uric acid in the plasma (i.e., > 7 mg/dL) and an elevated level of uric acid in the urine. The deposition and crystallization of uric acid crystals in joints and tissues produce the classic symptoms of gout. The pK_a of uric acid is 5.6; thus, in the blood and most tissues, uric acid is approximately

99% ionized. Because uric acid is an endogenous substance, its structure and pK_a cannot be altered. Additionally, it is not possible to alter the pH of the plasma, tissues, or joints without catastrophic consequences. As such, the treatment of gout requires either an increase in the elimination or a decrease in the synthesis of uric acid. As discussed with the above two examples, the pH of urine is quite different than that of plasma and can be altered. This is important in patients with gout because the excessive amount of uric acid in the plasma ultimately leads to an increase in urine concentrations, either naturally or through drug therapy that increases the renal elimination of uric acid. The formation of uric acid kidney stones occurs in approximately 10% to 25% of patients. As previously stated, the normal pH of the urine ranges from 5 to 6. At the lower end of that range, the pH of the environment is lower than the pK_a of uric acid; therefore, it is primarily unionized. Increasing the urine pH increases the ionization of uric acid and increases its saturation concentration. At a urine pH of 5.0, the saturation concentration of uric acid is 15 mg/dL, while at a urine pH of 7.0, the saturation concentration increases to 200 mg/dL. Thus, enhanced ionization decreases the chance of uric acid kidney stone formation.

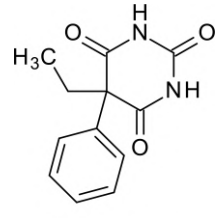
Rule of Nines Approximation for Uric Acid

The pK_a of uric acid is 5.6, while physiological pH is 7.4. These values are approximately two log units apart; hence, there is an approximate 99:1 ratio between the ionized and unionized forms. Since the pH is greater than the pK_a, the acid is approximately 99% ionized in this basic environment.

The Influence on the Duration of Action for a Drug Molecule

Most drug molecules are eliminated from the body via the kidneys or the gastrointestinal tract. For drugs that undergo significant renal elimination, there are four important renal processes to consider: glomerular filtration, active tubular secretion, active tubular reabsorption, and passive reabsorption. The following discussion focuses on this last process since the effects of pH, pK_a, and ionization on renal passive reabsorption are similar to those discussed for absorption.

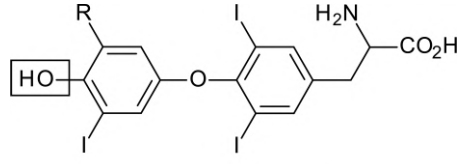
Passive reabsorption generally requires that the drug molecule be in its unionized form or be unable to be ionized (i.e., nonelectrolytes). Alterations of the urine pH can therefore either increase or decrease the percent ionization of a drug molecule. An increase in drug ionization hinders passive reabsorption, allows quicker elimination from the body, and can decrease its duration of action. Conversely, a decrease in ionization allows a larger percentage of the drug to be in its unionized form, which enhances passive reabsorption, decreases the rate of urinary excretion, and increases the duration of action. As an example, let us consider phenobarbital, a drug that was previously discussed. This drug contains an acidic imide functional group, and its renal elimination is pH dependent. If the urine pH is decreased, the percent of phenobarbital that is in the unionized form increases. This in turn increases its passive renal reabsorption, decreases its renal elimination, and increases its duration of action. Conversely, if the urine pH is increased, the percent of phenobarbital that is in an ionized form increases. This in turn decreases its passive reabsorption, enhances its renal elimination, and decreases its duration of action. This latter strategy has been used to increase the elimination of phenobarbital in the case of an overdose.



Phenobarbital

The duration of a drug molecule can also be altered by its ability to bind to plasma proteins. As mentioned in Chapter 3, acidic drugs bind to different plasma proteins than basic drugs. A variety of factors are involved in this binding, including the following important characteristics: the acid/base nature of the drug, the lipophilicity of the drug, and the degree of ionization of the drug. In general, drugs with enhanced lipophilicity and increased ionization tend to be bound tighter and to a greater extent than do analogous drugs that exhibit lower lipophilicity and decreased ionization. The following discussion focuses only on the relationship among ionization, plasma protein binding, and duration of action.

Since plasma protein binding occurs in the plasma, the pH of the environment remains constant. Thus, the only differences that can be considered when discussing the influence of ionization on the duration of action are the pK_a values of analogous drug molecules. As an example, let us consider the two naturally occurring thyroid hormones, liothyronine (T_3) and levothyroxine (T_4). The structures of these biomolecules are shown in **Figure 4-5**. They are structurally similar, with the only difference being that levothyroxine contains an additional iodine atom on the phenol containing aromatic ring. As mentioned in Chapters 2 and 3, iodine atoms, as well as all halogens (i.e., F, Cl, Br, and I), are electron withdrawing and thus increase the acidity of the phenol. Since the pH of the plasma remains constant at 7.4, increasing the acidity of this functional group increases its ionization and plasma protein binding. The phenol found within liothyronine has a pK_a of 8.5 and is approximately 10% ionized at a pH of 7.4, whereas the phenol within levothyroxine has a pK_a of 6.7 and is approximately 83% ionized in this same environment. Ionization of the amine and carboxylic acid is irrelevant in this scenario because it is identical for both drugs.



R = H, Liothyronine (T_3); pK_a = 8.4
R = I, Levothyroxine (T_4); pK_a = 6.7

FIGURE 4-5. Liothyronine and levothyroxine. The pK_a values are properties of the phenolic group highlighted in the box.

This increased ionization of the phenol is responsible for both the enhanced plasma protein binding and increased duration of action observed with levothyroxine. Both drug molecules are highly plasma protein bound, with liothyronine 99.7% bound and levothyroxine 99.97% bound. At first glance, the significance in this difference may not be readily apparent; however, if the percent unbound is examined, the significance is much easier to see. Levothyroxine is 0.03% unbound, while liothyronine is 0.3% unbound. Thus, liothyronine, which is less acidic (i.e., the phenol has a higher pK_a) and thus less ionized, has an unbound concentration that is 10-fold higher than that observed for levothyroxine. The significance of this difference can be seen in the relative half-lives of these drugs. The $t_{1/2}$ of liothyronine is 1 to 2 days, while the $t_{1/2}$ of levothyroxine is 6 to 7 days. For the sake of completeness, it should be noted that the extra iodine atom present on levothyroxine also increases its lipophilicity and therefore contributes to its enhanced plasma protein binding. Thus, the combination of increased ionization and increased lipophilicity provides levothyroxine with a significantly longer duration of action.

The Influence on Drug Binding Interactions

Ionized functional groups are capable of forming ionic bonds with binding sites located within their biological targets. As discussed in more detail in Chapter 6, an ionic bond is one of the strongest and most important types of noncovalent binding interactions. An ionic bond can form over the longest distance and is often the initial bonding interaction between a drug and its biological target. As shown in **Figure 4-6**, salicylic acid is initially attracted to its binding site on cyclooxygenase via an ionic interaction between its ionized carboxylic acid and a cationic site present within the active site of cyclooxygenase. The cationic site is most likely an ionized lysine or arginine side chain. The aromatic ring of salicylic acid also contributes to the overall binding; however, its interaction with the hydrophobic site of cyclooxygenase can only occur after the ionic attraction has drawn the two groups close to one another.

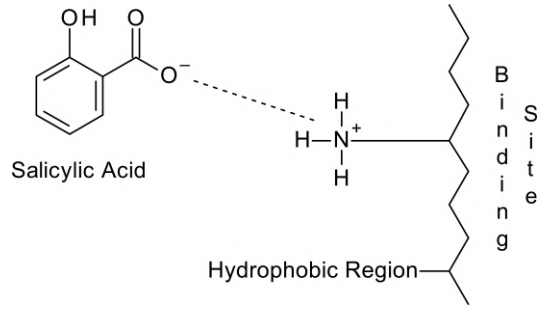


FIGURE 4-6. The initial ionic interaction between salicylic acid and its binding site on cyclooxygenase.

In exploring the interaction of drugs with their biological targets, it is important to note that the pH at the site of interaction almost always remains constant; therefore, ionic interactions are primarily affected by structural changes and altered pK_a values of acidic and basic functional groups. The following two examples use drug molecules previously discussed in this chapter to illustrate this concept.

Let us first look at methylsalicylate, an ester analog of salicylic acid (**Figure 4-7**). This drug does not contain a carboxylic acid and is unable to interact with cyclooxygenase because the initial interaction is no longer possible. The interaction between the aromatic rings also does not occur because the groups never get close enough. As a result, methylsalicylate, unlike salicylic acid, does not possess anti-inflammatory activity.

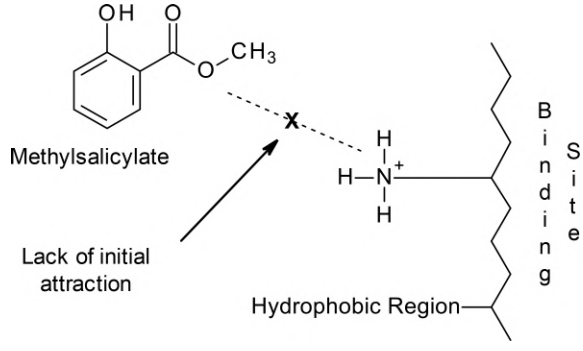
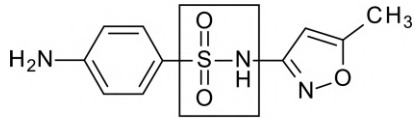


FIGURE 4-7. No initial interaction between methylsalicylate and the binding site on cyclooxygenase due to the lack of an ionized functional group.

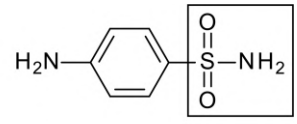
As a second example, let us revisit sulfamethoxazole and the sulfonamide class of antibiotics (**Figure 4-8**). This class of drugs exerts its antibiotic activity by structurally mimicking *para*-aminobenzoic acid (PABA). As a result, they act as antimetabolites and prevent the bacterial synthesis of folic acid, an essential cofactor associated with amino acid and DNA biosynthesis. To mimic PABA, the acidic sulfonamide group (highlighted with boxes in the structures of both sulfamethoxazole and sulfanilamide) must mimic the carboxylic acid of PABA. With a pK_a of 10.4, the acidic sulfonamide group of sulfanilamide is only approximately 0.1% ionized at a physiologic pH of 7.4. Because the carboxylic acid of PABA has a pK_a of 4.9, it is more than 99% ionized at a physiologic pH of 7.4. While sulfanilamide does interact with its target enzyme, its overall binding ability is hindered by the low ionization of the sulfonamide group and thus the inability to truly mimic the ionized form of PABA. In comparison, the sulfonamide group of sulfamethoxazole is adjacent to an electron withdrawing heterocyclic ring, causing it to be much more acidic and primarily ionized at a pH of 7.4. This allows it to be a much better mimic of PABA and have an increased ability to interact with the target enzyme. Thus, the pK_a and ionization of sulfonamide antibiotics is important for solubility, the prevention of adverse effects, and their overall antibacterial activity.

Rule of Nines Approximation for the Carboxylic Acid of PABA

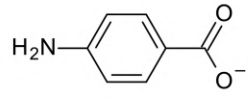
The pK_a of the carboxylic acid of PABA is 4.9, whereas physiological pH is 7.4. The absolute value of the pH minus the pK_a here is 2.5, and the carboxylic acid of PABA is in a basic environment. Thus, the ionization of this functional group lies between 99% (two log units) and 99.9% (three log units).



Sulfamethoxazole (pK_a = 5.6)



Sulfanilamide (pK_a = 10.4)

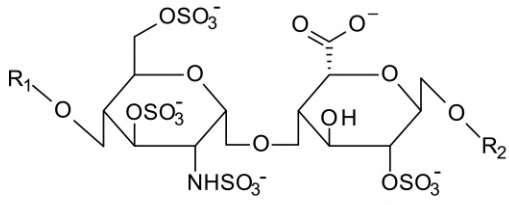


para-Aminobenzoic Acid (PABA)

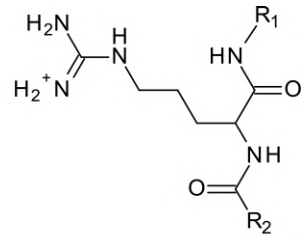
FIGURE 4-8. Sulfonamide antibiotics and *para*-aminobenzoic acid.

Ionization and Chemical Antagonism

Chemical antagonism occurs when a highly ionized acidic drug interacts with a highly ionized basic drug and the activities of both drugs are terminated. This antagonism is distinct from the acid/base incompatibilities that are discussed in Chapter 5. As defined here, chemical antagonism occurs among acidic and basic drugs in vivo. The acid/base incompatibilities discussed in Chapter 5 occur in prepared solutions and result in drug instability and precipitation. There are not many examples of chemical antagonism; however, a very important one is seen in the antagonism of heparin by protamine (**Figure 4-9**). Heparin is an acidic polysaccharide and is used as a parenteral anticoagulant. It is highly ionized due to the presence of multiple sulfate groups, each of which has a pK_a of 1 to 2. The major adverse effect of heparin is hemorrhage or excessive bleeding. This adverse effect can be



Partial Structure of Heparin



Partial Structure of Protamine

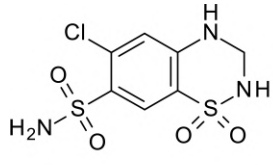
FIGURE 4-9. Heparin and protamine.

quickly reversed by the administration of protamine sulfate, a highly basic polypeptide that is rich in arginine units. The guanidine functional group present within arginine has a pK_a of 11 to 12. Due to their strong and opposing acid/base properties, these drug molecules have a strong ionic attraction for one another. Thus, protamine forms an acid–base complex with heparin that results in rapid inactivation of heparin.

Drug Interactions Based on pH and/or pK_a Changes

Alterations in the pH of an environment or the pK_a of a functional group can directly or indirectly cause or eliminate a drug interaction. In terms of physiologic environments, alterations in pH are most likely to be seen in the urine and the stomach. Let us look at an example for each of these environments.

Examples that illustrate how changes in urinary pH can affect the solubility and duration of action of drug molecules have already been discussed; however, it is important to note that these previous examples focused on the intentional alkalization of the urine to achieve a specific therapeutic benefit. Changes in the solubility and duration of action of a drug molecule can also occur when another concurrently used drug alters the urinary pH as part of its mechanism of action. The thiazide diuretics, a class of drugs commonly used to treat hypertension and other cardiovascular disorders, provide a good example of this. The diuretic action of this class of drugs is due to the ability to inhibit the Na^+/Cl^- symporter in first portion of the distal tubule; however, the thiazides can cause drug interactions based on their ability to also inhibit the enzyme carbonic anhydrase. Carbonic anhydrase catalyzes the reversible hydration of carbon dioxide (**Figure 4-10**). The primary functions of carbonic anhydrase in the kidney are to acidify the urine and to aid in the reabsorption of sodium bicarbonate. By inhibiting carbonic anhydrase, thiazides increase the normal urinary pH range. In general, this causes an increase in the ionization and water solubility of acidic drugs and decreases their ability to be passively reabsorbed. The opposite is true for basic drugs. The actual magnitude of this pH change on the solubility, reabsorption, and duration of a specific drug molecule depends on the pK_a values of its functional groups. The key point here is that thiazide diuretics can cause drug interactions due to their ability to alter urine pH.



Hydrochlorthiazide
(A Thiazide Diuretic)

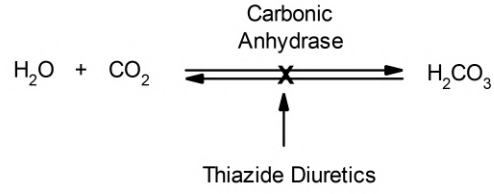
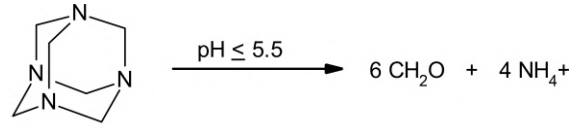


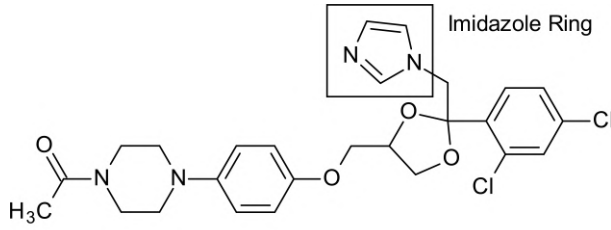
FIGURE 4-10. Hydrochlorothiazide and its inhibition of carbonic anhydrase.

Additionally, thiazide diuretics can decrease the activity of drugs requiring an acidic urine as part of their activation or mechanism of action. One example of this is observed with the antibacterial agent methenamine. Methenamine is a prodrug of formaldehyde and is used to treat urinary tract infections. The conversion of methenamine to formaldehyde requires that the urine pH be ≤ 5.5. Thus, if a patient taking a thiazide diuretic to treat hypertension develops a urinary tract infection, methenamine would be ineffective due to the elevated urine pH.



Methenamine

Similar to urinary pH, alterations in gastric pH can affect the dissolution of some drug molecules. Specifically, the use of antacids, histamine H_2 receptor antagonists, or proton pump inhibitors increases the gastric pH from its normal value of 1 to 2 up to 3.5 to 4, and decreases the oral bioavailability of drugs that require an acidic pH to dissolve. An example of this is seen with the antifungal agent ketoconazole shown below. Ketoconazole is highly lipid soluble and depends greatly on the ionization of the highlighted imidazole ring. Increases in gastric pH decrease the ionization of this basic functional group as well as its water solubility, dissolution, and absorption. As a result, patients requiring ketoconazole therapy should not concurrently use H_2 antagonists (e.g., cimetidine, ranitidine) or proton pump inhibitors (e.g., omeprazole, lansoprazole). Antacids can be used; however, doses must be appropriately spaced to ensure the gastric pH is in the normal range when the ketoconazole is administered.



Ketoconazole

The alteration of the molecular structure of a drug molecule can affect the pK_a values, and hence the ionization, of its acidic and/or basic functional groups. This particular topic is most likely to arise when discussing the structure activity relationships (SARs) of a specific drug class. While SARs are not a main focus of this chapter, a brief overview is provided in Chapter 9. *Within the context of the concepts discussed in this chapter, you should be aware that structural alterations that affect the pK_a of functional groups can cause or eliminate a specific drug interaction though the following processes:*

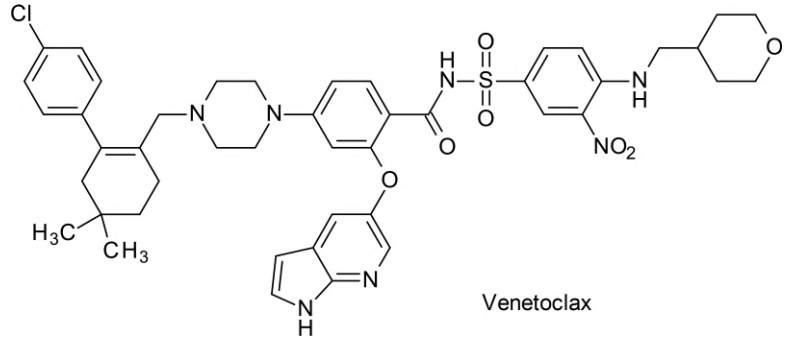
- Changes in the structure of a drug molecule may alter the acidity or basicity of a given functional group, which results in a lower or higher pK_a value for the affected functional group.
- Alterations in the pK_a value of a functional group may result in significant changes in its ionization, which may affect the overall water solubility of the drug molecule.
- Changes in ionization and solubility may affect the plasma protein binding of the drug molecule and may cause some drugs to be more or less susceptible to plasma protein displacement interactions than others.
- Changes in ionization and solubility may also affect the route of metabolism. Changes that increase ionization and water solubility may cause a drug molecule to rely more on renal elimination versus hepatic metabolism. Decreased hepatic metabolism may cause a drug molecule to be less susceptible to a drug interaction with cytochrome P450 (CYP450) metabolizing enzymes. Changes that decrease ionization and water solubility have just the opposite effect and may cause a drug molecule to be more prone to CYP450 related drug interactions.

Key Summary Points Involving the Importance of pH and pK_a in Drug Therapy

- The urinary and gastric pH values can be altered; however, the pH of the blood and other tissues is relatively constant and cannot be significantly altered.
- Drug molecules can be altered to increase or decrease the pK_a of their functional groups.
- Alterations of the pH of the environment or the pK_a of acidic and/or basic functional groups can affect.
  - the water solubility of a drug molecule.
  - the dissolution of a drug molecule.
  - the bioavailability of a drug molecule.
  - the prevalence of specific adverse drug reactions.
  - complications arising from specific disease states.
  - the duration of action of a drug molecule.
  - the ability of a drug molecule to bind to its biological target(s).
  - the therapeutic activity of a specific drug molecule.
  - the prevalence of specific types of drug interactions.

STRUCTURAL ANALYSIS CHECKPOINT

Checkpoint Drug 1: Venetoclax



Venetoclax

1. In Chapter 3, you were asked to identify all acidic and basic functional groups present within the structure of venetoclax. You should have identified a total of five functional groups, one that is acidic and four that are basic. Two of the basic functional groups have predicted pK_a values less than 1.

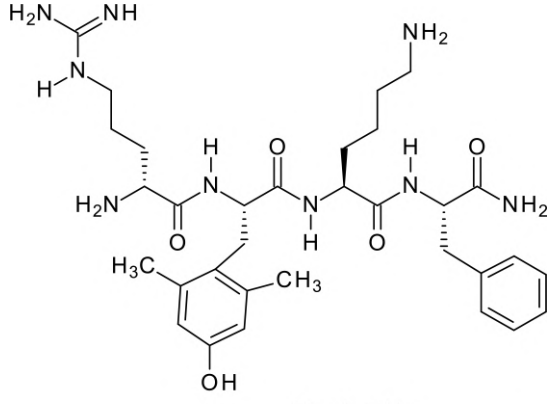
   A. Identify these very weakly basic functional groups and provide an explanation as to why they will not be appreciably ionized within the human body.

   B. The remaining three acidic and basic functional groups have predicted pK_a values of 3.5, 4.3, and 8.0. Match these pK_a values to the appropriate functional groups. If you need a hint, consult the Structural Analysis Checkpoint questions for Chapter 3.

2. Using your answers from Question 1B, individually identify if the functional groups will be primarily (> 50%) ionized or primarily (> 50%) unionized at the following pH levels:
   A. Stomach pH of 1.8
   B. Urinary pH of 5.3
   C. Cellular pH of 7.2
3. In looking at Question 2, there are nine scenarios.
   A. Which of these scenarios allows you to calculate the percent ionized using the Rule of Nines?
   B. Using the stomach pH of 1.8 and the pK_a values of the tertiary amine and the sulfonamide, explain how the Rule of Nines could be used to approximate the percent to which these two functional groups would be ionized.
4. Using the Henderson-Hasselbalch equation, calculate the following:
   A. The percent that the functional group with the pK_a of 4.3 is ionized at a gastric pH of 3.1.
   B. The percent that the functional group with the pK_a of 8.0 is ionized at a plasma pH of 7.3.
   C. The pH that is required for the functional group with the pK_a of 3.5 to be 30% ionized.

Checkpoint Drug 2: Elamipretide

In Chapter 3, you identified that the functional groups in boxes A, B, and C are either acidic or basic in character as well as the associated pK_a value/range. Identification of this information represents critical steps in solving pH/pK_a problems.



Elamipretide

	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range
A	Guanidine (arginine side chain)	Basic	~12.5
B	Primary amine (lysine side chain)	Basic	~10.5
C	Phenol (modified tyrosine side chain)	Acidic	~10.5

1. Evaluate the entire structure of elamipretide and determine if there are any additional acidic or basic functional groups.
   A. If the answer is "yes," then name the functional group(s), identify a pK_a value or range for each group, and add this information to the table above.
   B. If the answer is "no," then determine if either or both of the amino or carboxy termini of this tetrapeptide have been modified and provide a rationale for this modification. (HINT: consider normal degradation catalyzed by a variety of peptidases.)
2. In light of the fact that functional groups B and C have nearly the exact same pK_a value, provide a brief rationale for why it is important to identify the acid/base character of each functional group irrespective of the pK_a value and BEFORE trying to determine the percent ionized in a given physiologic environment.
3. Using a qualitative process, determine if each of the functional groups will be primarily (> 50%) ionized or primarily (> 50%) unionized in each of the physiologic environments listed below. Fill in the tables with the relevant information.
   A. Stomach pH of 2
   B. Urinary pH of 5
   C. Plasma pH of 7.4

	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range	Primarily Ionized (> 50%) Primarily Unionized (< 50%) pH = 2
A	Guanidine (arginine side chain)	Basic	~12.5	
B	Primary amine (lysine side chain)	Basic	~10.5	
C	Phenol (modified tyrosine side chain)	Acidic	~10.5	

	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range	Primarily Ionized (> 50%) Primarily Unionized (< 50%) pH = 5
A	Guanidine (arginine side chain)	Basic	~12.5	
B	Primary amine (lysine side chain)	Basic	~10.5	
C	Phenol (modified tyrosine side chain)	Acidic	~10.5	

	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range	Primarily Ionized (> 50%) Primarily Unionized (< 50%) pH = 7.4
A	Guanidine (arginine side chain)	Basic	~12.5	
B	Primary amine (lysine side chain)	Basic	~10.5	
C	Phenol (modified tyrosine side chain)	Acidic	~10.5	

4. Using a quantitative process, determine the extent to which each of these functional groups will be ionized in each of the physiologic environments listed below. Fill in the tables with the relevant information.

   A. Duodenum pH of 5.4

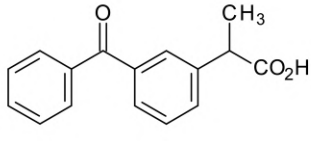
   B. Large intestine pH of 8.5

	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range	Percent Ionized pH = 5.4
A	Guanidine (arginine side chain)	Basic	~12.5	
B	Primary amine (lysine side chain)	Basic	~10.5	
C	Phenol (modified tyrosine side chain)	Acidic	~10.5	

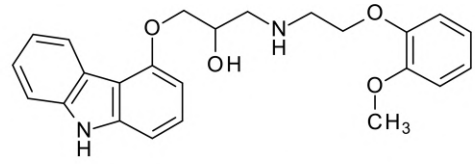
	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range	Percent Ionized pH = 8.5
A	Guanidine (arginine side chain)	Basic	~12.5	
B	Primary amine (lysine side chain)	Basic	~10.5	
C	Phenol (modified tyrosine side chain)	Acidic	~10.5	

REVIEW QUESTIONS

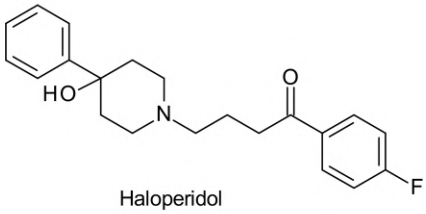
1. Each of the drug structures below contains at least one functional group that is either acidic or basic in character. Determine which functional group is associated with each pK_a value listed in the grid and whether the functional group (in its unionized or parent form) is acidic or basic.



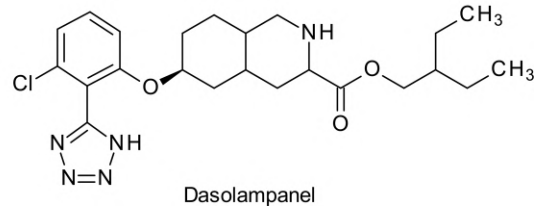
Ketoprofen



Carvedilol



Haloperidol



Dasolampanel

Drug (pK_a Value)	Name of Functional Group	Acidic/Basic
Carvedilol (7.8)		
Ketoprofen (5.94)		
Dasolampanel (3.93)		
Dasolampanel (6.73)		
Haloperidol (8.6)		

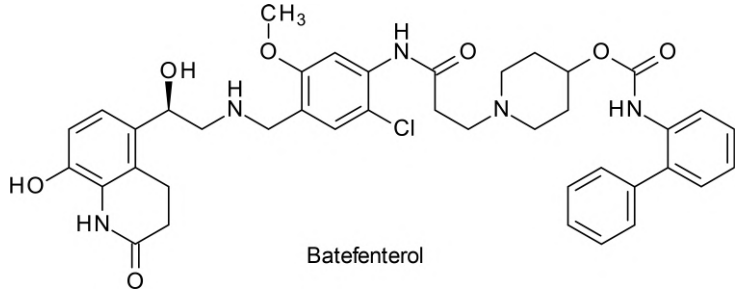
2. Each of these agents can be administered via an oral route and experiences several physiologic environments in its journey from administration to its target for biological action. Evaluate each physiologic environment to determine if the environment is acidic, basic, or neutral.

Saliva (pH = 6.4)	Stomach (pH = 2)	Duodenum (pH = 5.4)	Plasma (pH = 7.4)	Urine (pH = 5.7)

3. Evaluate each physiologic environment to determine if the functional group is predominantly (> 50%) ionized, unionized, or will be 50% ionized/50% unionized.

Drug (pK_a Value)	Saliva (pH = 6.4)	Stomach (pH = 2)	Duodenum (pH = 5.4)	Plasma (pH = 7.4)
Carvedilol (7.8)				
Ketoprofen (5.94)				
Dasolampanel (3.93)				
Dasolampanel (6.73)				
Haloperidol (8.6)				

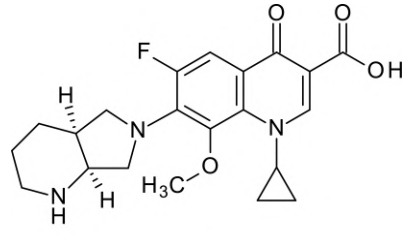
4. The structure of tolbutamide contains an acidic sulfonylurea group with a $pK_a = 5.4$. Using the Rule of Nines and the pH values provided in Question 3, determine the percentage ionization of this sulfonylurea in the duodenum, saliva, and plasma.
5. For the acidic and basic functional groups found in dasolampanel, use the Henderson-Hasselbalch equation or a qualitative approach to determine the following:
   A. Is the functional group predominantly ionized or unionized in the plasma?
   B. What is the percentage of ionized and unionized drug in the plasma?
6. Consider the answers provided in the Question 3 grid and determine which drug (based on ionization state only) is the least soluble in the duodenum. Which drug is the most soluble in the duodenum (based on ionization state only)?
7. In the active site of cyclooxygenase-1 (COX-1) there is an ionized arginine residue (pH = 7.4; pK_a ~12.5) that interacts via a critical ionic interaction with the nonsteroidal anti-inflammatory agents. Evaluate the structures of ketoprofen and haloperidol and determine which drug(s) can participate in this type of interaction with the COX-1 arginine residue.
8. Evaluate the structure of batefenterol and identify all acidic and basic functional groups and complete the table. Using the completed table, determine if there is a physiologic environment in which this drug is found predominantly in its unionized form.
   A. If the answer is "yes," then is this the most likely site for absorption?
   B. If the answer is "no," then provide a rationale for how this drug is absorbed.



Batefenterol

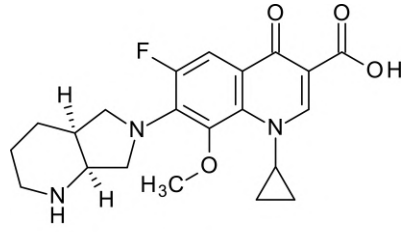
Functional Group Name	Acidic or Basic	Saliva (pH = 6.4)	Stomach (pH = 2)	Duodenum (pH = 5.4)	Plasma (pH = 7.4)	Urine (pH = 5.7)

9. Moxifloxacin is an antibacterial agent that is formulated as a solution for topical use in the treatment of bacterial conjunctivitis. Moxifloxacin has two pK_a values (6.3 and 9.3).
   A. Consider all of the acidic and basic functional groups found within the structure of moxifloxacin and match each pK_a to the correct group. Identify each functional group as either acidic or basic.



Moxifloxacin

B. In this formulation moxifloxacin is present as a hydrochloride salt. Modify the structure to show the hydrochloride salt form of the drug. Which is more water soluble: the salt form of the drug or the parent drug (i.e., the drug without salt)? Provide a brief rationale for your answer.



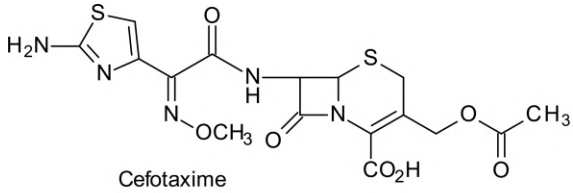
Moxifloxacin

C. The pH of the moxifloxacin hydrochloride formulation is ~7.4. Determine which, if any, of the functional groups will be predominantly ionized in this environment. Also, determine the percent to which the functional group(s) will be ionized.

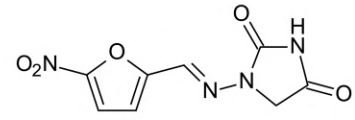
10. Shown below are the structures of cefotaxime, nitrofurantoin, atenolol, and ezetimibe. Each of these drug molecules contains one ionizable functional group. The pK_a values have been provided.

A. Match the pK_a values provided with the appropriate functional groups. For each functional group, identify the name of the group and whether it is acidic or basic.

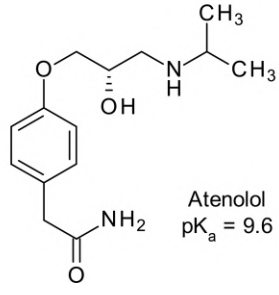
B. For each functional group, indicate whether it would be primarily ionized or primarily unionized at a stomach pH = 1.8, a urinary pH = 6.1, or a cellular pH of 7.4. Provide an explanation for each of your responses.



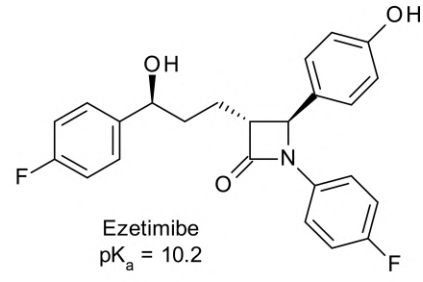
Cefotaxime
pK_a = 3.4



Nitrofurantion
pK_a = 7.1



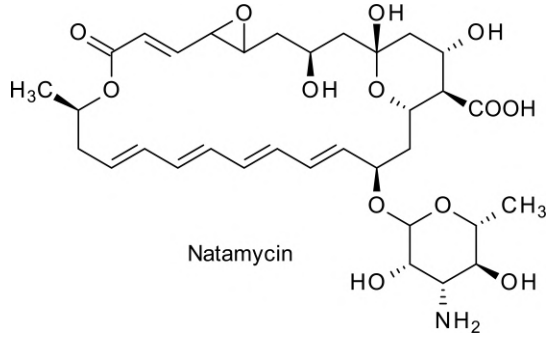
Atenolol
pK_a = 9.6



Ezetimibe
pK_a = 10.2

Drug (pK_a Value)	Stomach (pH = 1.8)	Urine (pH = 6.1)	Cell (pH = 7.4)
Cefotaxime (3.4)			
Nitrofurantoin (7.1)			
Atenolol (9.6)			
Ezetimibe (10.2)			

11. Shown below is the structure of natamycin. It contains two functional groups that could be potentially ionized. The pK_a values for natamycin are 4.6 and 8.4.

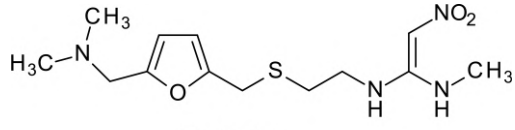


Natamycin

A. Match the pK_a values provided to the appropriate functional groups and identify if the functional group is acidic or basic.

B. Using the Henderson-Hasselbalch equation, calculate the percent ionization that would occur for each of these functional groups at an intestinal pH of 6.2.

12. The most basic functional group present within the structure of ranitidine has a pK_a value of 8.2. Identify this functional group and calculate the pH that is necessary for this functional group to be 70% ionized.



Ranitidine

5 SALTS AND SOLUBILITY

LEARNING OBJECTIVES

After completing this chapter, students will be able to

- Identify water-soluble inorganic salts as well as water-soluble and lipid-soluble organic salts.
- Explain the therapeutic advantages that can be achieved by using water-soluble salts and lipid-soluble salts.
- Explain how organic salt formation can lead to unwanted drug interactions.
- Explain how partition coefficients are determined and link these values to the water and lipid solubility of a drug molecule.
- Evaluate drug structures and identify those functional groups that contribute to the water solubility of the drug and those functional groups that contribute to the lipid solubility of the drug.
- Compare the relative water/lipid solubility of two or more structurally related drug molecules.
- Predict how structural alterations will alter the water/lipid solubility of a drug molecule.
- Explain the need for a drug molecule to have an adequate water/lipid balance.
- Discuss common strategies used to optimize the desired water or lipid solubility of a drug molecule.
- Explain how the overall water/lipid solubility of a drug molecule will influence its metabolism.
- Identify specific therapeutic advantages for enhancing either the water or lipid solubility of a drug molecule.

Drug molecules can be formulated as a wide variety of salts. Depending on the chemical nature, these salt forms can be used to enhance either the water or lipid solubility of the drug molecule and thus affect oral absorption, dosage formulation availability, and the route(s) of administration. Additionally, the metabolism, duration of action, and route(s) of elimination of a drug can be altered by structural modifications that alter water and lipid solubility (i.e., addition, deletion, or

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alteration of functional groups). This chapter reviews commonly used organic and inorganic salts, the effect these salts have on the overall solubility of a drug molecule, partition coefficients, advantages of increasing solubility (both lipid and water), the importance of a balance between lipid and water solubility, common strategies used to alter solubility in the desired direction, and the influence that water and lipid solubility can have on drug metabolism.

WHAT IS A SALT?

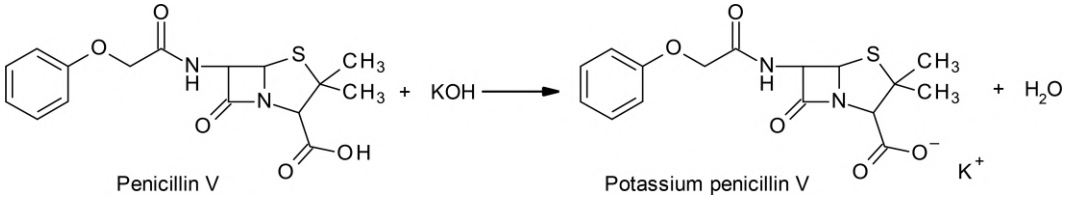
A *salt* is an ionic compound that is produced when an acid reacts with a base. Salts consist of a positively charged cation and a negatively charged anion and are electronically neutral. As an example, let us look at something very familiar: sodium chloride (aka table salt). This compound is formed by reacting sodium hydroxide, a base, with hydrochloric acid, an acid.

$$NaOH + HCl \longrightarrow Na^+ Cl^- + H_2O$$

It is important to understand the underlying chemistry that is occurring here. Hydrochloric acid and sodium hydroxide are strong acidic and basic chemicals, respectively. As a result, they rapidly dissociate into their respective ionic components. The positively charged sodium ion is attracted to the negatively charged chloride ion, resulting in the formation of sodium chloride, a salt. Additionally, the positively charged proton (H^+) and the negatively charged hydroxide ion (OH^-) are also attracted to one another, resulting in the formation of a water molecule. The key difference between these two compounds is that sodium chloride, the salt, can easily dissociate into its constituent ions while the water molecule cannot.

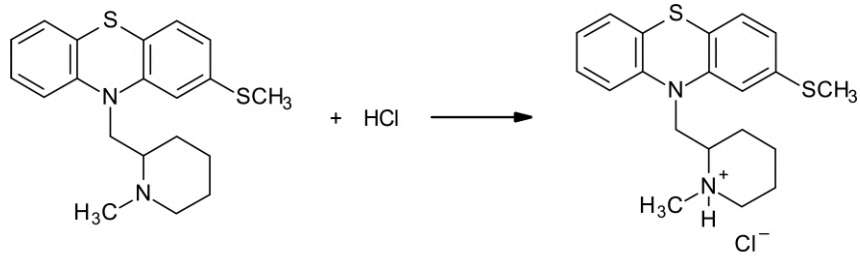
Application to Drug Molecules

The same type of reaction can be accomplished by using acidic and basic drug molecules. Penicillin V can be classified as an acidic drug molecule as it contains only one ionizable functional group, a carboxylic acid. Reaction of this functional group with the base, potassium hydroxide, produces the commercially available salt form of this drug, potassium penicillin V (aka Pen VK).



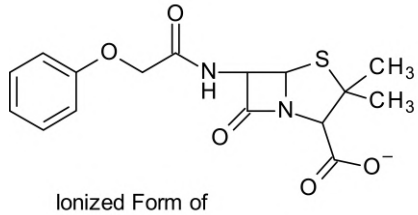
Penicillin V — Potassium penicillin V

A similar example can be seen with thioridazine, an antipsychotic agent that contains a basic tertiary amine. The major difference here is that a water molecule is not formed. In this example, hydrochloric acid dissociates, and the resulting proton binds to the basic functional group. The remaining chloride anion serves as the counterion to the positively charged amine, resulting in the hydrochloride salt of thioridazine.

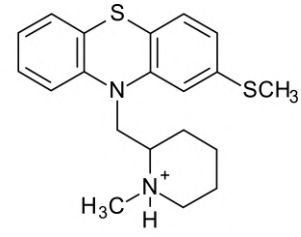


Thioridazine — Thioridazine Hydrochloride

Although similar, it is important to note that the ionized forms of acidic and basic drug molecules are distinctively different than their salt forms. The salt forms shown above are the ***products of reactions*** that occur between the drug molecules and other basic and acidic molecules, respectively. As a result, these salt forms contain a *counterion*, a positively or negatively charged inorganic ion that is opposite of the charge on the drug molecule (e.g., K^+ for penicillin V and Cl^- for thioridazine). In contrast, the ionized forms of penicillin V and thioridazine result from the removal or addition of a proton, respectively. Please note that the ionized forms of these drug molecules do not have counterions.



Ionized Form of Penicillin V



Ionized Form of Thioridazine

INORGANIC SALTS

With respect to chemical structures, the term *organic* pertains to a molecule that is either carbon-based or contains carbon as part of its structure whereas the term *inorganic* pertains to those molecules that do not contain carbon. Almost all drugs are carbon-based, or contain carbon, and thus can be classified as organic molecules. Hence, the terms *inorganic salt* and *organic salt* depend on the chemical nature of the molecule reacting with the drug. When inorganic acids or bases react with basic or acidic functional groups of drug molecules, respectively, the resultant products are termed *inorganic salts*. Thus, both penicillin V potassium and thioridazine hydrochloride can correctly be classified as inorganic salts. Additional inorganic salts can be seen in **Figure 5-1**. Inorganic salts of acidic drug molecules are commonly made using sodium hydroxide, potassium hydroxide, and calcium hydroxide, whereas inorganic salts of basic drug molecules are commonly made using hydrochloric acid, hydrobromic acid, sulfuric acid, and phosphoric acid.

The primary advantage of converting a drug molecule to an inorganic salt is that the inorganic salt form enhances solvation, dissolution, and water solubility. Because the positive and negative constituents of a salt are not covalently bound, they can easily and quickly dissociate in an aqueous environment. An example of this is illustrated in **Figure 5-2** with naproxen sodium. Once these ionic components separate, they can form multiple ion-dipole bonds with water molecules due to the partial charges on the oxygen and hydrogen atoms of the water molecules. These interactions enhance the rate and extent of solvation and dissolution in an aqueous environment. Because orally administered drug molecules in solid dosage formulations (i.e., tablets and capsules) must first undergo solvation and dissolution prior to absorption, the use of inorganic salts augments this initial step and allows for an increased rate and extent in oral absorption.

The same process can also occur without using an inorganic salt; however, as illustrated in **Figure 5-3**, this requires an additional step and thus occurs at a slower rate. The free acid, or unionized form, of naproxen must first undergo ionization of its carboxylic acid. This is different from the dissociation of salts in two ways. First, ionization of a carboxylic acid involves the breaking of a covalent bond as compared with a noncovalent ionic bond. Second, the ionization of acidic and basic functional groups involves an equilibrium. This process is both slower and less extensive (i.e., not 100%) than the dissociation of a salt. As a result, the overall effects on solvation and dissolution are slower and less extensive.

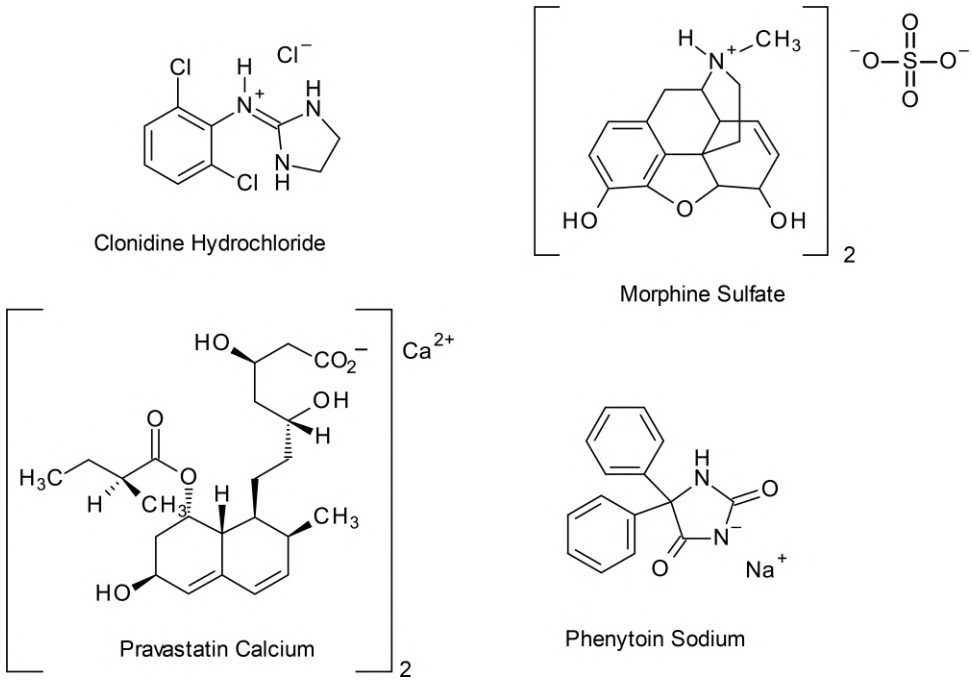


FIGURE 5-1. Examples of inorganic salts of acidic and basic drug molecules.

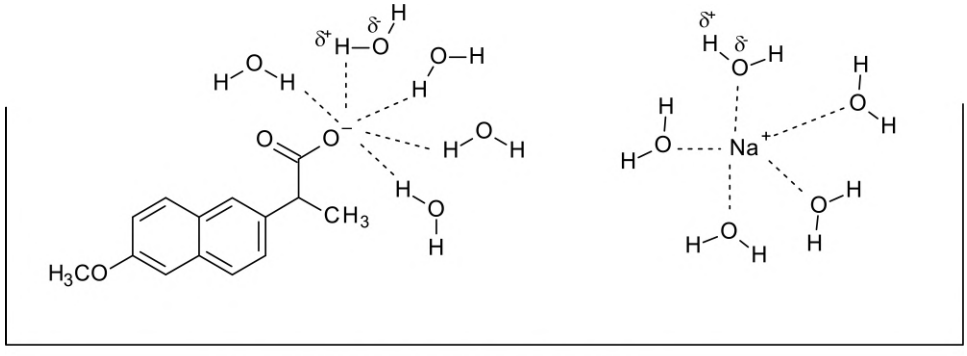


FIGURE 5-2. Solvation and dissolution of naproxen sodium.

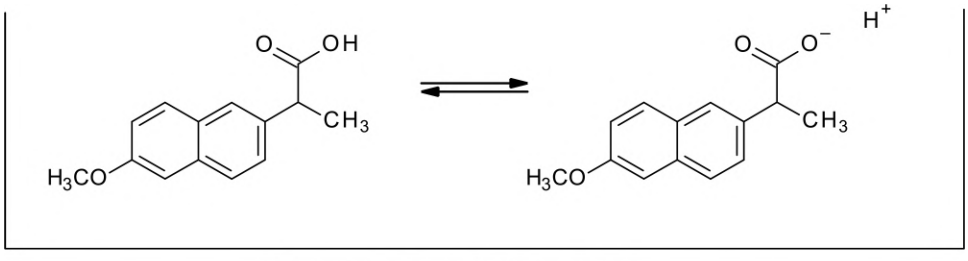


FIGURE 5-3. Solvation and dissolution of naproxen.

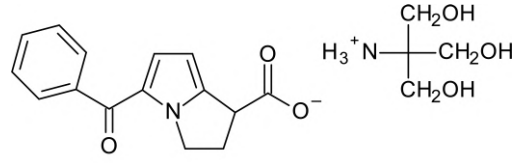
Although inorganic salts can provide the abovementioned beneficial effects, it is important to note that other factors contribute to the overall water solubility of a drug molecule. As discussed later in this chapter, the presence of other water-soluble functional groups also plays a key role in the ability of a drug molecule to dissolve in an aqueous environment. Thus, some drug molecules are marketed as their inorganic salts while others are marketed as their unionized free acids or free bases.

ORGANIC SALTS

Similar to inorganic salts, organic salts are simply the products of the reactions between acidic or basic drugs and other basic or acidic molecules, respectively. Two differences need to be noted. First, the drugs are combined with organic molecules containing acidic or basic functional groups rather than inorganic molecules. Second, the organic molecules used to produce these salts can be classified as either water soluble or lipid soluble depending on their chemical composition.

Water-Soluble Organic Salts

Similar to what was observed with inorganic salts, water-soluble organic salts can increase the solvation, dissolution, and water solubility of drug molecules. Sugars, analogs of sugars, and glycolysis intermediates are commonly used to produce water-soluble organic salts. All of these organic molecules contain multiple hydrophilic functional groups. As an example, let us consider ketorolac tromethamine. Similar to naproxen, ketorolac is a nonsteroidal anti-inflammatory drug (NSAID). It is indicated for the short-term treatment of acute moderate to moderately severe pain as well as a number of ocular conditions, including allergic conjunctivitis, ocular pain, ocular pruritus, and postoperative ocular inflammation. Ketorolac is marketed as its tromethamine salt and is available as an oral tablet, a nasal solution, an ocular solution, and a parenteral solution for either intravenous (IV) or intramuscular (IM) administration. The tromethamine salt possesses a positively charged primary amine as well as three primary hydroxyl groups. The ionized amine can form an ion–dipole interaction with a water molecule whereas each of the hydroxyl groups can form hydrogen bonds. All of these interactions enhance solvation, dissolution, and water solubility.



Ketorolac Tromethamine

Figure 5-4 highlights a number of other commercially available drug molecules that are formulated using water-soluble organic salts. Sulfisoxazole diolamine is a good example of how water-soluble, organic salts can provide enhanced water solubility compared with their analogous inorganic salts. Sulfisoxazole diolamine can be solubilized at physiologic pH whereas the analogous sulfisoxazole sodium requires a much more basic environment. Given the fact that the instillation or injection of basic solutions can produce burning and stinging in the eyes and at injection sites, the diolamine salt is much better suited for the preparation of parenteral and ophthalmic dosage forms than is a simple sodium or potassium salt.

An additional advantage of using either inorganic salts or water-soluble organic salts is that the enhanced solvation and dissolution allows these salts to be formulated in highly concentrated solutions. This advantage is extremely important for the development of ophthalmic, nasal, and parenteral solutions, in which the ability to deliver a significant amount of drug in a very small volume is often desired.

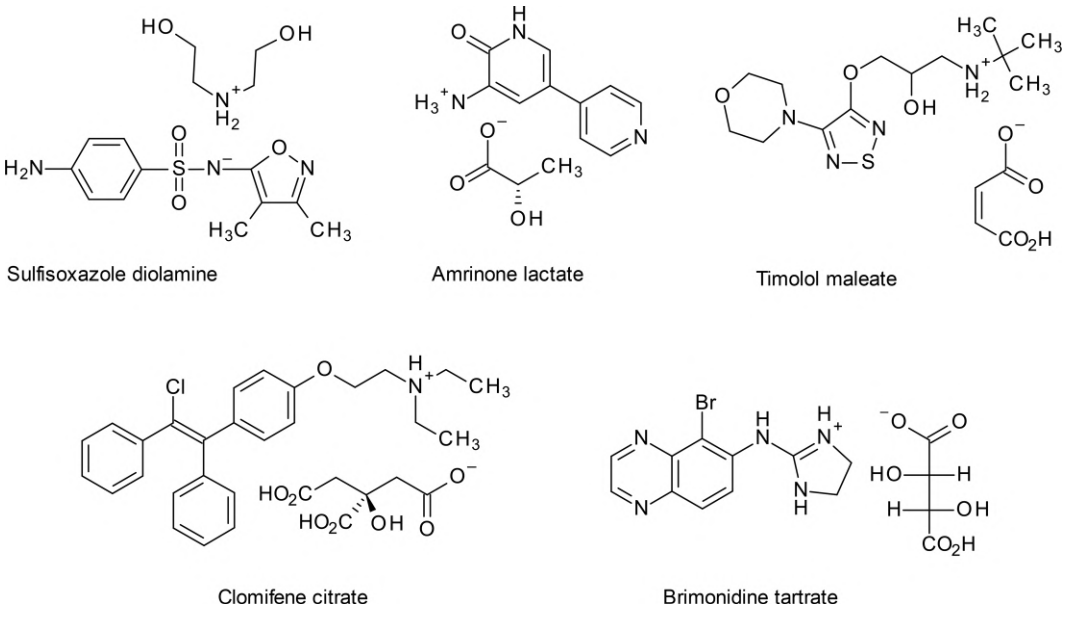
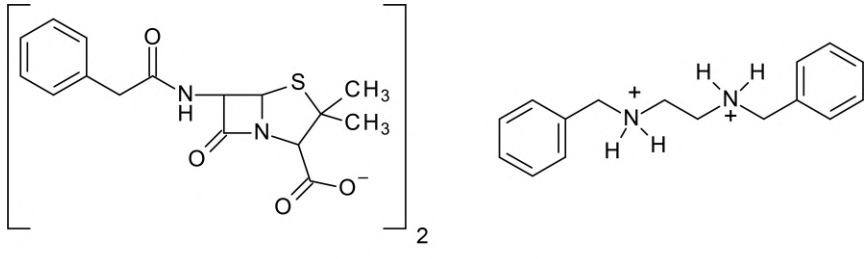


FIGURE 5-4. Examples of water-soluble organic salts.

Lipid-Soluble Organic Salts

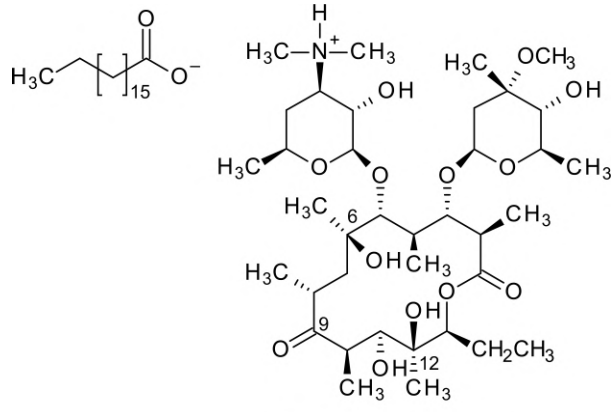
Contrary to water-soluble organic salts, lipid-soluble organic salts decrease the solvation, dissolution, and water solubility of drug molecules and enhance their lipid solubility. These salts are primarily used to form lipid-soluble suspensions or oil-based formulations that are administered as IM depot injections. They can also be used to enhance the oral bioavailability of acid labile drug molecules and increase the palatability of liquid formulations. Let us look at examples that highlight each of these beneficial effects.

Penicillin G can be used to treat a variety of moderate to severe infections, including lower respiratory tract infections, septicemia, bacteremia, and meningococcal infections caused by susceptible organisms. It can be administered via IV formulations as its sodium or potassium salt; however, due to rapid tubular secretion, which results in rapid elimination, penicillin G has a short half-life and must be administered every 4 to 6 hours. The frequency of administration can be greatly decreased by using penicillin G benzathine, a lipid-soluble organic salt of penicillin G. Due to its lipid solubility, this salt can be formulated as a suspension and administered as an IM depot injection. Once administered, penicillin G slowly dissolves and is released from its injection site in a consistent manner over an extended period of time. A key therapeutic advantage of the benzathine salt is a reduction in the frequency of dosing as well as a consistent blood concentration. One potential disadvantage of IM depot injections is that once administered, the effects of administered drug are not immediately reversible due to the slow and steady release.



A similar advantage is seen with NPH (or isophane) insulin. Regular, unmodified insulin is classified as a short-acting insulin and is often administered as a subcutaneous injection in conjunction with meals. If taken as the only insulin product, regular insulin would need to be administered up to four times daily, depending on the timing of meals and blood glucose levels. A more convenient and reliable dosing regimen involves the combination use of regular insulin with NPH insulin. NPH insulin is an organic salt that is formed when regular insulin is combined with protamine, a low molecular weight, arginine rich polypeptide obtained from salmon and other fish. Regular insulin has a net negative charge due the presence of more acidic amino acids than basic amino acids. The resulting salt, cocrystallized with zinc, can be formulated for injection. Similar to penicillin G benzathine, the protamine/insulin/zinc complex slowly dissolves and is released from its subcutaneous injection site in a consistent manner over an extended period of time. The resulting zinc insulin hexamer dissociates to release dimeric insulin. The dimeric insulin then further dissociates to the monomeric active form of insulin. The combination use of NPH insulin and regular insulin often reduces the frequency of administration and affords more consistent plasma glucose levels throughout the day.

A final example of the benefits of organic salts can be seen with erythromycin stearate. Similar to a number of antibiotics, erythromycin has a bitter taste. When formulated as a tablet or capsule, this undesired effect can easily be masked; however, young children and some adult populations have difficulties swallowing these types of formulations. A solution to this initial problem is to prepare a liquid formulation. Given that the antibiotic has a bitter taste, the use of a liquid solution, even a flavored one, may not be desirable. To increase the palatability of erythromycin, it can be converted to its lipid-soluble stearate salt and formulated as a suspension. When the suspension is given orally with water or another liquid, the lipid-soluble salt does not have time to dissolve in the saliva, and the patient is not able to taste the bitterness of this drug. In contrast, the patient tastes the water-soluble flavoring that is often added to these suspensions, thus increasing palatability and patient adherence.



Erythromycin Stearate

The lipid-soluble salt also provides a second advantage. Erythromycin is acid labile and can undergo degradation in the acid environment of the stomach. As shown in **Figure 5-5**, an acid-catalyzed attack of the C_6 hydroxyl group on the C_9 ketone results in an intramolecular cyclization to form a hemiketal. Subsequent dehydration and attack by the C_{12} hydroxyl group leads to the formation of a ketal and an inactivation of erythromycin. Similar to the fact that sweet or bitter tasting molecules must first dissolve in the saliva before a patient can experience this taste, the ability of any drug molecule to undergo an acid-catalyzed degradation in the stomach requires that the drug dissolve in the stomach. Due to its lipid solubility, erythromycin stearate dissolves much slower in the stomach than does the free base form of erythromycin. As a result, there is less acid-catalyzed degradation of the stearate salt and hence greater bioavailability as compared with the free base form of erythromycin. This lipid-soluble organic salt does eventually dissolve in the small intestine and erythromycin is absorbed there.

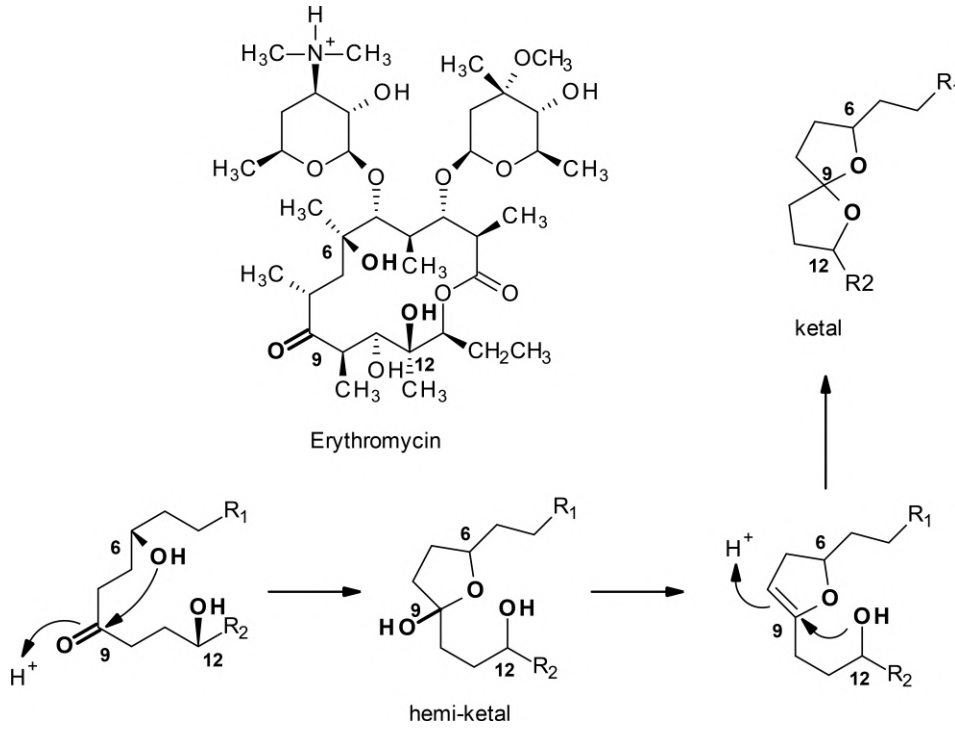


FIGURE 5-5. Acid-catalyzed degradation of erythromycin. The three-step reaction sequence involves the C_6 and C_{12} hydroxyl groups and the C_9 ketone. These oxygen atoms have been highlighted in bold. All other atoms have been removed for the purpose of simplicity and clarity.

Similarities Among Organic Salts and Esters

The advantages described above for both water- and lipid-soluble organic salts can also be achieved through the use of water- and lipid-soluble esters. A more complete discussion of water- and lipid-soluble esters, as well as examples, is provided later in this chapter; however, it is important to note that inorganic or organic salts are used more often to enhance the water solubility of a drug molecule than are water-soluble esters. In fact, although numerous acidic and basic molecules are used to make inorganic and organic salts, most water-soluble esters are produced by using only three molecules: succinic acid, sulfuric acid, or phosphoric acid. In contrast, lipid-soluble esters are used much more often than lipid-soluble salts.

Organic Salts and Drug Interactions

The beneficial effects of the organic salts discussed above are the direct result of specific planning and the application of a given salt's properties to a desired therapeutic need. In other words, specific types of organic salts are purposely designed and administered to meet specific therapeutic goals. In contrast, the formation of unwanted organic salts can lead to specific drug interactions and detrimental effects. This occurs most commonly in the preparation and administration of parenteral solutions and is best illustrated in the following two examples.

β-Lactam antibiotics, exemplified by cefepime (**Figure 5-6**), inhibit bacterial cell wall synthesis and hence provide a bactericidal effect for many systemic infections. In some cases, such as endocarditis and community-acquired pneumonia, combination antibiotic therapy with an aminoglycoside, exemplified by gentamicin (Figure 5-6), is recommended. The combination use of drugs from these

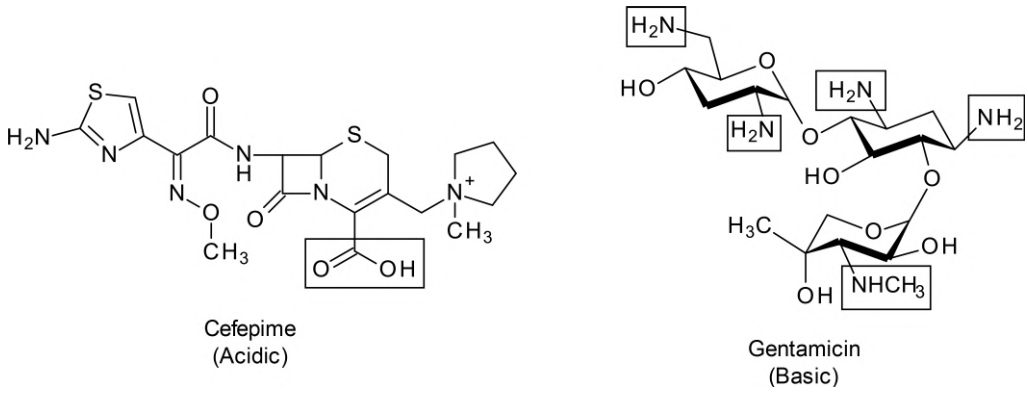
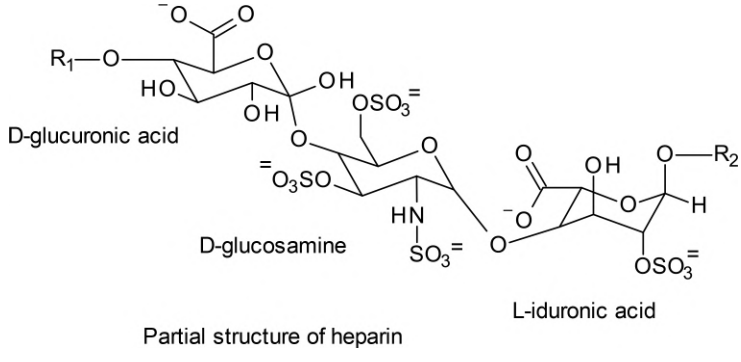


FIGURE 5-6. Cefepime and gentamicin. Acidic and basic functional groups have been highlighted with boxes.

two classes of antibiotics has been shown to produce a synergistic bactericidal effect along with a decrease in the ability of the microorganism to develop resistance. Although this combination provides beneficial therapeutic outcomes, caution must be used when administering these drugs in combination. As noted in Figure 5-6, cefepime contains an acidic carboxylic acid whereas gentamicin contains multiple primary and secondary amines. Thus, if these drugs are mixed in the same IV bag or are administered through the same IV line, it is possible for one molecule of gentamicin to form a salt complex with up to five cefepime molecules. The probability of this occurring depends on the concentrations of each drug; however, once formed, this organic salt is much less water soluble than either individual drug and may precipitate in the IV bag or tubing, leading to both decreased efficacy and potential harm to the patient.

A second example of this type of drug interaction is seen with heparin, an anticoagulant indicated for the treatment of a variety of thrombotic and embolic conditions. Chemically, heparin is a sulfated polysaccharide containing numerous negatively charged, ionized functional groups. A partial structure of heparin is shown below. Due to its chemical structure, heparin is highly water soluble, is not orally absorbed, and must be administered as either an IV infusion or a subcutaneous injection.



Partial structure of heparin

When given in IV form, it is important that solutions of basic drugs not be administered in the same IV bag or IV line as heparin. A hospital pharmacy in the Pittsburgh area provided the author with a list of medications that they have designated as "Cannot be administered in the same IV line as heparin." A sample of selected agents from this list is shown in **Figure 5-7**. Please note that all of these drugs are basic and would form organic salts with heparin. The resulting organic salts would be more lipid soluble than heparin and could lead to the precipitation in an IV administration line. Similar to what was previously discussed with gentamicin, one molecule of heparin can be involved in a salt complex with numerous molecules of any one of the drugs on this list.

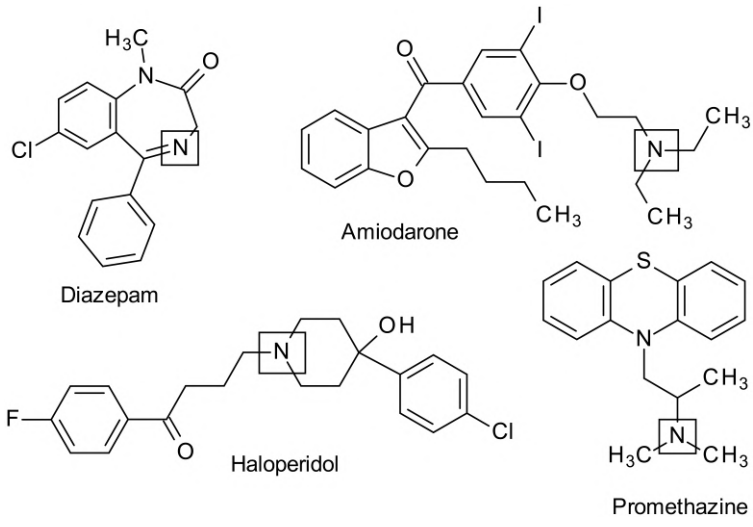


FIGURE 5-7. Basic drugs that would cause a drug interaction with heparin if infused simultaneously through the same IV line. The basic nitrogen atom has been highlighted with a box for each drug molecule.

USING NAMES OF SALTS TO IDENTIFY ACIDIC AND BASIC DRUG MOLECULES

Chapter 3 focused on the identification of acidic and basic functional groups; however, situations may arise in which it is necessary to identify the acidic and/or basic nature of a drug molecule without the benefit of viewing a specific drug structure. In these instances, if the drug is available as a salt form, it is possible to use the name of the salt to identify the acid/base nature of the drug molecule or the ionizable functional group. Please note that the following discussion is valid if the drug molecule has only one ionizable functional group or if all of its ionizable functional groups are either acidic or basic.

Remember that a salt is the product that results when an acid reacts with a base. Thus, if the organic or inorganic molecule used to make the salt is a base, then the functional group involved in the salt is an acid, and vice versa. As an example, let us consider heparin. Heparin is marketed as heparin sodium. If you find yourself in a situation when you simply can't remember anything about the structure of heparin, you can use the name of this salt to deduce the acidic nature of heparin. The sodium part of this salt comes from sodium hydroxide, a strong inorganic base; thus, the drug molecule must contain an acidic functional group. In the case of heparin, there are multiple acidic functional groups, each of which can form a salt with sodium hydroxide. Another way of correctly deducing this fact is to examine the charges on the molecule used to make the salt. The sodium ion has a positive charge, thus the ionizable functional group on the drug molecule must have a negative charge. As discussed in Chapter 3, ionized acids have a negative charge and ionized bases have a positive charge.

Using this method, it is possible to determine that ceftriaxone sodium, etidronate disodium, penicillin V potassium, leucovorin calcium, and pemirolast potassium are all salts of drug molecules that contain acidic functional groups. Similarly, it is easy to deduce that hydrochloride salts and hydrobromide salts are the product of hydrochloric or hydrobromic acid and drug molecules that contain a basic functional group (e.g., pseudoephedrine hydrochloride, citalopram hydrobromide).

A brief review of nomenclature is required when acids other than hydrochloric acid or hydrobromic acid are used to make salts of basic drug molecules. In most instances, the names of the salt

forms of these other acids follow the simple convention of removing the "-ic acid" from the end of the name and replacing it with an "-ate" suffix. For example, when sulfuric acid and phosphoric acid are used to make salts, the resulting products are termed sulfate and phosphate salts, respectively. The same is true for organic salts made with molecules containing carboxylic acids. Thus, the salt forms of acetic acid, citric acid, fumaric acid, and maleic acid are known as acetate, citrate, fumarate, and maleate salts, respectively.

Organic salts of drug molecules containing acidic functional groups are not as prevalent as organic salts of drug molecules containing basic functional groups; however, because they are normally produced from amines, the names of these salts often provide a helpful hint as to the acid/base nature of the drug molecule. This is exemplified by the tromethamine salt of ketorolac. The "amine" part of this name easily identifies it as a base; thus, the drug molecule must contain an acidic functional group.

The key point here is that the simple names of the acids and bases commonly used to prepare inorganic or organic salts can often help identify the acid/base nature of an unknown drug. While there are a multitude of drugs available for therapeutic use, there are a limited number of common acids and bases that are used to formulate salt preparations. It is strongly suggested that you become familiar with the acids and bases that are used to prepare the salt forms of drugs. A sample is provided in **Table 5-1**.

TABLE 5-1. A Sample of Salt Forms of Drug Molecules and the Acid/Base Nature of the Drug and the Molecule Involved in Salt Formation

Name of Salt Form of Drug Molecule	Inorganic or Organic Molecule Used to Formulate Salt	Acid/Base Nature of Drug Molecule
Carboprost tromethamine	Tromethamine (organic base)	Acidic
Ciclopirox olamine	Olamine (organic base)	Acidic
Clavulanate potassium	Potassium hydroxide (inorganic base)	Acidic
Clomipramine hydrochloride	Hydrochloric acid (inorganic acid)	Basic
Disopyramide phosphate	Phosphoric acid (inorganic acid)	Basic
Fosphenytoin sodium	Sodium hydroxide (inorganic base)	Acidic
Guanabenz acetate	Acetic acid (organic acid)	Basic
Metoprolol tartrate	Tartaric acid (organic acid)	Basic
Pitavastatin calcium	Calcium hydroxide (inorganic base)	Acidic
Quetiapine fumarate	Fumaric acid (organic acid)	Basic
Streptomycin sulfate	Sulfuric acid (inorganic acid)	Basic

SOLUBILITY AND PARTITION COEFFICIENTS

The *partition coefficient* of a drug molecule is defined as the ratio of the solubility of the unionized drug in an organic solvent to the solubility of the same unionized drug in an aqueous environment. A variety of organic solvents have been used in measuring partition coefficients; however, *n*-octanol is generally considered to be the standard. The aqueous environment should be buffered such that acidic and basic functional groups are primarily unionized. The partition coefficient, designated as *P*, is a dimensionless term (i.e., without units) and is often expressed as a log value, log *P*.

$$P = \frac{[\text{Drug}]_{\text{lipid environment}}}{[\text{Drug}]_{\text{aqueous environment}}} \text{ or } \log P = \log\left(\frac{[\text{Drug}]_{\text{lipid environment}}}{[\text{Drug}]_{\text{aqueous environment}}}\right)$$

While partition coefficients should be calculated at an aqueous pH in which the drug molecule is primarily unionized, this information is often not provided when partition coefficient values are published. In many cases, neutral (i.e., pH = 7.0) or physiologic (i.e., pH = 7.4) conditions are often used for partition coefficient determinations, and ionization is ignored. Additionally, a number of computer programs are available to calculate log *P* values. The designation cLog *P* (or clog *P*) is used to represent a calculated log *P*.

A related parameter, known as the distribution coefficient and designated as *D*, does account for the pH of the aqueous environment as well as the percent of the drug that is ionized. Similar to the partition coefficient, the distribution coefficient is a dimensionless term and is often expressed as a log value. Log *D* values for acidic and basic drug molecules are not constant and change according to the pH of the aqueous environment. For those drug molecules that do not contain ionizable acidic or basic groups, the log *D* value is constant and is the same as the log *P* value. The following discussion focuses solely on partition coefficients.

$$D = \frac{[\text{Drug}]_{\text{lipid environment}}}{[\text{Drug}]_{\text{ionized aq. envir.}} + [\text{Drug}]_{\text{unionized aq. envir.}}} \text{ or}$$

$$\log D = \log\left(\frac{[\text{Drug}]_{\text{lipid environment}}}{[\text{Drug}]_{\text{ionized aq. envir.}} + [\text{Drug}]_{\text{unionized aq. envir.}}}\right)$$

Partition coefficients are often used to compare the relative water or lipid solubility of a series of drug molecules. In reviewing the equation, it should be obvious that partition coefficients, or log *P* values, will be larger for those drug molecules that are more lipid soluble as compared with similar drug molecules that are either less lipid soluble or more water soluble. In contrast, log *P* values will be small for those drug molecules that are more water soluble. As an example, consider the following two hypothetical compounds. Compound A has a log *P* value of 0.38 while Compound B has a log *P* value of 1.26. Given this information, you should be able to determine that Compound B is more lipid soluble than Compound A. Moving from hypothetical compounds and log *P* values, let us examine the log *P* values of the seven commercially available HMG-CoA reductase inhibitors. These drugs are used to favorably alter plasma LDL and HDL levels and are indicated for several dyslipidemic conditions as well as the prevention of stroke and myocardial infarction. As seen in **Table 5-2**, pravastatin and rosuvastatin are much more water soluble than the other five drugs in this class of agents.

TABLE 5-2. The Calculated Log *P* Values for HMG-CoA Reductase Inhibitors[a]

Drug	Calculated Log *P* (cLog *P*)
Atorvastatin	4.13
Fluvastatin	3.62
Lovastatin	4.07
Pitavastatin	3.45
Pravastatin	1.44
Rosuvastatin	0.42
Simvastatin	4.42

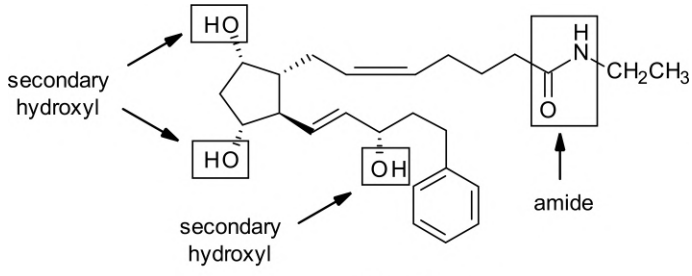
[a]The cLog *P* values were calculated using ACD/ChemSketch, version 12.01.

Analyzing a Drug Molecule for Its Water- and Lipid-Soluble Components

The term *hydrophilic* is commonly used to describe water-soluble molecules whereas the terms *hydrophobic* and *lipophilic* are commonly used to describe lipid-soluble molecules. The term *lipophobic* has also been used to describe water-soluble molecules; however, its use is not very common. It is strongly suggested that you become familiar with this terminology as these descriptions are often used interchangeably.

The partition coefficient of any given drug molecule is the result of the additive contributions and interrelationships of all of its functional groups. Each functional group helps to determine the overall hydrophobic or hydrophilic nature of the drug molecule. As such, the ability to recognize the hydrophobic and hydrophilic components of a drug molecule is an important skill. As discussed in Chapter 2, functional groups that are able to ionize and/or form hydrogen bonds contribute the most to the overall hydrophilicity of a drug molecule while halogens, aromatic rings, and hydrocarbon rings and chains contribute the most to the overall hydrophobicity of a drug molecule. A review of common hydrophilic and hydrophobic functional groups is shown in **Figure 5-8**. A more extensive list can be found in Chapter 2. Please note that each functional group is attached to one or more adjacent "R" groups. In most cases, these "R" groups represent the hydrocarbon ring or chain to which the functional group is attached. Examples of this are discussed in Chapter 2 with phenols, ethers, esters, and anilines. The hydroxyl group of a phenol, the oxygen atom of an ether, and the primary amine of an aniline are hydrophilic; however, the aromatic rings and alkyl chains to which they are attached are hydrophobic. The oxygen atoms of an ester are hydrophilic; however, esters are commonly designated as either water soluble or lipid soluble based on the composition of the "R" groups. Fluorine has been intentionally excluded from this list because its effect on solubility can vary. Fluorine can act as a hydrogen bond acceptor and, in some instances, can increase the water solubility of a drug molecule; however, studies have shown that the substitution of a fluorine atom for a hydrogen atom generally tends to slightly enhance the lipid solubility of a drug molecule. One specific fluorine-containing functional group that is known to enhance the lipid solubility of a drug molecule is the trifluoromethyl group (CF_3).

Using these functional groups, let us analyze a sample drug. Bimatoprost is a semisynthetic prostaglandin that is used to treat open angle glaucoma and ocular hypertension. This drug is formulated and administered as an ophthalmic solution. This drug contains four functional groups—three secondary hydroxyl groups and one amide—that are capable of forming hydrogen bonds with water. These functional groups contribute to the overall water solubility of the drug. The remainder of the molecule, or the "R" groups to which the hydrophilic groups are attached, consists exclusively of hydrocarbon chains, aromatic rings, and alicyclic rings that contribute to the overall lipid solubility of the molecule.



Bimatoprost

Although this type of analysis does not directly calculate a log *P* value, it does allow for a better understanding of published or calculated log *P* values. This type of analysis is useful in understanding the differences in log *P* values for a series of related drug molecules, in the comparison of specific structures and their log *P* values, and in prediction of log *P* values of analogs. Additionally, the actual

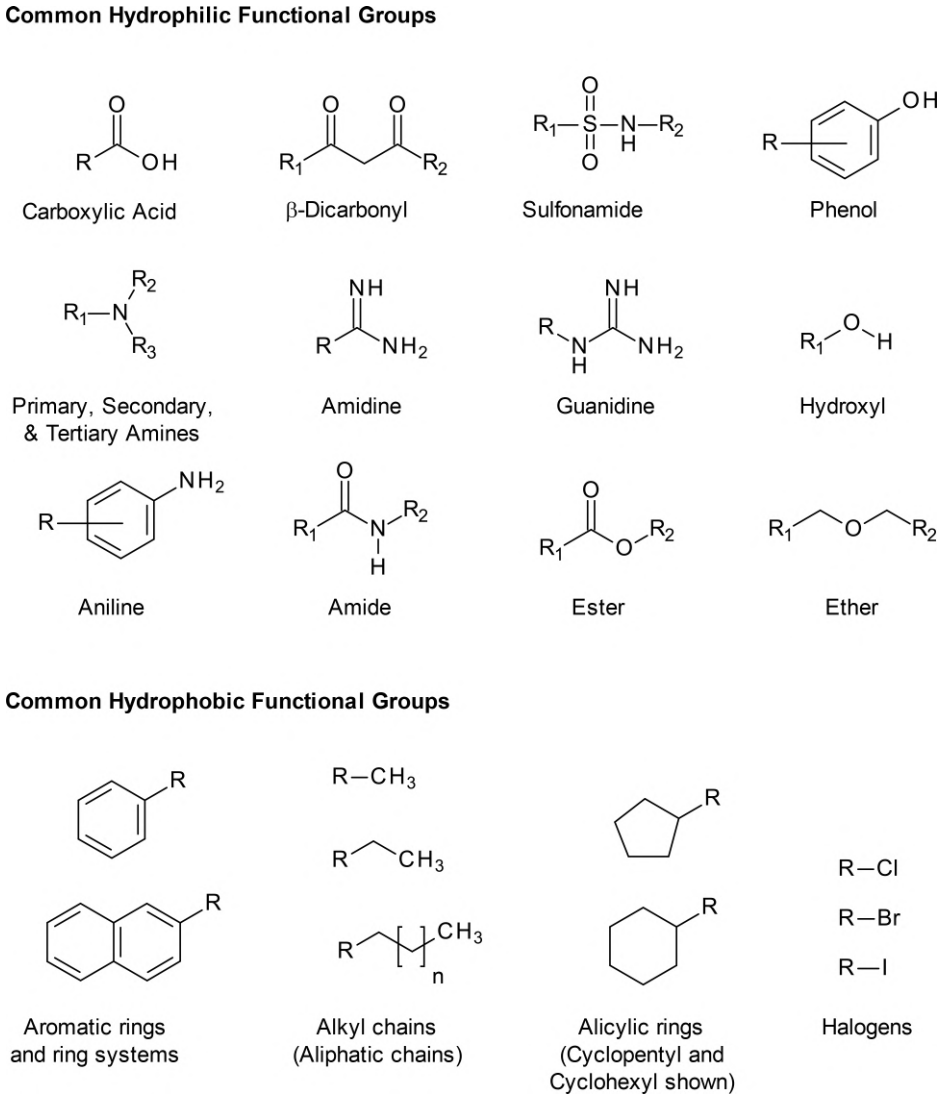


FIGURE 5-8. Common hydrophilic and hydrophobic functional groups. (Note: Fluorine has been intentionally omitted here; see text for an explanation of its role in solubility.)

log *P* values are often not as important as the relative hydrophilicity or lipophilicity of a given drug molecule within a series of drug molecules.

Important Skills to Master

First, you should be able to identify drug molecules that are either highly hydrophilic or highly hydrophobic. As an example, consider the four drug molecules shown in **Figure 5-9**. Alendronic acid is a small molecule that contains a tertiary hydroxyl group and three ionizable functional groups whereas tobramycin contains a large number of hydroxyl groups and primary amines. All of these functional groups are capable of undergoing ionization or participating in hydrogen bonds. As a result, both of these drug molecules are extremely hydrophilic. In contrast, vitamin K_1, also known

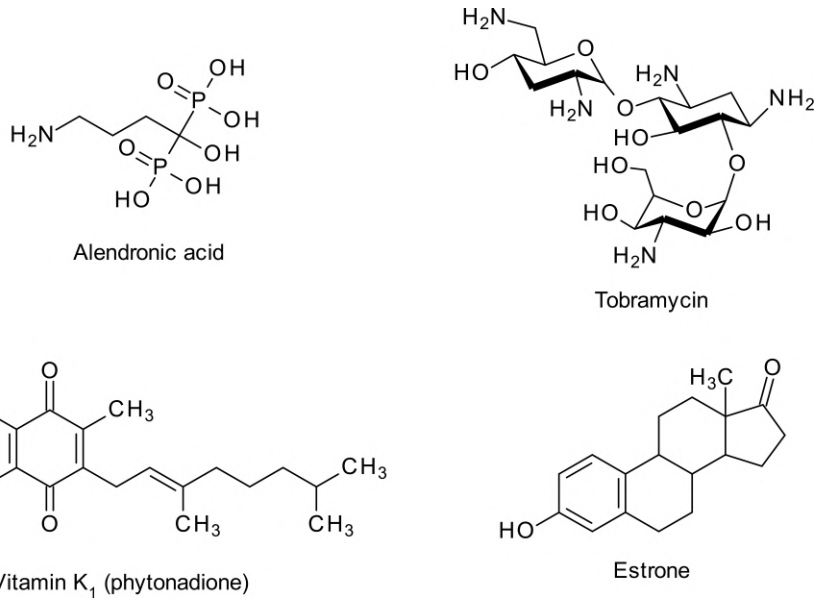


FIGURE 5-9. Examples of highly hydrophilic and highly hydrophobic drug molecules.

as phytonadione, and estrone lack any ionizable groups and are comprised primarily of hydrocarbons. Both drugs are highly hydrophobic.

Unlike the examples in Figure 5-9, most drug molecules contain a more balanced distribution of hydrophilic and hydrophobic functional groups and thus cannot be immediately categorized as either a hydrophilic or hydrophobic drug molecule. Therefore, a second important skill is the ability to compare the relative hydrophilicity or hydrophobicity of a specific drug molecule with structurally related drugs based on the relative solubilities of their respective functional groups. As an initial example, let us look at the series of hypothetical compounds shown in **Figure 5-10**. All of these compounds are structurally similar, with the only variation at the *ortho* position of the phenyl ring. Because Compound B in this series has an unsubstituted phenyl ring, we will use this compound as our reference point. In looking at the remaining three analogs, you can see that Compounds A and D contain hydrophilic functional groups at the *ortho* position, while Compound C contains a hydrophobic propyl group. Therefore, Compounds A and D are predicted to be more water soluble than Compound B, whereas Compound C is predicted to be more lipid soluble than Compound B. The only task remaining is to determine the relative water solubilities of Compounds A and D. Due to its ability to be primarily ionized in most physiologic environments, the carboxylic acid present

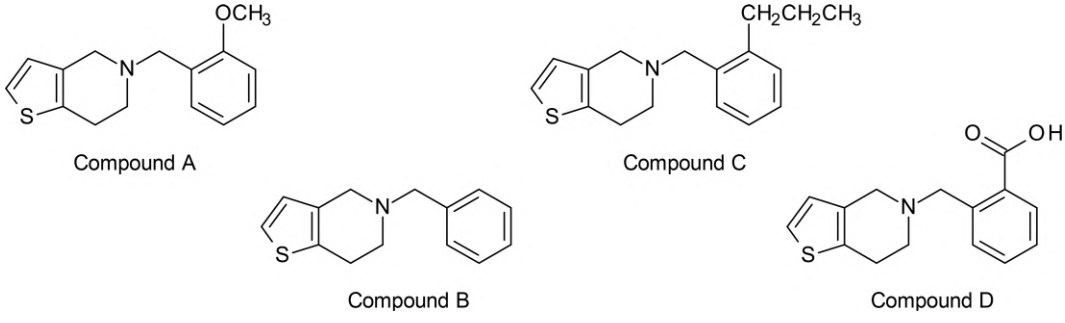


FIGURE 5-10. Structurally related compounds with differing water solubilities.

in Compound D is predicted to impart more water solubility than the methoxy group present on Compound A. Thus, the rank order of these four compounds in terms of their water solubility is Compound D > Compound A > Compound B > Compound C.

Let us now extend this type of evaluation to specific drug molecules by returning to Table 5-2 and examining the cLog *P* values for pravastatin (cLog *P* = 1.44) and simvastatin (cLog *P* = 4.42). Evaluation of these cLog *P* values reveals that simvastatin is approximately three log units more lipophilic than pravastatin. This significant difference can be explained by comparing their chemical structures. As shown in **Figure 5-11**, there are three sites of structural variation between these two drugs. At site A, simvastatin contains a lactone bond whereas in pravastatin, this bond has been broken to reveal an acidic carboxylic acid and a secondary hydroxyl group. While lactones can participate in hydrogen bonds, their capacity for increasing water solubility of a drug is far exceeded by an ionizable carboxylic acid and a hydroxyl group. At site B, simvastatin contains an extra methyl group that is not present on pravastatin, and at site C, simvastatin contains a methyl group whereas pravastatin contains a hydroxyl group. Compared with either a hydrogen atom or a hydroxyl group, a methyl group enhances lipid solubility. Thus, at all three of these sites, the functional group present in simvastatin is more lipophilic than the functional group present in pravastatin. This is very much consistent with the known log *P* values of these two drugs.

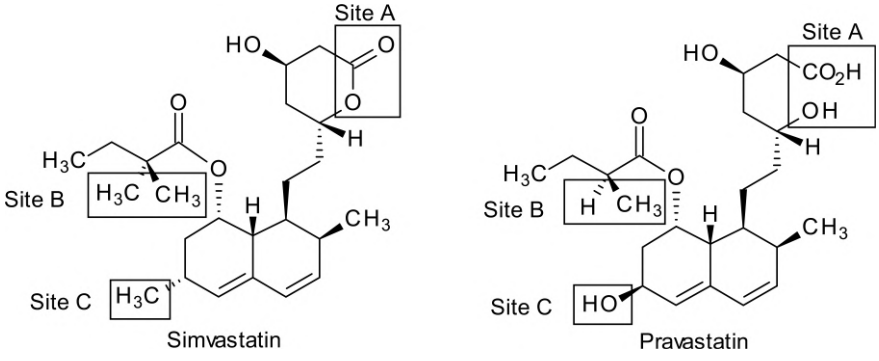


FIGURE 5-11. Simvastatin and pravastatin.

A second example of this type of evaluation is seen with temazepam and quazepam (**Figure 5-12**), two benzodiazepines used in the treatment of insomnia. Similar to lovastatin and pravastatin, there are four structural variations between these two drug molecules. At site A, temazepam contains a methyl group, while quazepam contains a trifluoroethyl group. The additional carbon atom, as

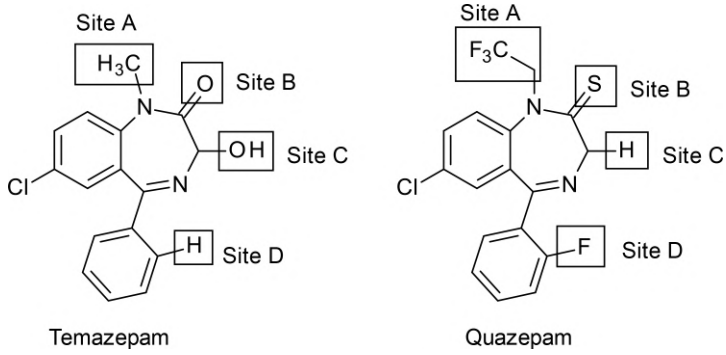


FIGURE 5-12. Temazepam and quazepam.

well as its three fluorine atoms, enhances the lipid solubility of quazepam as compared with temazepam. At site B, the substitution of the oxygen atom in temazepam with a sulfur atom in quazepam also enhances the lipid solubility of quazepam. This is because oxygen is more electronegative than sulfur, creates a stronger dipole, and is better able to interact with water than is sulfur. At site C, temazepam contains a hydrophilic hydroxyl group instead of the hydrogen present in quazepam, while at site D quazepam contains a fluorine atom instead of the hydrogen present in temazepam. In this case, the fluorine atom also enhances the overall lipid solubility of quazepam. At all four sites of variation, the functional groups present in quazepam are more lipophilic than those present in temazepam; thus, quazepam would be predicted to be more lipophilic (or hydrophobic) than temazepam. This is verified by comparing the log *P* values of these two drugs. Quazepam has a reported log *P* value of 4.1 ± 0.8, while temazepam has a reported log *P* value of 2.2 ± 0.6. Although both drug molecules could be considered lipophilic, this relative comparison reveals that temazepam is considerably less lipophilic than quazepam.

Both of the above examples illustrate the third important skill you need to learn and master. Because the log *P* value of any given drug molecule is the result of the additive contributions of all of its functional groups, you should be able to explain the log *P* differences between two or more drug molecules by comparing the chemical differences of their respective functional groups.

A final skill to master is prediction of how structural alterations alter the hydrophilic and hydrophobic nature of a drug molecule. Also implicit in this skill is the ability to match log *P* changes with structural changes. As an example, let us revisit bimatoprost and compare its hydrophobic nature with the two hypothetical analogs shown in **Figure 5-13**. Analog 1 lacks the aromatic ring seen in bimatoprost while Analog 2 contains a *meta* trifluoromethyl group on the aromatic ring. The cLog *P* value for bimatoprost is 3.25, and the cLog *P* values for the analogs, from highest to lowest, are 4.13 and 1.64 (Accelrys Draw 4.0). Because an aromatic ring adds lipid solubility to a drug molecule, Analog 1 would be predicted to be less hydrophobic than bimatoprost; thus, the log *P* value of 1.64 would be consistent with this analysis. The trifluoromethyl group of Analog 2 is more hydrophobic than an unsubstituted hydrogen atom; thus, Analog 2 would be predicted to be more hydrophobic than bimatoprost, and the log *P* value of 4.13 would be consistent with this analysis.

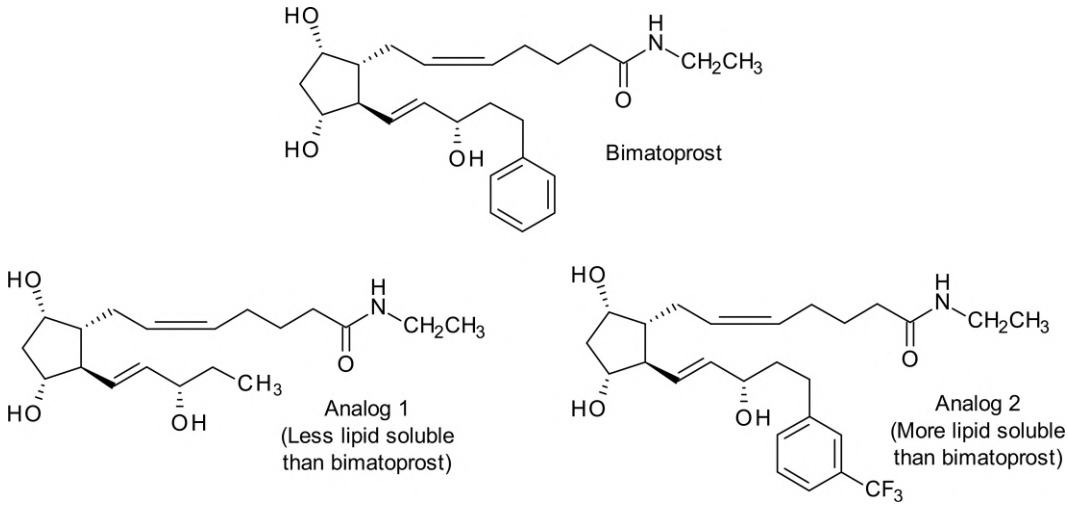


FIGURE 5-13. Bimatoprost and two hypothetical analogs.

Summary of Key Skills to Master When Evaluating the Solubility of a Drug Molecule

Based upon a chemical analysis of the functional groups that comprise a drug molecule, you should be able to

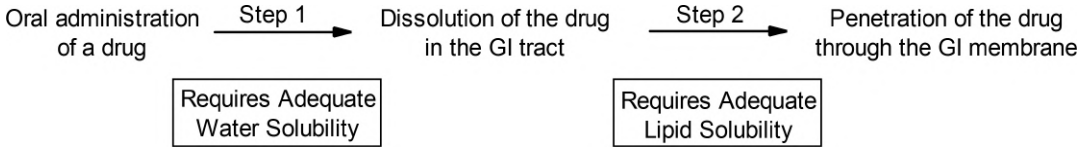
- Identify those drug molecules that are comprised almost exclusively of water- or lipid-soluble functional groups.
- Rank order a series of structurally related drug molecules in terms of their predicted water or lipid solubility.
- Explain the log *P* differences between two or more drug molecules based on the chemical differences of their respective functional groups.
- Predict how a specific structural alteration will alter the overall water and lipid solubility of the drug molecule.

A Balance Between Water and Lipid Solubility

The previous discussions focused upon an understanding of partition coefficients and the ability to analyze the functional groups of drug molecules to either predict or explain the differences in partition coefficient values. Given this information, a common question often arises, "What is more important for a drug molecule, water solubility or lipid solubility?" The simple answer is both; however, this answer requires some additional explanation. Although some drug molecules have enhanced pharmaceutical properties based on the fact that they are either highly lipid soluble or highly water soluble, *most drug molecules need to have a balance between these two extremes.* The therapeutic benefits of enhancing lipid and water solubility are discussed in subsequent sections; however, let us first consider the need for a balance between lipid and water solubility.

In very general terms, the human body can be described as a group of hydrophilic regions (e.g., the blood, the gastrointestinal [GI] tract, the cytosol of individual cells) separated by hydrophobic barriers (e.g., lipid bilayers, the blood brain barrier). When viewed in this context, it should be easy to recognize that most drug molecules require a balance of water and lipid solubility to traverse these hydrophilic and hydrophobic regions. In instances in which a drug molecule is either too lipid soluble or too water soluble, specific transport proteins are required to allow drugs to move through the blood or cross specific lipid barriers, respectively.

The need for a balance between water and lipid solubility is best exemplified by drugs that are orally administered as either a tablet or capsule. Once swallowed, the drug molecules present in either of these dosage formulations must first dissolve in the aqueous environment of the saliva, stomach, or small intestine to be absorbed. This initial step requires that the drug molecule have sufficient water solubility for adequate solvation and dissolution to occur. If there are problems in this initial step due to the lack of sufficient water solubility, it is possible that a substantial amount of the drug will not undergo dissolution and absorption but rather will be excreted in the feces. Once the drug has dissolved, it must possess adequate lipid solubility to be able to cross the lipid bilayer that comprises the GI mucosal membrane. If a drug molecule lacks sufficient lipid solubility, it will have difficulty being absorbed into the bloodstream. Consequently, a highly water-soluble drug may instead be primarily excreted in the feces.



For orally administered drugs, studies have shown that there is a parabolic relationship between lipid solubility and drug action. Within a series of drug molecules, lipid solubility enhances the activity only to a certain point, beyond which it begins to decrease activity. This is illustrated in **Figure 5-14**, Graph A. Whereas some drug molecules possess a lipid solubility that is at or near their optimal lipid solubility, many others possess lipid solubility that lies on either the ascending or descending portions of this parabolic curve. Drug molecules that possess a lipid solubility corresponding to either position 1 or position 2 of Graph A tend to have a more hydrophilic nature. Although they should have no problems dissolving within the aqueous contents of the GI tract, they may have difficulty getting across the GI mucosal membranes and into the bloodstream. In these two scenarios, structural analogs with increased lipid solubility (i.e., a larger log *P* value) are predicted to have enhanced oral absorption and activity. In contrast, drug molecules that possess a lipid solubility corresponding to position 3 have exceeded their optimal lipid solubility and have a high hydrophobic nature. These types of molecules can easily pass through GI mucosal membranes; however, they may exhibit poor absorption due to a failure to adequately dissolve in the aqueous environment of the GI tract. In this scenario, structural analogs with increased water solubility (i.e., a smaller log *P* value) would be expected to have greater oral absorption and overall activity.

You may occasionally encounter a graph that depicts a linear relationship between lipid solubility and drug activity, as illustrated in Figure 5-14, Graph B. It is important to note that these types of graphs are consistent with the parabolic relationship shown in Graph A and are simply magnifications of a specific subsection of this parabolic curve. As an example, there are some instances in which an entire chemical or pharmacological class of drugs is much more hydrophilic than hydrophobic. In this instance, the lipid solubility of all of the drug molecules in this class lies at or around position 1or between positions 1 and 2 of Graph A. Magnification of this small subsection of Graph A can result in a linear depiction of lipid solubility and drug action, as seen in Graph B. The only caution here is that at some point the maximum lipid solubility will be exceeded, and both absorption and drug action will decrease.

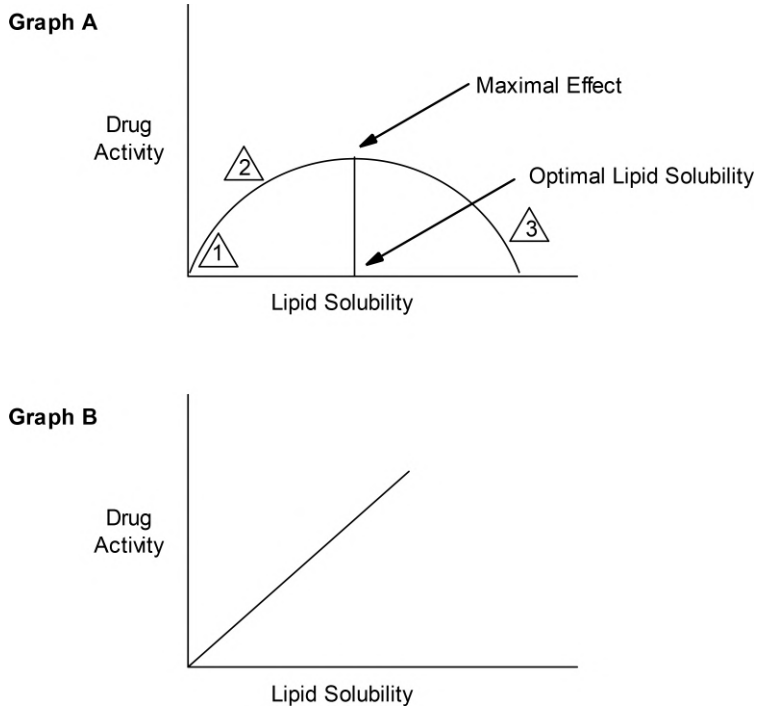


FIGURE 5-14. The parabolic (Graph A) and linear (Graph B) relationships between lipid solubility and the action of orally administered drugs.

STRATEGIES FOR OPTIMIZING THE DESIRED WATER OR LIPID SOLUBILITY

Up to this point, we have discussed partition coefficients, the analysis of a drug molecule to identify its water- and lipid-soluble functional groups, specific skills that help to associate differences in log *P* values with structural differences between and among specific drug molecules, and the need to have an appropriate balance between water and lipid solubility based upon a given therapeutic need. In this section, we discuss three common strategies that are used to favorably alter the overall water/lipid solubility of a specific drug molecule to meet specific therapeutic goals.

Strategy 1: Use Inorganic and Organic Salts

This simple strategy to enhance either water or lipid solubility has already been discussed in this chapter. Inorganic salts and water-soluble organic salts enhance the water solubility of drug molecules by enhancing their solvation and dissolution. These properties alter the water/lipid balance and can result in an increase in the rate and extent of oral absorption. Additionally, the enhanced water solubility allows for the preparation of concentrated parenteral, ophthalmic, or otic solutions. Examples of water-soluble salts can be found in Figures 5-1 and 5-4. Lipid-soluble organic salts decrease water solubility and enhance lipid solubility. As previously discussed with penicillin G benzathine, NPH insulin, and erythromycin stearate, lipid-soluble salts can enhance the duration of action, decrease the acid degradation in the stomach, and/or increase the palatability of a drug molecule.

Strategy 2: Convert the Parent Drug Molecule to a Water- or Lipid-Soluble Ester Prodrug

A *prodrug* is defined as a drug molecule that has been covalently modified to either an inactive or weakly active analog to achieve a specific therapeutic benefit. Once the prodrug is administered, it undergoes metabolic activation to release the original active drug molecule. This process is also known as bioactivation (or programmed metabolism). Esterification of carboxylic acids, hydroxyl groups, and phenol groups is often used to produce a prodrug with an enhanced water or lipid solubility compared with the parent drug molecule. The resulting ester prodrugs are cleaved in vivo by either esterase enzymes or acid/base catalyzed hydrolysis within the GI tract. Esterase enzymes are ubiquitous within the human body, and the active drug molecules are released in the liver, the bloodstream, the GI tract, or the target tissue.

In comparing the use of water- and lipid-soluble salts to water- and lipid-soluble esters, there is one main similarity—both types of modification eventually release the parent or active drug molecule. There are also two main differences. First, esters require covalent modification and in vivo bioactivation whereas salts simply require the dissociation of noncovalently bound ionic molecules. Second, salts are used more often than esters to enhance water solubility while esters are used more often than salts to enhance lipid solubility.

The two most commonly used water-soluble ester prodrugs are those that contain a sodium phosphate or a sodium succinate ester. Compared with the hydroxyl group present on the parent drug molecule, a phosphate or succinate ester contains ionizable functional groups that will further enhance water solubility. Examples of each of these are shown in **Figure 5-15**. Please note that the names directly indicate that these are water-soluble esters. As previously discussed, the inclusion of the term *sodium* in the name indicates the presence of an inorganic salt, specifically a sodium salt of an acidic functional group. The sodium salts have been highlighted in the figure. Water-soluble formulations of prednisolone sodium phosphate are used for ophthalmic purposes whereas

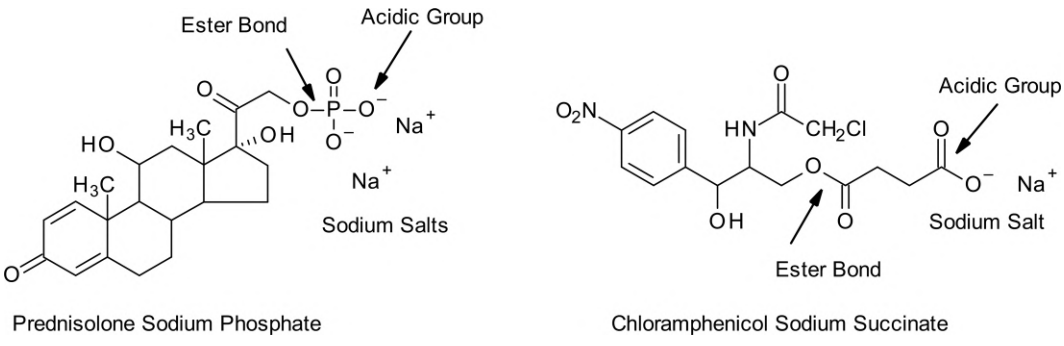
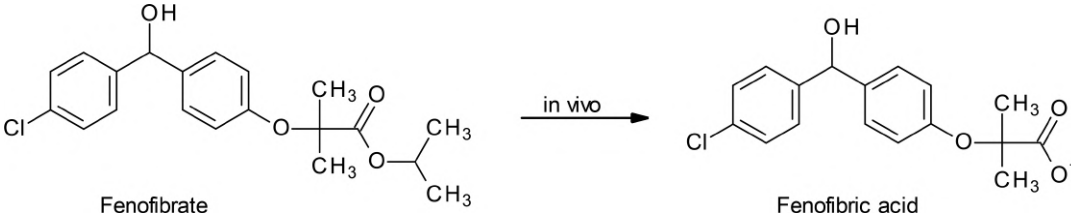


FIGURE 5-15. Examples of water-soluble esters.

water-soluble formulations of chloramphenicol sodium succinate are available for IV, ophthalmic, or otic use.

Lipid-soluble esters are commonly used to produce prodrugs with enhanced oral bioavailability. In these situations, the parent (or active) drug molecule possesses sufficient water solubility for dissolution but lacks sufficient lipid solubility to allow it to pass through the GI mucosal membrane. An example of this is seen below with fenofibrate, which is used for the treatment of several dyslipidemias. It is administered orally as an inactive isopropyl prodrug and is metabolized in vivo to the active agent, fenofibric acid.



In general, lipid-soluble esters of carboxylic acids tend to be somewhat chemically simplistic, as the key strategy involves masking an ionizable and hydrophilic group as a more hydrophobic ester. As such, carboxylic acids are generally converted to their respective methyl, ethyl, propyl, isopropyl, or *tert*-butyl esters. In some situations, more complex esters, such as that seen with candesartan cilexetil (**Figure 5-16**), are used to optimize the overall water/lipid solubility of the drug molecule.

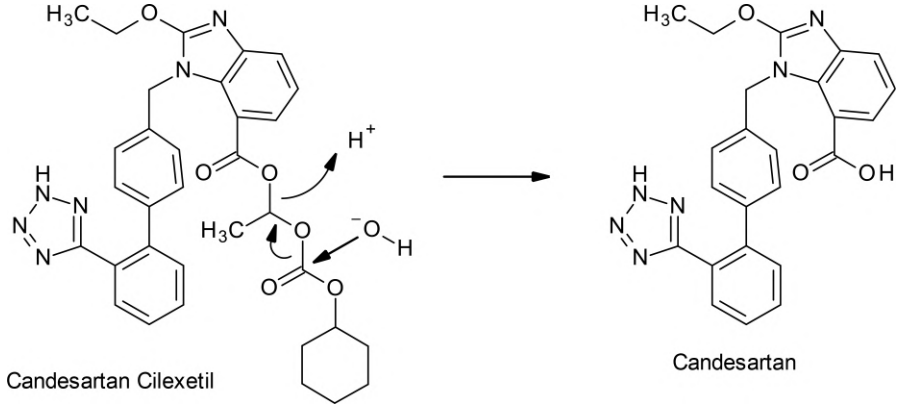


FIGURE 5-16. Candesartan cilexetil and its conversion to candesartan.

Another common use of lipid-soluble esters is in the preparation of depot injections. Similar to penicillin G benzathine and NPH insulin, the addition of a lipid-soluble ester to a drug molecule significantly decreases its water solubility and allows it to be formulated as a suspension for IM or subcutaneous injection. After injection, the lipid-soluble ester dissolves slowly and moves from its initial injection site to the plasma in a consistent manner over an extended period of time. Once in the plasma, the ester is hydrolyzed and releases the active drug molecule. These types of esters are commonly made by combining hydroxyl groups or phenols present on the parent drug molecule with carboxylic acids containing various hydrocarbons chains and/or rings. Carboxylic acids commonly used for this purpose are illustrated in **Figure 5-17**, and two examples of this concept are illustrated by haloperidol decanoate and estradiol valerate, shown in **Figure 5-18**. In both examples, a hydrophilic hydroxyl group has been replaced with a lipid-soluble ester. Both of these drug molecules are highly lipophilic and greatly enhance the duration of action of the parent drug molecules when administered as an IM injection. Both drug molecules can be used either once a month or every 4 weeks. Haloperidol decanoate is used for the treatment of a number of psychotic disorders whereas estradiol valerate is used to treat the symptoms of menopause or is used as estrogen replacement therapy.

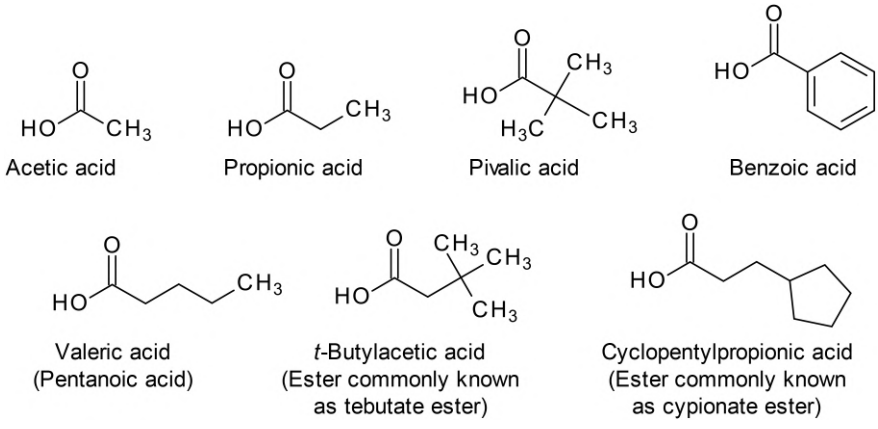


FIGURE 5-17. Carboxylic acids commonly used to prepare lipid-soluble esters.

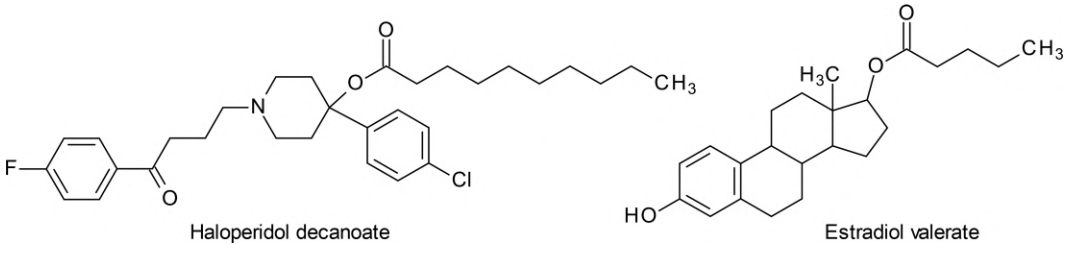


FIGURE 5-18. Examples of lipid-soluble ester prodrugs: haloperidol decanoate and estradiol valerate.

Finally, lipid-soluble esters can be used to enhance either the pulmonary or topical absorption of drug molecules. Two examples of this can be seen within the glucocorticoid class of drugs (**Figure 5-19**). These drugs are used to treat a variety of disorders, including allergic rhinitis, asthma, inflammation, and a variety of skin disorders. Hydrocortisone is available over-the-counter (OTC) as a 1% cream for the topical treatment of pruritus, inflammation, eczema, psoriasis, and other skin disorders. It is also available as a variety of lipid-soluble esters, including hydrocortisone butyrate.

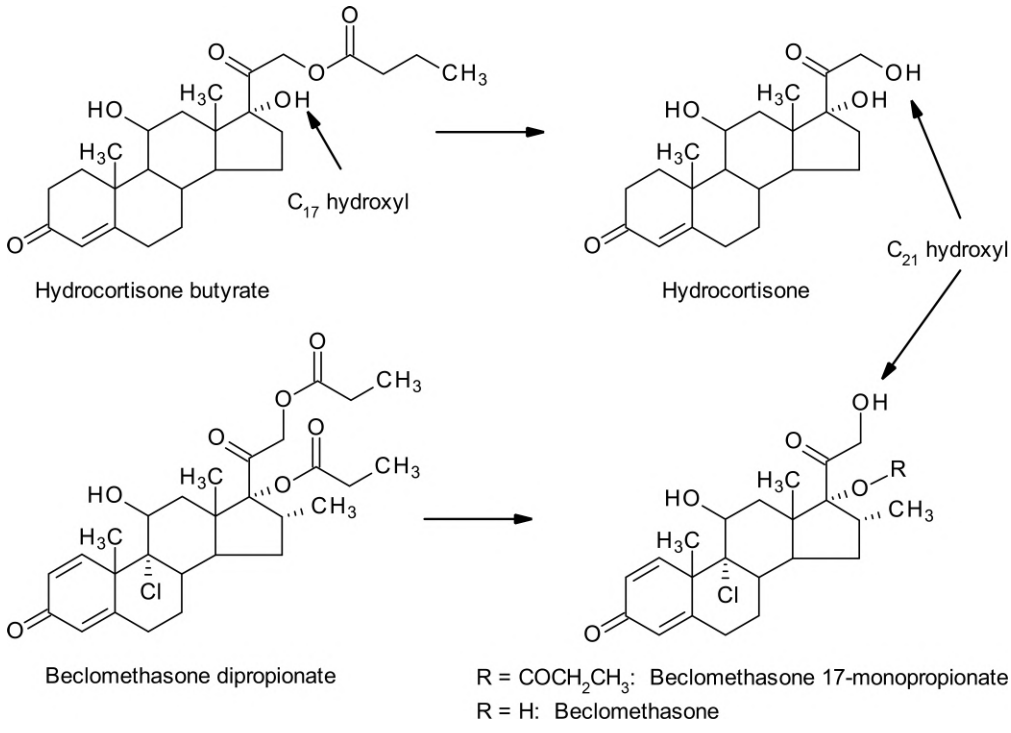


FIGURE 5-19. Lipid-soluble ester prodrugs of glucocorticoids. Esters are formed using the C_{17} and C_{21} hydroxyl groups.

The butyrate ester enhances the topical penetration of the drug through the skin and increases its topical potency. Instead of using a 1% formulation, the lipid-soluble butyrate ester can be used as a 0.1% cream. Beclomethasone dipropionate is used for the treatment of asthma and allergic rhinitis and is administered via oral or nasal inhalation. The two propionate esters enhance the lipid solubility of beclomethasone. Once inhaled, this prodrug is rapidly absorbed in either the pulmonary tract or nasal sinus cavity, depending upon the route of administration. Once absorbed, the C_{21} ester is rapidly cleaved to produce beclomethasone 17-monopropionate. This metabolite is pharmacologically active and can directly produce the desired therapeutic response or be subsequently converted to beclomethasone that is also able to produce the desired response.

Strategy 3: Add or Alter Functional Groups

This strategy involves replacement of existing functional groups with those that are either more water soluble or more lipid soluble, depending on the specific pharmaceutic or therapeutic need. Common types of modifications include the replacement of a hydrogen atom with either a lipid- or water-soluble functional group, replacement of a water-soluble functional group with a lipid-soluble functional group or vice versa (e.g., replacement of a hydroxyl group with a methyl group or replacement of a methyl group with a hydroxyl group), and modification of existing functional groups (e.g., conversion of a $-CH_2CH_3$ group to a more water-soluble $-CH_2CH_2OH$ group or conversion of a primary amine, RNH_2 to a more lipid-soluble secondary amine, $RNHCH_2CH_2CH_2CH_3$).

There is an important difference between this strategy and the two strategies previously discussed. While salts and esters can enhance either the water or lipid solubility of the drug molecule, once the salt or ester is administered to the patient, *the parent drug molecule will eventually be released*. For example, timolol maleate (Figure 5-4) separates into its two components, and timolol binds to its target receptor and produces the therapeutic effect. The same is true for haloperidol

decanoate (Figure 5-18). The decanoate ester is hydrolyzed, and haloperidol provides the therapeutic action. In contrast, the replacement or alteration of functional groups *results in a permanent change* in the original drug molecule and the creation of an entirely new drug entity. Additionally, and as discussed in Chapter 2, the alteration of a functional group not only changes the water/lipid solubility but also may affect the electronics and the overall size of the drug molecule. As such, there are limitations to the types of alterations that can be done because the altered drug molecule must still be able to bind to its biological target.

Let us look at two examples of this strategy. As previously discussed, increasing the lipid solubility of glucocorticoids can enhance their topical, nasal, and/or pulmonary absorption. While lipid-soluble ester prodrugs can provide this advantage, another strategy involves masking the C_{16} and C_{17} hydroxyl groups. The reaction of triamcinolone with acetone produces triamcinolone acetonide **(Figure 5-20)**. The acetonide analog is more lipid soluble than triamcinolone and is used topically to treat pruritus and a variety of dermatological disorders, as a nasal inhalation to treat allergic rhinitis, and as an oral inhalation to treat asthma. Unlike ester prodrugs, the acetonide analog is active and is not metabolized back to triamcinolone. Other glucocorticoids that are used as their acetonide analogs are also shown in Figure 5-20.

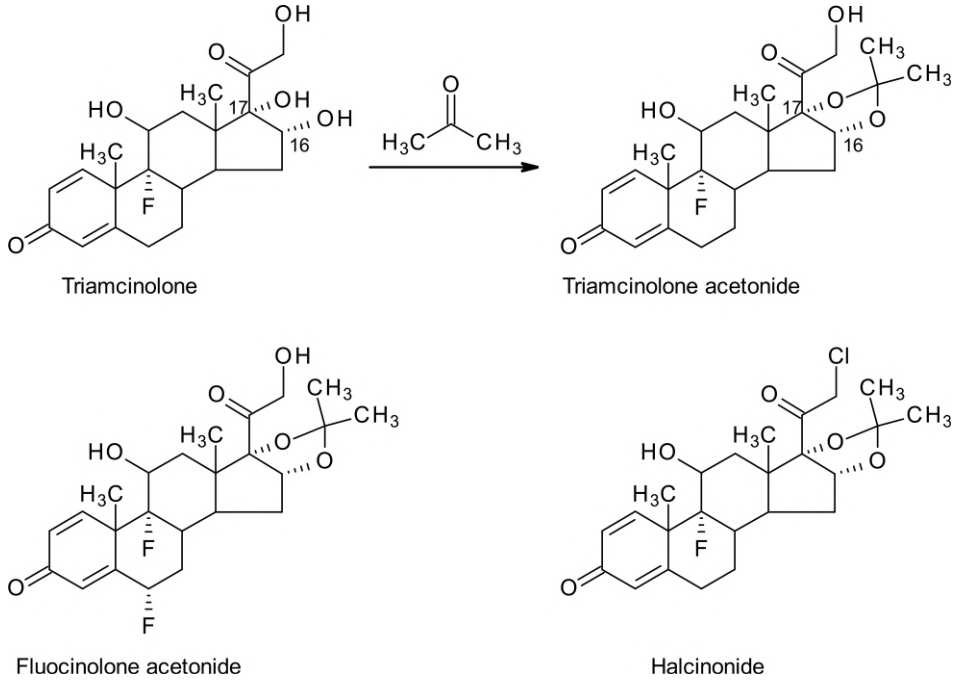


FIGURE 5-20. Acetonide analogs of selected glucocorticoids.

A second example involves modifications of penicillin G, a naturally occurring β-lactam antibiotic that was previously discussed in this chapter, to ampicillin and amoxicillin (**Figure 5-21**). Penicillin G is useful in treating a variety of microbial infections; however, it is only active against a limited number of bacteria and has a narrow spectrum of action. One way to extend its spectrum of action is to add hydrophilic functional groups at the carbon atom between the phenyl ring and the carbonyl group. This modification is seen in ampicillin. The increased water solubility attained by the addition of this primary amine allows ampicillin to access water soluble channels, also known as porins, that are present in gram-negative bacteria. This allows ampicillin to treat bacterial infections that are not susceptible to penicillin G.

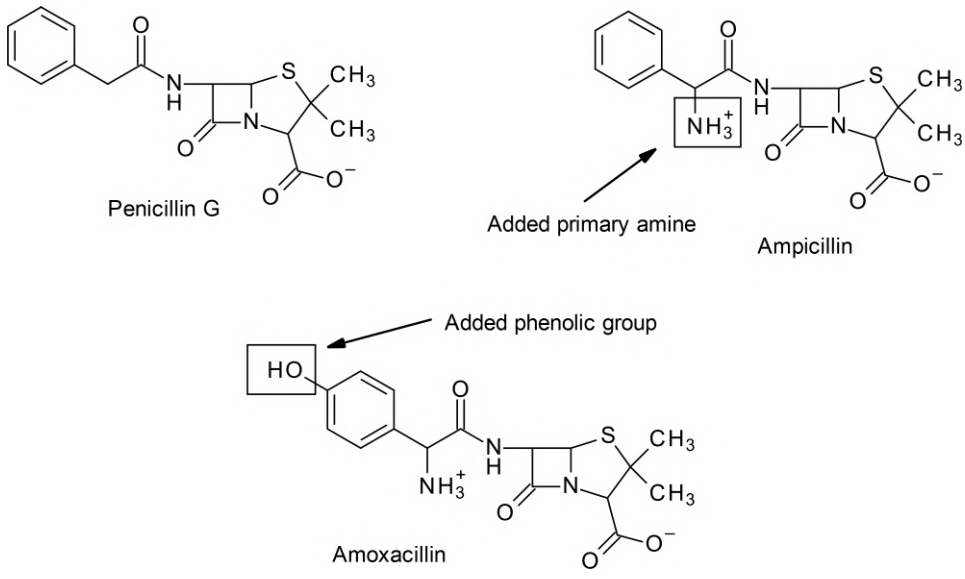


FIGURE 5-21. Penicillin G and two analogs: ampicillin and amoxicillin.

While the addition of this primary amine is useful in treating specific bacterial infections, it also converts penicillin, an acidic drug, to ampicillin, an amphoteric drug. Within the small intestine, both the carboxylic acid and the primary amine are primarily ionized, and ampicillin will exist in what is known as its *zwitterion form*. In the zwitterion form, both the acidic and basic functional groups are ionized, and the molecule has an overall net charge of zero. In some instances, such as the example here with ampicillin, zwitterions have inadequate water solubility, a much slower dissolution within the GI tract, and an overall decrease in oral absorption. To enhance the oral absorption, a phenol was added at the *para* position of the aromatic ring to produce amoxicillin. Amoxicillin, by virtue of the hydrogen bonding capacity of the phenol, is more water soluble than ampicillin, contains a better balance between lipid and water solubility, and has a much higher oral absorption. Because the structure of amoxicillin retains the primary amine, it has an extended spectrum of action compared with penicillin G. The oral absorption of ampicillin ranges from 30% to 55% while the oral absorption of amoxicillin ranges from 74% to 92%. It should be noted that not all zwitterions have dissolution problems and that the key attribute is an appropriate balance between water and lipid solubility. Amoxicillin and ampicillin both exist as zwitterions within the GI tract; however, amoxicillin has a better balance of water/lipid solubility and thus has better oral absorption.

Factors Affecting the Oral Absorption of Drug Molecules

This chapter highlights the importance of both water and lipid solubility on the oral absorption of drug molecules. *It is important to recognize that other reasons, beyond an inadequate balance between water and lipid solubility, can alter the oral absorption, oral bioavailability, and oral efficacy of a drug molecule.* The following list highlights some of the major factors that can cause a drug molecule to be orally inactive or have poor oral absorption or oral bioavailability.

- The drug molecule has insufficient water solubility, which results in poor dissolution of the drug molecule in the GI tract.
- The drug molecule has insufficient lipid solubility, which results in a decreased ability to traverse GI mucosal membranes.

- The drug molecule is unstable in the acid environment of the stomach, which can cause a significant amount of a given dose to be destroyed or inactivated before it can be absorbed.
- The drug molecule is subject to enzymatic degradation within the GI tract, which is seen with insulin and other small proteins and peptides.
- The drug molecule is too large to pass through the GI mucosal membranes.
- The drug molecule undergoes extensive first-pass metabolism prior to reaching the circulation. In this case, the drug is absorbed but a significant percentage is inactivated prior to reaching the general circulation.

THE INFLUENCE OF SOLUBILITY ON DRUG METABOLISM

A full discussion of metabolism and metabolic pathways is provided in Chapter 8; however, it is important to recognize that the overall water/lipid solubility of a drug molecule plays a key role in the extent to which it is metabolized. Most drug molecules administered for therapeutic purposes are not normally found in the human body. As such, the human body views these as foreign molecules, commonly known as xenobiotics. The two major purposes of metabolism are to detoxify these foreign molecules and ensure that they can be eliminated from the body.

Drug molecules that possess sufficient lipid solubility are able to reside longer in the body than drug molecules that are more water soluble. Drug molecules with sufficient lipid solubility can be passively reabsorbed from the nephron tubule back into the blood. Additionally, drug molecules with sufficient lipid solubility can undergo *enterohepatic recycling*, a process by which the drug molecules are secreted from the liver into the small intestine and then reabsorbed into the blood. Finally, lipid-soluble drug molecules have a greater affinity for plasma proteins than do analogous water-soluble drugs. An example of this can be seen with pravastatin and simvastatin (Figure 5-11), two drugs that were previously discussed in this chapter. Simvastatin is more lipid soluble than pravastatin and is 95% bound to plasma proteins. In contrast, the more water-soluble pravastatin is only 43% to 55% plasma protein bound. While bound to a plasma protein, a drug molecule is less likely to be eliminated from the body.

The goals of metabolism are thus met through biotransformation reactions that enhance the water solubility of drug molecules and make them easier to be eliminated in either the urine or the feces. As such, drug molecules that are already highly water soluble are often eliminated unchanged (i.e., without requiring metabolism). Examples of this are seen with alendronic acid and tobramycin (Figure 5-9) and zanamivir (**Figure 5-22**). In contrast, drug molecules that are highly lipid soluble often undergo extensive hepatic metabolism prior to entering the general circulation. This process is known as first-pass metabolism and often significantly reduces the amount of orally administered drug that is available (i.e., the drug's bioavailability). An example of this can be seen with propranolol (Figure 5-22), a nonselective β receptor antagonist (or β blocker). When given orally, more than

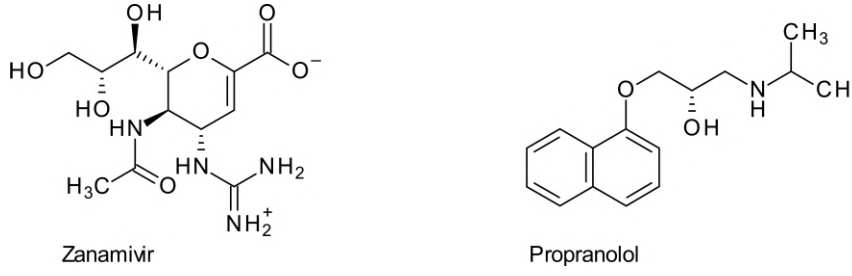


FIGURE 5-22. Zanamivir and propranolol.

90% of the given dose is orally absorbed; however, 50% to 70% of the absorbed drug is inactivated through first-pass metabolism.

In general, the extent of metabolism increases as lipid solubility increases. Within a given class of drugs, those with higher log *P* values often require more extensive metabolism than those with lower log *P* values. An example of this can be seen with temazepam and quazepam. As previously mentioned, quazepam has a log *P* value of 4.1 ± 0.8 while temazepam has a log *P* value of 2.2 ± 0.6. Due to its higher lipid solubility, quazepam undergoes multiple oxidative transformations prior to being conjugated with glucuronic acid (**Figure 5-23**). In contrast, temazepam requires only a single nonoxidative metabolic transformation to be eliminated from the body.

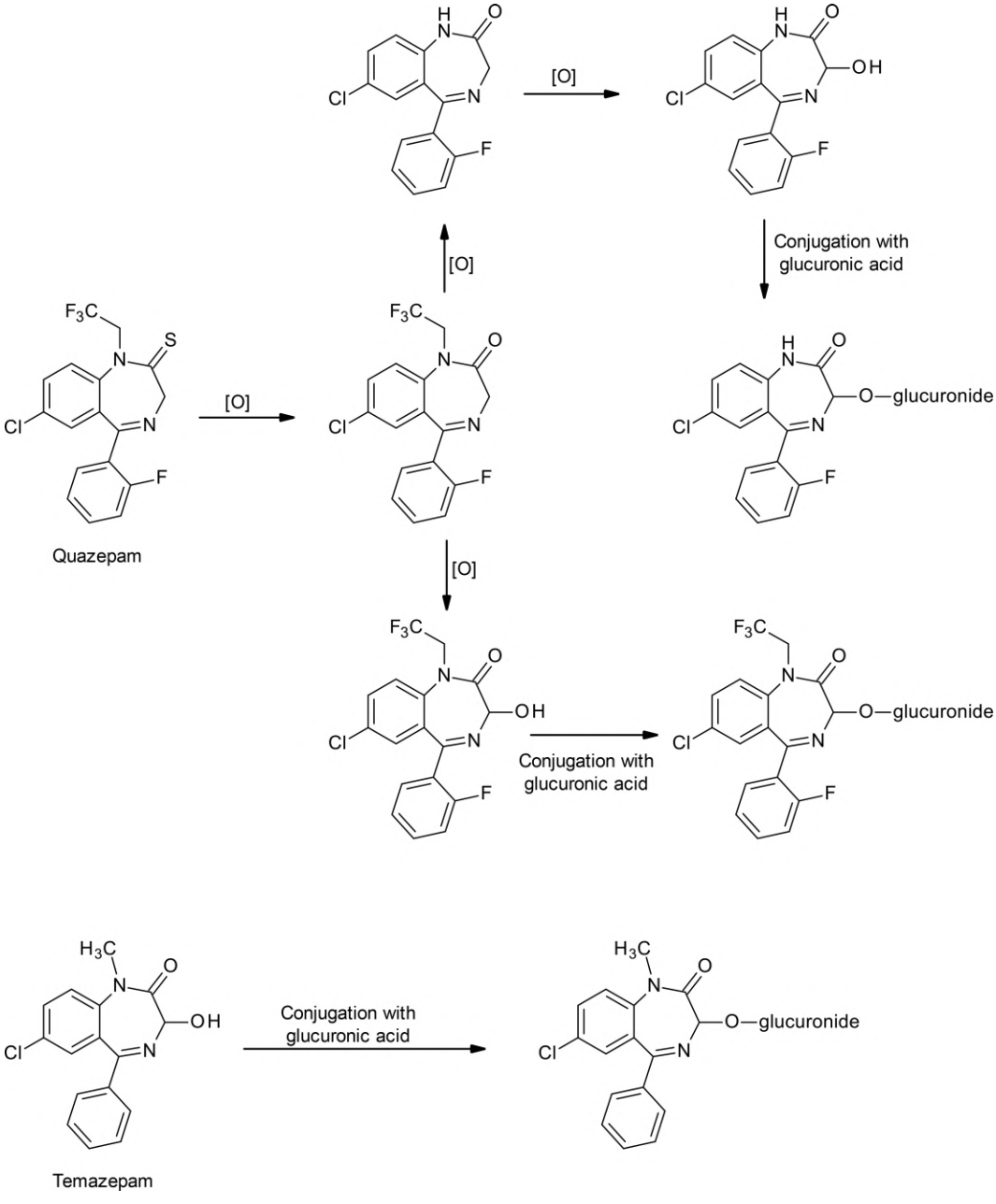


FIGURE 5-23. The metabolism of quazepam and temazepam. Oxidative metabolic pathways are designated by the [O] abbreviation.

As will be discussed in Chapter 8, a common type of drug interaction can occur when a coadministered drug either induces or inhibits the activity of hepatic oxidative enzymes. In looking at the example given here, quazepam, the more lipid-soluble drug, requires oxidative metabolism and could be subject to this type of drug interaction, whereas temazepam, the more water-soluble drug, does not require oxidative metabolism and would not be subject to this type of drug interaction. Thus, the overall water/lipid solubility of a drug molecule affects both the extent to which it is metabolized and its potential to be involved in a certain type of drug interaction.

A SUMMARY OF THE ADVANTAGES OF ENHANCING EITHER WATER OR LIPID SOLUBILITY

Throughout this chapter, examples of specific drugs have been used to illustrate specific therapeutic benefits that can be gained through the enhancement of their respective water or lipid solubility. These advantages are summarized here.

Therapeutic Advantages of Enhancing Water Solubility

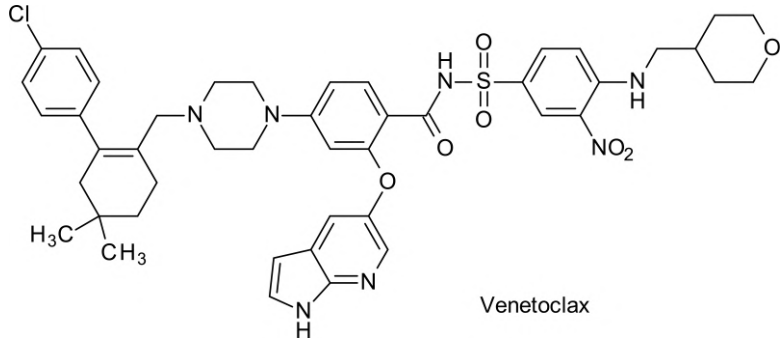
- Water solubility enhances the solvation and dissolution of drug molecules in the GI tract, which is required for oral absorption.
- Water solubility allows for the preparation of concentrated IV, ophthalmic, and otic solutions.
- Water solubility allows certain drug molecules (e.g., diuretics, antibiotics) to reach adequate concentrations in the urine.
- Drug molecules with higher water solubility require less metabolism and are less likely to have drug interactions with other drugs that alter hepatic oxidative enzymes (i.e., CYP450 enzymes).

Therapeutic Advantages of Enhancing Lipid Solubility

- Lipid solubility is required for drug molecules to pass through the lipid bilayer present within the GI mucosal membrane.
- Adequate lipid solubility is required for drug molecules to cross the blood brain barrier and have effects within the central nervous system.
- Lipid solubility allows for the use of IM or subcutaneous depot injections. This type of administration allows for a slow release from the injection site and an extended duration of action.
- Drugs that are more lipid soluble have greater plasma protein binding, which may provide a longer duration of action compared with analogous drugs that are more water soluble.
- Lipid-soluble salts and esters can be used to prepare more palatable oral suspensions.
- Lipid-soluble salts and esters can delay the dissolution of drug molecules in the stomach and therefore decrease the degradation of acid labile drugs.
- Lipid solubility can enhance the absorption of drugs that are administered via oral or nasal inhalation.
- Lipid solubility can enhance the absorption of drugs that are administered as topical creams or ointments.

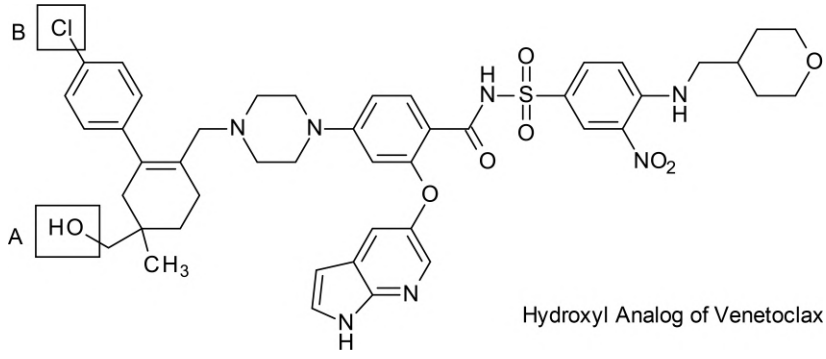
STRUCTURAL ANALYSIS CHECKPOINT

Checkpoint Drug 1: Venetoclax



Venetoclax

1. Draw the potassium salt of venetoclax.
   A. In Chapter 3, you were asked to identify if venetoclax is an acidic drug, a basic drug, an amphoteric drug, an electrolyte, or a nonelectrolyte. You should have determined that this is an amphoteric drug. Given this information, explain why the salt is only formed at one functional group.
   B. Using the same functional group, draw a water-soluble organic salt of venetoclax.
   C. In comparison with the potassium salt, what advantages does the organic salt you drew in question 1B provide?
2. Venetoclax is administered orally. Evaluate the functional groups present within the structure of venetoclax and explain how these functional groups allow venetoclax to be orally active.
3. How could the addition of a hydroxyl group (Box A) provide opportunities to alter both the water and lipid solubility of venetoclax?

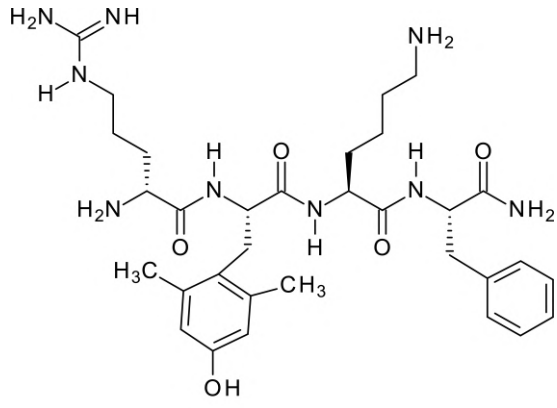


Hydroxyl Analog of Venetoclax

4. If the chlorine group (Box B) is removed, how would his affect the overall solubility of venetoclax? What other chemical properties would also be affected?

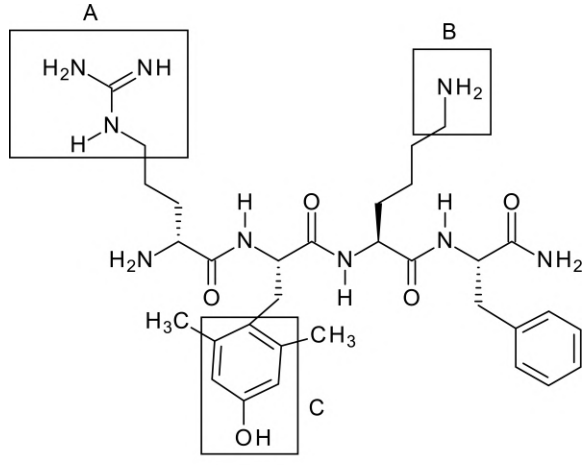
Checkpoint Drug 2: Elamipretide

Elamipretide is a peptide-based drug molecule and cannot be administered orally due to instability in the GI tract. You identified in a previous chapter that there are both acidic (substituted phenol) and basic (guanidine and primary amine functional groups) present within the molecule.



Elamipretide

1. This drug has not yet been formulated into a salt for drug formulation and marketing purposes.
   A. Identify two different forms of inorganic salts that could be formed with elamipretide.
   B. Modify the structure below to show each of the two forms of salts that you identified.



Elamipretide

   C. Consider the parent drug and either of the salt forms of the drug from Question 1B and determine which form of the drug will have improved solvation, dissolution, and water solubility. Provide a brief rationale for your answer.
2. This drug will likely need to be administered via injection. Based on your structural evaluation of this molecule, determine whether formulation as a water-soluble organic salt is beneficial. Provide a brief rationale for your answer.
3. Log *P* for elamipretide is calculated to be 0.3677 (Accelrys Draw 4.2).
   A. Based on the cLog *P* values in Table 5-2 for the HMGCoA reductase inhibitors, determine if elamipretide is likely to be more water soluble or more lipid soluble than this class of drugs.
   B. Based on the structure evaluation that you conducted in Chapter 2, does your functional group evaluation agree with or disagree with your answer to Question 3A?

C. Predict what types of structural modifications would be necessary to increase the lipophilic character of this drug.

4. Elamipretide is subject to degradation by amino and carboxy peptidases. To protect peptide-based drugs from this type of degradation, the amino and carboxy termini can be "protected" via the use of lipid-soluble prodrugs. In this case, the carboxy terminus has already been protected as an amide, which makes the drug resistant to degradation by carboxy peptidases.

   A. What kind of modification could occur at the amino terminus of elamipretide?

   B. Identify which physicochemical properties of elamipretide will change if a lipid-soluble prodrug is in fact developed. Don't forget the impact on cLog *P*!

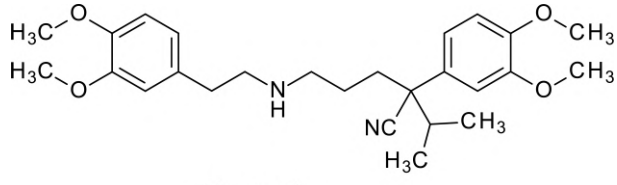
REVIEW QUESTIONS

1. Verapamil is marketed as a hydrochloride salt, and rosuvastatin is marketed as a calcium salt.

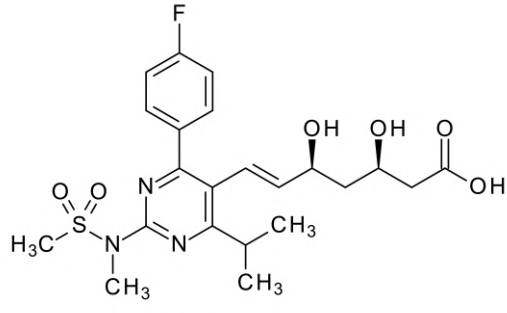
   A. Circle the functional group in verapamil that reacts with HCl to form a salt. Circle the functional group in rosuvastatin that reacts with $Ca(OH)_2$ to form a salt.

   B. Modify the structures of verapamil and rosuvastatin to show the salt forms of each drug.

   C. Are the salts that are formed organic or inorganic salts?

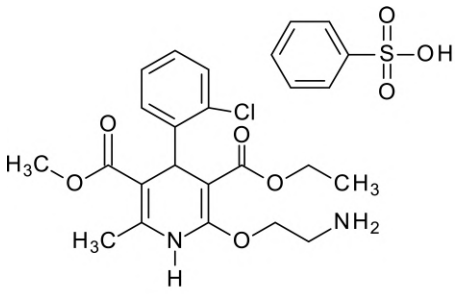


Verapamil

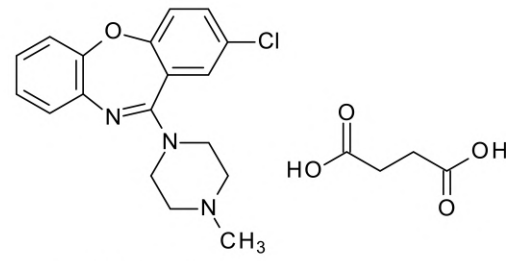


Rosuvastatin

2. Consider the structures of amlodipine besylate and loxapine succinate and determine if they are examples of organic or inorganic salts. For each drug, modify each component of the salt to show the form that is relevant at pH = 7.4.



Amlodipine besylate



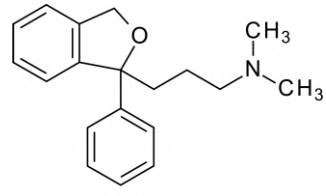
Loxapine succinate

3. Citalopram is marketed as a hydrobromide salt. Complete the following steps:

   A. Complete the grid for citalopram drawn below.

   B. Based on your knowledge related to the need for a drug molecule to contain a balance between hydrophobic and hydrophilic functional group character, provide a brief rationale for why this drug can be administered via an oral route.

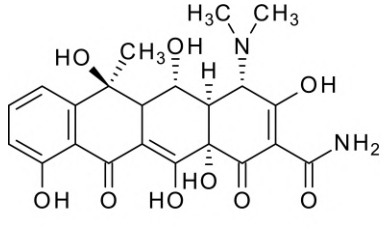
   C. Which is more hydrophilic, citalopram or citalopram HBr? Provide a brief structural rationale for your answer.



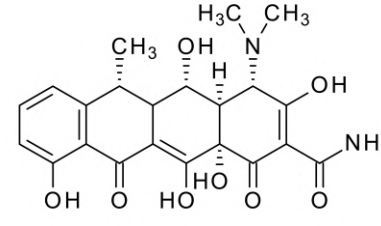
Citalopram

Name of Functional Group	Hydrophilic and/or Hydrophobic	Acidic, Basic, or Neutral	Contribution to Aqueous Solubility and/or Absorption

4. Consider the two tetracyclines drawn below and their respective log *P* values. Compare the structural features between oxytetracycline and doxycycline. Provide a structural rationale for the changes in log *P* observed.



Oxytetracycline (log P = -1.12)



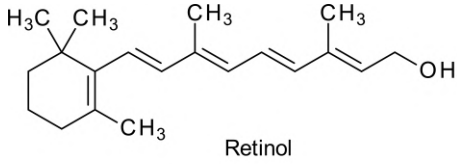
Doxycycline (log P = -0.02)

5. Each of the following three agents is used in the treatment of acne.

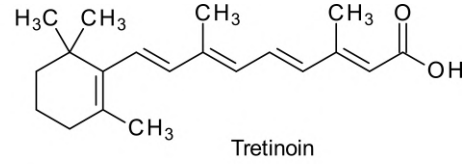
Retinol is a naturally occurring vitamin A that is applied topically.

Tretinoin is a naturally occurring vitamin A that is administered topically and orally.

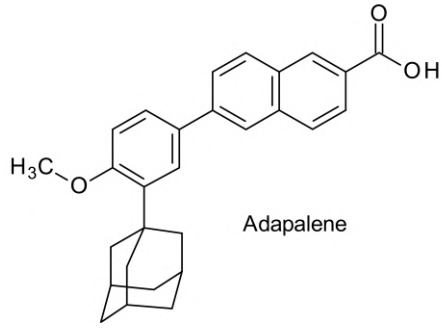
Adapalene is a third-generation retinoid/vitamin A analog that is applied topically.



Retinol



Tretinoin



Adapalene

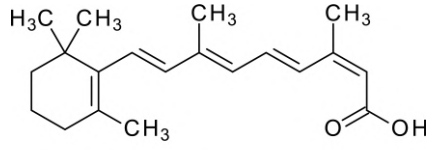
A. For each of these drug molecules, complete the structure evaluation in the grid below.

Name of Functional Group	Hydrophilic and/or Hydrophobic	Acidic, Basic, or Neutral	Contribution to Aqueous Solubility and/or Absorption
Retinol			
Tretinoin			
Adapalene			

B. Based on your structural evaluation of hydrophobicity and hydrophilicity, provide a brief structural rationale for why retinol and adapalene can be administered topically. Why is it appropriate to treat acne with a topical agent?

C. Based on your structural evaluation of hydrophobicity and hydrophilicity, provide a brief structural rationale for why tretinoin can be administered orally.

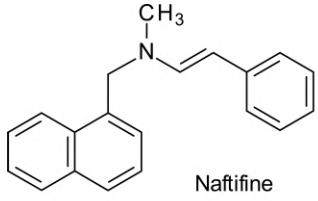
D. Some patients prefer to use an oral product for the treatment of acne. Isotretinoin is an orally available isomer of tretinoin. The following statement is found within the isotretinoin capsule package insert. Based on this statement, evaluate isotretinoin's structural features and provide a rationale for why it is capable of being systemically distributed.



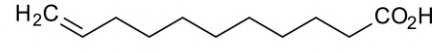
Isotretinoin

Package Insert Statement: "*Accutane must not be used by female patients who are or may become pregnant. There is an extremely high risk that severe birth defects will result if pregnancy occurs while taking Accutane in any amount, even for short periods of time. Potentially any fetus exposed during pregnancy can be affected. There are no accurate means of determining whether an exposed fetus has been affected. Pregnancy category X.*"

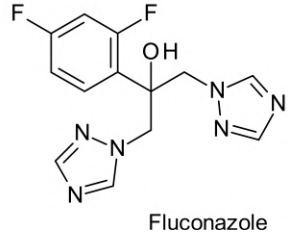
6. An 85-year-old patient presents you with three containers, each containing one of the drugs shown. The patient knows that each of these medications is used to treat different types of fungal infections and that some of them can be applied topically. Now that they have returned from a Caribbean cruise, he and his wife both must treat the athlete's feet that they contracted. She wants to take a medication orally because she doesn't like to have messy fingers. He could not care less what type of medication he uses, as long as it fixes the problem.



Naftifine



Undecylenic acid



Fluconazole

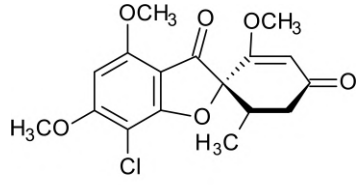
A. For each of these drug molecules, complete the structure evaluation in the grid on the next page.

Functional Group Name	Hydrophilic and/or Hydrophobic	Contribution to Aqueous Solubility and/or Absorption
Naftifine (marketed as HCl salt)		
Undecylenic Acid (marketed as calcium salt [powder] or zinc salt [cream])		
Fluconazole		

B. Using the information that you obtained in the structure evaluation process on the previous page, provide a structural rationale for why naftifine and undecylenic acid can be administered topically. Why is it appropriate to treat athlete's foot with a topical agent?

C. Fluconazole is administered orally but is not an effective treatment for athlete's foot. What kind of characteristics (properties) must a drug have to be given orally? Provide a structural rationale for why fluconazole can be administered via an oral route.

D. Griseofulvin is used orally to treat superficial fungal infections (e.g., fingernail and toenail). It does not penetrate the skin or nails if used topically. Provide a structural rationale for why this agent cannot be used topically to treat fungal infections.

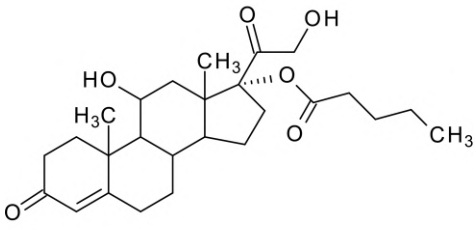


Griseofluvin

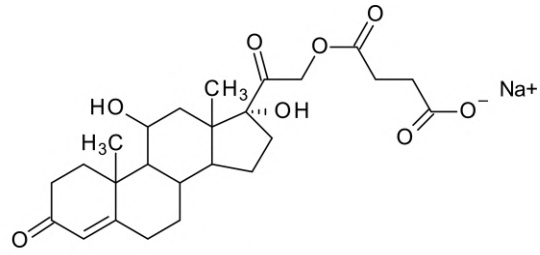
7. Hydrocortisone is marketed in a variety of forms, including as a sodium succinate C-21 ester and a C-17 valerate ester. These agents are used in the management of several different disease states/conditions, including systemic treatment of acute flares of inflammatory conditions and topical treatment of dermatological inflammation and itching. Based on your functional group evaluations of these two forms of hydrocortisone, answer the following questions:

   A. Which of these forms of hydrocortisone do you anticipate can be administered via an IM or IV route? What strategy was used to create a more water-soluble form of hydrocortisone?

B. Provide a rationale for why the other form of hydrocortisone is available only as a topical cream or ointment. What strategy was used to create a more lipid-soluble form of hydrocortisone?

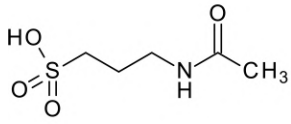


Hydrocortisone valerate

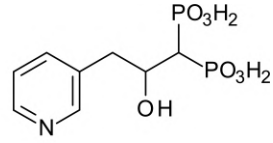


Hydrocortisone sodium succinate

8. Acamprosate (calcium salt) and risedronate (sodium salt) do not require systemic metabolism to be eliminated. Modify each structure below to show the salt form of the drug and provide a brief structural rationale for why these agents are able to be "excreted unchanged."



Acamprosate

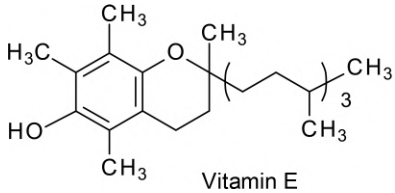


Risedronate

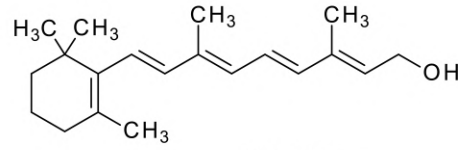
9. Each of the following drugs contains one functional group that can be ionized or contains several functional groups that are either all either basic or acidic. Based on the name of the salt that is marketed, determine whether the parent drug molecule (in its unionized form) is acidic or basic in character.

   A. Orphenadrine citrate
   B. Losartan potassium
   C. Atorvastatin sodium
   D. Bazedoxifene acetate
   E. Abacavir sulfate
   F. Enalapril maleate
   G. Tetracycline phosphate
   H. Omeprazole magnesium
   I. Tramadol hydrochloride
   J. Doxycycline calcium
   K. Butaconazole nitrate

10. Both vitamin E and vitamin A (in the form of retinol) are used in the management of skin conditions, including acne, wound healing, and the effects of aging.



Vitamin E

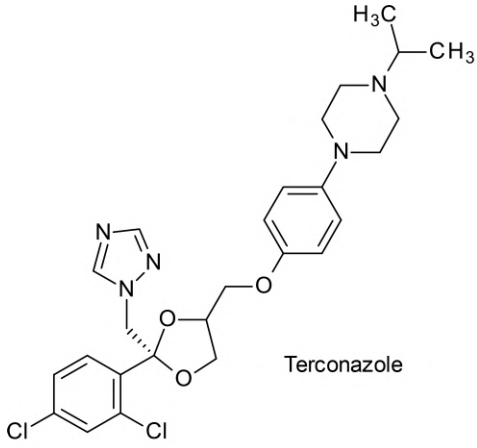


Vitamin A

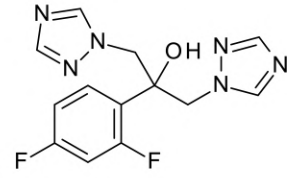
A. Vitamin A has a cLog $P = 4.6$. Vitamin E has a cLog $P = 8.4$. Provide a structural rationale for why these vitamins can be administered topically.

B. Oral administration of these agents is a challenge because vitamin A is considered "insoluble" and vitamin E is only dispersible in aqueous solution. Given the information presented in this chapter, identify two ways that each of these molecules could be structurally modified to improve aqueous solubility.

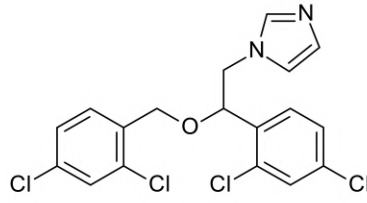
11. A 25-year-old woman approaches the counter and asks for a private consultation. During the consultation, she reports that she suspects that she has a vaginal yeast infection. From doing some Internet searching, she knows that creams, suppositories, and tablets are available and has a box of each formulation in her hands. She really wants to take the tablet form because it is the least messy but doesn't understand how that could possibly treat an external infection.



Terconazole



Fluconazole



Miconazole

Terconazole is available as a cream or suppository.

Fluconazole is available as a tablet.

Miconazole is available as a cream or ointment.

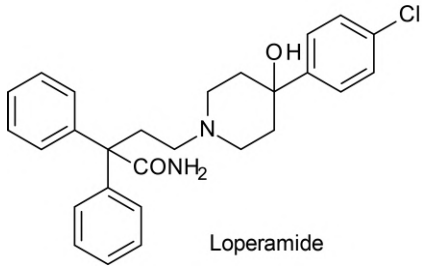
A. For each of these drug molecules, complete the structure evaluation in the grid on the next page:

Functional Group Name	Hydrophilic and/or Hydrophobic	Contribution to Aqueous Solubility and/or Absorption
Terconazole		
Fluconazole		
Miconazole		

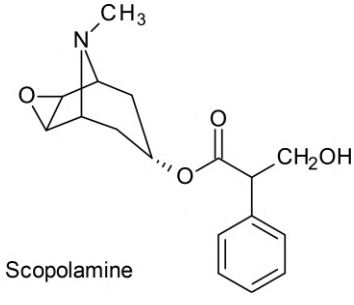
B. Using the information that you obtained in the structure evaluation process on the previous page, provide a structural rationale for why miconazole and terconazole can be administered topically. Why is it appropriate to treat a vaginal yeast infection with a topical agent?

C. Fluconazole is administered orally and is an exceptionally effective treatment for vaginal yeast infections. What kind of characteristics (properties) must a drug have to be given orally? Provide a structural rationale for why fluconazole can be administered via an oral route.

12. Each of these drug molecules treats a particular ailment by either managing a symptom or modulating a biochemical pathway. In either case, the drug has to get to its biological target. Using your knowledge about functional group character, describe how both of the drugs get to their respective biological targets. Consider the concepts of solubility, absorption, distribution, and route of administration in your answer [pH (stomach) = 1; pH (intestine) = 8; pH (plasma) = 7.4].



Loperamide



Scopolamine

Biological Targets and Routes of Administration

Loperamide: μ opioid receptors located in the large intestine; oral administration.

Scopolamine: muscarinic acetylcholine receptors (M1) located in the peripheral nervous systems; transdermal administration.

DRUG BINDING INTERACTIONS

LEARNING OBJECTIVES

After completing this chapter, students will be able to

- **Explain the differences between covalent bonds and noncovalent bonds.**
- **Identify the major types of covalent bonds and explain both the beneficial and detrimental effects that these types of bonds can have on the activity of drug molecules.**
- **Explain why each type of noncovalent bond has a unique distance requirement and bond energy.**
- **Analyze a drug molecule and identify the types of bonds that can be formed by each of its functional groups.**
- **Identify specific amino acids, nucleotides, and functional groups present on biological targets that are capable of forming specific types of bonds.**
- **Explain how specific situations, such as adjacent functional groups or steric hindrance, can alter the formation of specific types of bonds.**

Drug molecules can form numerous types of covalent and noncovalent bonds (**Table 6-1**) with specific biological targets. These biological targets can be organized into four general categories (receptors, enzymes, nucleic acids, and excitable membranes/other biopolymers) and are typically composed of proteins or nucleic acids. Drug molecules can also form bonds with chemical entities, including trace metals, and other coadministered drugs, vitamins, and/or herbal products. The number of bonds, as well as the types and strengths of these bonds, depends on the chemical nature of the functional groups present in the drug molecule as well as the biological target or xenobiotic. Minor variations in the structure of a drug molecule can tremendously alter the types and strengths of binding interactions and contribute to the overall potency, efficacy, and duration of the drug. This chapter reviews the major types of binding interactions that can be formed between a drug molecule and its biological target(s), the functional groups that are capable of forming these bonds, the relative strengths and requirements for these bonds, and the functional groups present on the biological targets that are involved in these interactions.

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TABLE 6-1. Types of Bonds Available for Drug Molecules

Covalent Bonds
- Alkylation reactions
- Acylation reactions
- Phosphorylation reactions
- Rearrangement reactions

Noncovalent Bonds
- Ionic bonds
- Dipole interactions
  - Ion–dipole interactions
  - Dipole–dipole interactions
  - Hydrogen bonds
- van der Waals interactions
  - Debye forces
  - London dispersion forces
- Hydrophobic effects
- Additional aromatic interactions
  - π–π stacking interactions
  - Cation–π interactions
  - Charge transfer interactions
- Chelation and complexation

COVALENT BONDS

A *covalent bond* is the strongest bond that can be made between a drug molecule and its biological target. The overall strength of a covalent bond can vary but is generally in the range of 40 to 150 kcal/mol. As such, most covalent bonds are too strong to be spontaneously cleaved in normal physiologic environments and are generally considered to be irreversible bonds. It is possible for some covalent bonds to be cleaved by enzymatic or acid/base catalyzed reactions; however, depending on the specific covalent bond, this is not always possible. In most cases, when a drug forms a covalent bond with its biological target, the only way to reverse this action is by the normal metabolic turnover of enzymes and receptors and the cellular replenishment or replacement of irreversibly inactivated enzymes or proteins. Please note that this turnover requires nuclear transcription, cellular translation, and posttranslational modification to produce a new protein or enzyme. Additionally, it is possible for some covalent bonds between drug molecules and DNA to be reversed by normal DNA repair enzymes.

Concerns With the Use of Covalent Bonds

Only a small number of drugs act by forming covalent bonds with their biological target. This is not due to a lack of therapeutic efficacy because covalently bound drugs are as effective and, in some cases, more effective than noncovalently bound drugs. For example, aspirin, thienopyridine antithrombotic agents, β-lactam antibiotics, and alkylating agents (**Figure 6-1**) all act through the formation of covalent bonds and are very effective for the treatment/prevention of thrombotic events, bacterial infections, and cancer, respectively. In fact, the ability of aspirin to irreversibly acetylate platelet cyclooxygenase-1 (COX-1) is responsible for the selective, beneficial cardiovascular effects of low-dose aspirin therapy.

There are several reasons why covalently bound drugs are not used as frequently as noncovalently bound drugs. First, due to the bond strength, the actions of covalently bound drugs cannot be readily reversed. As discussed above, the reversal of effects can occur only through specific catalytic processes or the formation of new proteins and enzymes. These processes take longer than the simple dissociation of a noncovalently bound drug from its biological target. As such, the overall duration of action of a covalently bound drug may be too long for a given therapeutic indication. Noncovalently bound drugs generally have durations of actions appropriate for daily dosing

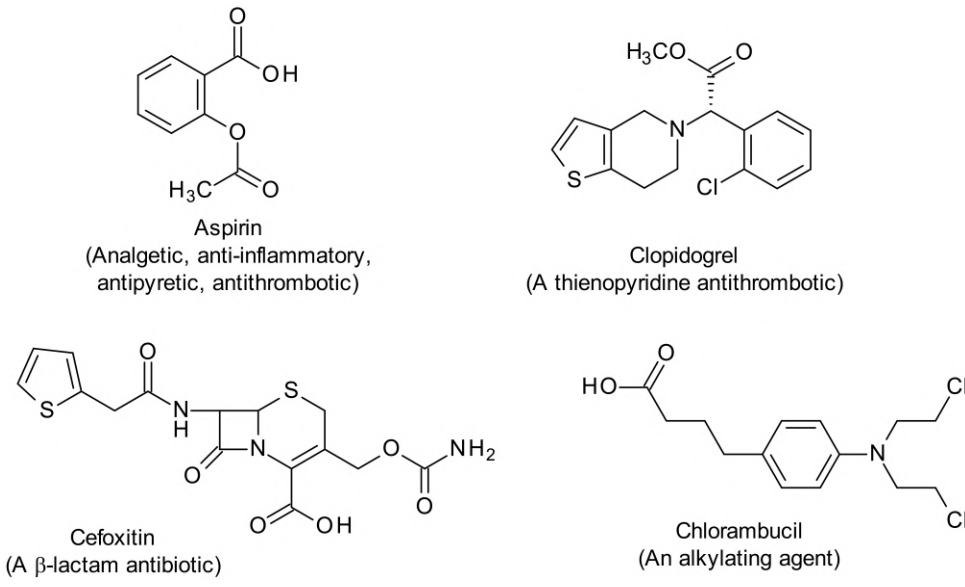


FIGURE 6-1. Examples of drugs that form covalent bonds with their target receptor.

regimens; however, it should be noted that in some cases covalently bound drugs can provide an optimal duration of action. As an example, rapid tubular secretion very quickly eliminates β-lactam antibiotics from the body; however, due to the covalent binding of these drugs, their overall actions last much longer than their half-lives would suggest. Many β-lactam antibiotics, specifically the cephalosporins (e.g., cefoxitin in Figure 6-1), can be dosed once or twice daily due to their covalent, irreversible binding to transpeptidase, a bacterial enzyme that is required in the cross-linking of bacterial cell walls. In conclusion, the duration of action of covalently bound drugs is a valid concern but does not always prevent their use.

Another concern with covalently bound drugs is their specificity for their biological target. Although binding specificity is important for all drugs, it is even more crucial with covalently bound agents because it is highly undesirable for a drug molecule to indiscriminately form covalent bonds throughout the body. A lack of sufficient specificity and irreversible bonds could lead to prolonged adverse effects. Based on the severity of these adverse effects, the ability to either reverse them or limit their duration may be a critical choice in the decision to use a covalently bound drug or not. Finally, some drugs that form irreversible, covalent bonds may require specific handling and guidelines for their administration. This is true for certain alkylating agents used to treat specific types of cancer (e.g., chlorambucil in Figure 6-1). These drug molecules are highly reactive and require special preparation and administration guidelines. Whereas all drugs that form covalent bonds possess some degree of reactivity greater than that of noncovalently bound drugs, it is incorrect to make a blanket statement that all covalently bound drugs are difficult to administer. Aspirin, β-lactam antibiotics, and thienopyridine antithrombotic agents (Figure 6-1) can all be safely administered as oral tablets and capsules without any additional warnings or precautions.

Specific Types of Covalent Bonds

The four most common mechanisms by which drug molecules can form covalent bonds are as follows:

- alkylation
- acylation

- phosphorylation
- rearrangement reactions that reveal a highly reactive intermediate

The following discussions examine these mechanisms and provide specific examples of drugs that use these mechanisms to produce their therapeutic effects.

Alkylation

Alkylation occurs when a nucleophilic atom or functional group present in receptors, DNA, or other biological targets attacks a highly electrophilic atom or functional group present in the drug molecule. As discussed in Chapter 2, nucleophiles are nucleus loving and generally contain either a negative charge or a lone pair of electrons, whereas electrophiles are electron loving and generally contain a positive charge, a conjugated double bond system, or a functional group that can be easily displaced and released. Chlorambucil is used to treat Hodgkin's disease, non-Hodgkin's lymphoma, and a variety of other neoplastic disorders. It is chemically classified as a β-chloroethylamine but is often referred to as a nitrogen mustard. Chlorambucil alkylates DNA via the mechanism shown in **Figure 6-2**. The initial step involves the nucleophilic attack of the lone pair of electrons present on the tertiary amine on either one of the β-carbon atoms. This attack causes the chloro group to leave and results in a highly reactive electrophile known as an aziridinium ion. In the second step, a nucleophile from a biological target attacks the aziridinium ion, resulting in an alkylated macromolecule. The N_7 atom of a guanine residue present on DNA usually serves as the nucleophile; however, the aziridium ion is highly reactive and can also react with other biological targets. This reaction can then repeat using the other chloro group. The end result is a cross-link in DNA that ultimately leads to the anticancer effect.

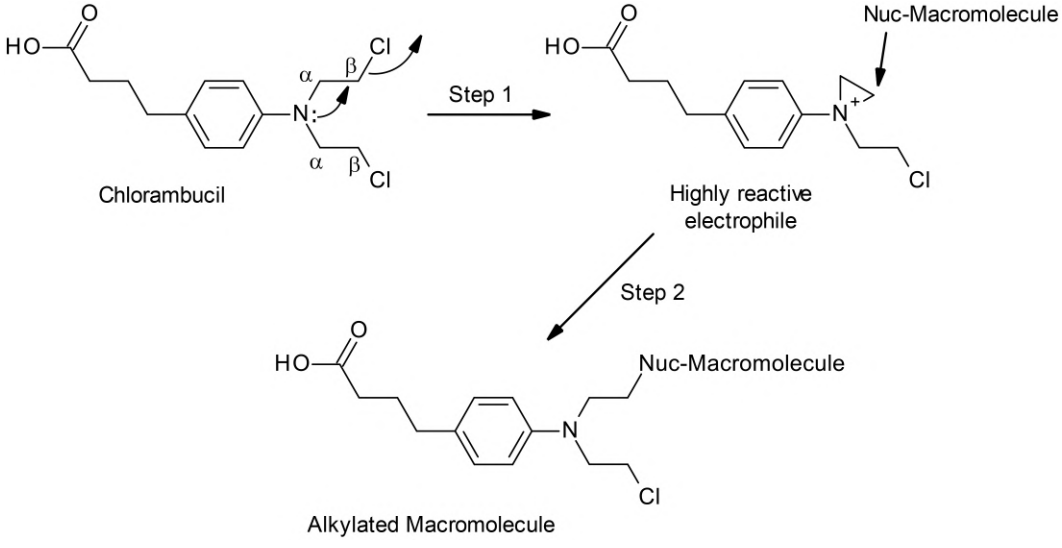
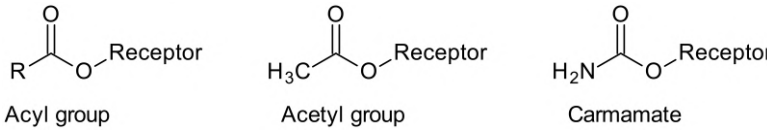


FIGURE 6-2. The mechanism of alkylation of a macromolecule by chlorambucil. (Nuc-Macromolecule = nucleophile present on a biological macromolecule; e.g., DNA, protein, enzyme.)

Acylation

Acylation occurs when a nucleophilic atom or functional group present on a protein, enzyme, or other biological target attacks an ester, lactone, amide, lactam, or carbamate functional group present on a drug molecule. The resulting product contains an acyl group. The general structure of an

acyl group is shown below. Depending on the R group, the acyl group may also have a specific name. For example, if the R group is a methyl group, the acyl group is commonly known as an acetyl group. Additionally, if the R group is an amine (primary, secondary, or tertiary), the acyl group is commonly known as a carbamate. The formation of an acetyl group is also known as acetylation, and the formation of a carbamate is also known as carbamylation.



Let us look at three examples of acylation. The β-lactam class of antibiotics exerts their mechanism of action by inhibiting transpeptidase, a bacterial enzyme responsible for cross-linking bacterial cell walls (**Figure 6-3**). Because human cells do not have cell walls, this biological target is found only in bacteria. A serine residue found within transpeptidase is responsible for recognizing D-Ala-D-Ala dipeptide sequences on newly synthesized cell wall precursors. The serine initially cleaves D-Ala-D-Ala bonds and forms an esterified intermediate that is eventually used to cross-link the cell wall precursors. β-Lactam antibiotics, exemplified by cefoxitin in Figure 6-3, mimic the D-Ala-D-Ala sequence of cell wall precursors. As a result, transpeptidase binds to β-lactam antibiotics, cleaves the β-lactam bond, and becomes irreversibly acylated.

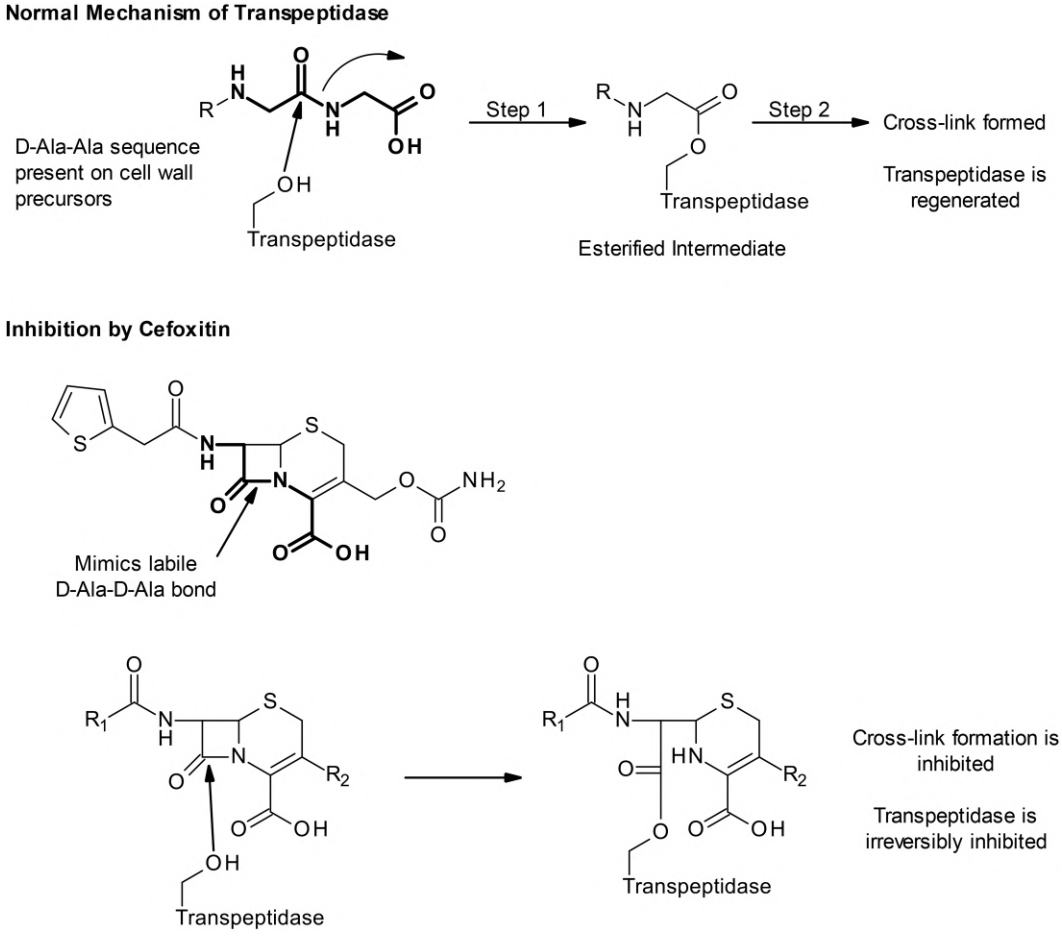


FIGURE 6-3. The acylation of transpeptidase by cefoxitin, a β-lactam antibiotic.

A second example of acylation is seen with aspirin. Aspirin is a nonsteroidal anti-inflammatory drug (NSAID) and is unique from all other drugs in this class in that it is the only one that acts in a covalent manner. All other NSAIDS exert their actions via noncovalent interactions. Additionally, most NSAIDs exert their mechanism of action by nonselectively inhibiting both COX-1 and -2 enzymes whereas aspirin is 170-fold more selective for COX-1. Cyclooxygenase enzymes are involved in the biosynthesis of prostaglandins. Prostaglandins are endogenous molecules that produce a variety of beneficial therapeutic effects but also play a role in inflammation, pain, and fever. As such, inhibitors of COX enzymes are useful as anti-inflammatory agents, analgesics, and antipyretics. As shown in **Figure 6-4**, once aspirin binds to COX-1, the primary hydroxyl group of this serine can act as a nucleophile and attack the acetyl group of aspirin. This results in the release of salicylic acid and the irreversible acetylation of COX-1. The covalent, irreversible nature of this mechanism is the reason why low-dose aspirin is used as an antithrombotic agent for certain cardiovascular disorders. Among the various prostaglandins, thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) are responsible for maintaining an appropriate balance between the aggregation of platelets and the inhibition of this process. Thromboxane A_2 is synthesized in the platelets and requires COX-1, and its release enhances platelet aggregation, whereas PGI_2 is synthesized in the blood vessels, requires COX-2, and acts to inhibit platelet aggregation. The key difference here is that platelets, unlike most cells, do not have a nucleus. Thus, a low dose of aspirin when given once daily has a more profound effect on platelets than it does on blood vessels. Because the platelets do not have a nucleus, they cannot synthesize new copies of COX-1 to replace the irreversibly inhibited acylated enzymes. Thus, aspirin's effect lasts the lifetime of the platelet, normally 7 to 11 days. In contrast, cells that comprise blood vessels contain a nucleus and can synthesize more copies of COX-2. The overall net effect is a decrease in platelet activity and an antithrombotic effect.

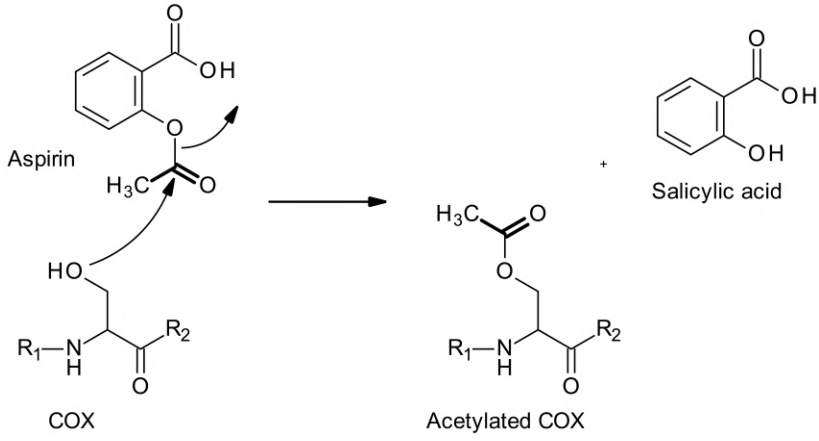


FIGURE 6-4. The acetylation of COX-1 by aspirin.

Unlike the previous two examples, there are some instances in which the acylation of a biological target is not truly irreversible. Over time, some acylated groups can be hydrolyzed, thus restoring the original receptor or enzyme function. In these cases, the bond is said to be pseudo-irreversible. A good example of this is seen with the third example using neostigmine, a carbamate inhibitor of acetylcholinesterase indicated for treatment of myasthenia gravis. As shown in **Figure 6-5**, the serine hydroxyl group of acetylcholinesterase is nucleophilic and is responsible for attacking the acetyl carbonyl of acetylcholine. The resulting acetyl group is rapidly hydrolyzed, and acetylcholinesterase is regenerated almost immediately. Neostigmine is structurally similar to acetylcholine; however, it contains a carbamate group instead of the normal acetyl group. Acetylcholinesterase binds neostigmine, and the serine hydroxyl group of the enzyme again acts as a nucleophile and attacks the

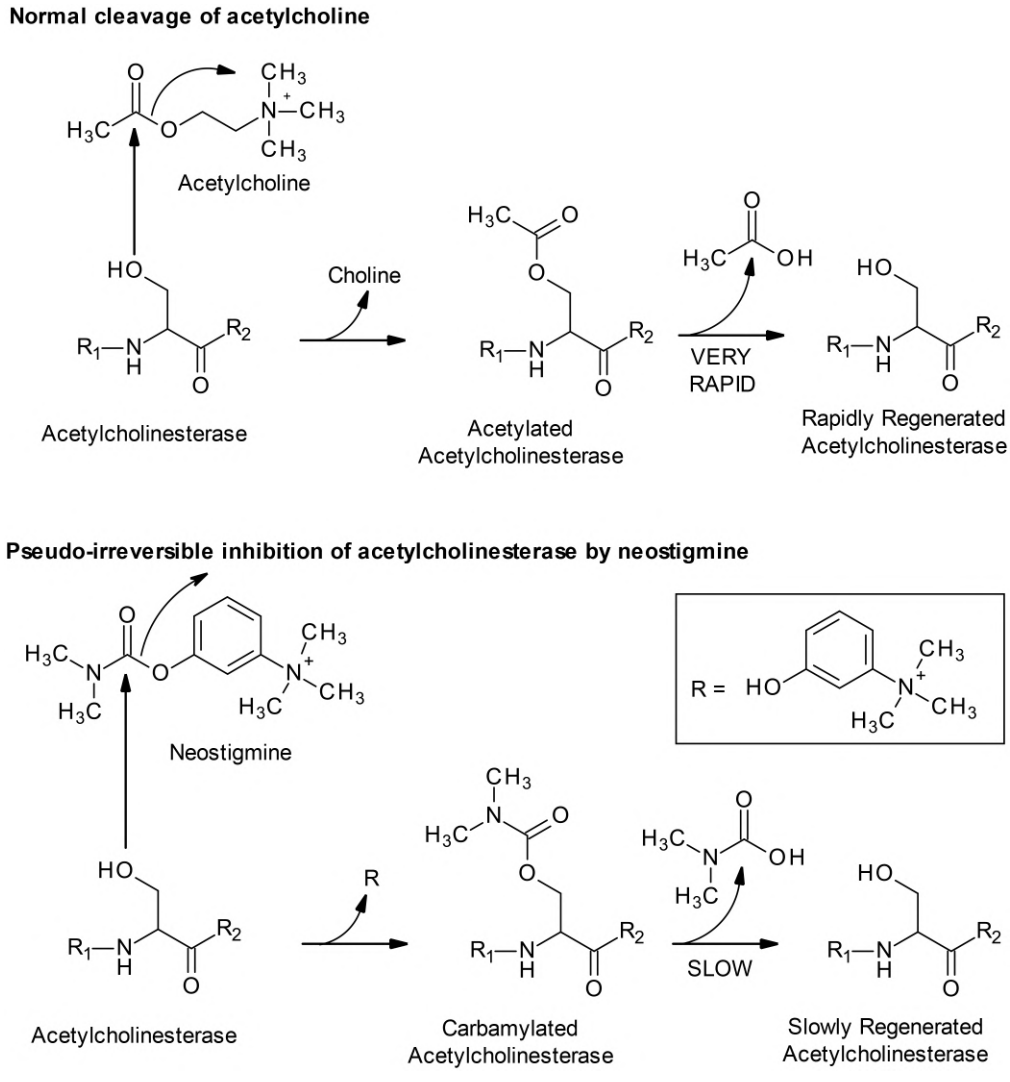


FIGURE 6-5. Acetylcholinesterase: normal mechanism and pseudo-irreversible inhibition by neostigmine.

carbamate group of neostigmine. The carbamylated enzyme is much more resistant to hydrolysis, resulting in a pseudo-irreversible bond. Over time, the carbamate is hydrolyzed and acetylcholinesterase is regenerated. For neostigmine, the time needed for enzyme regeneration is long enough for it to be dosed three or four times daily.

Phosphorylation

This type of covalent bond can be formed with organophosphates. Isofluorophate and other molecules in this chemical class were once used as ophthalmic agents to decrease intraocular pressure associated with glaucoma. Today, organophosphates are primarily used as insecticides or as military nerve gases and are quite toxic due to the irreversible inhibition of acetylcholinesterase. The mechanism of action is very similar to the pseudo-irreversible carbamates, with the main difference being that phosphorylation of the serine hydroxyl group can become truly irreversible. As shown in **Figure 6-6A**, the initial phosphorylation produces an intermediate with three phosphoester bonds.

Hydrolysis of either of the isopropyl phosphoester bonds creates a negative charge, makes the remaining phosphoester bonds much less electrophilic, greatly decreases the likelihood that the bond to the serine hydroxyl group of acetylcholinesterase will be broken, and irreversibly inhibits the enzyme. This process is known as *aging* and, as the name implies, it occurs over a period of time. Prior to the hydrolysis of either of the isopropyl phosphoesters, the administration of pralidoxime chloride (2-PAM) can reverse the phosphorylation, as shown in **Figure 6-6B**. Pralidoxime is an antidote for organophosphate poisoning and works by displacing the di-isopropyl phosphate from the active site of acetylcholinesterase. This reaction occurs because the oxime hydroxyl group of 2-PAM has a higher affinity for the phosphate than does the serine hydroxyl group of acetylcholinesterase. Thus, 2-PAM readily displaces di-isopropyl phosphate and regenerates acetylcholinesterase.

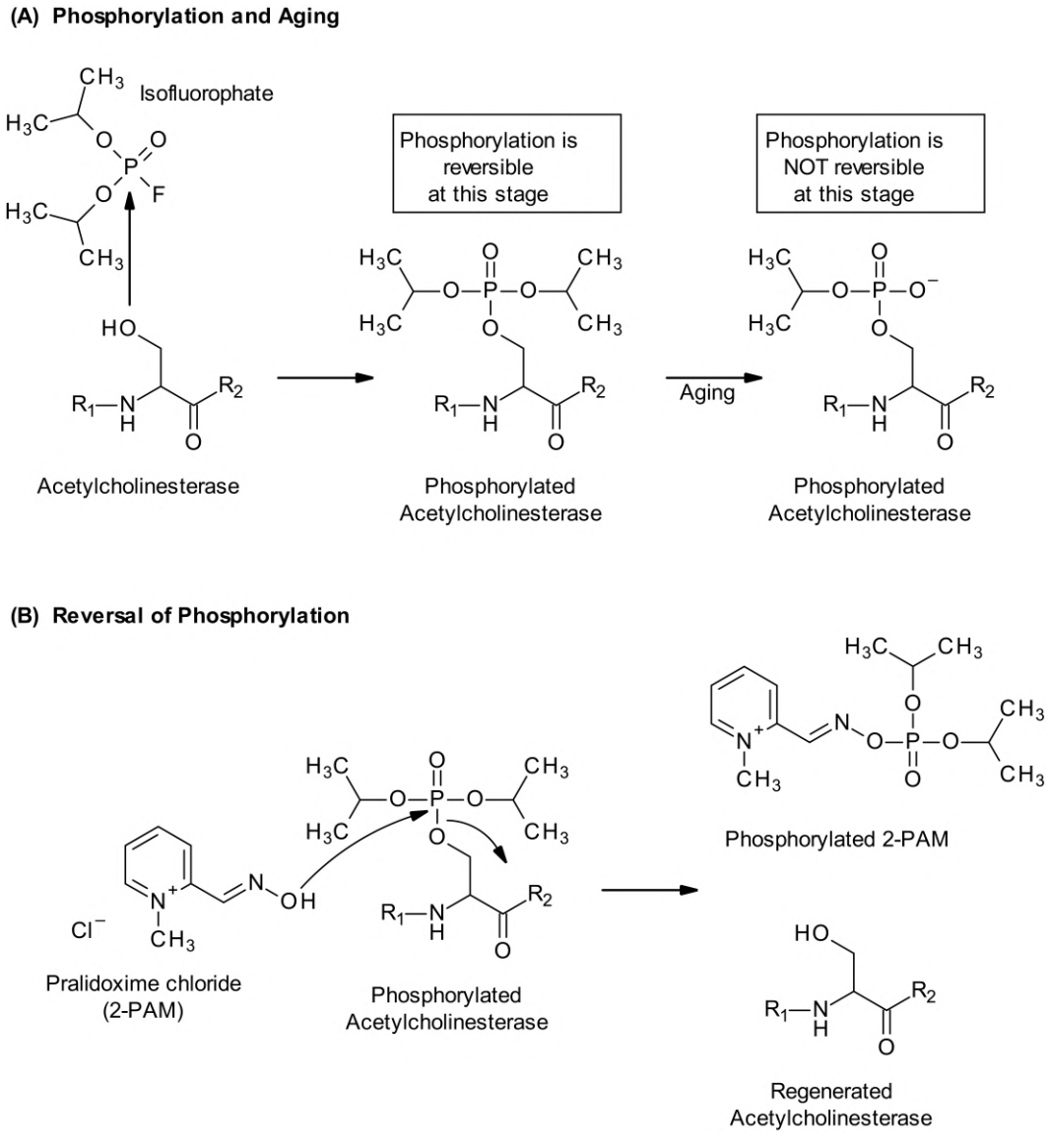


FIGURE 6-6. (A) The phosphorylation of acetylcholinesterase by isofluorophate. (B) Reversal of phosphorylation by 2-PAM.

Rearrangement Reactions

A handful of other drugs form covalent bonds with their biological targets as a result of specific metabolic activation and the rearrangement of existing functional groups to produce a reactive intermediate. A good example of this can be seen with clopidogrel, a thienopyridine antithrombotic drug (**Figure 6-7**). Clopidogrel is initially oxidized to 2-oxoclopidogrel. This is followed by a keto-enol tautomerization and the hydrolytic opening of the thiophene ring to produce the active metabolite. The thiol group forms a disulfide bond with a specific receptor found on platelets. This covalent bond prevents adenosine diphosphate (ADP) from binding to the platelet receptor and inducing platelet aggregation. Clopidogrel and other drugs within this chemical class are useful for the treatment of a variety of cardiovascular disorders, including acute myocardial infarction and unstable angina.

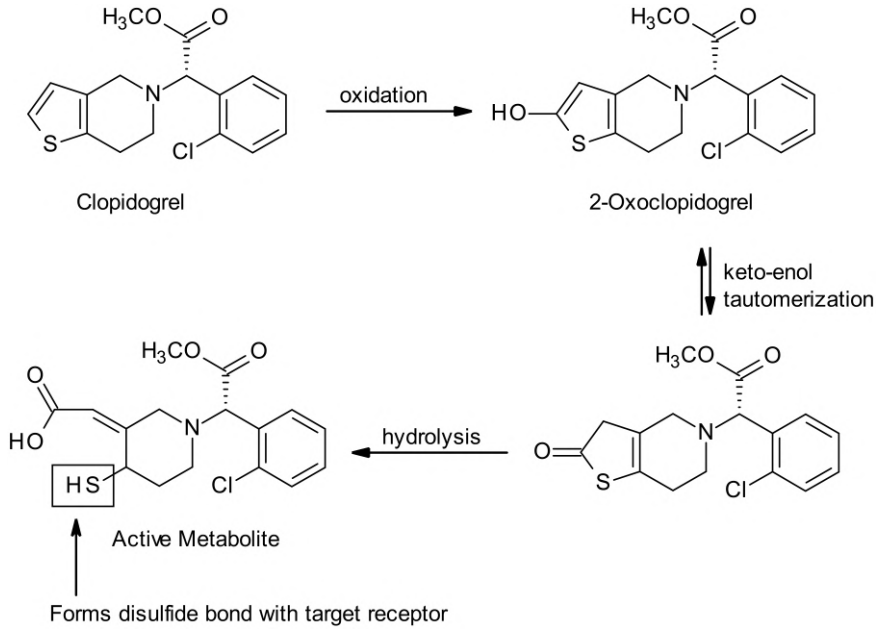


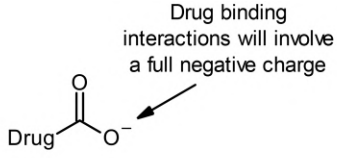
FIGURE 6-7. The metabolic conversion of clopidogrel to its active metabolite.

NONCOVALENT BONDS

Most drugs bind to their biological targets through the formation of noncovalent bonds. Compared with covalent bonds, noncovalent bonds are much weaker, with the bond strengths (or the bond energies) of individual noncovalent bonds generally ranging from 0.5 to 10 kcal/mol. Although each individual drug–biological target interaction may appear weak, drugs that bind in a noncovalent manner form multiple interactions with their biological target. *Thus, the overall binding strength is a summation of all of the individual noncovalent interactions.* This binding strength is related to the overall affinity, or attraction, of a drug molecule to its biological target. In pharmacological terms, the affinity of a drug molecule for its biological target is often represented as an equilibrium between the bound versus unbound drug. Dissociation constants are used to compare the relative affinities of specific drugs to a specific biological target and inherently include the overall binding strengths of the drugs. As long as appropriate plasma levels are maintained, drugs that bind in a noncovalent manner are able to bind to their biological targets and provide their desired therapeutic effects.

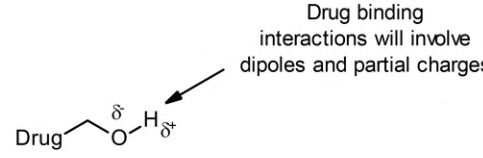
The ability of any single noncovalent bond to form is inversely proportional to the distance between the functional group present on the drug molecule and a complementary functional group present on the biological target. In other words, the respective functional groups must be close enough to one another for recognition to occur and the noncovalent interaction to occur. Each type of noncovalent bond has a unique distance requirement. Ionic bonds can form over the greatest distance whereas certain types of van der Waals interactions can only form if the respective functional groups are extremely close. This is also true for the covalent bonds that were previously discussed. For covalent bonds to form, the reactive functional groups must be close enough to one another for the reaction to occur. As an example, let's review the reaction in Figure 6-6. Please note that the serine hydroxyl group must be close enough to isofluorophate to be phosphorylated. The same is true for the alkylations and acylations shown in Figures 6-2 through 6-5. The distance requirement for a covalent bond is usually the same as that for an ion–dipole or a dipole–dipole interaction. Specific distance requirements are discussed with each type of noncovalent bond.

The ionization state of a functional group plays a key role in determining what types of noncovalent bonds it can and cannot form. Because most drug binding interactions occur at a physiologic pH of 7.4, this is the primary focus for this chapter. Therefore, prior to evaluating the types of binding interactions that a functional group can participate in, *it is first necessary to determine if the functional group is primarily ionized or primarily unionized at a pH of 7.4*. Functional groups that are primarily ionized at physiologic pH can undergo binding interactions in which the functional group has a full positive or negative charge. It is *extremely unlikely* that they would participate in binding interactions in which the functional group needs to be unionized or carry a partial charge. In contrast, functional groups that are primarily unionized at physiologic pH can participate in binding interactions in which the functional group provides a dipole, partial charge, or induced partial charge. It is *extremely unlikely* that they would participate in binding interactions that require the functional group to have a full positive or negative charge. As an example of this, let's look at two functional groups: a carboxylic acid and a primary aliphatic hydroxyl group. As discussed in Chapter 3, carboxylic acids are acidic functional groups and have a pK_a range of 2.5 to 5. Applying the concepts discussed in Chapter 4, we can conclude that this functional group will be primarily ionized at physiologic pH and will participate in drug binding interactions in which it provides a full negative charge. It does not participate in drug binding interactions that require it to be unionized and have a partial charge. In contrast, a primary aliphatic hydroxyl group is neither acidic nor basic and is unionized at physiologic pH. As discussed in this chapter, it can participate in drug binding interactions in which it provides a dipole bond with partial charge separation. It is not able to participate in drug binding interactions that require it to be ionized.



Carboxylic acid (Acidic)

Primarily ionized at
physiological pH



Primary aliphatic hydroxyl (Neutral)

Primarily unionized at
physiological pH

At the end of Chapter 3, Table 3-1 provides the approximate pK_a ranges for each acidic and basic functional group. Using these ranges, the approximate ionization state for each of these functional groups at a physiologic pH of 7.4 can be determined. This information is provided in **Table 6-2**. In examining this table, the acidic and basic functional groups can be divided into three categories: (1) those that will almost always be primarily ionized at a physiological pH of 7.4, (2) those that will almost always be unionized at a physiological pH of 7.4, and (3) those whose pK_a ranges span both

TABLE 6-2. Approximate Ionization States for Common Acidic and Basic Functional Groups at a Physiologic pH of 7.4

Functional Group	Acidic or Basic	pK_a Range and Primary Ionization State at pH of 7.4
Carboxylic acid	Acidic	2.5-5 (primarily ionized)
β-Dicarbonyl groups (includes imides)	Acidic	4.5-8.5 (varies depending on pK_a value)
Sulfonamides	Acidic	4.5-11 (varies depending on pK_a value)
Sulfonylureas	Acidic	5-6 (primarily ionized)
Tetrazoles	Acidic	4.5-6 (primarily ionized)
Phenols	Acidic	9-10 (primarily unionized)
Thiols	Acidic	10-11 (primarily unionized)
Sulfates	Acidic	1-2 (primarily ionized)
Phosphates and phosphonates	Acidic	1.5-2.5 (first phosphate; primarily ionized) 6.5-7.5 (second phosphate; primarily ionized but could vary)
Aliphatic amines and alicyclic amines (aka saturated heterocycles)	Basic	9-11 (primarily ionized)
Aromatic amines (aka anilines)	Basic	2-5 (primarily unionized)
Imine	Basic	3-5 (primarily unionized)
Hydrazine	Basic	7.5-8.5 (primarily ionized but could vary)
Amidine	Basic	10-11 (primarily ionized)
Guanidine	Basic	12-13 (primarily ionized)
Nitrogen containing aromatic heterocycles	Basic	1-6 (primarily unionized)

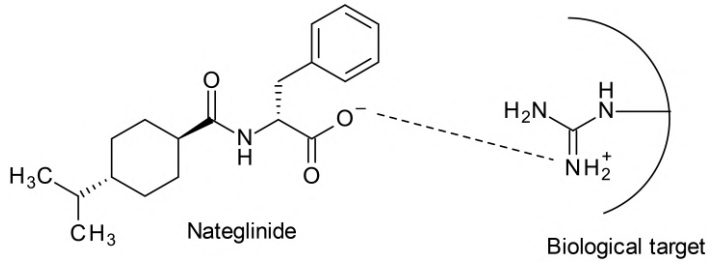
sides of 7.4 and require individual evaluation. The term *almost* is included in the previous statements because the steric and electronic factors discussed in Chapter 3 can result in rare outliers.

Steric factors and the stereochemistry of drug molecules are also important for the interactions between a drug and its biological target. These topics are discussed in more detail in Chapter 7; however, based on the distance requirements for drug-target binding, drug molecules that cannot make close contact with their respective targets would be expected to exhibit decreased binding. If steric factors preclude a functional group on a drug molecule from getting close enough to a functional group on its biological target, the bond will be either weak or nonexistent. Additionally, based on the distance requirements for drug-target binding, one should expect a sequential process for multiple bond formation. Because ionic bonds can form over the longest distance, they generally occur first, followed by ion–dipole interactions, and then dipole–dipole interactions, dipole-induced dipole interactions, and finally induced dipole-induced dipole interactions.

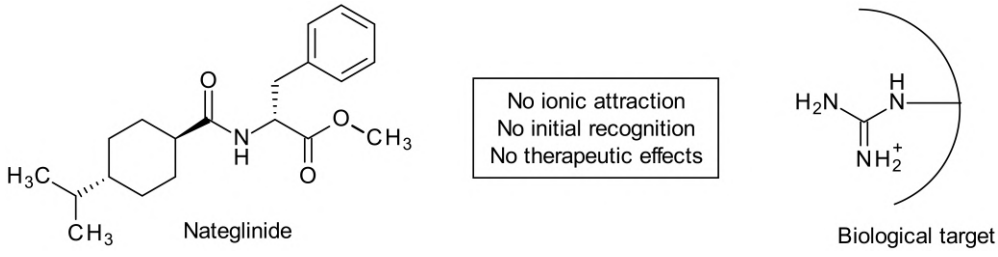
Ionic Bonds

Ionic bonds occur between ionized acidic and basic functional groups or between ionized acidic and quaternary ammonium functional groups. In other words, an ionic bond occurs between two atoms that bear opposite full charges (i.e., a "+" and a "–" charge). The ability of these bonds to form depends on the pK_a of the functional groups and their ionization at a specific physiologic pH. Ionic bonds have a bond strength of approximately 5 to 10 kcal/mol and are the strongest of all noncovalent interactions. The ability to form an ionic bond is proportional to 1/r, where r represents the

distance between the ionized functional groups. An example of an ionic bond between nateglinide and a positively charged guanidine group present on its biological target is shown below.



Nateglinide binds to SUR1, a specific receptor on the pancreatic β cell, and stimulates the release of insulin in a glucose dependent fashion. Because ionic bonds can form over the greatest distance, the electronic attraction between the positively charged guanidine group on SUR1 and the negatively charged carboxylic acid on nateglinide serves here as the initial recognition between the drug and its biological target. The importance of this initial recognition can be seen with the methyl ester analog of nateglinide. There is no ionic attraction between this analog and SUR1, so this drug is unable to recognize its biological target and completely lacks any anti-inflammatory or analgesic activity.



Since drugs primarily bind to proteins and enzymes, the acid/base nature of the amino acids comprising these macromolecules is essential to their ability to form ionic bonds. **Figure 6-8** highlights the naturally occurring basic functional groups that are the most likely to be present on an enzyme or protein.

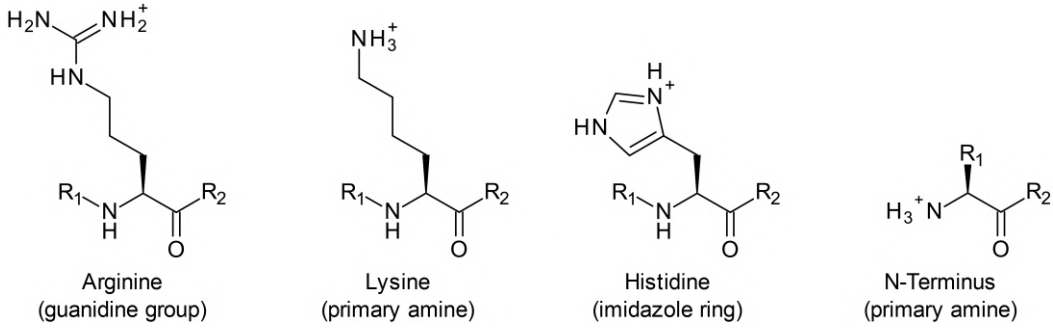


FIGURE 6-8. Amino acids containing basic functional groups.

The side chains of both arginine and lysine contain strong, basic functional groups. The pK_a value of the guanidine group of arginine is approximately 12.5, while the pK_a of the primary amine of lysine is approximately 10.5. Both of these functional groups are primarily ionized in all physiologic environments. The imidazole ring of histidine is significantly less basic with a pK_a of 6.0. As such, it is less

than 10% ionized at physiologic pH. Despite this fact, it is capable of forming ionic bonds, especially if it is in close proximity to other functional groups. An example of this is seen in **Figure 6-9**.

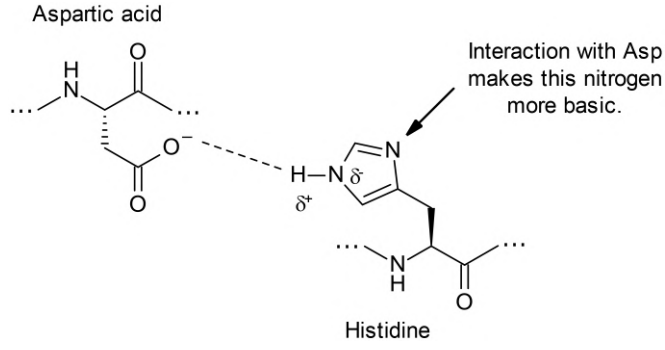


FIGURE 6-9. Enhanced ionization of histidine by an adjacent aspartic acid.

The interaction between the side chain of histidine and an adjacent aspartic acid enhances the dipole between the imidazole nitrogen atom and the hydrogen atom. The hydrogen atom becomes more partially positive and the ring becomes more partially negative, which allows the electrons on the other imidazole nitrogen atom to become more available and hence more basic and more capable of forming ionic bonds. This is an example of what is known as a local environment within the structure of a protein or enzyme. As seen in this example, local environments can provide situations where functional groups may be ionized to a greater extent than that predicted by comparing their pK_a values to a pH of 7.4.

The *N*-terminal primary amine is also capable of forming an ionic bond with an acidic functional group; however, this amine is often acetylated or otherwise modified for the purpose of forming a neutral, unionized functional group. This common posttranslational modification masks the amino end of the protein and prevents degradation by aminopeptidases, exopeptidases that sequentially remove amino acids from the *N*-terminus of the protein or enzyme.

Figure 6-10 highlights the acidic functional groups present on enzymes and proteins. Both aspartic acid and glutamic acid have side chains that contain a carboxylic acid. The pK_a values for these two acidic functional groups are 3.6 and 4.2, respectively, and therefore are primarily ionized at physiologic pH. The difference in the pK_a values of these two carboxylic acids is due to differences in adjacent functional groups. Within the structure of glutamic acid, there is an additional adjacent methylene (CH_2) group that is not present in aspartic acid. Because hydrocarbons are electron donating, this additional methylene group decreases the acidity of the side chain carboxylic acid in glutamic acid, compared with the analogous carboxylic acid in aspartic acid. The C-terminal carboxylic acid has the lowest pK_a (range = 1.8 to 2.4); however, similar to the *N*-terminal amine, it is often

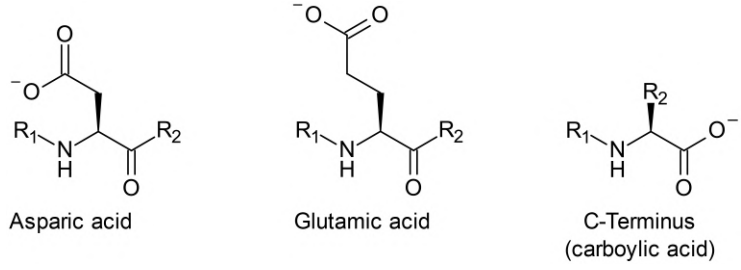


FIGURE 6-10. Amino acids containing acidic functional groups.

esterified or otherwise modified to form a neutral, unionized functional group that protects it from carboxypeptidase degradation. Thus, it is often unavailable to form ionic bonds.

Similar to what was discussed with histidine, adjacent functional groups can create local environments that can either enhance or detract from the ionization of aspartic acid or glutamic acid. **Figure 6-11A** illustrates a glutamic acid moiety present in a polar binding pocket. The functional groups on the adjacent lysine and serine amino acids help to enhance the ionization of glutamic acid. This enhancement increases the tendency of the carboxylic acid to become ionized and hence decrease its pK_a. In contrast, **Figure 6-11B** illustrates a glutamic acid moiety present in an otherwise nonpolar binding region. The surrounding amino acids, indicated in the figure by valine and phenylalanine, are not able to either stabilize or share in the ionic charge. This decreases the tendency of the carboxylic acid to become ionized and increases its pK_a. Please note that the same effects would occur with an aspartic moiety.

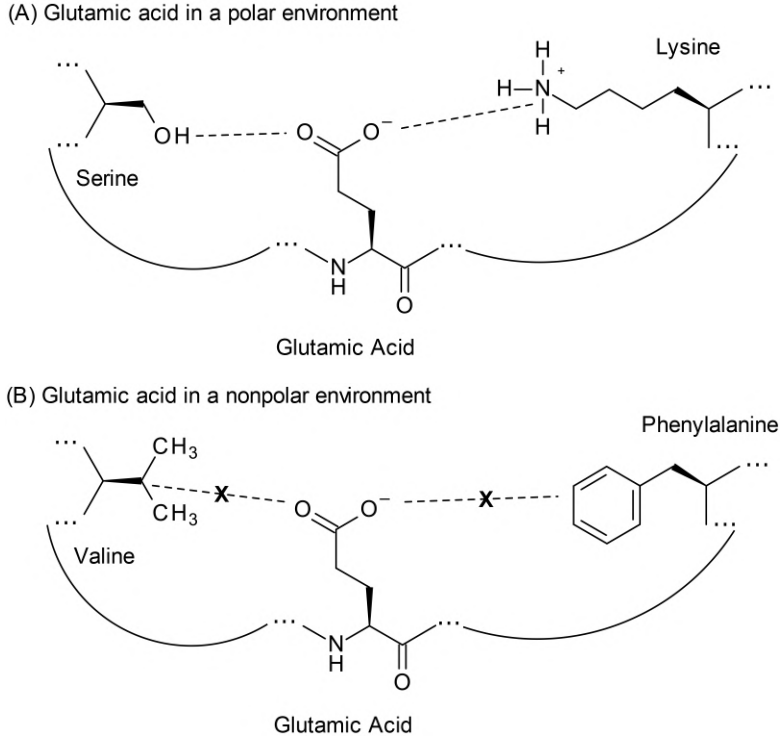


FIGURE 6-11. The effects of local environments on the ionization of glutamic acid.

Another Way to Look at Local Environments

Within any given biological target, adjacent functional groups can interact with one another and thus influence the ability to form specific types of bonds. This was illustrated in Figures 6-9 and 6-11. To help understand this concept, let's step outside the realm of chemistry and look at an ordinary social interaction. Consider a student who just received his first "A" grade in a very challenging course. In the ***friendly environment*** of his fraternity's lunch table, he is ***more than willing*** to share this good news with his friends, especially those who have helped him learn and study the course material. Later in the day, this same student is

traveling home on a bus and is seated beside a total stranger who is somewhat engrossed in the local newspaper. In this environment, the student is much ***less likely*** to share his good news about his exam grade. The key point in this analogy is that the only thing that has changed is the environment. The student is the same, the good news is the same, but the environment or the people surrounding him is different. In the examples using histidine and glutamic acid, the functional groups remained the same; however, the environments, or the surrounding functional groups, altered their ionization and ability to form ionic bonds.

Dipole Interactions

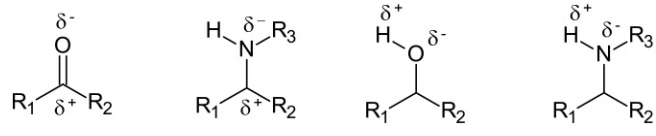
Dipole interactions occur between functional groups in which there is a partial charge separation between the atoms involved in the individual bonds of the functional group. This partial separation of the charge is due to the varying electronegativity values of the atoms comprising the functional groups. These electronegativity values were introduced in Chapter 2, in which the concept of intrinsic induction was discussed. They are reproduced here in **Table 6-3**, but we examine them with a slightly different emphasis.

TABLE 6-3. Electronegativity Values for Atoms Commonly Seen in Drug Molecules

Atom	Electronegativity Value
F	3.98
O	3.44
Cl	3.16
N	3.04
Br	2.96
I	2.66
S	2.58
C	2.55
H	2.20
P	2.19

Listed below are the key points to learn in terms of applying electronegativity values to dipole interactions. *Items 1 and 2 are the most significant due to the relative prevalence of oxygen, nitrogen, carbon, and hydrogen atoms in drug molecules*.

1. Oxygen and nitrogen are more electronegative than carbon and hydrogen; thus, whenever an oxygen or nitrogen atom is bound to either a carbon or hydrogen atom, the oxygen or nitrogen atom has a partial negative charge and the carbon or hydrogen atom has a partial positive charge.



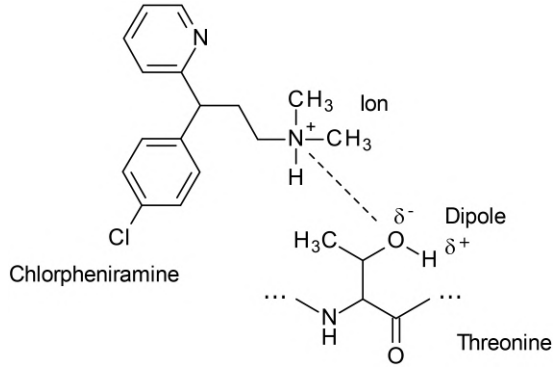
2. The electronegativity values of carbon and hydrogen atoms are similar to one another. As such, there is no significant dipole in a C—H bond.

3. The electronegativity values of sulfur and hydrogen atoms are also similar to one another; however, due to the larger size of the sulfur atom, a dipole does exist in a S—H bond, with the sulfur atom having a partial negative charge and the hydrogen atom having a partial positive charge. This dipole is not as great as that seen for an O—H bond.
4. Similar to a carbon atom, a phosphorus atom has a much lower electronegativity than an oxygen atom; thus, a phosphorous atom has a partial positive charge when bound to an oxygen atom, and the oxygen atom has a partial negative charge.
5. All halogen atoms (i.e., F, Cl, Br, and I) are much more electronegative than carbon, so all C—halogen bonds contain a partially positive carbon atom and a partially negative halogen atom. With the exception of interactions with a C—F bond, interactions with C—halogen bonds are not that common in terms of dipole interactions between a drug and its biological target.

Ion–Dipole and Dipole–Dipole Interactions

Dipole interactions can be subdivided into ion–dipole interactions, dipole–dipole interactions, and hydrogen bonds. Dipole–dipole interactions are also known as *Keesom forces*, and hydrogen bonds are a specialized type of dipole–dipole interaction. The bond strength of a dipole interaction varies from 1 to 7 kcal/mol, depending on the specific interaction. Dipole interactions require functional groups to be closer in proximity than that for an ionic bond. This is reflected in the fact that the ability to form an ion–dipole interaction is proportional to $1/r^2$ whereas the ability to form a dipole–dipole interaction is proportional to $1/r^3$. Similar to ionic bonds, ***r*** represents the distance between the interacting functional groups. The mathematical relationships (e.g., 1/r, $1/r^2$) provided in this chapter may vary slightly among references; however, the overall trends remain the same. *As the electronic interactions become smaller, the distance required to form the bond becomes more stringent.*

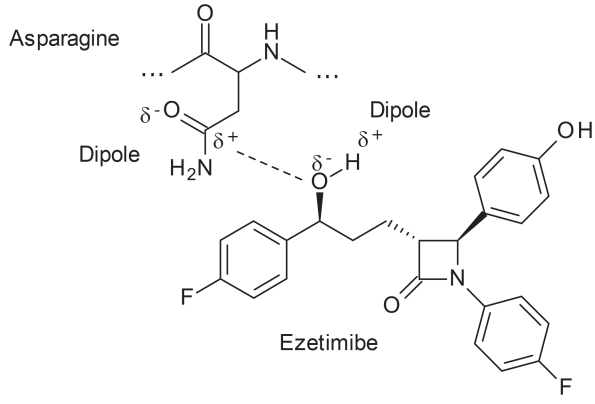
An ion–dipole interaction occurs between an ionized acidic or basic functional group (full charge) and a functional group with a partially charged dipole. An example of an ion–dipole interaction is shown below. Chlorpheniramine is an antihistamine indicated for the treatment of allergic conjunctivitis, allergic rhinitis, hay fever, and symptoms of the common cold. It contains a basic tertiary amine that is primarily ionized at physiologic pH. It is capable of forming an ion–dipole interaction with the hydroxyl group of threonine. In this interaction, the full positive charge on the tertiary amine is attracted to the partially negative charge of oxygen atom of the hydroxyl group. In identifying ion–dipole interactions, it is important that you identify which functional group is participating as the ion and which functional group is participating as the dipole.



A disclaimer is appropriate at this point. Because the emphasis of this chapter is to identify the types of bonds that can be formed between drug molecules and their biological targets, the example here and those that follow are simply examples of what is possible. Unless otherwise stated, these examples are not meant to portray any specific known binding interactions. In other words, the example here is simply showing that chlorpheniramine could form an ion-dipole bond with a

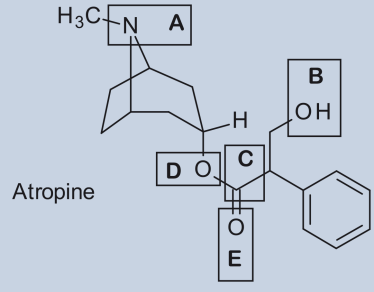
threonine residue on a biological target; it is not stating that this is the actual interaction that occurs between chlorpheniramine and the histamine H_1 receptor. For the sake of illustrating what is possible, various amino acids and functional groups are used as examples.

A dipole–dipole interaction occurs between functional groups that have complementary, or opposite, partial charges. The interaction of the hydroxyl oxygen of ezetimibe with the carbonyl carbon of an asparagine residue present on a biological target of ezetimibe provides an example of a dipole–dipole interaction.



Application of Concepts

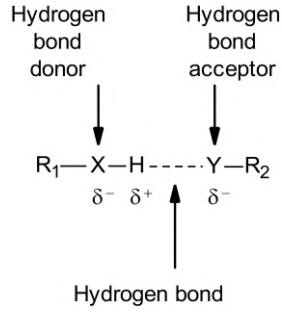
An important skill to master is the ability to examine the structure of a drug molecule and identify the possible types of interactions that it can form with its biological targets. Thus far, we have discussed the following noncovalent bonds: ionic bonds, ion–dipole bonds, and dipole–dipole bonds. Using only these three types of bonds, let's evaluate atropine. The tertiary amine (Group A) is primarily ionized at physiological pH and thus is able to participate in either ionic bonds or ion–dipole bonds (as the ion). The hydroxyl group (Group B) can participate in either ion–dipole bonds (as the dipole) or dipole–dipole bonds similar to what was seen with chlorpheniramine and ezetimibe. The three atoms of the ester functional group can all participate in either ion–dipole bonds (as the dipole) or dipole–dipole bonds. The carbon atom (Group C) is partially electropositive whereas the carbonyl and ester oxygen atoms (Groups D and E) are partially electronegative.



Hydrogen Bonds

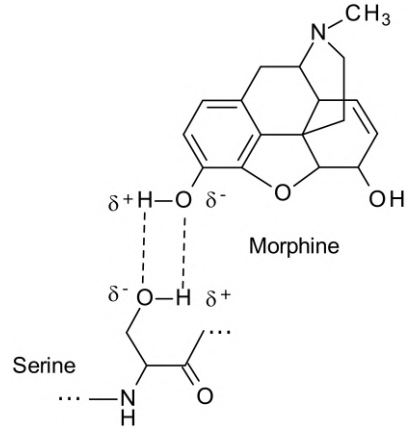
A *hydrogen bond* is a specialized type of dipole–dipole interaction and in general is stronger than the dipole–dipole bonds shown above. A discussion of hydrogen bonds and their role in the water solubility of a drug molecule can be found in Chapter 2. As initially discussed in that chapter, a hydrogen bond is one that occurs whenever a hydrogen atom serves as a bridge between two electronegative

atoms. In this type of bond, the hydrogen atom is covalently bound to one atom and noncovalently bound to the other. A general representation of a hydrogen bond is shown below.



In a hydrogen bond, the atom that is covalently bound to the hydrogen atom is known as the *hydrogen bond donor*, and the atom that is noncovalently bound to the hydrogen atom is known as the *hydrogen bond acceptor*. Thus, in the representation above, atom X is the hydrogen bond donor and Y is the hydrogen bond acceptor. Oxygen, nitrogen, and sulfur can serve as both hydrogen bond donors and acceptors, with oxygen and nitrogen being the most common and providing the strongest hydrogen bonds. Fluorine atoms can serve as hydrogen bond acceptors. A representative summary of the most common functional groups that are capable of forming hydrogen bonds is shown in **Figure 6-12**. *A key point in identifying hydrogen bonds is to designate which functional group is acting as the donor and which functional group is acting as the acceptor.*

An example of hydrogen bonding is shown below with morphine and a serine residue that could be present within a biological target. In this example, the phenolic group of morphine is able to form two individual hydrogen bonds with the hydroxyl group of serine. In the leftmost interaction, the hydrogen atom of the hydroxyl group of morphine is the hydrogen bond donor, and the oxygen atom of the hydroxyl group of serine is the hydrogen bond acceptor. In the rightmost interaction, the roles of these two hydroxyl groups are reversed, with the oxygen atom of morphine serving as the hydrogen bond acceptor and the hydrogen atom of serine serving as the donor.



The bond strength of a hydrogen bond ranges from 3 to 7 kcal/mol, and two major factors affect the overall bond strength. First, the bond strength depends on the alignment of the atoms involved in the hydrogen bond. Using the R_1—X—H---Y—R_2 designation, the strongest hydrogen bonds occur when X, H, and Y are collinear. Second, the bond strength depends on which atom serves as the donor and which serves as the acceptor. In comparing oxygen and nitrogen, the two most common electronegative atoms involved in a hydrogen bond, it is found that oxygen is a better hydrogen bond donor than nitrogen, and nitrogen is a better hydrogen bond acceptor than oxygen. Using the

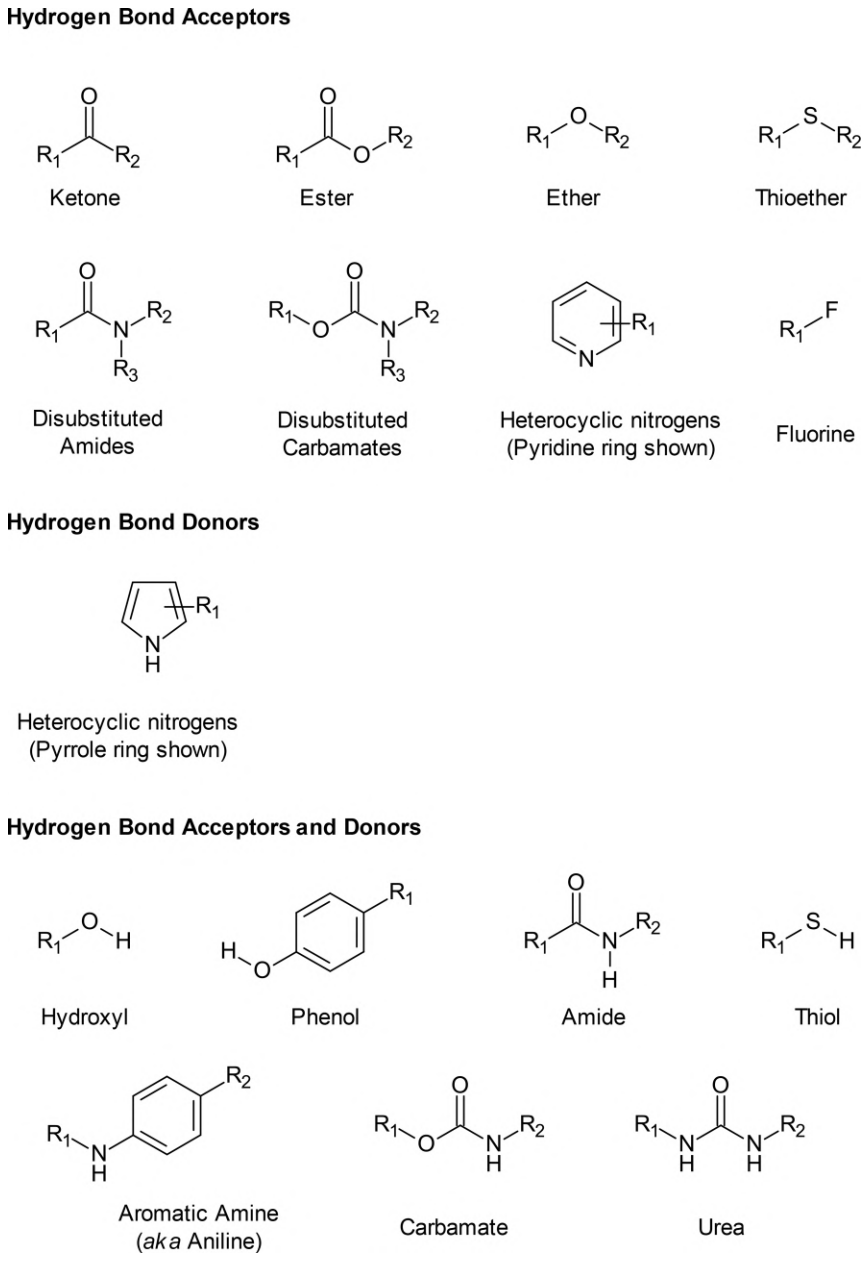


FIGURE 6-12. Common functional groups capable of hydrogen bonds.

above XHY sequence, in which XH is the hydrogen bond donor and Y is the hydrogen bond acceptor, the approximate rank order of hydrogen bond strength is OHN > OHO > NHN ≥ NHO. **Figure 6-13** provides an example that includes both of these factors. In this example, the catechol hydroxyl groups of isoproterenol can form hydrogen bonds with a nitrogen atom present on the backbone of the protein (hydrogen bond 1) as well as a hydroxyl group present on a tyrosine residue of that same protein (hydrogen bond 2). Based on the above information, hydrogen bond 2 would be expected to be stronger based on the fact that the OHO atoms are collinear, whereas the NHO atoms are at a 120° angle. Additionally, an OHO hydrogen bond is stronger than an NHO bond.

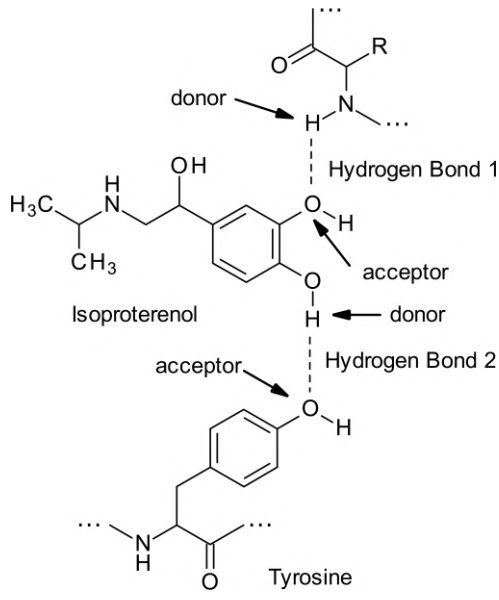


FIGURE 6-13. Hydrogen bonding interactions of isoproterenol and a biological protein target.

The importance of collinearity in forming hydrogen bonds can be further exemplified by examining DNA base pairing. Shown in **Figure 6-14** is the base pairing of thymidine with adenosine and guanosine with cytidine. Notice the collinear nature of the hydrogen bonds between these nucleotide bases. Additionally, note that the oxygen, nitrogen, and hydrogen atoms of the nucleotide bases can interact with functional groups present within the structures of drug molecules via ion–dipole interactions (as the dipole), dipole–dipole interactions, or hydrogen bonds. The sugar-phosphate backbone of DNA can also participate in a variety of drug binding interactions. The phosphate groups are primarily ionized and can participate in ionic bonds and ion–dipole bonds with functional groups

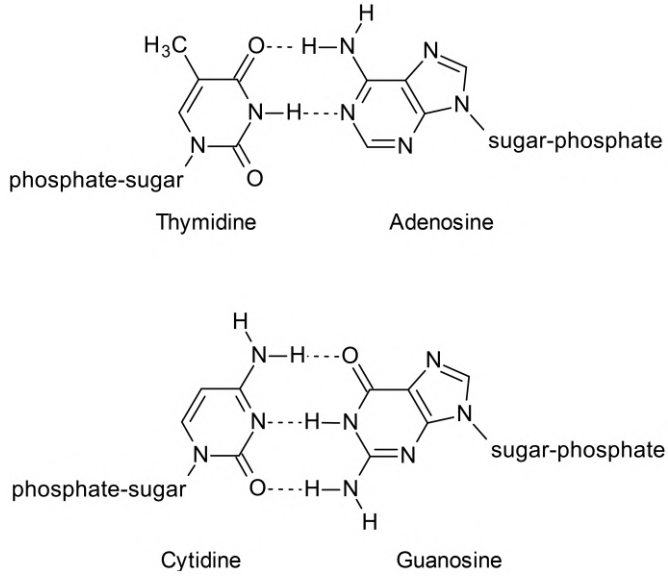
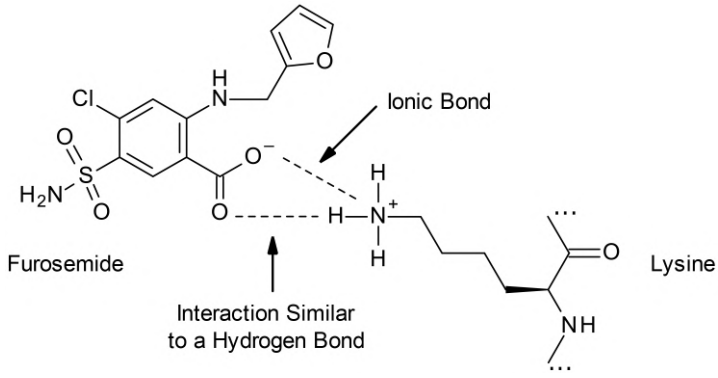


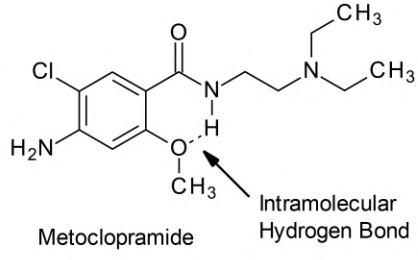
FIGURE 6-14. The importance of hydrogen bonding in nucleotide base pair formation.

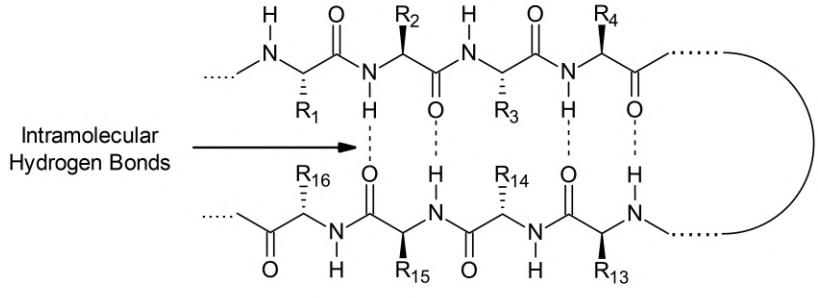
present on drug molecules whereas the oxygen atoms in the sugars can participate in ion–dipole bonds (as the dipole) and hydrogen bonds.

Binding interactions analogous to hydrogen bonds can also enhance the strength of an ionic bond. The resulting attraction has been referred to as a *reinforced ionic bond* and has the potential to enhance the binding energy to the original ionic bond by approximately 5 kcal/mol. Shown below is an example of a reinforced ionic bond. In this example, the carboxylic acid of furosemide initially forms an ionic bond with the primary amine of lysine. Once this occurs, a hydrogen atom from the primary amine can form a second interaction with the carbonyl oxygen. This second interaction is similar to a hydrogen bond and does enhance the overall interaction; however, due to the resonance delocalization of the negative charge and the resulting changing interactions, it is not a true hydrogen bond.



Finally, intramolecular hydrogen bonds can play *a significant role in the overall conformation of a drug molecule* or a biological macromolecule. Two examples are shown in **Figure 6-15**. The first example is seen with metoclopramide, an antiemetic agent that is indicated for the prophylactic treatment of chemotherapy-induced nausea and vomiting as well as for the treatment of





Partial structure of a β-sheet

FIGURE 6-15. Examples of intramolecular hydrogen bonds.

gastroesophageal reflux disease. The intramolecular hydrogen bond between the amide hydrogen atom and the oxygen atom of the methoxy group locks the conformation of this portion of the molecule and allows for the formation of a virtual ring system. The second example is seen with a β-sheet (also known as a β-pleated sheet), which is a common structure found in various types of proteins. Multiple intramolecular hydrogen bonds between carbonyl oxygen atoms and amide hydrogen atoms stabilize these structures.

Summary of the Advantages of Hydrogen Bonds

- Hydrogen bonds are generally stronger than other types of dipole–dipole interactions.
- Hydrogen bonds can enhance the strength of an ionic bond.
- Hydrogen bonds are important for enhancing the water solubility of a drug molecule.
- Intramolecular hydrogen bonds can be important for determining the preferred conformation for a drug molecule or a biological macromolecule.

Other functional groups are capable of forming hydrogen bonds; however, their prevalence in drug structures is not common. To be complete, these additional functional groups are shown in **Figure 6-16**. Please note the following key points. Once an amide nitrogen atom is substituted with

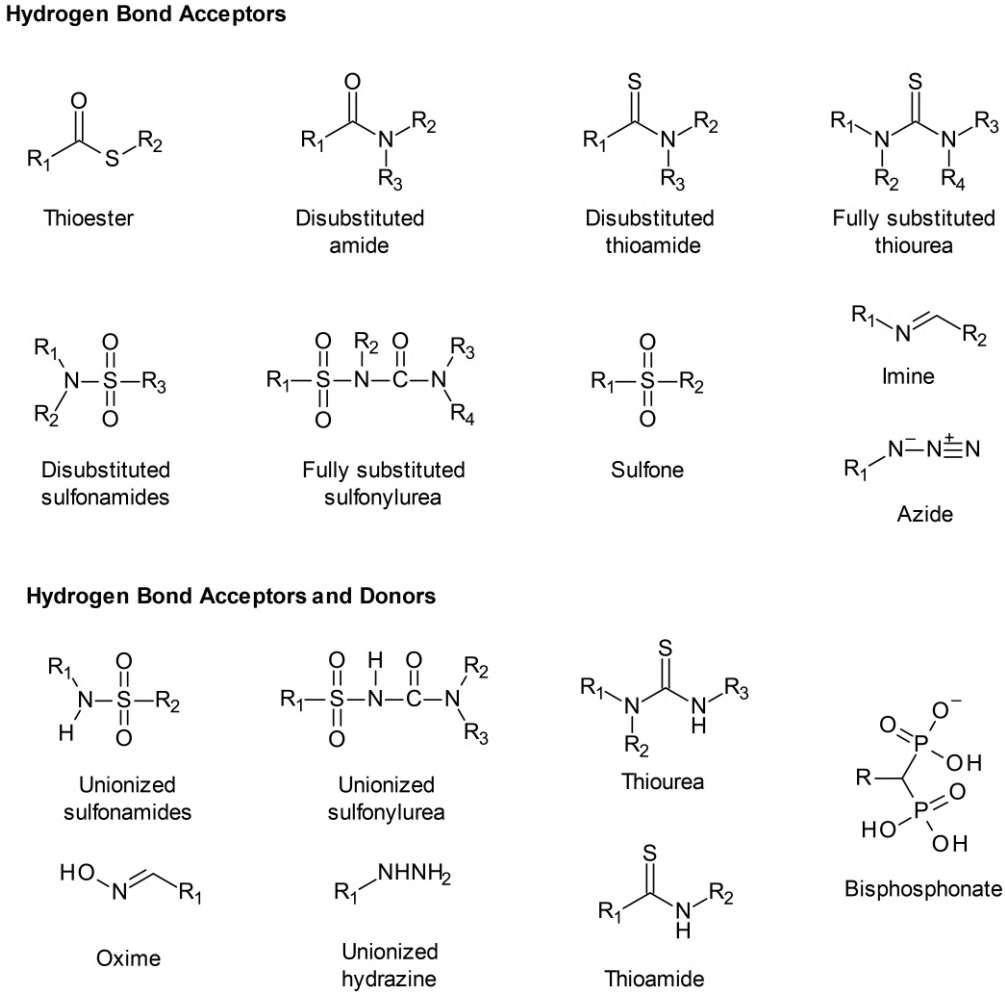
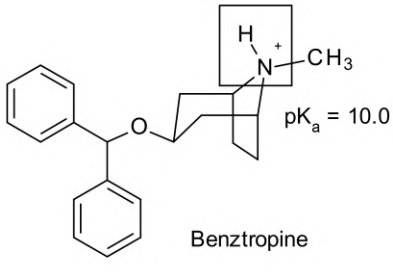


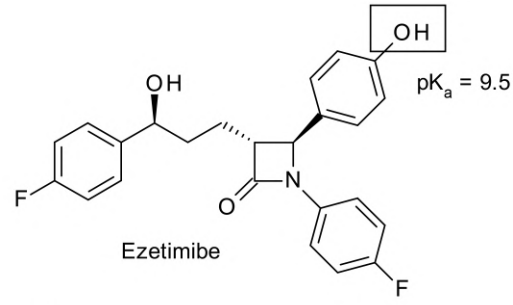
FIGURE 6-16. Additional functional groups capable of forming hydrogen bonds.

two additional groups, it no longer has a hydrogen atom and cannot function as a hydrogen bond donor. The same is true with sulfonamides and sulfonylureas. Sulfones, imines, and azides also lack a hydrogen atom and can only function as hydrogen bond donors. As described in Chapter 3, the acidity and ionization of sulfonamides can vary widely depending on adjacent functional groups. Therefore, sulfonamide functional groups that are primarily unionized can participate in hydrogen bonds as either the acceptor or the donor. Similarly, due to their respective pK_a ranges and adjacent functional groups, it is possible for a sulfonylurea or a hydrazine to be primarily unionized and participate in a hydrogen bond. As discussed in Chapter 3, the pK_a values for the two phosphonates of a bisphosphonate are very different. The pK_a value for the first hydrogen atom is approximately 1.5 to 2.5 whereas the pK_a value for second hydrogen atom is higher, ranging from 6.5 to 7.5. Thus, while one phosphonate is primarily ionized, the second phosphonate may be able to participate in hydrogen bonds if it is significantly unionized.

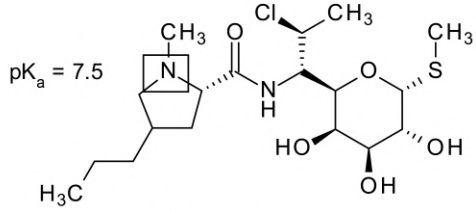
Prior to discussing binding interactions with functional groups containing hydrocarbon, let's evaluate three drugs and apply what has been discussed thus far. Shown below are the structures of benztropine, ezetimibe, and clindamycin. Their respective acidic and basic functional groups have been highlighted and the pK_a values have been provided. As discussed above, prior to determining the types of binding interactions that these acidic and basic functional groups can undergo, it is essential to *first consider their predominant ionization states*.



Benztropine



Ezetimibe



Clindamycin

Let us first evaluate benztropine. Its tertiary amine is greater than 99% ionized at a physiologic pH of 7.4 and can participate in an ionic bond, a reinforced ionic bond, or an ion–dipole interaction (as the ion) with its biological target. The ether oxygen atom within the structure of benztropine can participate in an ion–dipole interaction (as the dipole), a dipole–dipole interaction, or a hydrogen bond (as the hydrogen bond acceptor). In evaluating the structure of ezetimibe, its aromatic hydroxyl group (or phenol) is approximately 99% unionized at a cellular pH of 7.4 and most likely participates in an ion-dipole interaction (as the dipole), a dipole–dipole interaction, or a hydrogen bond (as either the donor or acceptor) with its biological target. The secondary hydroxyl group and the amide can form similar types of interactions although the amide can only act as a hydrogen bond acceptor. The two fluorine atoms can also participate in hydrogen bonds as an acceptor.

Clindamycin provides an example in which the pK_a of the functional group is close to the physiologic pH of 7.4. Its tertiary amine has a reported pK_a of 7.5. In a physiologic pH of 7.4, this basic functional group is 56% ionized and 44% unionized; thus, the probability that it will form an ionic bond, a reinforced ionic bond, or an ion–dipole interaction with its biological target is similar to the probability that it will form a hydrogen bond (as an acceptor), an ion–dipole interaction (as the

dipole), or a strict dipole–dipole interaction that doesn't involve a hydrogen bond. The hydroxyl groups and the amide can participate in ion–dipole interactions (as the dipole), dipole–dipole interactions, and hydrogen bonds (as either the donor or acceptor). The sulfur atom can participate in a hydrogen bond as an acceptor. *As a key and closing point here: you should be able to provide similar evaluations for any given drug molecule.*

van der Waals Interactions

The term *van der Waals interactions*, or *van der Waals forces*, actually refers to three different types of interactions or forces: Keesom forces, Debye forces, and London dispersion forces. As previously mentioned, *Keesom forces* is another name for a dipole–dipole interaction. Many texts, including this one, discuss dipole–dipole interactions, or Keesom forces, separate from Debye and London dispersion forces. In terms of the following discussion, the term van der Waals interactions pertains only to Debye and London dispersion forces. These two types of interactions involve induced dipoles and are thus different than the interactions between two permanent dipoles. *Debye forces* are those that occur between a permanent dipole (e.g., a hydroxyl group) and an induced dipole whereas *London dispersion forces* are those that occur between two induced dipoles. In terms of drug binding interactions, London dispersion forces are much more common than Debye forces. As such, the following discussion focuses primarily on London dispersion forces.

Unlike polar groups, such as a hydroxyl or an amide functional group, hydrocarbon rings and chains do not have measurable dipoles. This, however, does not mean that the electrons between a carbon–carbon bond are static. On the contrary, there is a rapid fluctuation of partial positive and partial negative charges between the two carbon atoms, which is illustrated below. At any given moment, the electron movement within the carbon–carbon bond could result in the carbon atom on the left having a partial positive charge and the carbon atom on the right having a partial negative charge. In the next instant, the opposite could be true. Both carbon atoms have the same electronegativity, so they equally share the electrons back and forth.

Polarity 1 Polarity 2

While hydrocarbon chains and rings lack a measurable dipole, a dipole is induced whenever these functional groups are aligned with either a polar functional group, such as a hydroxyl group, or another nonpolar hydrocarbon chain or ring. Shown in **Figure 6-17A** is an example of a dipole-induced dipole interaction between the *n*-butyl side chain of losartan, an angiotensin II receptor blocking agent used to treat hypertension, and a hypothetical serine hydroxyl group present at the angiotensin II receptor. As previously discussed, a permanent dipole exists within a hydroxyl group, with the oxygen atom possessing a partially negative charge and the hydrogen atom possessing a partially positive charge. When this functional group is close enough to the *n*-butyl alkyl chain, it induces a dipole in the carbon–carbon bond resulting in the formation of a dipole-induced dipole bond. Again, this is just a hypothetical example. **Figure 6-17B** shows a more likely interaction of this *n*-butyl chain with a leucine residue present on the angiotensin II receptor. As the two hydrophobic chains approach one another, a mutual polarization of the carbon–carbon bonds occurs. This induces the formation of complementary dipoles, resulting in numerous induced dipole-induced dipole interactions.

Individual van der Waals interactions are weak, with bond strengths ranging from 0.5 to 1.0 kcal/mol. A value of 0.7 kcal/mol has been used as an approximate bond strength between two carbon atoms. The ability to form van der Waals interactions depends highly on the distance between the participating functional groups. The ability for a dipole-induced dipole interaction to occur is proportional to $1/r^4$, where r represents the distance between the interacting functional groups.

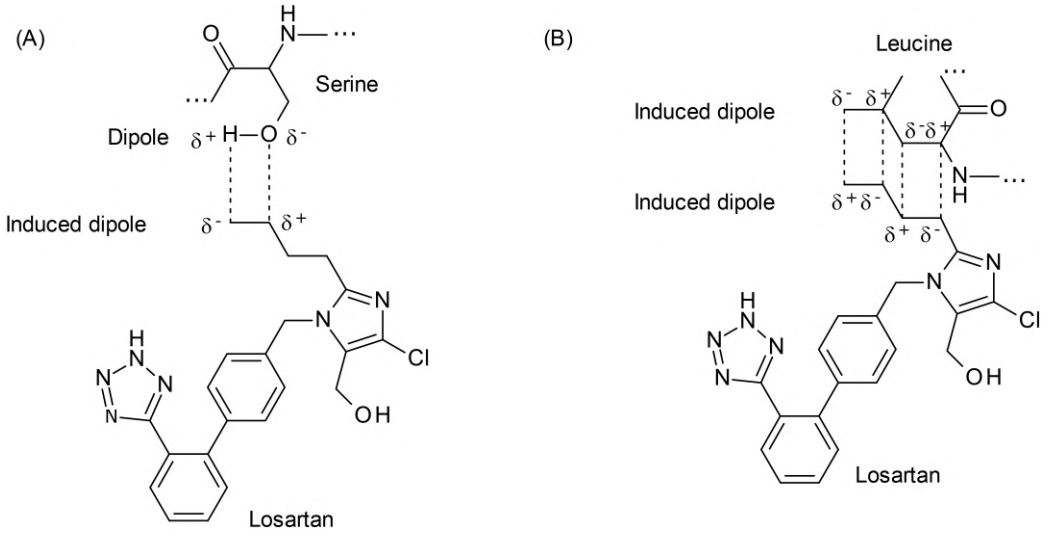


FIGURE 6-17. Examples of (A) dipole-induced dipole interactions and (B) induced dipole-induced dipole interactions.

Similarly, the ability for an induced dipole-induced dipole interaction to occur is proportional to $1/r^6$. If only a single carbon–carbon interaction is examined, it is easy to dismiss this single van der Waals interaction as relatively insignificant to the overall binding ability or affinity of the drug molecule for its biological target. However, when one considers that alkyl chains and aromatic rings are capable of multiple van der Waals interactions, the overall contribution can become significant. Consider the *n*-butyl alkyl chain of losartan shown in **Figure 6-17B**. If all four carbon atoms are able to interact with the side chain of a leucine residue (or other hydrophobic amino acid), then this functional group has the potential to provide an overall bond strength of 2.8 kcal/mol. Another example of this can be seen with the unsubstituted aromatic ring of meperidine shown in **Figure 6-18**. If this aromatic ring were able to closely interact with the aromatic ring of a phenylalanine residue, there would be a total of six individual interactions and a potential bond strength of 4.2 kcal/mol.

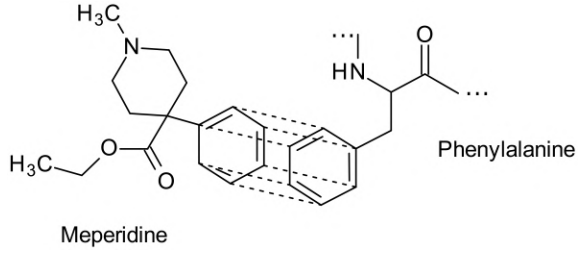


FIGURE 6-18. Example of van der Waals interactions possible for the aromatic ring within the structure of meperidine.

The above examples with losartan and morphine are meant to illustrate how van der Waals interactions can provide meaningful binding interactions. Additional examples can be seen with salmeterol and tamoxifen (**Figure 6-19**). These drugs contain multiple aromatic rings, aliphatic chains, and/or alicyclic rings. As a result, the total contribution of van der Waals interactions to the binding of these drugs to their biological targets can be quite substantial.

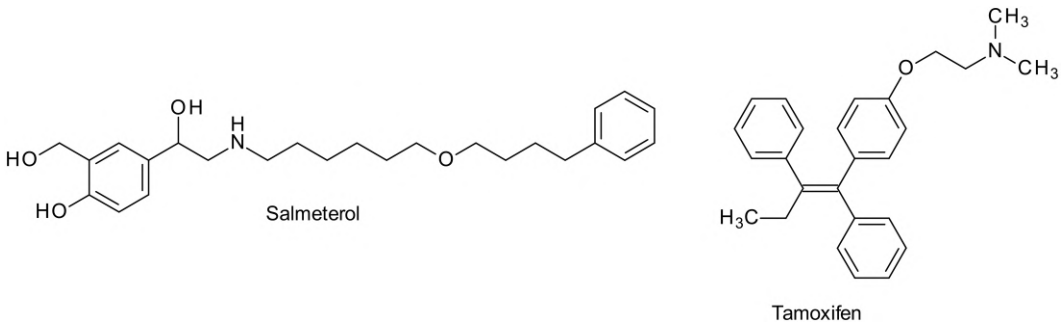
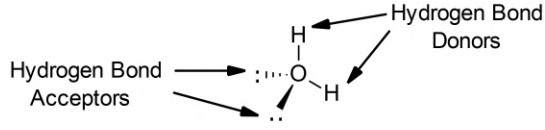


FIGURE 6-19. Salmeterol and tamoxifen.

An additional consideration for induced dipole-induced dipole interactions is that these interactions are most favorable when they occur between similar types of functional groups. In other words, an aliphatic chain present on a drug molecule is able to form stronger van der Waals interactions with other aliphatic chains as compared with an aromatic ring. The reason here is that similar types of functional groups are better able to make close contact with one another. This is especially important for van der Waals interactions due to their distance requirements. The previous examples of losartan and meperidine illustrate this concept of similar groups interacting with each other.

Hydrophobic Effects (aka Hydrophobic Interactions)

The term *hydrophobic effects* refers to the tendency of lipid-soluble, or hydrophobic, molecules to interact with one another in an aqueous, or hydrophilic, environment. In terms of drug binding interactions, a hydrophobic effect (or a hydrophobic interaction) is not directly related to bond formation but rather to the gain in entropy that occurs when two hydrophobic functional groups are attracted to one another. As described above, the actual binding interaction between two hydrophobic groups is known as a van der Waals interaction; however, when these types of bonds occur, water is displaced and there is a gain of entropy that results in the release of energy. Water molecules have the ability to become highly ordered. As shown below, each individual water molecule can form hydrogen bonds with four other water molecules.



Within the body, drug molecules as well as their biological targets reside in an aqueous environment. Even biological targets that are embedded into cell membranes have some portion of the macromolecule exposed to the aqueous physiologic environment. As shown in **Figure 6-20A**, water molecules can become highly ordered whenever they encounter nonpolar functional groups. For these two nonpolar groups to participate in van der Waals interactions, they must be very close to one another. Whenever this occurs, there is a subsequent "squeezing out" or release of water molecules from their original, highly ordered state (**Figure 6-20B**). The overall result of this action is an increase in entropy and a release of energy. This process is often referred to as a hydrophobic effect, or a hydrophobic interaction; however, please note that this designation actually describes the exclusion of water rather than the attraction between two functional groups.

The release of energy through this process enhances the bond strength of the initial van der Waals interaction. The hydrophobic effect, or interaction, between two carbon atoms has the potential to add up to 0.7 kcal/mol in binding energy and theoretically double the bond strength of a van der Waals interaction. Thus, the maximum bond strength of a single carbon–carbon interaction is

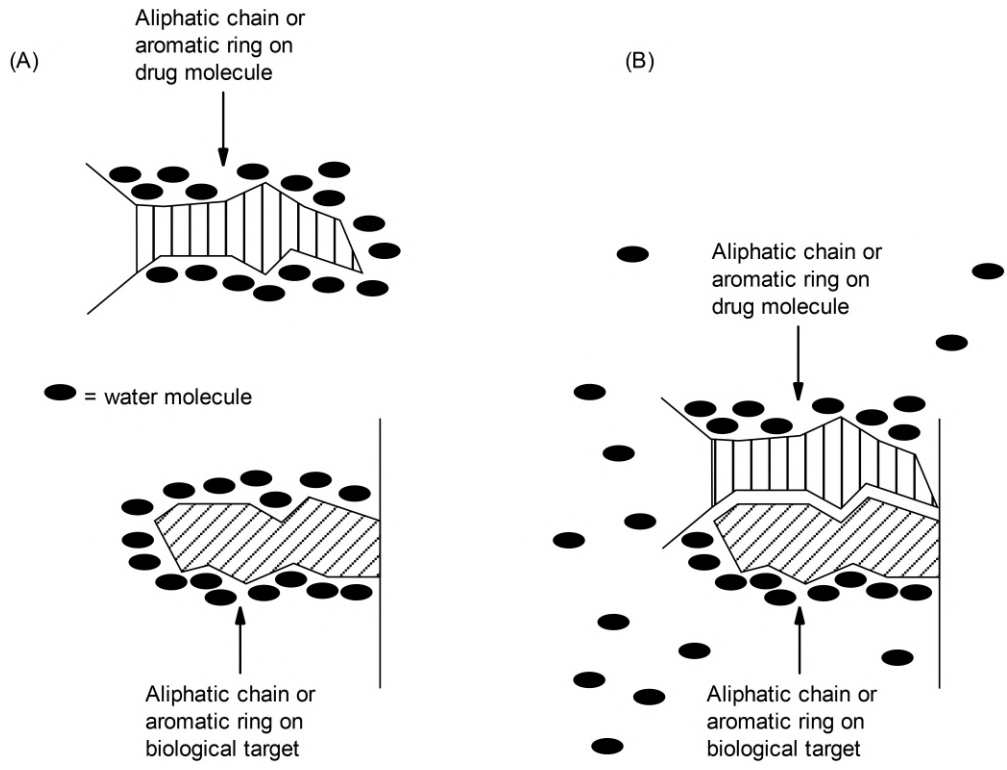


FIGURE 6-20. An illustration of hydrophobic effects. (A) Highly ordered water molecules initially surround the lipid/water interface of hydrophobic functional groups. (B) When the hydrophobic groups form a van der Waals interaction, they displace the water molecules and increase the entropy of the system.

1.4 kcal/mol. In applying this concept to meperidine (Figure 6-18), it is found that the total binding energy of meperidine's phenyl ring with the phenyl ring of phenylalanine can approach 8.4 kcal/mol. This finding reinforces the previous statement that the overall contribution of van der Waals interactions to drug binding can be substantial.

Caution is warranted in this situation. In theory, each carbon atom present in an aromatic ring, aliphatic chain, or aliphatic ring of a drug molecule has the potential to contribute a maximum of 1.4 kcal/mol toward the overall binding strength associated with the interaction of the drug to its biological target. *In reality, this potential maximum value cannot always be attained due to steric factors and/or the location of the hydrocarbon groups.* To illustrate this fact, let us evaluate meperidine a little closer. The phenyl ring of meperidine shown in Figure 6-17 is a good example of a functional group that lacks any significant steric hindrance and has an optimal location within the drug molecule. It is a terminal substituent of the piperidine ring as opposed to being located in the middle of the molecule. As such, it has more conformational flexibility, has a greater chance to align itself with a complementary region on its biological target, and can significantly contribute to meperidine's binding. Shown in **Figure 6-21** is another view of morphine that highlights four additional carbon atoms that could potentially contribute to meperidine's binding. Carbon A and its adjacent methylene carbon are part of a side chain ester and have significant conformational freedom. Similar to the phenyl ring, they should be able to align with complementary functional groups and be involved in van der Waals interactions and hydrophobic effects. Carbon B is also located at an external position and is sterically unhindered. A potential problem with this carbon is that it is directly adjacent to a basic nitrogen atom. In all likelihood, this amine will be ionized and the methyl group will reside

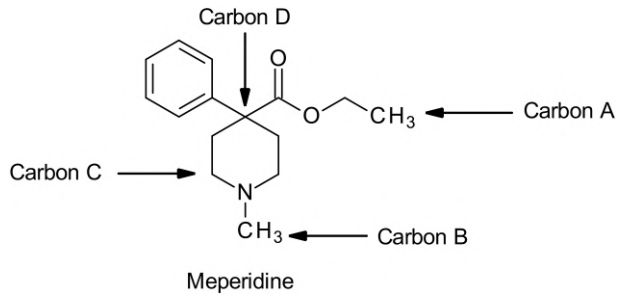


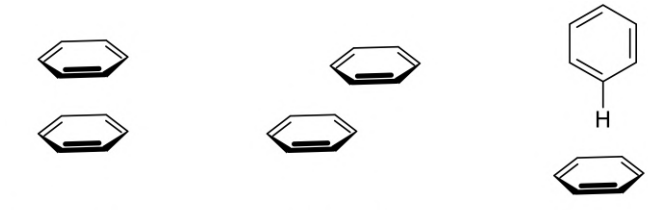
FIGURE 6-21. Additional carbons atoms present within the structure of morphine that could undergo van der Waals and hydrophobic interactions.

in a polar region of the binding site. Because polar regions of a binding site are less likely to contain complementary nonpolar functional groups, the probability that carbon B will participate in a van der Waals interaction and a hydrophobic effect is diminished. Carbon C has a similar problem as it is also adjacent to the basic amine. Further, this methylene is part of a piperidine ring. Due to the conformation of the ring as well its substituents, steric factors could prevent this group from attaining the required distance to participate in van der Waals interactions. Carbon D is also located within the piperidine ring and has bonds to four different carbon atoms. Although it may very well reside in a nonpolar region of a binding site, the steric factors would eliminate the possibility of this carbon atom getting close enough to form a van der Waals interaction.

In summary, each aromatic, aliphatic, and alicyclic carbon atom can theoretically contribute up to 1.4 kcal/mol of binding energy by virtue of van der Waals interactions and hydrophobic effects. Due to steric hindrance and the location of the carbon atom within the drug molecule, some carbon atoms are not able to contribute to the overall binding or are only able to contribute a portion of the maximum binding energy. Functional groups that are most likely to contribute the maximum binding energy are those that are the *least sterically hindered, have the most conformational flexibility, and have the ability to be located in a nonpolar region* of the biological target's binding site.

Additional Aromatic Interactions

Due to the overall electron distribution and density in an aromatic ring, these functional groups can provide additional binding interactions beyond the van der Waals and hydrophobic interactions discussed above. The first type of interaction involves the electron clouds of adjacent aromatic rings and is known as a π-π or aryl-aryl stacking interaction. This interaction is similar to a van der Waals interaction but also involves polar movements, or uneven charge distributions, of interacting rings. *As shown below, parallel stacking interactions can be either face-to-face (aka sandwiched) or displaced. Additionally, it is possible for an edge-to-face, or T-shaped, interaction to occur.*



A large number of drug molecules contain aromatic rings and ring systems that can potentially form π-π stacking interactions with their biological targets. For drugs that bind to a protein receptor, π-π stacking interactions can occur with the side chains of phenylalanine, tyrosine, tryptophan,

and histidine residues. Additionally, many antineoplastic drugs provide their therapeutic effects by intercalating with DNA. In this process, the aromatic portion of the drug molecule is inserted in between stacked nucleotide bases and can form several types of interactions. **Figure 6-22A** shows an example of a π-π stacking interaction between the unsubstituted phenyl ring of tolterodine and the side chain of a tyrosine residue within the structure of the cholinergic muscarinic receptor, the biological target for tolterodine. The structure of mitoxantrone, a drug that binds to DNA through intercalation, is shown in **Figure 6-22B**.

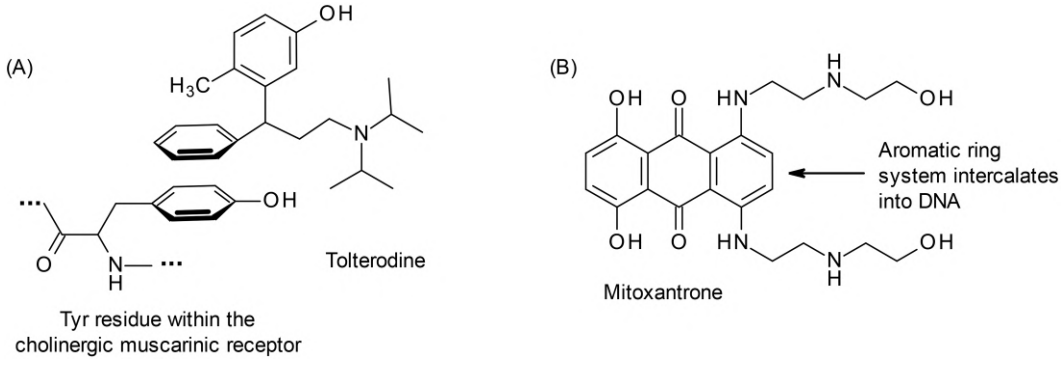


FIGURE 6-22. Examples of (A) a π-π stacking interaction and (B) a drug that interacts with DNA through intercalation.

The second type of interaction is a cation–π interaction that occurs due to the attraction of a positively charged group to the electrons in the π cloud of an aromatic ring. Within the structure of a protein or enzyme receptor, the ionized side chains of arginine, lysine, and histidine can function as the cation, while the side chains of phenylalanine, tyrosine, tryptophan, and histidine can provide the π electrons. Two examples of this type of interaction are shown in **Figure 6-23** using the antidepressant atomoxetine. Atomoxetine produces its antidepressant effects by blocking the presynaptic norepinephrine transporter. The secondary amine is primarily ionized at physiologic pH and could undergo a cation–π interaction with a phenylalanine residue located within the structure of the norepinephrine transporter, while either of the aromatic phenyl rings could interact with a lysine residue within the transporter.

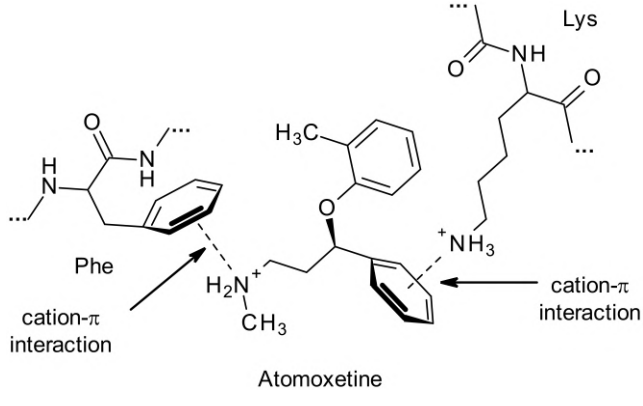


FIGURE 6-23. Examples of cation–π interactions.

The third type of interaction occurs between electron rich and electron deficient aromatic rings and is known as a charge transfer interaction. These types of interactions are, in essence, dipole–dipole interactions; however, due to the overall number of atoms and electrons involved in these

interactions, they have the ability to provide a much greater bond energy than the dipole–dipole interactions previously discussed. Electron rich aromatic rings include five-membered aromatic heterocyclic rings and phenyl rings that have electron donating functional groups (**Figure 6-24**). Similar to an unsubstituted phenyl ring, pyrrole, furan, and thiophene, have six π electrons; however, the π electrons in these latter three rings are delocalized over five atoms instead of six. Thus, compared with an unsubstituted phenyl ring, pyrrole, furan, and thiophene are electron rich. This concept is also applicable for other five-membered heterocyclic rings and five-six fused, bicyclic rings. Examples of these are shown in Figure 6-24. The addition of an electron donating group to a phenyl ring enhances the electron density of the ring and makes it electron rich. Functional groups that can donate electrons through resonance, such as an amine, an ether, or a hydroxyl group, can provide a greater effect than those that can only donate through induction, such as an alkyl group.

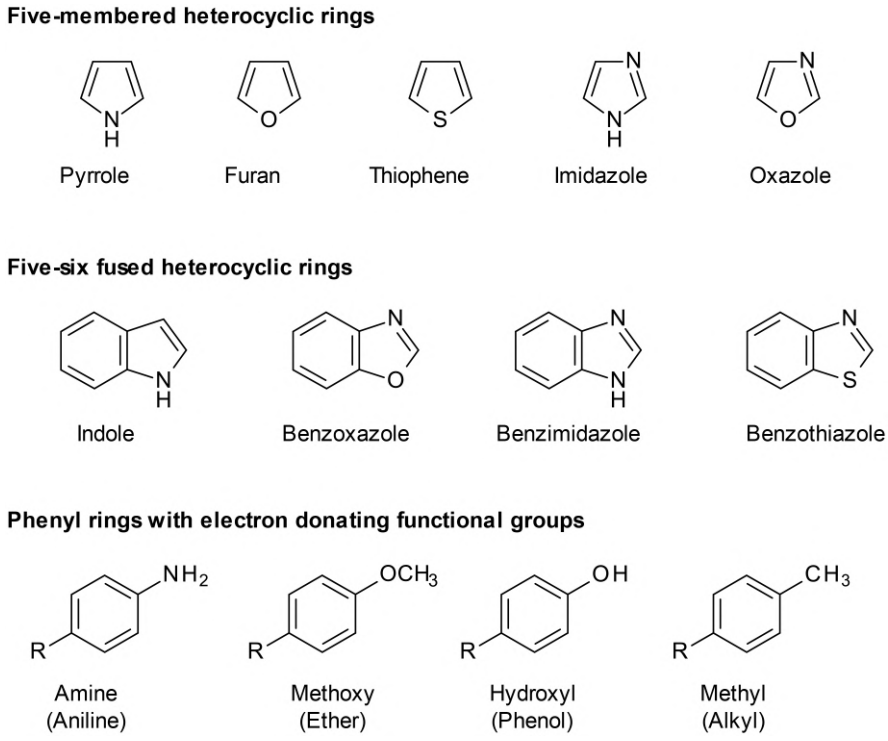


FIGURE 6-24. Examples of electron rich aromatic rings.

Electron deficient, or electron poor, aromatic rings include six-membered aromatic heterocyclic rings and phenyl rings that have electron withdrawing functional groups (**Figure 6-25**). Six-membered aromatic heterocycles, such as pyridine, pyrimidine, pyridazine, and pyrazine, are electron poor due to the electronegativity of the nitrogen atoms. The higher electronegativity of the nitrogen atoms draws the π electrons toward them and away from the other carbon atoms. This unequal sharing of electrons causes the aromatic ring as a whole to be electron deficient. This concept extends to six-six fused, bicyclic rings such as quinolone, quinazoline, and pteridine. The addition of an electron withdrawing group to a phenyl ring decreases the electron density of the ring and makes it electron deficient. Similar to what is seen with electron donating functional groups, those that can withdraw electrons through resonance, such as a nitrile, nitro, or a carbonyl group, can provide a greater effect than those that can only withdraw through induction, such as halogen.

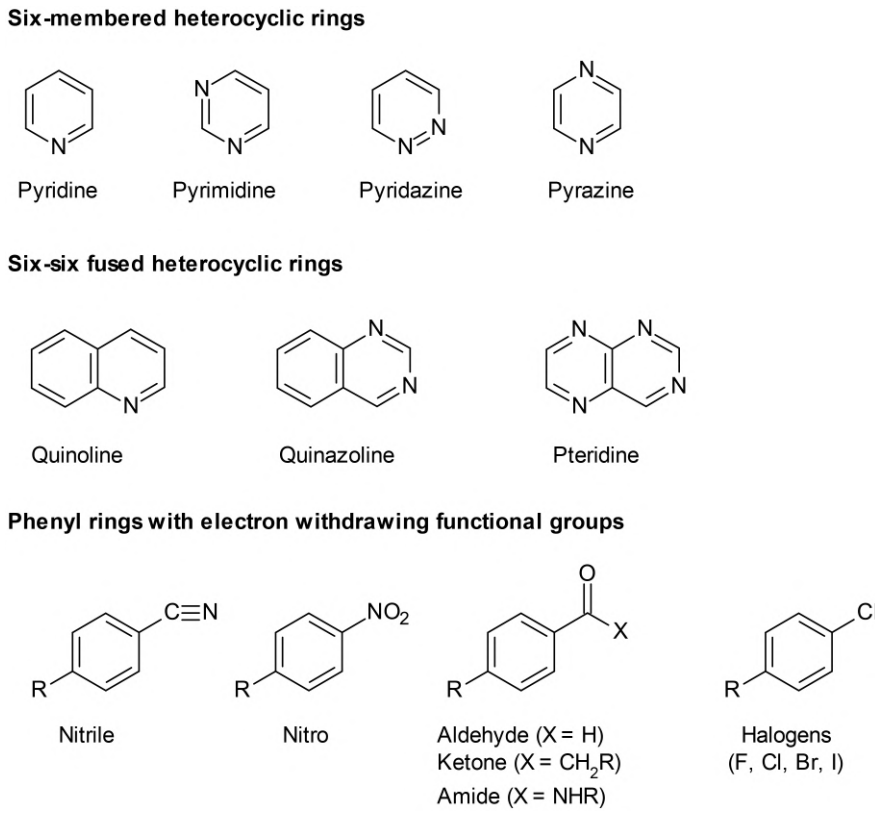


FIGURE 6-25. Examples of electron deficient aromatic rings.

An example of a charge transfer reaction is shown in **Figure 6-26** with nefazodone, an antidepressant drug that acts as an antagonist at serotonin 5-HT_2 postsynaptic receptors. The chloro substituted phenyl ring is electron deficient and could form a charge transfer reaction with an electron rich tryptophan residue within the 5-HT_2 postsynaptic receptor binding site.

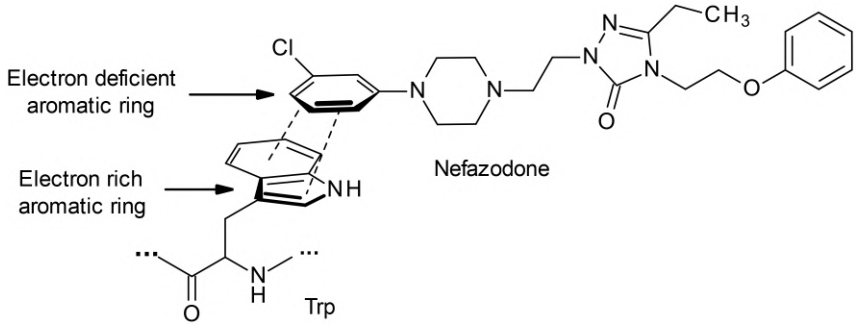


FIGURE 6-26. Example of a charge transfer interaction.

Summary of Biological Targets and Drug Binding Interactions

As mentioned at the beginning of this chapter, the primary biological targets for drug molecules are proteins and nucleic acids. Throughout this chapter, various examples have been used to emphasize the possible types of binding interactions that can occur between these major biological targets and

the functional groups within the structure of a drug molecule. To aid in future application of these concepts, **Table 6-4** has been developed to provide a summary of the types of binding interactions that are possible for amino acids and nucleic acids.

TABLE 6-4. Summary of Binding Interactions for Amino Acids and Nucleic Acids at Physiologic pH

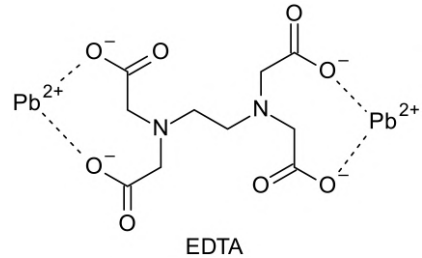
Amino Acid	Side Chain Functional Group[a]	Types of Possible Binding Interactions at pH = 7.4
Aspartic acid and Glutamic Acid	Carboxylic acid (acidic)	Ionic, reinforced ionic, ion-dipole (as the ion)
Asparagine and Glutamine	Amide[b]	Dipole-dipole, hydrogen bond (as either donor or acceptor), ion-dipole (as the dipole)
Lysine	Primary amine (basic)	Ionic, reinforced ionic, ion-dipole (as the ion), cation-π (as the cation)
Arginine	Guanidine (basic)	Ionic, reinforced ionic, ion-dipole (as the ion), cation-π (as the cation)
Histidine	Imidazole ring (basic)	***If ionized:*** Ionic, reinforced ionic, ion-dipole (as the ion), cation-π (as the cation) ***If unioinized:*** Dipole-dipole, hydrogen bond (as either donor or acceptor), ion-dipole (as the dipole)
Serine and Threonine	Primary hydroxyl	Dipole-dipole, hydrogen bond (as either donor or acceptor), ion-dipole (as the dipole)
Tyrosine	Phenolic aromatic ring	***Phenol (aromatic hydroxyl):*** Dipole-dipole, hydrogen bond (as either donor or acceptor), ion-dipole (as the dipole) ***Aromatic Ring:*** van der Waals, hydrophobic, π–π stacking, charge transfer (electron rich), cation-π (as the π -cloud)
Phenylalanine	Aromatic ring	van der Waals, hydrophobic, π–π stacking, cation-π (as the π -cloud)
Tryptophan	Aromatic ring (indole)	van der Waals, hydrophobic, π–π stacking, charge transfer (electron rich), cation-π (as the π -cloud), hydrogen bond (as the donor)
Cysteine	Sulfhydryl	Dipole-dipole, hydrogen bond (as either donor or acceptor)
Methionine	Thioether	Hydrogen-bond (as an acceptor), van der Waals, hydrophobic
Alanine, Valine, Leucine, Isoleucine, and Proline	Hydrocarbon	van der Waals, hydrophobic
Glycine	Hydrogen atom	None
Nucleic Acid Component	**Functional Group**	**Types of Possible Binding Interactions at pH = 7.4**
Purine and Pyrimidine Rings	Heterocylic, substituted aromatic rings	π–π Stacking, hydrophobic, hydrogen bonds (as either donor or acceptor), dipole-dipole
Sugar (ribose or deoxyribose)	Hydroxyl group, hemiacetal oxygen atom	***Hydroxyl group:*** Hydrogen bonds (as either donor or acceptor), dipole-dipole, ion-dipole (as the dipole) ***Hemiacetal oxygen:*** Same as hydroxyl, but only a hydrogen bond acceptor
Phosphate	Phosphate (acidic)	Ionic, reinforced ionic, ion-dipole (as the ion)

[a]This excludes the carboxylic acid and primary amine present on every amino acid because they would be involved in peptide bonds.

[b]The peptide bond (or amide) could participate in the same types of binding interactions.

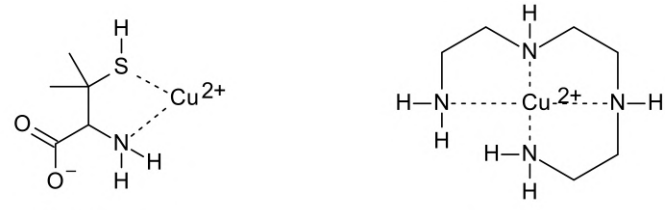
Chelation and Complexation

The final two binding interactions are chelation and complexation. *Chelation* is a process that occurs whenever two distinct electron donating groups, present on the same molecule, bind to a metal ion and form a ring structure. An example of this is shown below with ethylenediaminetetraacetic acid (EDTA), a drug that is used for the treatment of lead toxicity, hypercalcemia, and cardiac glycoside-induced arrhythmias. The negative charges on the adjacent carboxylic acid groups can bind to, or chelate, positively charged metal ions. Shown here is the chelation with lead, but EDTA can also chelate calcium, iron, mercury, and other metals.



EDTA

Chelation therapy is also important in the treatment of Wilson's disease, a genetic disorder resulting in inappropriate copper transport and the accumulation of copper in the liver, brain, kidneys, and eyes. This accumulation leads to tissue damage and prevents the affected organs from functioning normally. Penicillamine and trientine (**Figure 6-27**) can chelate copper, increase its elimination in the urine, and restore copper levels to their normal values. Due to the lack of a sulfhydryl group, trientine has been shown to produce fewer adverse effects than penicillamine. Please note that the chelation of trientine and penicillamine with copper occurs when their respective amine nitrogen atoms are in their unionized form.



Penicillamine chelated with copper

Trientine chelated with copper

FIGURE 6-27. The chelation of copper by penicillamine and trientine.

In addition to treating certain toxicities and elevated levels of specific metal ions, chelation and complexation are important for the mechanisms of action of a select number of individual drugs and classes of drug molecules. Most chelates involve oxygen, nitrogen, and/or sulfur atoms that are present within the functional groups of a drug molecule. Additionally, most chelates form five- or six-membered rings, although as evidenced with EDTA, larger as well as smaller chelates are capable of forming. In general, four-membered rings occur only if sulfur is involved. Let us look at a few examples.

Zileuton, shown in **Figure 6-28A**, is indicated for the treatment of asthma. It acts by inhibiting the enzyme 5-lipoxygenase and thus prevents the formation of leukotrienes, endogenous compounds that are known to contribute to inflammatory responses related to certain disease states. In the initial step of leukotriene formation, 5-lipoxygenase catalyzes the addition of a peroxide group to the C_5 atom of arachidonic acid. This reaction requires both oxygen and an iron atom that is

normally present within the enzyme's active site. Zileuton inhibits 5-lipoxygenase due in part to its ability to chelate iron. A second example is seen in the mechanism of action of the tetracycline class of antibiotics. These drugs inhibit bacterial protein synthesis by binding to the bacterial 30S ribosome and preventing the binding of transfer RNA (tRNA). Resident within the 30S binding site is a magnesium ion that is important for tRNA binding. As shown in **Figure 6-28B**, tetracyclines, by virtue of their β-dicarbonyl group, can form a chelate with this magnesium ion. This interaction is important for their ability to bind to the 30S ribosome and produce their antibacterial effect.

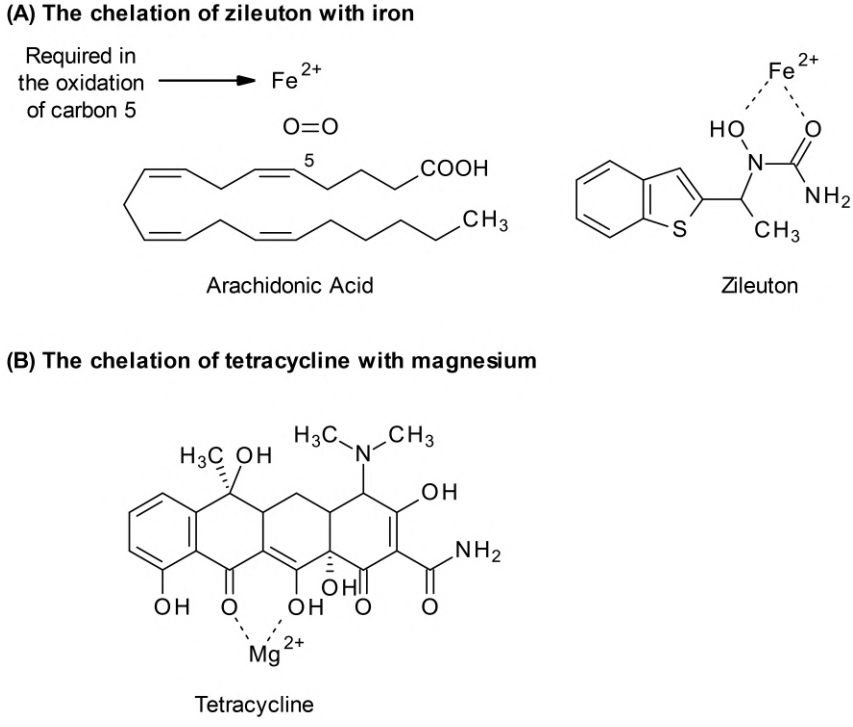
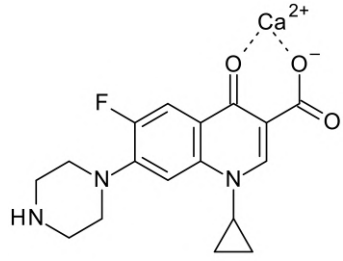


FIGURE 6-28. Additional examples of chelation.

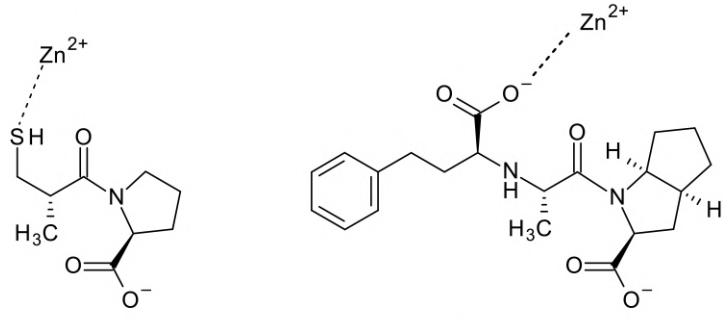
Chelation is also responsible for some specific drug interactions. A good example of this is seen with the tetracyclines. The β-dicarbonyl group discussed above can also form chelates with calcium, magnesium, aluminum, and iron in the GI tract. These chelates have very poor water solubility; hence, their formation significantly decreases the absorption of tetracyclines. Antacids, dairy products, vitamins, and other preparations containing these metal ions can therefore cause drug interactions if taken concurrently with a tetracycline. As such, preparations containing these metals need to be taken at least 1 to 2 hours before or after the administration of a tetracycline. This same drug interaction also occurs with the fluoroquinolone (e.g., ciprofloxacin) class of antibiotics. As shown below, the quinolone oxygen atom and the carboxylic acid of ciprofloxacin can form a chelate with a metal ion. Similar to tetracyclines, products containing aluminum, magnesium, calcium, iron, or

other trace metals need to be administered at least 1 to 2 hours before or after the administration of a fluoroquinolone.



Ciprofloxacin

A related type of bond occurs whenever a metal ion interacts with a single atom. In this case, the metal ion does not form a ring, and the process is known as *metal complexation*. Similar to chelation, this type of interaction is important in the binding of drug molecules to specific enzymes. Angiotensin-converting enzyme (ACE) is a zinc protease that catalyzes the conversion of angiotensin I to angiotensin II. Angiotensin II, when bound to its biological target, has a number of effects on the cardiovascular and renal systems, including an increase in blood pressure, a release of aldosterone, and an increase in sodium reabsorption. Inhibitors of ACE are used to treat hypertension, heart failure, and other cardiovascular disorders. As shown in **Figure 6-29**, a key binding interaction between these drugs and ACE involves the complexation of either a sulfhydryl group or an ionized carboxylic acid with the zinc atom. The term *complexation* is used here because a metal ion is involved; however, the interaction between the zinc ion and sulfhydryl group of captopril could also be designated as an ion-dipole bond whereas the interaction involving the carboxylic acid of ramiprilat could also be designated as an ionic bond.



Captopril Ramiprilat

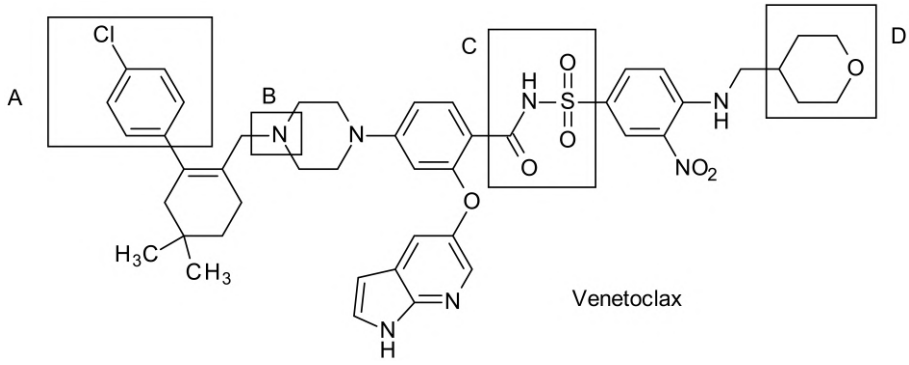
FIGURE 6-29. The complexation of the ACE inhibitors captopril and ramiprilat with a zinc atom present in the enzyme active site.

Summary of Key Concepts for Noncovalent Drug Binding Interactions

- The overall binding strength of a drug to its biological target is a summation of all individual noncovalent interactions.
- The formation of each noncovalent bond is inversely proportional to the distance between the functional groups present on the drug molecule and on the biological target.
  - Steric factors that would prevent the required distance would hinder or prevent drug binding.
- The ionization state of acidic and basic functional groups plays a key role in determining what types of noncovalent bonds can and cannot be formed.
  - Prior to evaluating the types of binding interactions that an acidic or basic functional group can form, it is first necessary to determine if the functional group is primarily ionized or primarily unionized at a pH of 7.4.
- Ionic bonds involve full positive and negative charges, can occur at the longest distance, and often provide the initial recognition between a drug and its biological target.
- Dipole–dipole interactions between functional groups involve partial charge separations between atoms due to their varying electronegativity values.
  - The most common type of dipole–dipole interaction is a hydrogen bond.
  - When identifying a hydrogen bond, it is important to indicate which functional group is the donor and which functional group is the acceptor.
- Ion–dipole interactions occur between a functional group with a full charge and a functional group with a partial charge.
  - When identifying an ion–dipole interaction, it is important to indicate which functional group has the ionic charge and which functional group has the partial charge.
- Hydrocarbon chains and rings do not have measurable dipoles but can form van der Waals interactions.
  - These interactions include dipole-induced dipole interactions and induced dipole-induced dipole interactions, with the latter being more common.
  - Induced dipole-induced dipole interactions are most likely to occur when the functional groups have minimal steric hindrance, are conformationally flexible, and are located in nonpolar regions.
  - A hydrophobic effect is associated with an induced dipole-induced dipole interaction) and is due to the gain of entropy.
- Aromatic rings and ring systems can participate in π–π stacking interactions, cation–π interactions, and/or charge transfer interactions.
  - When identifying a cation–π interaction, it is important to indicate which functional group has the π electrons and which functional group has the cation.
  - When identifying a charge transfer interaction, it is important to indicate which aromatic system is electron rich and which is electron poor.
- Metal ions can form binding interactions with functional groups on drug molecules through the processes of chelation and complexation.

STRUCTURAL ANALYSIS CHECKPOINT

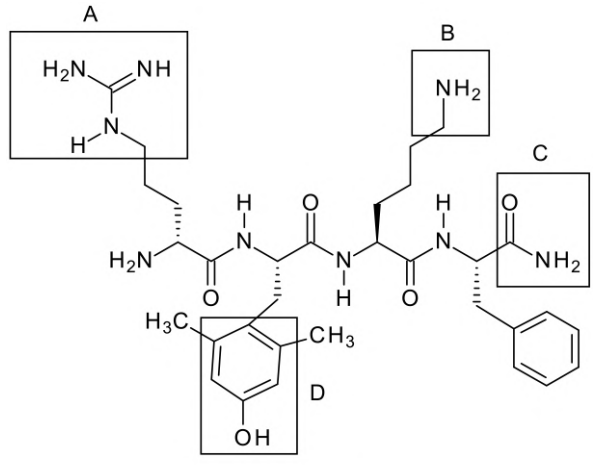
Checkpoint Drug 1: Venetoclax



1. Shown above is the structure of venetoclax. Four of its functional groups have been boxed. For each functional group, complete the following tasks:
   A. Identify all possible drug binding interactions. Assume that these drug interactions are occurring at a physiologic pH of 7.4.
   B. Identify one amino acid that could participate in each of the drug binding interactions you listed in part 1A. Try to identify a wide variety of amino acids rather than using the same one multiple times.
2. If venetoclax interacted with DNA, which of drug interactions you listed in part 1A would be possible?
3. Based on the functional groups present within the structure of venetoclax, is it possible for this drug to form covalent bonds with its biological target? Why or why not?

Checkpoint Drug 2: Elamipretide

1. Four of the functional groups within the structure of elamipretide have been boxed. Complete the following tasks for each of these functional groups and fill in the table provided.
   A. Identify two possible drug binding interactions with an amino acid side chain (as the drug molecule is drawn).
   B. Identify two possible drug binding interactions with an amino acid side chain (in an environment in which pH = 7.4).
   C. List one unique amino acid that could participate in each of the drug binding interactions you identified (in an environment in which pH = 7.4).



Elamipretide

	Functional Group Name	Interactions Possible (as Drawn)	Interactions Possible (at pH = 7.4)	Amino Acid Whose Side Chain Can Interact with the Functional Group at pH = 7.4
A		1.	1.	1.
		2.	2.	2.
B		1.	1.	1.
		2.	2.	2.
C		1.	1.	1.
		2.	2.	2.
D		1.	1.	1.
		2.	2.	2.

2. Which functional groups (A–D) can participate in the following types of interactions (at pH = 7.4)? List all correct answers.

   A. Cation-π

   B. Ionic

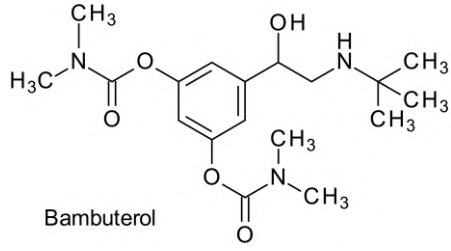
   C. Chelation

   D. van der Waals

   E. Ion-dipole (as the dipole)

REVIEW QUESTIONS

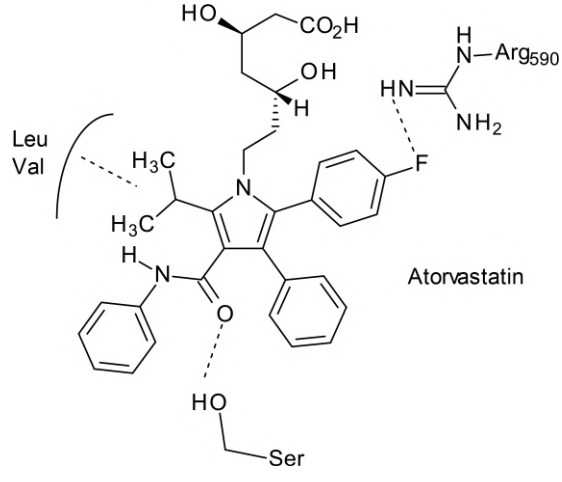
1. Bambuterol is a β_2 receptor agonist that is a long-acting bronchodilator. Evaluate the structure below to determine what type of interactions might be possible between this drug molecule and its biological target. As a reminder, a review of amino acids can be found in Chapter 2 and Table 6-4.



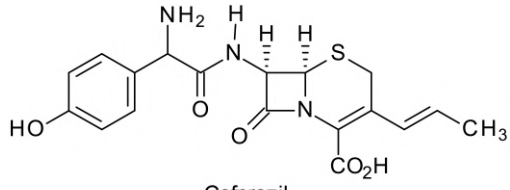
Name of Functional Group	Acidic, Basic, or Neutral (as Drawn)	Ionized, Unionized or not Ionizable (at pH = 7.4)	Hydrogen Bond Acceptor, Donor, Both, or Neither (at pH = 7.4)	Amino Acids Whose Side Chain Can Interact with the Functional Group via Hydrogen Bonding (at pH = 7.4)[a]

[a]"None" is a possible answer.

2. Consider the molecule of atorvastatin drawn below. The *para* fluoro substituent, the amide, and the isopropyl groups can participate in different types of interactions with the amino acids found in the active site of HMG CoA reductase. Modify the diagram below to show the interactions of these functional groups with the relevant amino acid side chain. Label each interaction type.



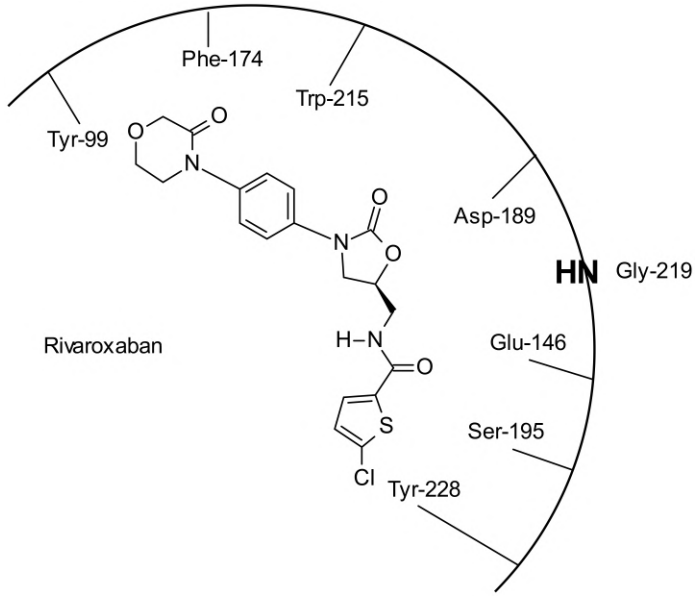
3. Evaluate cefprozil and complete the grid to determine which interactions are possible with the side chain of each of the four amino acids. Be careful to pay attention to the ionization state of each functional group and amino acid side chain in your evaluation.



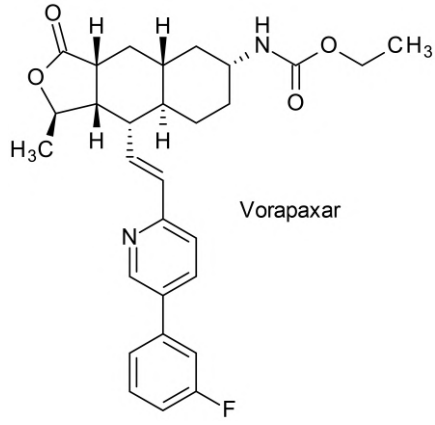
Cefprozil

Name of Functional Group	Acidic, Basic, Neutral (as Drawn)	H-bond Acceptor, Donor, Both, or Neither (at pH = 7.4)	Interaction Possible with Serine (at pH = 7.4)	Interaction Possible with Glutamic Acid (at pH = 7.4)	Interaction Possible with Lysine (at pH = 7.4)	Interaction Possible with Tryptophan (at pH = 7.4)

4. Rivaroxaban is a direct Factor Xa inhibitor. It interacts with several amino acids in several active site pockets. In the S1 pocket, a tyrosine (228) side chain participates in a hydrophobic interaction. In the S4 pocket, a tryptophan (215) side chain participates in a π-π stacking interaction. The backbone NH of glycine (219) participates in a hydrogen bonding interaction. Using the figure below, circle and name the functional groups within rivaroxaban that can participate in these interactions, keeping in mind that you should be selecting functional groups that are in close proximity to the identified amino acids.



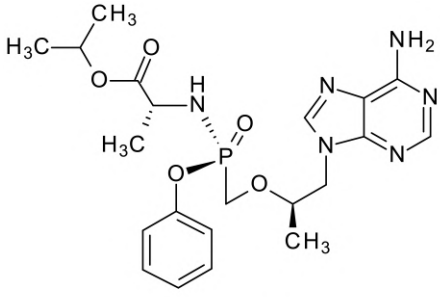
5. Vorapaxar is a PAR-1 receptor (also known as thrombin receptor) antagonist. Conduct a functional group evaluation of this molecule to determine which amino acids may be present in the PAR-1 receptor binding site.



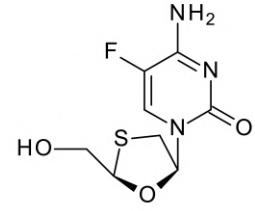
Vorapaxar

Name of Functional Group	Acidic, Basic, or Neutral	Hydrogen Bond Acceptor, Donor, Both, or Neither	Interaction Possible with the PAR-1 (Thrombin) Receptor	Name of One Amino Acid That Can Participate in the Interaction Identified in the Previous Column with the Functional Group (at pH = 7.4)

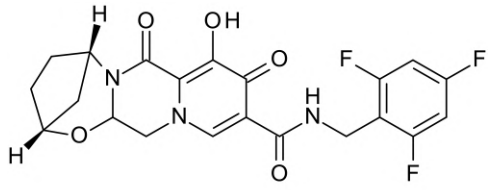
6. Biktarvy® is considered a complete regimen for the treatment of HIV-1 in adult and pediatric patients. This once daily orally administered triple therapy includes bictegravir, emtricitabine, and tenofovir. Each of the components must be dissolved in the aqueous contents of the stomach prior to absorption. For each of the drugs drawn below, choose TWO functional groups that could participate in hydrogen bonding interactions with water. For each interaction, indicate if the functional group is participating as the hydrogen bond donor or acceptor.



Tenofovir



Emtricitabine

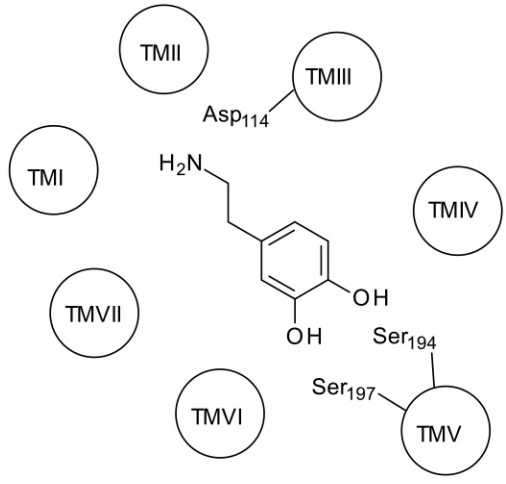


Bictegravir

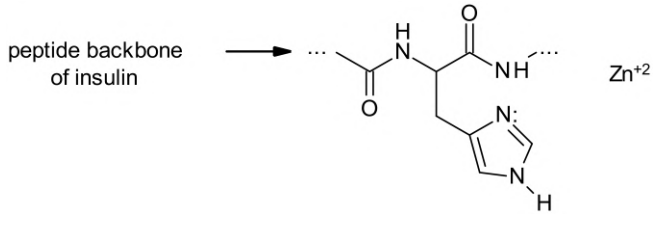
7. Dopamine is a catecholamine that interacts with the D_1 receptor via interactions with three amino acids that are found in two of the seven transmembrane domains that make up the receptor. The key amino acids have been identified in the diagram below. Determine which types of interactions are possible between the following pairs of amino acids and the functional groups found within dopamine:

   A. Asp^{114} and primary amine

   B. Ser^{194} and Ser^{197} and catechol



8. In the presence of zinc, insulin forms a stable, inactive zinc-insulin hexamer. Within this hexamer, a histidine residue found within each of the insulin molecules complexes with a pair of zinc atoms. For the insulin to be biologically active, the hexamer must break up and each monomer must diffuse away from the zinc atoms. Using the structure below, draw a diagram that shows how the side chain of histidine complexes with zinc.



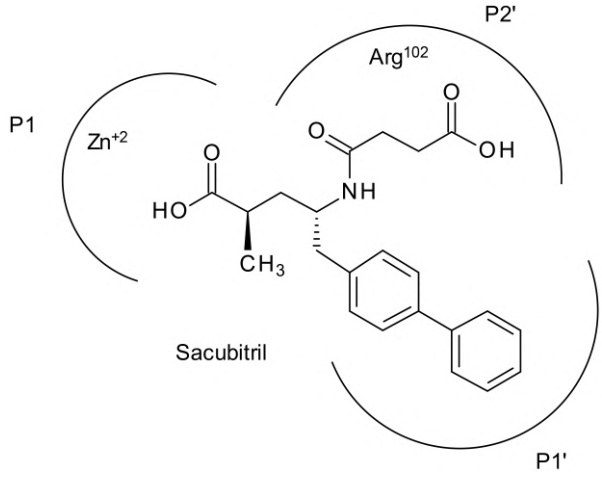
9. Sacubitril is a neprilysin inhibitor used in the management of heart failure. It interacts with the enzyme via three pockets (P1, P1′, and P2′). In the P1 pocket, an active site zinc atom is present; in the P2′ pocket, an active site arginine residue is present.

   A. Determine the ionization state of each eligible functional group within sacubitril at physiologic pH 7.4 as well as with the active site arginine residue.

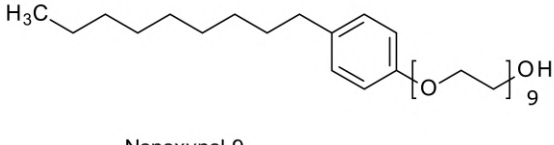
   B. Determine the interactions possible with the functional groups within sacubitril and the zinc atom and the arginine residue.

   C. Circle the functional groups within sacubitril that can participate in ionic interactions with the zinc atom and the arginine residue.

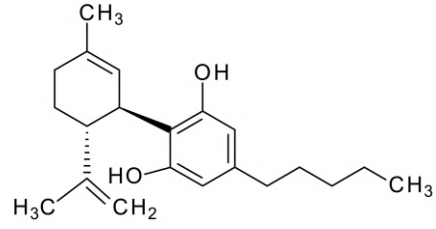
   D. What type of binding interaction do you anticipate occurring in the P1′ pocket? Name three amino acids that could participate in that interaction with the biphenyl functional group positioned in that pocket.



10. Consider the structural features found in nonoxynol-9 and cannabidiol drawn below. Provide a brief rationale for why it is likely that these drugs participate in van der Waals or hydrophobic interactions.



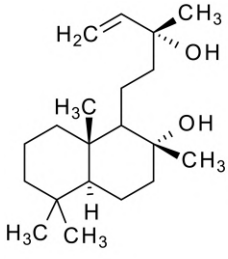
Nonoxynol 9



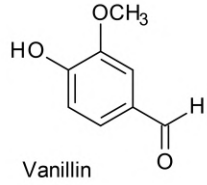
Cannabidiol

11. In the management of peripheral artery disease, the active form of clopidogrel binds irreversibly to the platelet P_2Y_{12} (ADP) receptor. Describe the advantages and disadvantages associated with drugs that covalently bind to their biological target.

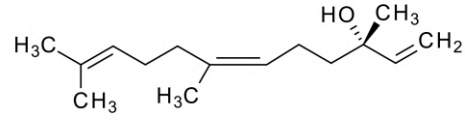
12. Each of the three odorant molecules drawn below produces a unique scent upon interaction with the olfactory receptors. Unlike most biological targets for drug action, olfactory receptors typically have an affinity for a wide variety of odorant molecules and can adopt unique conformations to enhance the affinity of a given odorant for the receptor. Based on the structural features found in each molecule, indicate which functional group(s) can participate in each of the interactions identified.



Sclareol
(herbal scent)



Vanillin



Nerolidol
(green woody scent)

Sclareol		Vanillin		Nerolidol	
Interaction Type	Functional Group	Interaction Type	Functional Group	Interaction Type	Functional Group
van der Waals, hydrophobic		van der Waals, hydrophobic		van der Waals, hydrophobic	
Hydrogen bond (acceptor and donor)		Hydrogen bond (acceptor)		Hydrogen bond (acceptor and donor)	
Ion-dipole (as the dipole)		Hydrogen bond (donor)			
		Ion-dipole (as the dipole)			

STEREOCHEMISTRY AND DRUG ACTION

LEARNING OBJECTIVES

After completing this chapter, students will be able to

- **Identify chiral and prochiral carbon atoms that are present on drug molecules.**
- **Explain the similarities and differences between enantiomeric drugs, including the various ways in which the enantiomers of a drug molecule could affect its pharmacological actions.**
- **Compare and contrast the following stereochemical designations: (+)/(–), *d*/*l*, D/L, and *R*/*S*.**
- **Explain how *R*/*S* designations are assigned and be able to apply this information to a given chiral center.**
- **Explain why one enantiomer would have a greater affinity for a given biological target compared with the other enantiomer.**
- **Identify diastereomers and geometric isomers and explain how these stereoisomers differ from enantiomers.**
- **Explain how conformational isomers differ from configurational isomers.**
- **Draw conformational and configurational isomers of a given drug molecule.**
- **Explain the difference between the active and preferred conformations of a drug molecule and how this affects the ability of drug molecules to bind to their biological targets.**
- **Explain how conformational restriction and flexibility can provide therapeutic advantages.**

Textbooks, drug prescribing information, online drug information sources, and course lecture notes often represent drug molecules as flat, two-dimensional objects, when in fact these drug molecules, as well as the enzymes, receptors, and other biological targets with which they interact, are actually three-dimensional objects. Thus, these overall binding interactions can only occur if there is a complementary spatial or steric orientation of the required functional groups.

This chapter focuses on two major topics areas: stereoisomers, which are also known as configurational isomers, and conformational isomers. *Stereoisomers* occur whenever a molecule possesses a chiral or asymmetric center. *Conformational isomers* can exist in almost all drug molecules and are the result of the rotation of single bonds. The following concepts are discussed in this chapter: the identification and designation of chiral centers, enantiomers, diastereomers, geometric isomers,

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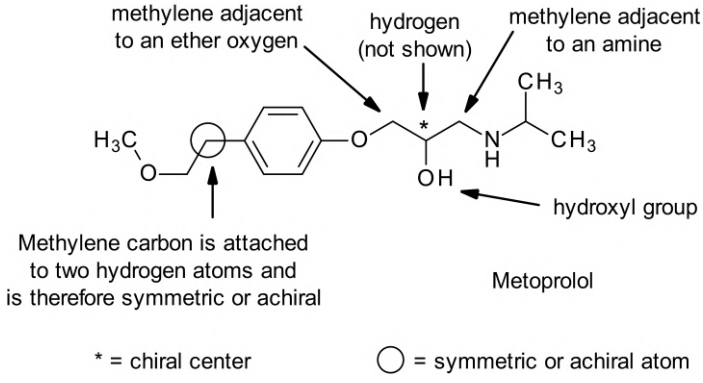
conformational isomers, the influence of stereochemistry on drug action, preferred versus active conformations, and the advantages that conformational restriction or flexibility can impart on drug action.

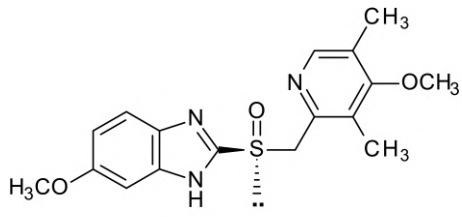
CHIRALITY AND ASYMMETRIC CARBON ATOMS

In a broad sense, the term *chiral* can be used to describe any object that cannot be superimposed with its mirror image. For this to occur, the object must lack a plane of symmetry. The simplest and most commonly seen example of chirality is the human hand. Your right and left hands are nonsymmetrical and mirror images of one another. Regardless of how much you try, it is impossible to superimpose them on one another. Interestingly, the term *chiral* comes from the Greek word for hand, and chiral molecules have been occasionally referred to as having a "handedness."

Similar to the human hand, a chiral drug molecule lacks a plane of symmetry and cannot be superimposed upon its mirror image. The drug molecule must have at least one asymmetric atom that is attached to four different substituents. An example of this is shown below with metoprolol, a selective β_2-adrenergic antagonist. The carbon atom highlighted with an asterisk is attached to a hydrogen atom, a hydroxyl group, a methylene adjacent to an amine, and a methylene adjacent to an ether oxygen. Please note that hydrogen atoms attached to carbon atoms are often not displayed; therefore, if only three bonds are shown, the fourth bond must be to a hydrogen atom. Using this same concept, you should be able to recognize that the circled methylene carbon of metoprolol is attached to two hydrogen atoms and is thus symmetric or achiral (i.e., lacks chirality). Please note that the terms *asymmetric atom* or *center, stereogenic atom* or *center*, and *chiral atom* or *center* are all synonymous.

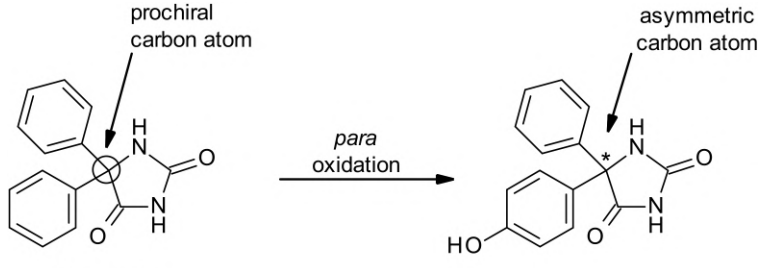


Carbon atoms are, by far, the most common chiral centers present in drug molecules; however, nitrogen, phosphorous, and sulfur atoms can also serve as chiral centers. An example of a sulfur atom serving as a chiral center can be seen below with omeprazole, a proton pump inhibitor that is used to treat a number of gastrointestinal disorders, including gastric ulcers and gastroesophageal reflux disease. The sulfur atom is attached to an oxygen atom, a methylene carbon, and a benzimidazole ring. Due to the valence of the sulfur atom, it also has an unpaired set of electrons. Thus, the spatial orientation of these three bonds plus the pair of nonbonded electrons creates an asymmetric center. Because sulfur, nitrogen, and phosphorus are the exception rather than the rule, the remainder of the chapter focuses solely on chiral, or asymmetric, carbon atoms.



Omeprazole

In Chapter 5, the concept of using a metabolic pathway to convert a water- or lipid-soluble prodrug into its active metabolite was introduced. Metabolic pathways can also transform prochiral carbon atoms into chiral or asymmetric carbon atoms. The term *prochiral* applies to a tetrahedral carbon atom that can be converted to a chiral center by changing only one of its attached groups. An example of this is seen below with phenytoin, an antiepileptic agent that is used to treat a variety of types of seizures. Phenytoin lacks an asymmetric carbon atom; however, the highlighted carbon atom has a prochiral nature. It is attached to a carbonyl carbon atom and a nitrogen atom as well as two unsubstituted phenyl rings. Normal oxidative metabolism of phenytoin introduces a *para* hydroxyl group to one of the unsubstituted phenyl rings and converts the prochiral carbon atom to an asymmetric carbon atom.



Phenytoin

A second example of the prochiral nature of specific symmetrical carbon atoms can be seen with metoprolol (**Figure 7-1**). As previously discussed, metoprolol is a chiral molecule and contains an asymmetric carbon atom. It also contains several symmetrical carbon atoms. Two of these atoms, carbon atoms A and B, can be converted to asymmetric carbon atoms through normal metabolic processes. Carbon atom A is attached to a hydrogen atom, a secondary amine, and two methyl carbon atoms. Oxidation of one of these methyl groups converts carbon atom A to an asymmetric

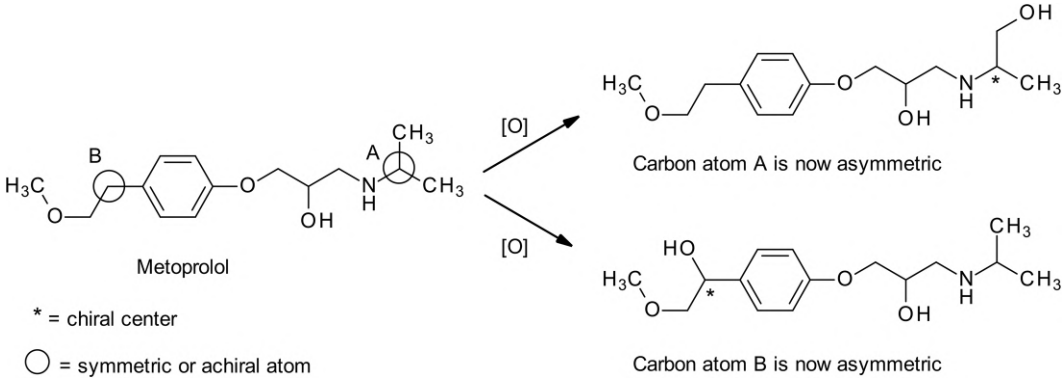
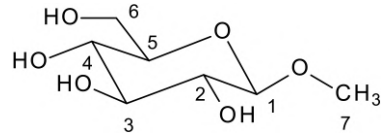


FIGURE 7-1. Metabolic conversion of prochiral carbon atoms to asymmetric chiral centers.

carbon atom because it is now attached to four different substituents. The same is true of carbon atom B. This methylene group is initially attached to two hydrogen atoms; however, oxidation at this carbon atom replaces one of the hydrogen atoms with a hydroxyl group and converts the carbon atom to an asymmetric center. The key point of all of this is that some drug molecules are initially nonchiral but can be transformed in vivo to either active or inactive chiral molecules. Additionally, chiral drugs can undergo metabolism to introduce additional asymmetric centers. Because the steric orientation of complementary functional groups that are present on a drug molecule and its biological target(s) play an important role in drug binding interactions, the conversion of prochiral centers to asymmetric carbon atoms can play a large role in the activity and/or toxicity of a drug metabolite.

A key skill to master is the ability to identify asymmetric, or chiral, carbon atoms. In many cases, such as that seen with metoprolol, the chiral center is attached to four separate functional groups, making identification relatively easy. Other situations require a more systematic evaluation of similar functional groups to discern if they are identical or different. To this end, let's evaluate each of the carbon atoms present in β-methyl-D-glucose.



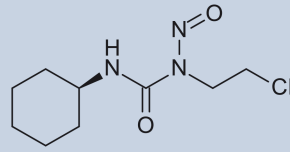
- ***Carbon atom 1*** is attached to four different substituents: an oxygen atom involved in a glycosidic bond with the methyl group (carbon atom 7), an oxygen atom within the ring, a hydrogen atom, and a carbon atom (carbon atom 2). Please note that even though carbon atom 1 is attached to two oxygen atoms, the oxygen atoms are not identical. Thus, carbon atom 1 is a chiral center.
- ***Carbon atom 2*** is attached to four different substituents: a hydrogen atom, a hydroxyl group, and two nonidentical carbon atoms. To verify that the carbon atoms are nonidentical, please note that when you move clockwise around the ring, you encounter three carbon atoms before the oxygen. When you move counterclockwise, you only encounter one carbon atom before the oxygen. There are other differences as well; however, you need to find only one difference to conclude that the carbon atoms are not identical. Thus, carbon atom 2 is a chiral center.
- ***Carbon atom 3*** is attached to four different substituents: a hydrogen atom, a hydroxyl group, and two carbon atoms that are not identical. Starting at carbon atom 3, you should notice that carbon atoms 2 and 4 are identical; however, carbon atoms 1 and 5 are different. Carbon atom 1 is attached to two oxygen atoms whereas carbon atom 5 is attached to only one oxygen atom. Because moving clockwise around the ring is different than moving counterclockwise, carbon atom 3 is a chiral center.
- ***Carbon atom 4*** is similar to carbon atom 2. It is attached to a hydrogen atom, a hydroxyl group, and two carbon atoms located at different distances from the ring oxygen. Therefore, carbon atom 4 is a chiral center.
- ***Carbon atom 5*** is attached to four different substituents: a hydrogen atom, an oxygen atom, a hydroxymethyl group, and a carbon atom that is part of the ring. Therefore, carbon atom 5 is a chiral center.
- ***Carbon atom 6*** is a methylene group and is bonded to two hydrogen atoms. Therefore, it is not a chiral center.
- ***Carbon atom 7*** is part of a methyl group that contains three hydrogen atoms and is thus not a chiral center.

In summary, β-methyl-D-glucose contains five chiral centers.

Application Question

Shown below is the structure of lomustine, an alkylating agent used for the treatment of Hodgkin's disease.

Question: Does this drug contain a chiral carbon atom?



Lomustine

Answer: No, it does not. Lomustine contains two methylene carbon atoms present in the chloroethyl chain on the right. As previously discussed, methylene carbon atoms are attached to two hydrogen atoms and are thus symmetrical and achiral. The cyclohexane ring similarly has five methylene carbon atoms as well as a carbon atom attached to a urea nitrogen atom. Despite the wedged bond, this carbon atom is not a chiral center. The cyclohexane ring contains a plane of symmetry. Regardless of whether you move clockwise or counterclockwise around the ring, the atoms are exactly the same. Thus, this carbon atom is not attached to four different substituents and is not a chiral center. This example, along with that of β-methyl-D-glucose, illustrates a key point. Aliphatic rings with multiple substituents often have multiple chiral centers whereas monosubstituted aliphatic rings are often achiral. A systematic analysis of each carbon atom allows for the correct identification of chiral centers.

ENANTIOMERS

Enantiomers, as well as diastereomers and geometric isomers, can be classified as *stereoisomers* or *configurational isomers*. Stereoisomers, or configurational isomers, are compounds that have the same molecular formula and differ only in the stereochemical arrangement of functional groups attached to one or more atoms. These types of isomers cannot be interconverted without the breaking and reforming of specific bonds.

Enantiomers, or an enantiomeric pair of compounds, are nonsuperimposable mirror images of one another. To meet these criteria, enantiomers must contain at least one chiral center as part of their structure. Let's look at two examples. As shown in **Figure 7-2**, warfarin contains a chiral center and can exist in one of two enantiomeric forms. The *R* and *S* designations are discussed later in this chapter. Please note that these stereoisomers are mirror images. When the *S* enantiomer of warfarin is flipped 180°, it cannot be superimposed on the *R* enantiomer due to the chiral center. The phenyl ring and the bicyclic coumarin ring can be superimposed but the ketopropyl chain cannot.

In contrast to warfarin, irbesartan, shown in **Figure 7-3**, does not contain a chiral center. Although it is possible to draw the mirror image of irbesartan, this does not represent its enantiomer. When irbesartan "B" is flipped 180°, it is completely superimposable with irbesartan "A." *The key point is that enantiomers must meet both criteria, being mirror images of one another and not superimposable.*

While enantiomers must contain at least one chiral center, there is no upper limit to the number of chiral centers that enantiomers can contain within their structures. To illustrate this, consider enalaprilat, an angiotensin-converting enzyme inhibitor used to treat hypertension, heart failure, and other cardiovascular disorders. As shown at the top left of **Figure 7-4**, enalaprilat contains three chiral centers. The mirror image of enalaprilat is shown on the right. Similar to what was done in the

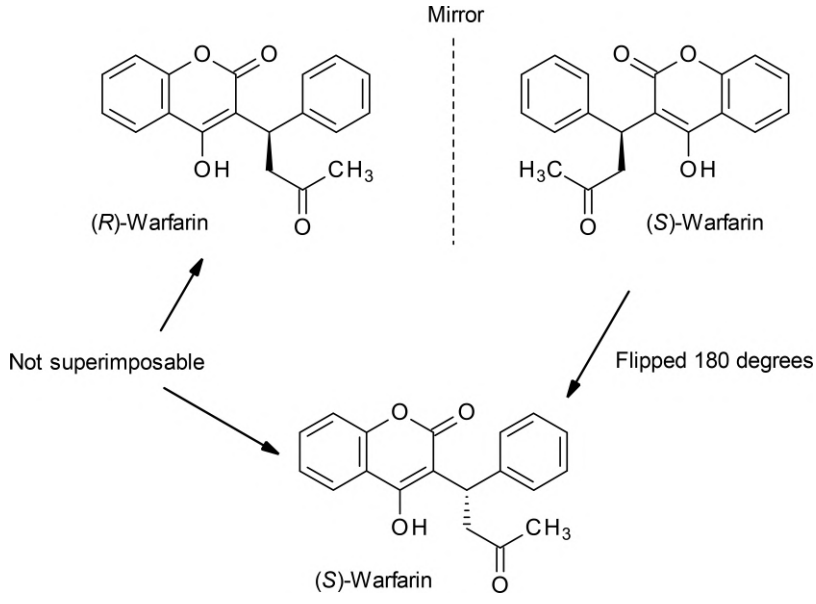


FIGURE 7-2. **The *R* and *S* enantiomers of warfarin.**

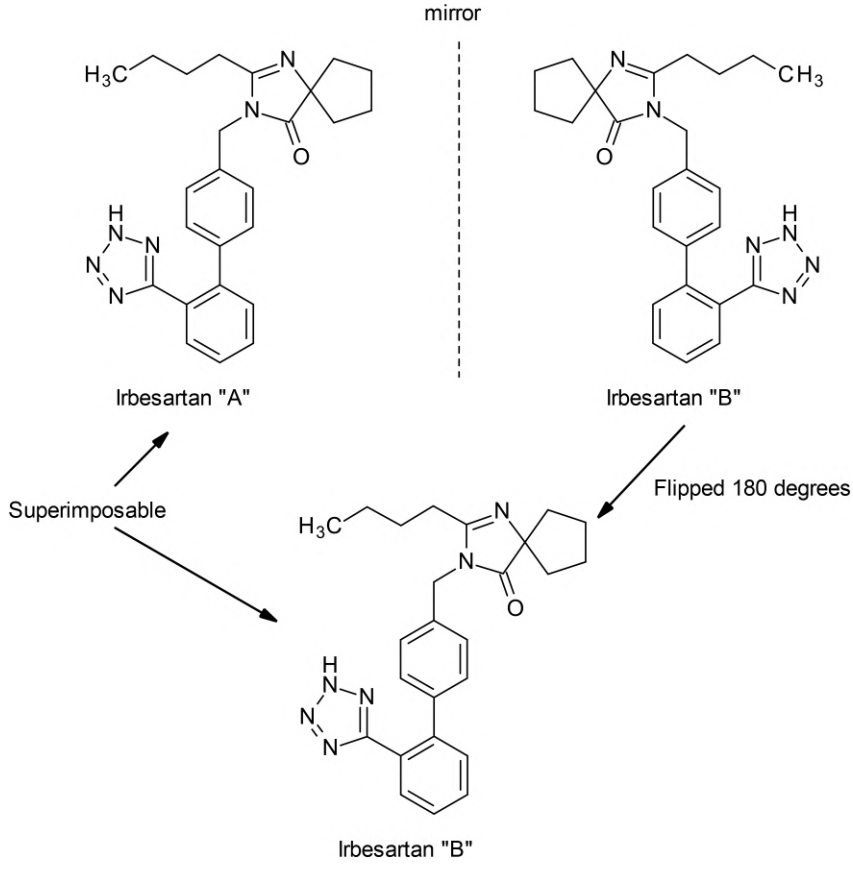


FIGURE 7-3. **Mirror images of irbesartan.**

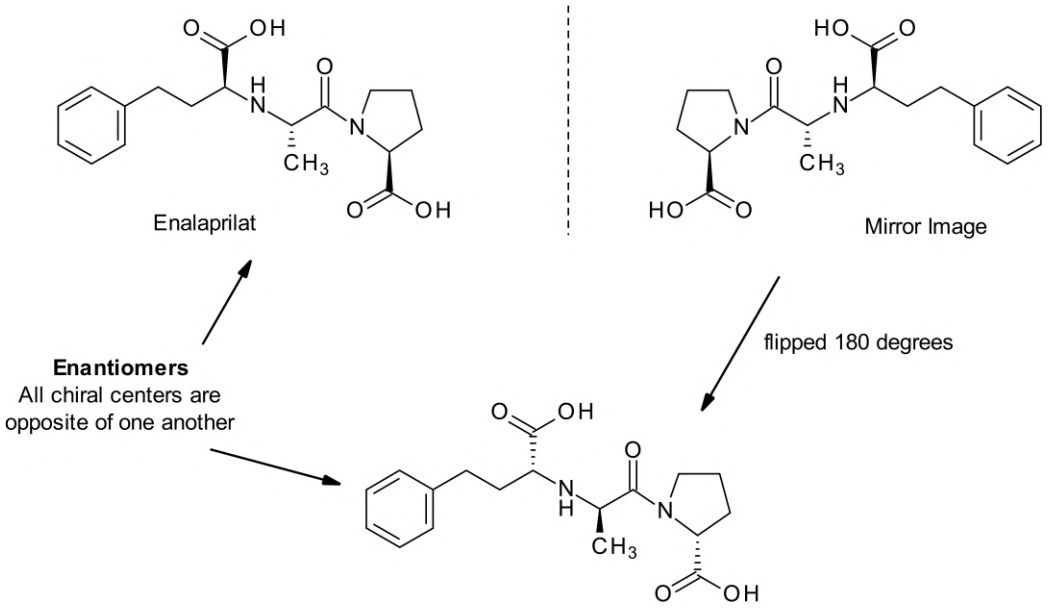


FIGURE 7-4. Enalaprilat and its enantiomer.

previous examples, flipping this mirror image 180° clearly shows that the orientations of all chiral centers are opposite to one another. As such, this mirror image cannot be superimposed on enalaprilat and is indeed its enantiomer.

This example serves to illustrate two key points:

1. First, regardless of the number of chiral centers present in a molecule, enantiomers must possess the opposite stereochemistry at every chiral center.
2. Second, while enantiomers can be drawn as their mirror images, this generally is not the norm. Most enantiomers are represented by simply changing the stereochemistry at each chiral center. The mirror images were used in the above examples to visually illustrate that the compounds either met or failed the required criteria.

Test-taking tip: If you are ever given a drug molecule and asked to draw its enantiomer, it is much easier to simply alter the stereochemistry at each chiral center than to try to draw the mirror image.

Chemical and Physical Properties

With one exception, the chemical and physical properties of enantiomers are identical. Therefore, the *R* and *S* enantiomers of warfarin that were previously discussed have identical molecular weights, IR and NMR spectral properties, log *P* values, water/lipid solubility balance, dissolution rates, pK_a values of the β-dicarbonyl group, and percent ionization at any given pH value. They differ solely in the direction, but not the magnitude, in which they rotate plane polarized light. As such, they are also known as *optical isomers*.

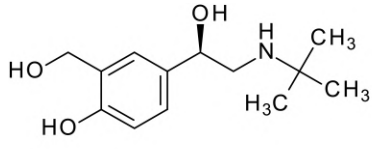
From a pharmacy perspective, the ability to rotate plane polarized light to the right or to the left is irrelevant. It does not confer any specific chemical, pharmacological, or therapeutic advantage to drug action; however, it does serve to distinguish one enantiomer from another. The enantiomer that rotates plane polarized light clockwise, or to the right, is known as the (+) *isomer*, and the enantiomer that rotates plane polarized light counterclockwise, or to the left, is known as the (–) *isomer*. Each enantiomer rotates plane polarized light to the same magnitude or extent. For example, if the

(+) isomer of a drug molecule rotates plane polarized light 15° to the right, the (–) isomer will rotate the light 15° to the left. Some drug molecules are marketed as a single, pure enantiomer whereas others are marketed as a racemic mixture: a 50:50 mixture of both enantiomers. Racemic mixtures do not have any net effect on the rotation of plane polarized light because one half of the molecules rotates the light in one direction and the other half rotates the light to the exact same extent in the other direction.

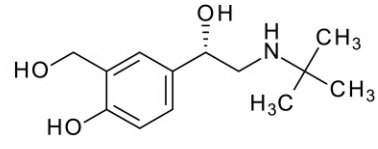
Designations for Enantiomers and Chiral Centers

Four stereochemical designations can be used to describe enantiomers: (+)/(–), *d/l*, D/L, and *R/S*. The (+)/(–) and *d/l* designations are similar in that they identify only the direction in which the enantiomer rotates plane polarized light. The *d* designation is an abbreviation for dextrorotatory and, similar to the (+) designation, indicates that the enantiomer rotates plane polarized light to the right, or clockwise. The *l* designation is an abbreviation for levorotatory and, similar to the (–) designation, indicates that the enantiomer rotates plane polarized light to the left, or counterclockwise. Of the two designations, the (+)/(–) designations are highly preferred because the *d/l* designations can be confused with the D/L designations described below.

Neither the (+)/(–) nor the *d/l* designations provide any information about the actual configuration of a chiral center and by themselves do not provide enough information to discern which structure is responsible for a specific optical rotation. Given only the optical activity and the structures shown below, it is not possible to determine which enantiomer is the most pharmacologically active. The pharmacological activity can be determined only by studies looking at receptor interactions. As an example, let's examine albuterol. In evaluating its structure, it contains one chiral center and can exist as one of two isomers, designated below as enantiomer 1 and enantiomer 2. It is known that the (–), or *l*, isomer of albuterol is primarily responsible for its beneficial bronchodilating effects whereas the (+), or *d*, isomer has little therapeutic activity and may even contribute to undesirable bronchoconstriction. Without any additional information beyond (+)/(–) and *d/l* designations, it is not possible to determine if enantiomer 1 or enantiomer 2 represents the active, (–) isomer of albuterol.

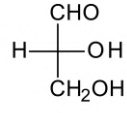


Albuterol: Enantiomer 1

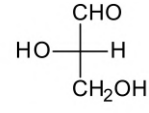


Albuterol: Enantiomer 2

In contrast to the (+)/(–) and *d/l* designations, the D/L designations, as well as the *R/S* designations that follow, refer to the absolute configuration, or steric arrangement, of the atoms about a given chiral carbon. The D/L designations trace back to the work of Hermann Emil Fischer in the late 19th century. Using the enantiomers of glyceraldehyde, Fischer assigned the D designation to the (+) enantiomer and the L designation to the (–) enantiomer.



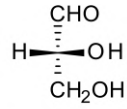
D-(+)-Glyceraledhyde



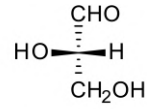
L-(-)-Glyceraldehyde

The structures shown above are drawn in what is known as a *Fischer projection*. Fischer used this two-dimensional depiction to more simply represent the three-dimensional nature of the chiral carbon atom. In a Fischer projection, the horizontal bonds are assumed to project toward the viewer

whereas vertical bonds are assumed to project away from the viewer. The actual configurations of both enantiomers are shown below.



D-(+)-Glyceraledhyde

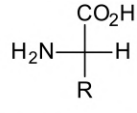


L-(-)-Glyceraldehyde

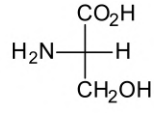
There are two major disadvantages to using the D/L designations. First, to use these designations, it is necessary to chemically convert or correlate the structure back to either D- or L-glyceraldehyde. Situations can arise in which this conversion or correlation becomes ambiguous, resulting in two different correlations and two opposite assignments. Second, if there is more than one chiral center in a molecule, it is possible that one chiral center can be correlated back to D-glyceraldehyde while the other can be correlated back to the L isomer. For the most part, the D/L designations are rarely used outside of amino acids, sugars, and their analogs.

Similar to the fact that the (+)/(–) designations provided no information regarding the configuration of a chiral center, the D/L designations (as well as the *R/S* designations that follow) provide no information regarding the direction of rotation of plane polarized light. In other words, there is absolutely no direct relationship between a (+)/(–) designation and either a D/L or *R/S* designation. To illustrate this key point, let us look at two amino acids. Shown below and on the left is a Fischer projection for the general structure of all naturally occurring L-amino acids. Please note that there is no (+)/(–) designation assigned to the general structure. The structures of serine and alanine shown below have the same configuration of atoms about the chiral carbon atom and are both designated as L. Interestingly, L-serine rotates plane polarized light to the left whereas L-alanine rotates it to the right. Both biomolecules have the same configuration but differ in the direction in which they rotate plane polarized light. *There are three key concepts to remember here.*

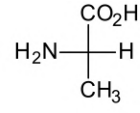
1. The (+)/(–) and *d/l* designations only identify the direction in which the enantiomer rotates plane polarized light.
2. The D/L and *R/S* designations refer to the absolute configuration, or steric arrangement, of the atoms about a given chiral carbon.
3. A complete stereochemical designation requires both a (+)/(–) or *d/l* designation **and** a D/L or *R/S* designation.



General Structure
for L-Amino Acids



L-(-)-Serine



L-(+)-Alanine

In 1966, Robert Cahn, Christopher Ingold, and Vladimir Prelog published an unambiguous procedure to describe individual chiral centers. The Cahn-Ingold-Prelog system (or CIP system) involves the following general rules. First, the atoms or functional groups attached to the chiral center are given priorities according to atomic number and various sequence rules. Second, the molecule is rotated so that the group with the lowest priority is directed away from the viewer. Third, the established priorities are used to determine if the remaining three groups are oriented in a clockwise or a counterclockwise manner, as shown in **Figure 7-5**. The *R* designation, which comes from the Latin word for right, is assigned if the orientation is clockwise whereas the *S* designation, which comes from the Latin word for left, is assigned if the orientation is counterclockwise.

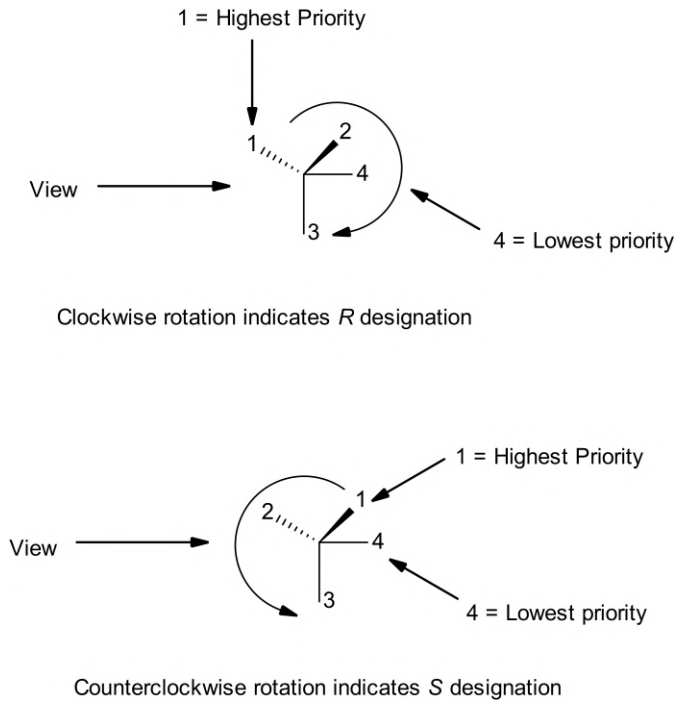


FIGURE 7-5. Assignment of *R* and *S* designations.

The assignment of priorities follows three sequence rules. The rules themselves are somewhat simple; however, based on the complexity of a drug molecule, multiple steps are sometimes required to correctly designate a chiral center.

Sequence rule 1: Prioritize the four atoms attached to the chiral center in terms of their atomic number. Although it is not necessary to memorize the periodic table, it is important to know the relative atomic numbers and priorities of the atoms commonly found on drug molecules. These are shown in **Table 7-1**. There are additional rules for chiral centers that contain different isotopes of the same atom (e.g., hydrogen, deuterium, tritium); however, this is extremely rare in drug molecules and is not discussed here.

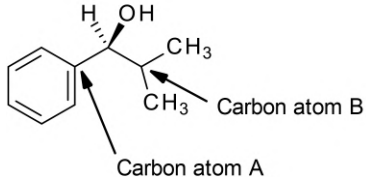
TABLE 7-1. Relative Atomic Numbers and Priorities of Atoms Commonly Found on Drug Molecules

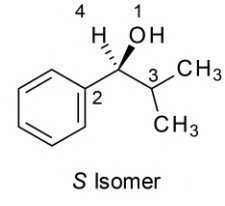
Priority	Atom	Atomic Number
Highest ↕ Lowest	I	53
	Br	35
	Cl	17
	S	16
	P	15
	F	9
	O	8
	N	7
	C	6
	H	1

Sequence rule 2: If the chiral center is attached to two or more atoms that are the same, then sequence rule 1 is applied to the atoms that are the same. This continues until a difference is found among these atoms. As an example, if a chiral center is attached to a chlorine atom, a nitrogen atom, and two carbon atoms, then the chlorine atom would have the first priority, the nitrogen atom would have the second priority, and the two carbon atoms would be tied for the third priority. Using sequence rule 2, these two "tied" carbon atoms would be examined. If one of these carbon atoms was part of a methyl group and the other was part of an alkyl chain, then the carbon that was part of the alkyl chain would have a higher priority (attached to C,H,H) than the methyl group (attached to H,H,H).

Sequence rule 3: When a double or triple bond is encountered, then the atoms are duplicated or triplicated, respectively, when looking at priority.

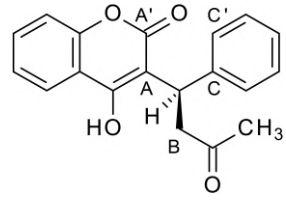
Using these sequence rules, let us first look at a hypothetical example. The compound shown below has a chiral center that is attached to an oxygen atom, two carbon atoms, and a hydrogen atom. According to sequence rule 1, oxygen has the highest priority and hydrogen has the lowest priority. The carbon atoms are initially "tied," so we need to invoke sequence rule 2. Carbon atom A is part of an aromatic ring. As drawn, it has a double bond to one carbon atom and a single bond to another carbon atom. Using sequence rule 3, the carbon atom involved in the double bond is duplicated, so carbon atom A is designated as being attached to three additional carbon atoms. Carbon atom B is part of an isopropyl alkyl chain. It is attached to two carbon atoms and one hydrogen atom. Carbon atom A (C,C,C) therefore has priority over carbon atom B (C,C,H). Thus, the overall priority for this molecule is oxygen atom > carbon atom A > carbon atom B > hydrogen atom. Note that the hydrogen atom is projected into the paper and thus located away from the viewer. Applying the previously mentioned priorities, it is seen that the other three atoms are oriented in a counterclockwise manner; thus, the correct designation for this chiral center is *S*.





S Isomer

The *R* and *S* isomers of warfarin were previously shown in Figure 7-2. As a second example, let us further examine the *R* isomer of warfarin by applying the above-mentioned sequence rules to verify that this designation is correct.



Using sequence rule 1, it is seen that the chiral carbon in the above molecule is attached to a hydrogen atom and three carbon atoms. The hydrogen atom thus has the lowest priority. Using sequence rule 2, we now need to examine carbon atoms A to C. Carbon atom A is attached to two carbon atoms, one by a single bond and one by a double bond. According to sequence rule 3, the double bond counts twice, so carbon atom A is attached to three carbon atoms (C,C,C). Carbon atom B is attached to two hydrogen atoms (not shown) and a carbon atom (C,H,H). Carbon atom C is similar to carbon atom A because it is attached to one carbon atom by a single bond and to another by a double bond (C,C,C). At this point, carbon atom B can be designated as having a lower priority than either carbon atoms A or C. To resolve the priority between carbon atoms A and C, we need to move to the next adjacent carbon atoms. These have been identified above as carbon

atoms A′ and C′. Carbon atom A′ is attached to two oxygen atoms, again one by a single bond and the other by a double bond. Carbon atom C′ is attached to a carbon atom by a double bond and to a hydrogen atom. Thus, carbon atom A′ (O,O,O) has priority over carbon atom C′ (C,C,H), and the side chain emanating from carbon atom A has priority over that emanating from carbon atom C. The final priority is carbon atom A > carbon atom C > carbon atom B > hydrogen. Similar to the previous example, the hydrogen atom is projected into the paper and thus away from the viewer. Using the established priority, it is seen that the other three atoms are oriented in a clockwise manner, thus verifying that this is the *R* enantiomer of warfarin.

Summary of Key Points Regarding Stereochemical Designations

- For drug molecules, the *R/S* or D/L designations are much more important than the (+)/(–) designations because the former provides information regarding the configuration or steric arrangements of atoms about a chiral center. For this reason, the (+)/(–) designations are often omitted when a single pure enantiomer is discussed.
- The *R/S* and D/L designations provide absolutely no information regarding what direction an enantiomer will rotate plane polarized light. Similarly, the (+)/(–) designations provide absolutely no information regarding the configuration of atoms about a chiral center.
- A pure enantiomer, regardless of how many chiral centers it contains, only has one (+)/(–) designation that designates the net rotation of plane polarized light.
- Sugars, amino acids, and their analogs still use the D/L system to designate a ***specific chiral center***. Regardless of how many chiral centers are present, a pure enantiomer would have only one D/L designation. As an example, naturally occurring glucose molecules have four chiral centers but are designated as D-glucose.
- The *R/S* designations unambiguously identify the stereochemical arrangement of atoms about a chiral center. Each *R/S* designation is assigned individually to each chiral center; thus, if a drug molecule had three chiral centers, it would have three *R/S* designations. If one enantiomer had an *R,R,S* designation, the other enantiomer would have an *S,S,R* designation.

Pharmacological and Therapeutic Differences Between Enantiomers

The above discussions focused on the stereochemical arrangement of atoms about a chiral center and the proper designations for these chiral atoms. It has already been noted that enantiomers have identical chemical and physical properties, with the exception of the direction of rotation of plane polarized light, and that this rotation provides no therapeutic advantage or disadvantage. The subsequent discussion focuses on the most important aspect of enantiomers: *their ability to bind and interact with their three-dimensional biological targets*. Specifically, due to the different stereochemical arrangement of atoms, enantiomers differ in their abilities to bind to protein receptors, DNA, enzyme sites, transport proteins, and other biological targets.

From a pharmacological and/or therapeutic perspective, enantiomers can be divided into four groups based on their potency and activity.

Group 1: These enantiomers have identical potency and pharmacological action; there is really no significant pharmacological or therapeutic difference between the two enantiomers. This situation is extremely rare.

Group 2: These enantiomers have similar activities but different potencies. This is very common among drug molecules in which one enantiomer is primarily responsible for the pharmacological activity and the other enantiomer is less active. An example of this is seen with

warfarin, a drug previously discussed in this chapter. The *S* enantiomer of warfarin is three to five times more potent as an anticoagulant than is the *R* enantiomer.

Group 3: These enantiomers have different pharmacological properties. This is a common occurrence and can be further subdivided. First, some enantiomers produce different effects, but the different effects work together for a beneficial response. An example of this is seen with methacholine (**Figure 7-6**), an acetylcholine analog used for the diagnosis of bronchial airway hyper-reactivity. The *S* enantiomer is equipotent with acetylcholine, while the *R* enantiomer is 20-fold less active; however, the *R* enantiomer inhibits acetylcholinesterase and prevents the rapid degradation of the *S* enantiomer. Second, in some situations, one enantiomer produces the desirable effect while the other contributes to adverse effects and toxicity. Additionally, there are situations in which both enantiomers produce the desired effect, but the adverse effect is due primarily to one enantiomer. An example of this latter situation is seen with disopyramide (Figure 7-6). While both enantiomers provide a beneficial antiarrhythmic effect, the *S* enantiomer is responsible for QT wave prolongation and most anticholinergic adverse effects. Finally, one enantiomer may partially counteract the beneficial effects of the other. An example of this is seen with albuterol (Figure 7-6). As previously mentioned, the (–) isomer (which is the *R* enantiomer) of albuterol is responsible for the beneficial bronchodilation effects, while the (+) isomer (which is the *S* enantiomer) enhances bronchoconstriction and opposes the beneficial effects of the *R* enantiomer. For this reason, albuterol is available as its pure *R* enantiomer.

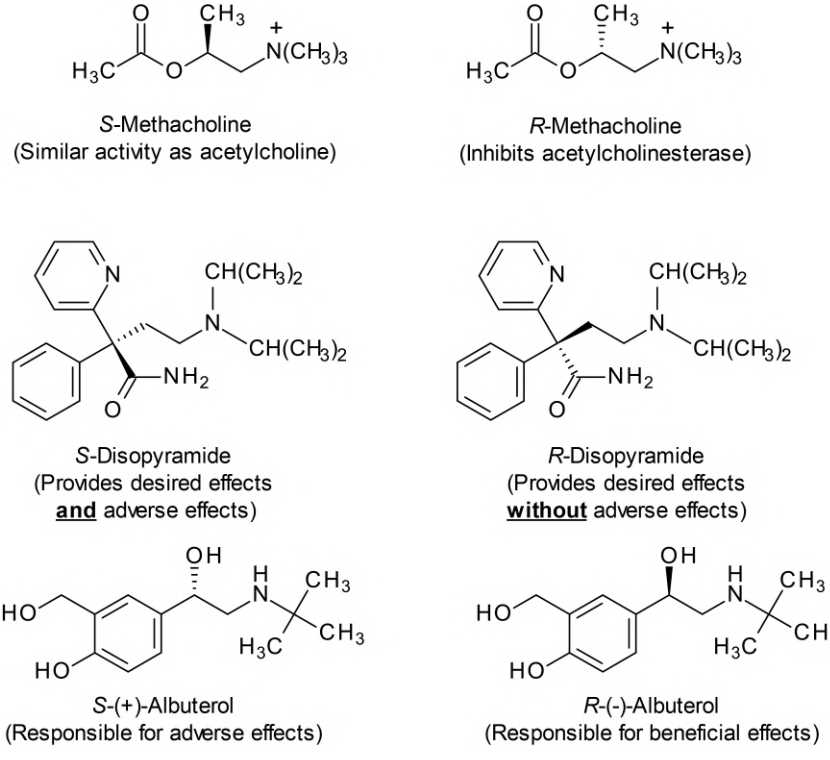
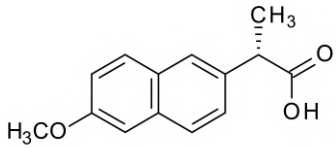


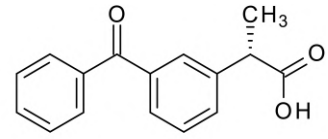
FIGURE 7-6. Examples of enantiomers that have different pharmacological properties.

Group 4: In this group, essentially all of the activity resides in only one enantiomer and the other is simply an inert compound. Similar to group 1, this is uncommon; however, it is seen in some drug classes. Within the α-methyl acetic acid subclass of nonsteroidal anti-inflammatory drugs (NSAIDs), the anti-inflammatory activity resides solely in the *S* enantiomer. Drugs within this subclass include ibuprofen, naproxen, flurbiprofen, and ketoprofen.

Similar to diclofenac and fenoprofen (discussed in Chapter 2), these NSAIDs nonselectively inhibit cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes.



S-Naproxen



S-Ketoprofen

The differences in potency and activity seen with the above examples are directly related to the relative abilities of the enantiomers to bind to their biological targets. The enantiomer that is able to form more interactions with its biological target is predicted to have enhanced activity compared with the enantiomer that is not able to form all of these interactions. A good example of this concept is seen in the work of Leslie Easson and Edgar Stedman. The *R*-(–) enantiomer of epinephrine was known to have a higher affinity for adrenergic receptors than the *S*-(+) enantiomer. In 1933, Easson and Stedman put forth a simple hypothesis that states that the differences in the activity of these two stereoisomers are due to differences in their receptor binding. As shown in **Figure 7-7**,

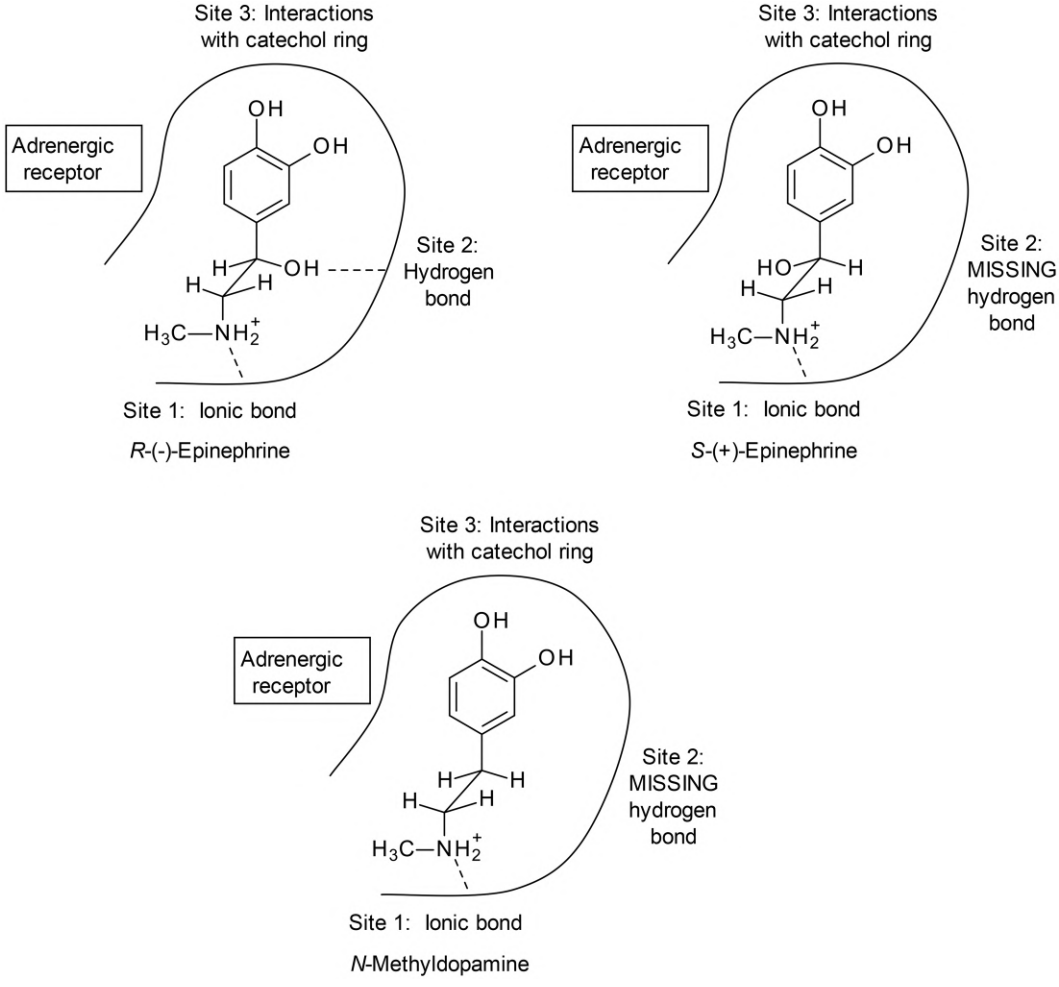


FIGURE 7-7. A comparison of the binding of *R*-(–)-epinephrine, *S*-(+)-epinephrine, and *N*-methyldopamine to the adrenergic receptor.

R-(–)-epinephrine is able to make three key binding interactions with the adrenergic receptor. The positively charged amine can form an ionic bond, the β-hydroxyl group can form hydrogen bonds, and the catechol ring can form several interactions based on the aromatic hydroxyl groups and the aromatic ring. In contrast, *S*-(+)-epinephrine can only form two key binding interactions with the adrenergic receptor. Due to the stereochemistry of the chiral center, the β-hydroxyl group is oriented away from the required binding region and does not participate in the drug-receptor binding interaction. As such, the *S* enantiomer can still interact with the receptor but provides less than 10% of the activity of the *R* enantiomer. To ensure that this loss of activity was due to this missing binding interaction, Easson and Stedman also examined *N*-methyldopamine (i.e., the desoxy analog of epinephrine). This desoxy analog has the same two binding interactions as *S*-(+)-epinephrine but lacks the binding interaction of a β-hydroxyl group. It was found to have an activity similar to that of *S*-(+)-epinephrine, thus supporting the hypothesis. The key point in this example is that *the chiral center of the most active enantiomer orients the functional groups in such a manner that the maximum number of binding interactions can be achieved*.

Steric hindrance can also play a key role in the activity of enantiomers. Any given biological target has a finite amount of space that is available for drugs to bind. Some portions of a biological target may tolerate only small groups while other portions may be able to accommodate larger groups. The orientation of small and large functional groups at a chiral center may dictate which has the better fit. The α-methyl acetic acid subclass of NSAIDs provides a good example of this concept. As discussed above, the activity of this subclass of drugs resides solely in the *S* enantiomer. The inability of the *R* enantiomer to produce an anti-inflammatory effect is due to its inability to sterically fit into the enzyme binding pocket of cyclooxygenase enzymes. As shown in **Figure 7-8**, both *S*-flurbiprofen and diclofenac are active NSAIDs whereas *R*-flurbiprofen and the dimethyl analog of flurbiprofen are inactive. Because diclofenac lacks an α-methyl group but still retains anti-inflammatory activity, the differences in the activity of the enantiomers of flurbiprofen cannot be explained

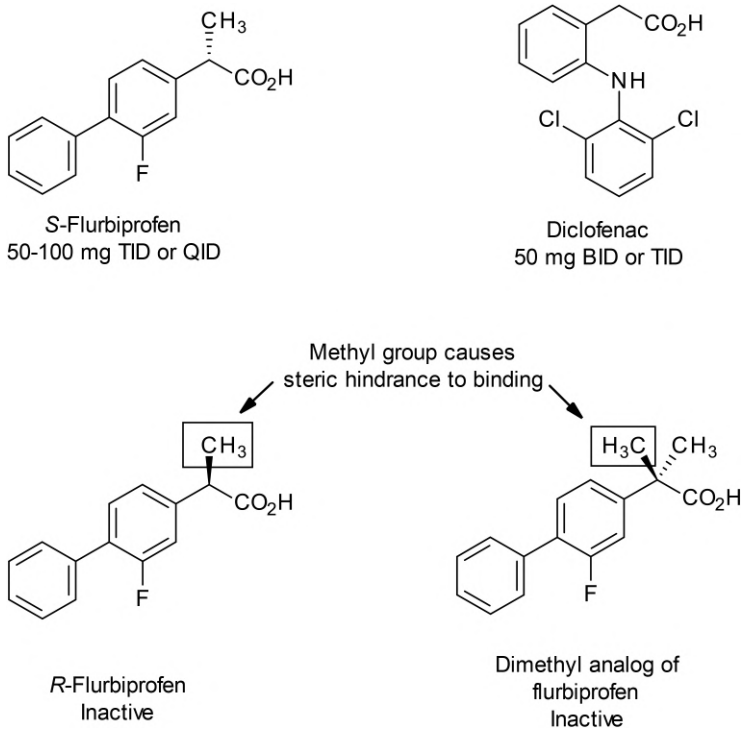


FIGURE 7-8. Structures and activities of α-methyl acetic acids, a subclass of NSAIDs.

by the loss of a binding interaction. Instead, the inactivity of the dimethyl analog substantiates the fact that the inactivity of the *R* enantiomer of flurbiprofen, and other α-methyl acetic acids, is due to steric hindrance. There simply isn't room within the enzyme binding site to accommodate a methyl group that is oriented in that direction.

Similar to the interaction with its desired biological target (e.g., a receptor protein, enzyme, DNA), the ability of an enantiomer to bind to three-dimensional objects also affects its interactions with metabolizing enzymes and transport proteins. Drug metabolism can selectively alter the chemical structure of one enantiomer while leaving the other enantiomer unchanged. The resulting metabolite could be more active or less active or could contribute to unwanted adverse effects. Likewise, active transport processes can selectively enhance the transport of one enantiomer into a cell while leaving the other enantiomer on the outside of the membrane. Again, this could be beneficial (the drug molecule has access to its target cell) or detrimental (the drug molecule is transported into a cell, which leads to an adverse reaction).

DIASTEREOMERS

Diastereomers must contain at least two chiral centers. Similar to enantiomers, there is no upper limit to the number of chiral centers that diastereomers can contain within their structures. Diastereomers are similar to enantiomers in that they are not superimposable but differ in that they are not mirror images. To meet these conditions, at least one chiral center must remain the same and one must have the opposite stereochemical orientation. This is best illustrated by looking at an example. Shown in **Figure 7-9** is Compound A, a hypothetical drug molecule with two chiral centers. If the compound is simply flipped 180°, neither of the chiral centers has actually changed. This is simply a different depiction of Compound A. It is included here as a reminder to thoroughly evaluate chemical structures and chiral centers prior to designating a stereoisomer. If both chiral centers are opposite, as shown on the bottom left, then this is the enantiomer of Compound A; however, if one chiral center remains the same and the other is opposite, as shown on the bottom right, then this is a diastereomer of Compound A.

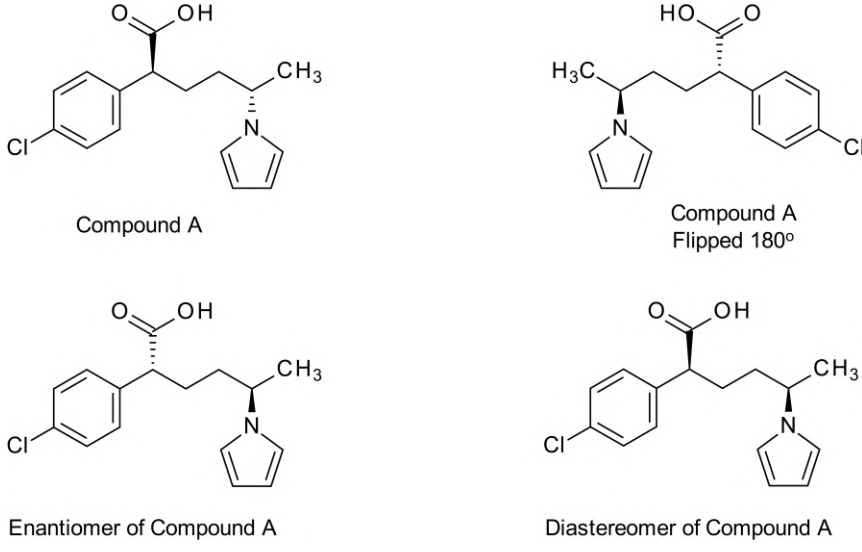
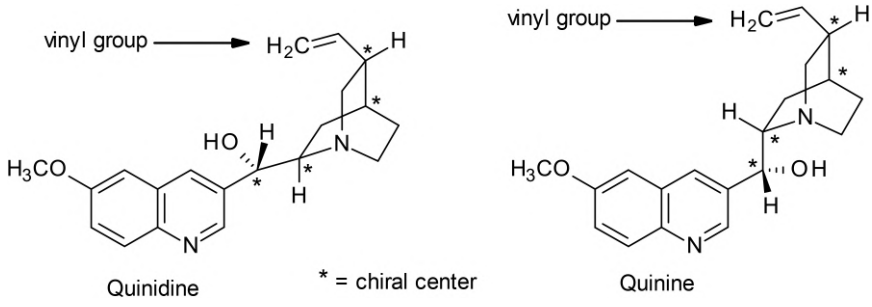


FIGURE 7-9. The enantiomer and a diastereomer of a hypothetical compound.

Study/learning tip: If you are evaluating two structures with multiple chiral centers, evaluate each chiral center separately. If all of the chiral centers are opposite, then the structures are enantiomers. If all of the chiral centers are the same, then the two structures are two different depictions of the same enantiomer. If at least one chiral center is the same and at least one chiral center is different, then the structures are diastereomers.

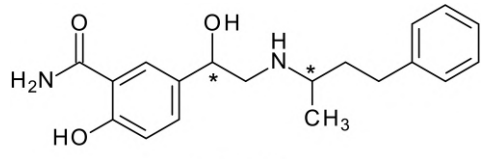
Unlike enantiomers, diastereomers have different physical and chemical properties and often exhibit very different pharmacological properties. As an example, quinidine is an antiarrhythmic agent; however, quinine, a diastereomer with opposite stereochemistry at two of the four chiral centers, is an antimalarial agent (**Figure 7-10**).



Quinidine Quinine

FIGURE 7-10. An example of diastereomers: quinidine and quinine.

Although many enantiomers are marketed as racemic mixtures, it is uncommon for diastereomers to be used in combination with one another. This is primarily because most diastereomers do not produce similar or complementary pharmacological effects. There are always exceptions, one of which is seen with labetalol, a mixed α-/β-adrenergic blocking agent. As shown below, labetalol has two chiral centers. The *R*(OH),*R*(CH_3) diastereomer blocks the β-adrenergic receptor, whereas the *R*(OH),*S*(CH_3) diastereomer blocks the α_1-adrenergic receptor. Both actions are beneficial in treating hypertension and other cardiovascular disorders. The respective *S,S* and *S,R* enantiomers are inactive. Again, this is a rare situation in which diastereomers are used in combination to produce a beneficial effect.



Labetalol
(chiral centers are indicated)

Geometric Isomers

Geometric isomers are similar to diastereomers in that they are neither superimposable nor mirror images of one another, have different physical and chemical properties, and normally have different pharmacological actions. They differ from the above diastereomers in that they result from the restricted rotation about a carbon-carbon bond. There are two types of geometric isomers, those that occur due to the presence of an alicyclic ring and those that occur due to the presence of a double bond.

Shown in **Figure 7-11** are examples of geometric isomers that occur due to the presence of an aliphatic ring. The structures of hypothetical compounds A and B contain a cyclopentyl alicyclic ring

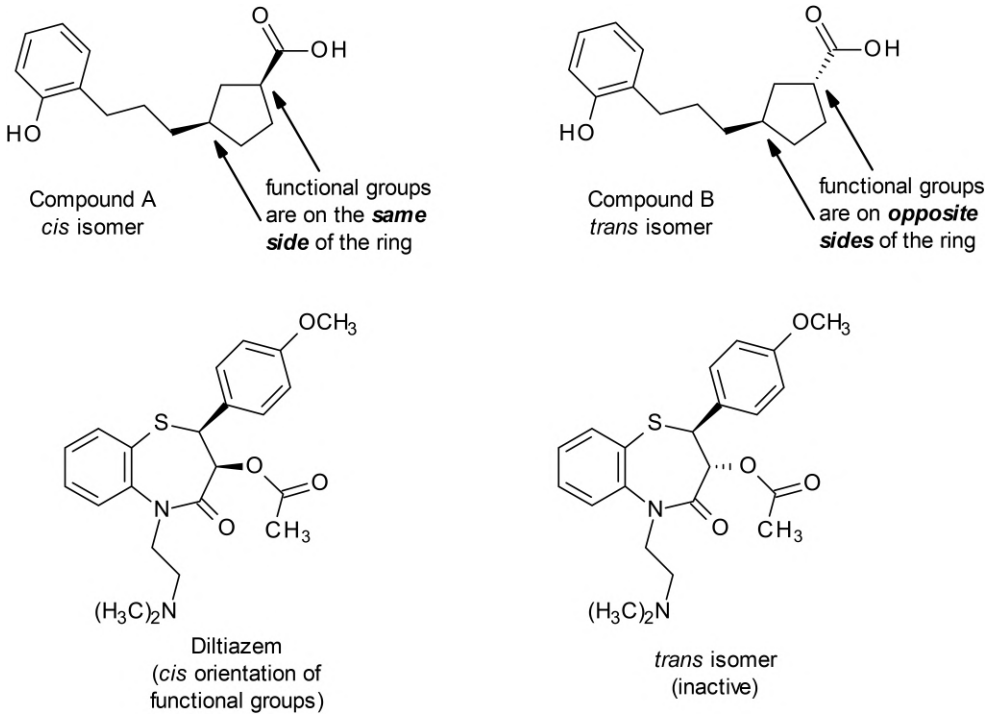
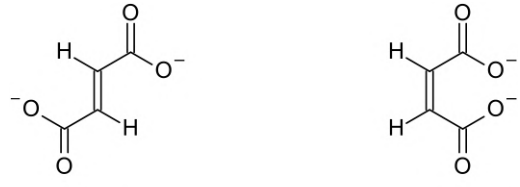


FIGURE 7-11. Examples of alicyclic *cis* and *trans* isomers.

and differ only in the orientation of one of the two chiral centers. The *cis* designation for compound A is used to indicate that both functional groups, relative to one another, are located on the same side of the ring whereas the *trans* designation is used to indicate that both functional groups, relative to one another, are located on different sides of the ring. Also shown in Figure 7-11 is the structure of diltiazem. It contains two chiral centers within its thiazepine ring. Because both the phenyl ring and the ester are on the same side of the ring, diltiazem is the *cis* isomer. The *trans* diastereomer of diltiazem is inactive. Please note that these geometric isomers differ in the orientation of some (but not all) chiral centers and thus can also correctly be designated as diastereomers.

The second type of geometric isomers involves the presence of a double bond. Similar to the above examples, the *cis*/*trans* designations can be used to designate the stereochemistry, especially if a hydrogen atom is present at each end of the double bond. As an example, let us look at fumarate and maleate. In evaluating the structures of these two compounds, it is easy to see that the two carboxylic acids are on opposite sides of the double bond in fumarate and on the same side of the double bond in maleate. Hence, fumarate is the *trans* isomer and maleate is the *cis* isomer.



When the complexity of a drug molecule increases and hydrogen atoms are not present on both ends of the double bond, the *cis*/*trans* designations can become difficult to assign. In these instances, *E*/*Z* designations are used. Similar to the *R*/*S* designations for enantiomers, the *E*/*Z* designations use

the CIP system to unambiguously assign the stereochemical designations. The *E* designation is used when the groups with the highest priority according to the CIP system are on the opposite sides of the double bond, and the *Z* designation is used when the groups with the highest priority are on the same side of the double bond. The *E* and *Z* designations come from the German words *entgegen* and *zusammen,* which mean "opposite" and "together," respectively. Using this system, fumarate can be designated as the *E* isomer and maleate can be designated as the *Z* isomer.

Figure 7-12 shows two examples of drug molecules that use the *E*/*Z* designations. Tamoxifen, an antiestrogen used to treat breast cancer, contains a double bond with a *Z* configuration. Let's verify this assignment. In evaluating the structure, it is seen that carbon atom A is attached to a substituted phenyl ring and an unsubstituted phenyl ring. According to the CIP system, the substituted phenyl ring has priority (designated as 1) over an unsubstituted phenyl ring (designated as 2). Carbon atom B is attached to a phenyl ring and an ethyl side chain. According to the CIP system, the phenyl ring has priority (designated as 1′) over the ethyl side chain (designated as 2′). Because the groups with the highest priority reside on the same side of the double bond, the *Z* designation for tamoxifen is correct. Doxepin is an antidepressant that is marketed as an 85:15 mixture of its *E* and *Z* isomers. The *E* isomer is responsible for blocking the reuptake of norepinephrine and producing a beneficial antidepressant effect. In a similar fashion, let's verify this assignment. Carbon atom A is part of a tricyclic ring system that is nearly symmetrical; however, because there is an oxygen atom located on the right side of the ring system, the carbon atom to the right of the double bond has priority (designated as 1) over the carbon atom to the left (designated as 2). The evaluation of carbon atom B is much easier because the ethylamine side chain has priority (designated as 1′) over the hydrogen atom (designated as 2′). Because the groups with the highest priority reside on opposite sides of the double bond, the *E* designation is correct for the isomer shown. The structure of amitriptyline is also shown in Figure 7-12 to emphasize a key point. Similar to the fact that a chiral center cannot occur unless the atom is attached to four different groups, a geometric isomer cannot occur unless each atom involved in the double bond is attached to two different groups. Amitriptyline is

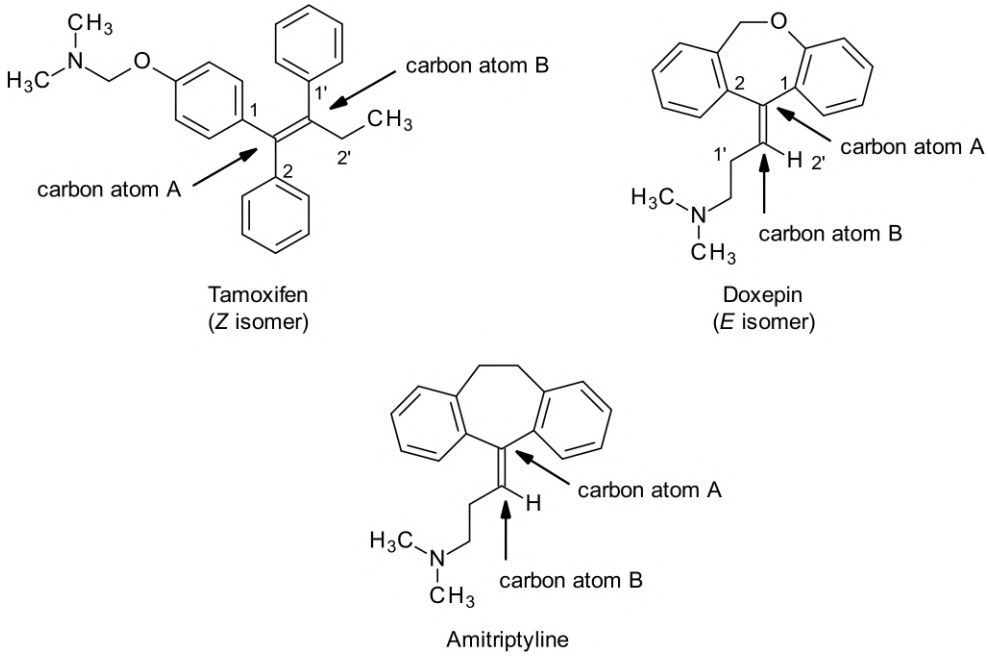
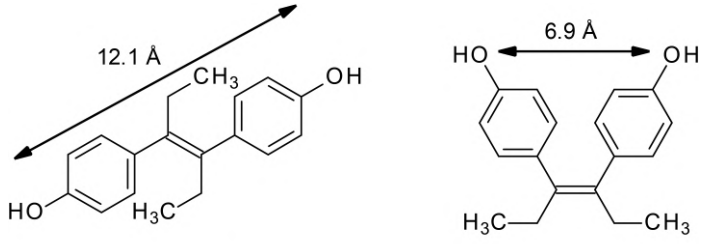


FIGURE 7-12. Examples of *E*/*Z* designations of double bonds. Priorities are shown for the carbon atoms of tamoxifen and doxepin (1 and 1′ signify higher priority than 2 and 2′).

similar in structure to doxepin; however, because the tricyclic ring system is symmetrical, carbon atom A is not attached to two different groups and amitriptyline does not have a geometric isomer. Another example of this concept is seen with quinidine and quinine (Figure 7-10). Although these diastereomers have four chiral centers, the vinyl group does not add another stereochemical center. This is because one of the carbon atoms involved in this double bond is attached to two hydrogen atoms or, in other words, two identical groups. Please note that these geometric isomers involve the orientation of functional groups about a double bond and not a chiral center. Thus, they are different from diastereomers.

Pharmacological and Therapeutic Differences Between Diastereomers and Geometric Isomers

The pharmacological differences seen with diastereomers and geometric isomers occur for two main reasons. First, these compounds have different physical and chemical properties that may account for their differences in pharmacological and/or pharmacokinetic activity. Second, one isomer may have a better fit with its biological target than the other. This is similar to what was discussed with enantiomers and could be due to the orientation of functional groups, the number of available bonds, and/or steric factors. For geometric isomers containing double bonds, the difference in activity may be due to the interatomic differences of functional groups essential for biological activity. As an example of this, let's examine the isomers of diethylstilbestrol (**Figure 7-13**). The *trans* isomer is able to mimic the structure and actions of estradiol primarily because the distance between its two phenolic hydroxyl groups is similar to that seen with estradiol (12.1 Å). In contrast, the *cis* isomer is inactive, primarily because the phenolic hydroxyl groups are much closer together (6.9 Å), they cannot mimic the structure of estradiol, and thus they cannot bind to the estrogen receptor. Please note that the *cis/trans* designations are appropriate here because there are only two different functional groups. The phenol rings or the ethyl chains are either on the same side of the double bond (i.e., *cis*) or on opposite sides (i.e., *trans*).



trans-Diethylstilbestrol *cis*-Diethylstilbestrol

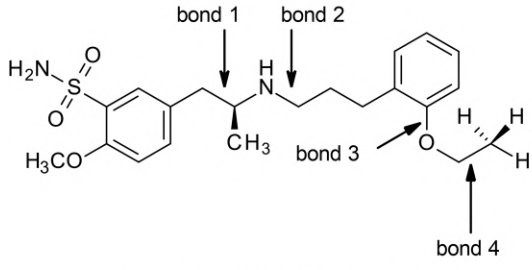
FIGURE 7-13. The *trans* and *cis* isomers of diethylstilbestrol.

CONFORMATIONAL ISOMERS

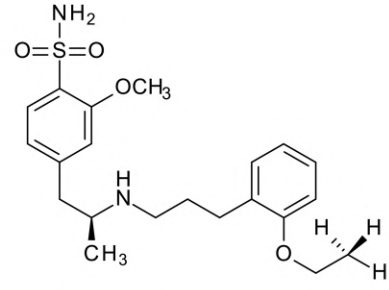
Conformational isomers (or conformers) are nonsuperimposable orientations of a molecule that result from the free rotation of atoms about single bonds. A molecule must meet two criteria to possess conformational isomers. First, it must possess at least one single bond that can freely rotate. In general, single bonds that are part of an alkyl chain have more freedom of rotation as compared with single bonds that are part of an alicyclic ring system. Second, neither of the atoms that are joined by this single bond can contain three identical substituents (e.g., three hydrogen atoms, three methyl groups) or else the rotation about the bond is irrelevant. Because almost every drug molecule meets these criteria, conformational isomers can exist for almost every drug molecule. Both the number of rotatable single bonds as well as their position determine whether a drug is classified

as *conformationally flexible* or *conformationally rigid*. In general, a rotatable bond located in the middle of a molecule allows for much more flexibility than one located at either end.

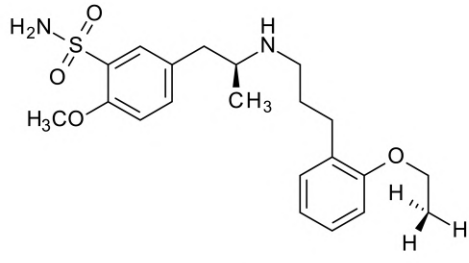
Let us look at two examples to illustrate these key concepts. Shown in **Figure 7-14** is the structure of tamsulosin, a selective α_1 adrenergic receptor antagonist used for the treatment of benign prostatic hyperplasia. Tamsulosin has a significant amount of conformational flexibility due to the presence of numerous single bonds that can undergo rotation. Four of these have been highlighted. Rotation about bonds 1, 2, and 3 produces three additional conformational isomers of tamsulosin. Please note that the overall conformation of tamsulosin is altered to a greater extent when bonds 1 and 2 are rotated as compared with bond 3. This is because bonds 1 and 2 are located in the middle of the molecule whereas bond 3 is located more at the end of the molecule. Rotation of bond 4 fails to produce a conformational isomer because the methyl group is attached to three identical hydrogen atoms. Although the individual hydrogen atoms can move from one position to another, this does not change the overall conformation of tamsulosin.



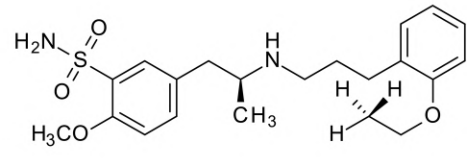
Tamsulosin: Original Conformation



Tamsulosin: Conformation A
(Rotation about bond 1)



Tamsulosin: Conformation B
(Rotation about bond 2)

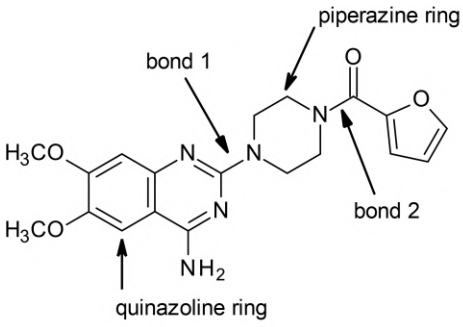


Tamsulosin: Conformation C
(Rotation about bond 3)

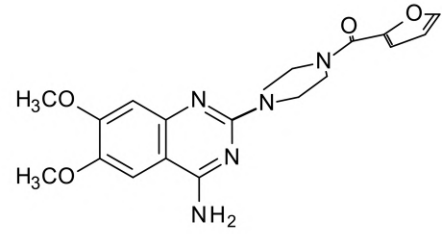
FIGURE 7-14. Conformational isomers of tamsulosin.

Prazosin, shown in **Figure 7-15**, is similar to tamsulosin in that it is also a selective α_1 adrenergic receptor antagonist. As compared with tamsulosin, prazosin is much more conformationally rigid. The bicyclic quinazoline ring, as well as the piperazine ring, limits the number of rotatable bonds and conformations.

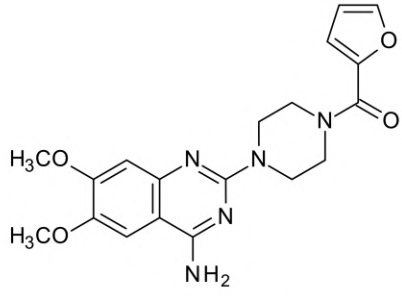
Please note that the rotations seen here for tamsulosin and prazosin alter the spatial arrangement of the functional groups and not the configuration of atoms about a chiral center. Unlike configurational isomers, no bonds need to be broken and reformed to convert one conformer to another. *Therefore, conformational isomers are not distinct molecules but rather different orientations of the same molecule*.



Prazosin: Original Conformation



Prazosin: Conformation A
(Rotation about bond 1, rings
are more perpendicular to one another)



Prazosin: Conformation B
(Rotation about bond 2)

FIGURE 7-15. Conformational isomers of prazosin.

Terminology

A few key terms are used to describe the orientation of functional groups present on adjacent carbon atoms. These are illustrated in **Figure 7-16** using the structure of acetylcholine and its Sawhorse and Newman projections. Similar to tamsulosin, acetylcholine has a number of rotatable single bonds. Rotation of the single bonds between atoms 2 and 3, atoms 3 and 4, and/or atoms 4 and 5 produces conformational isomers. Rotation of the single bonds between atoms 1 and 2 and/or atoms 5 and 6 does not produce conformational isomers because atoms 1 and 6 have three identical substituents. The conformational isomers that are shown in Figure 7-16 highlight the rotation about carbon atoms 4 and 5. When the acetyl (Ac) group and the quaternary nitrogen are situated 180° apart, as shown in both the Sawhorse and Newman projections, the molecule is said to be in the *trans* or *anti* conformation. Rotation of the *trans* Sawhorse and Newman projections by 120° in the counterclockwise direction gives rise to the *gauche* or *skew conformation*. Continued rotation by another 60° in the same direction provides the *fully eclipsed conformation*. An estimation of this is shown in the Newman projection.

Preferred Conformation

There are many other conformational isomers of tamsulosin and acetylcholine beyond those shown in Figures 7-14 and 7-16. In fact, rotation about bonds 1 and 2 of tamsulosin can theoretically produce a very large number of conformers; however, not all of these conformers are energetically desirable. It has been observed that the rotation about carbon-carbon single bonds is not really "free" but rather subject to an energy barrier. This energy barrier is due to both steric repulsions and electronic interactions among the atoms or groups on adjacent carbon atoms. Thus, those conformations that

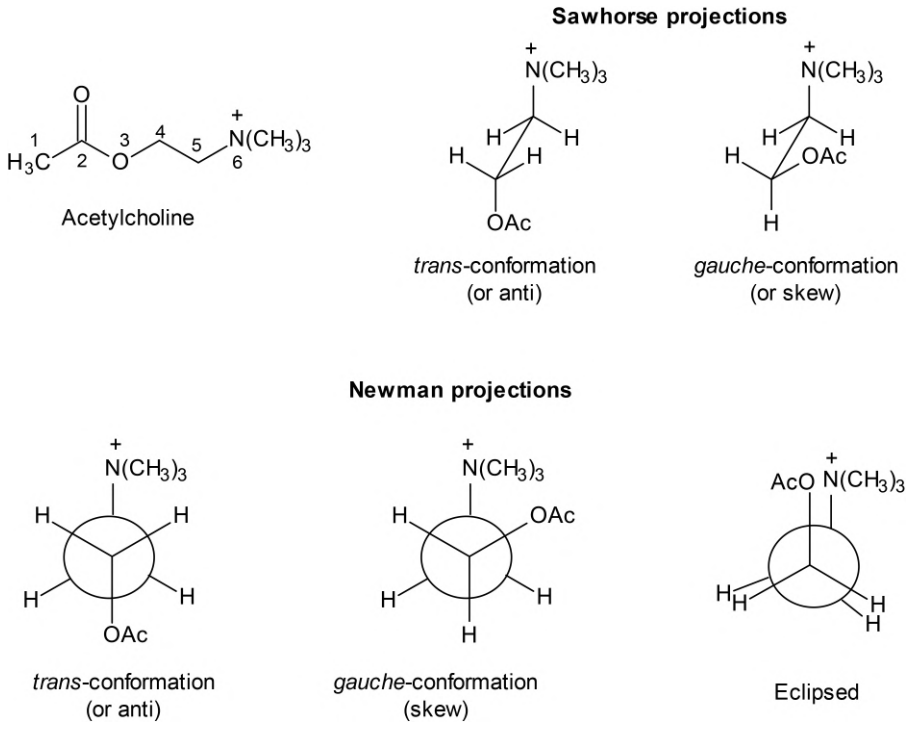
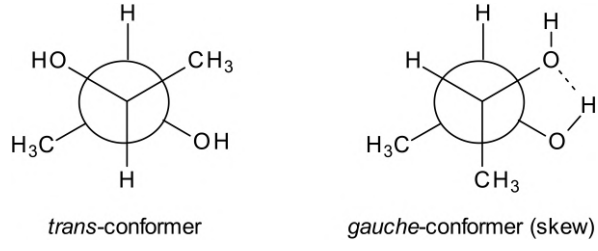


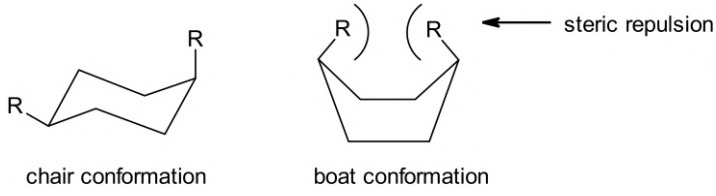
FIGURE 7-16. Conformations of acetylcholine (the eclipsed view has been slightly offset to view the atoms).

minimize any repulsive interactions and maximize all attractive interactions are more energetically favorable than other conformations and are said to be preferred.

From a strictly steric point of view, *gauche* or skew conformations are less energetically favorable than *trans* conformations. Therefore, as a general rule, conformations in which the larger groups are staggered and separated from one another by as great a distance as possible are more energetically favorable than those in which a significant number of skew interactions occur. Exceptions to this general rule occur when forces of electronic attraction more than compensate for any steric repulsion. An example of this is shown below. The skew or *gauche* form of 2,3-dihydroxybutane is more energetically favorable than the *trans* form because of the intramolecular hydrogen bonding, which is present in the *gauche* conformation. As an additional example, consider the structures of acetylcholine shown in Figure 7-16. Looking only at steric factors, one would most likely predict that the *trans* conformation would be preferred. A variety of spectrographic studies have shown, however, that actually the *gauche* conformer is preferred. The intramolecular attractive force between the quaternary nitrogen and the ester carbonyl overcomes steric barriers and stabilizes the *gauche* conformer through intramolecular ion-dipole interactions. These examples illustrate the importance of examining both steric and electronic factors before making any predictions on preferred conformations.



The above examples focused on the rotation of single bonds found in an aliphatic chain. These same concepts also apply to alicyclic rings. Cyclohexane and other six-membered, nonaromatic, heterocyclic ring systems (e.g., piperidine, piperazine) are present in several drug molecules. Cyclohexane can exist in several conformations; however, only two, the boat form and the chair form, maintain the proper tetrahedral bond angles.



Of these two conformations, the chair conformer is of much lower energy than the boat conformer. The reason for this is 2-fold. First, all of the bonds are staggered in the chair conformer, while two sets of eclipsed interactions are present in the boat conformer. Second, depending on the respective "R" groups (i.e., side chains, functional groups), steric repulsive interactions can occur because the groups are directed toward each other in the boat conformation. Thus, under most circumstances, cyclohexane rings adopt a chair conformation.

Cyclohexane rings, as well as other six-membered, nonaromatic, heterocyclic ring systems, can undergo what is known as *chair-chair inversion* or *flipping*. **Figure 7-17** shows two simple examples. Whenever cyclohexane undergoes chair-chair inversion, all hydrogen atoms that were originally axial (e.g., H_1) become equatorial, and all hydrogen atoms that were originally equatorial (e.g., H_2) become axial. Thus, there are two possible chair conformations of cyclohexane. Admittedly, this is only of theoretical interest when dealing with cyclohexane; however, it becomes extremely important when considering substituted cyclohexane rings present in drug molecules. In this case, inversion does not simply interchange hydrogen atoms but instead switches axial substituents to equatorial and vice versa. As an example, consider *cis*-1,3-dimethylcyclohexane. Chair-chair inversion of this compound produces two conformations, one in which both methyl groups are axial and one in which both methyl groups are equatorial. Due to steric hindrance caused by the 1,3-diaxial interaction, the conformation in which both methyl groups are equatorial is more favorable than the one in which both methyl groups are axial.

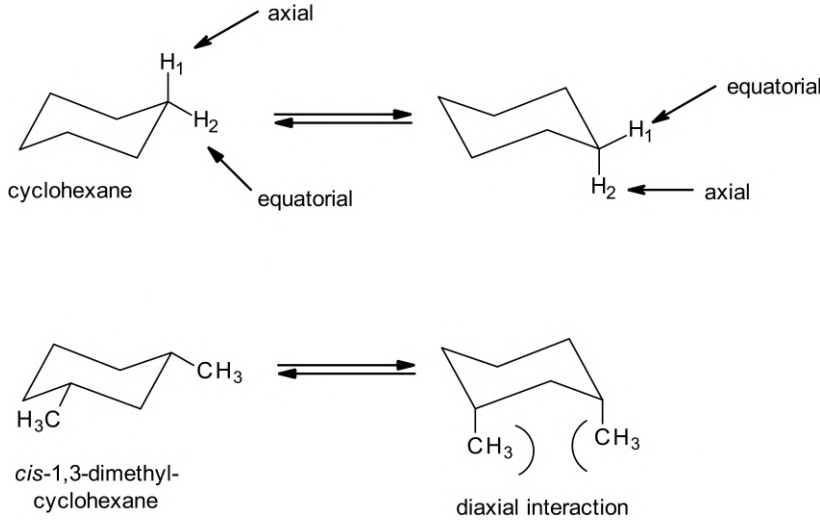
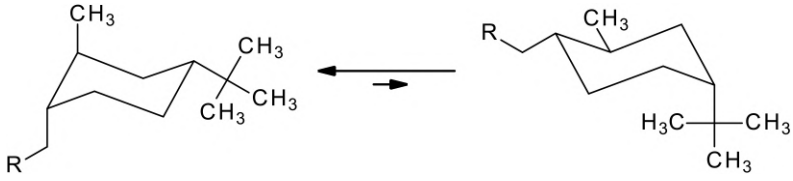


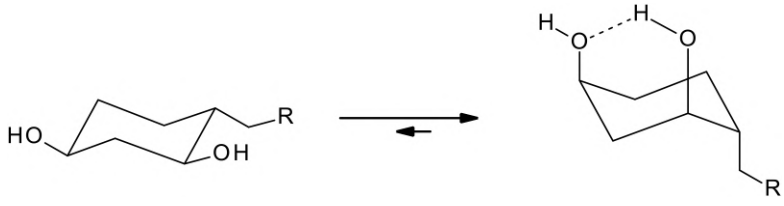
FIGURE 7-17. Chair-chair inversions of cyclohexane and *cis*-1,3-dimethylcyclohexane.

Although there are always exceptions, a few generalizations can be made concerning the conformation of substituted cyclohexane and other six-membered, nonaromatic, heterocyclic ring systems.

- In most instances, the chair conformation is favored over the boat conformation.
- Due to 1,3-diaxial interactions, the chair conformation in which most substituents are equatorial is favored.
- An exception to the above rule can occur if the cyclohexane ring contains an extremely bulky functional group, such as a *t*-butyl group. In this case, the conformation that allows this bulky group to be equatorial is favored.



- In some instances, chair conformations with axial substituents are favored if attractive forces between the groups are present. This is similar to the *gauche* conformation of acetylcholine.



Active Conformation

The conformation of a drug molecule that binds to its desired biological target is known as its *active conformation*. This active conformation contains the correct spatial arrangement of all essential binding groups but is not necessarily the same as the most energetically preferred conformation. This is illustrated in the binding of acetylcholine (Figure 7-16) to the muscarinic receptor. Although the *gauche* conformer of acetylcholine is preferred, the *trans* conformer is required for acetylcholine to bind to the muscarinic receptor.

The energy required to change a drug molecule from its preferred solution conformation to its required active conformation *can be provided by the energy released when the drug molecule binds to its biological target*. As discussed in Chapter 6, energy is released whenever a drug forms a bond with its biological target. This bond energy or bond strength varies among the different types of bonds that can form as well as the number of binding interactions that can occur; however, the overall summation of all of these bond energies is often sufficient to allow the preferred conformation of a drug molecule to be transformed into its required active conformation. In some cases, the energy barriers to rotation are too prohibitive, and the drug molecule is inactive (i.e., does not bind to its biological target). These ideas are consistent with the induced fit theory of drug-receptor binding. This theory postulates that the binding of a molecule to its receptor is a dynamic process that results in a mutual plastic molding of both the molecule and its biological target. In a stepwise manner, bonds are formed, energy is released, small energy barriers are overcome, and conformations are altered to allow for additional binding interactions. This process then continues to maximize the interactions between the drug and its biological target. Unlike preferred conformations, in which the chemical structure of the drug molecule can be used to predict which conformer would be more favorable, active conformations are much more difficult to predict unless you have a crystallographic structure of the drug's biological target.

Benefits of Conformational Restriction

Restriction of conformational flexibility is a commonly used drug development strategy to "lock" a drug molecule into a desired conformation. To illustrate the potential benefits that could possibly be gained by this strategy, let us consider the following hypothetical example.

Assume a drug molecule contains two functional groups, X and Y, which are located on adjacent carbon atoms and are essential for pharmacological activity. Further assume that the drug is capable of binding to three different receptor types or subtypes. Given this scenario, it would be beneficial to determine if the drug is binding to the three receptors in similar or distinct conformations. Conformational restriction could help to provide this information. Additionally, there are many conformations of X and Y relative to one another. Of these, let us consider the four conformations (A–D) shown in **Figure 7-18**.

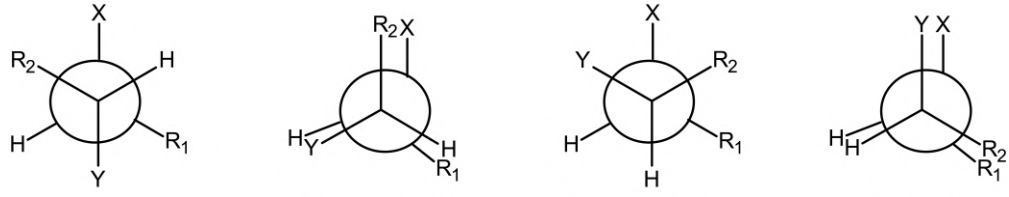


FIGURE 7-18. Conformations of a hypothetical drug molecule (the eclipsed views of conformations B and D have been slightly offset to view the atoms).

As an idealized case, let us assume that previous research has determined that the drug acts on its three receptors in three different conformations to produce two beneficial effects (antihypertensive and hypoglycemic effects) and one very prevalent side effect (dry mouth). We also assume that the following information is true: when the molecule is in conformation A, it binds to receptor 1 and produces an antihypertensive effect; when the molecule is in conformation B, it binds to receptor 2 and produces a hypoglycemic effect; and when the molecule is in conformation C, it binds to receptor 3 and produces the side effect. Because the active conformations are known, it should be possible to use conformational restriction to develop specific agonists and/or antagonists for these receptors. By locking the molecule in either conformation A or B, it should be possible to elicit only the desired beneficial effect without the side effect or any other unnecessary effects. Carrying this idealistic example one step further, assume that the drug has a high affinity for hepatic metabolic enzymes when it is in conformer D. Conformationally restricted analogs of conformers A or B would then be expected to have an increased duration of action over the parent compound in addition to the above-stated advantages.

Although the hypothetical example is an idealized case, it is not that far-fetched. Small, conformationally flexible neurotransmitters such as acetylcholine, norepinephrine, dopamine, and histamine are known to bind in different conformations to different receptor subtypes to produce different effects. As an example, acetylcholine binds to its metabolizing enzyme, acetylcholinesterase, in its *trans* conformation. This information, as well as the structures of naturally occurring acetylcholinesterase inhibitors, led to the development of pyridostigmine bromide and neostigmine bromide (**Figure 7-19**). The aromatic rings present in these two drugs provide conformational restriction and lock the positively charged ammonium group in a *trans* orientation to the carbamate group, a functional group that is analogous to the acetyl ester found in acetylcholine. Due in part to this conformational restriction, these drugs can bind to acetylcholinesterase and inhibit this enzyme. The binding and inhibition indirectly increase the concentration of acetylcholine and can be used to treat myasthenia gravis.

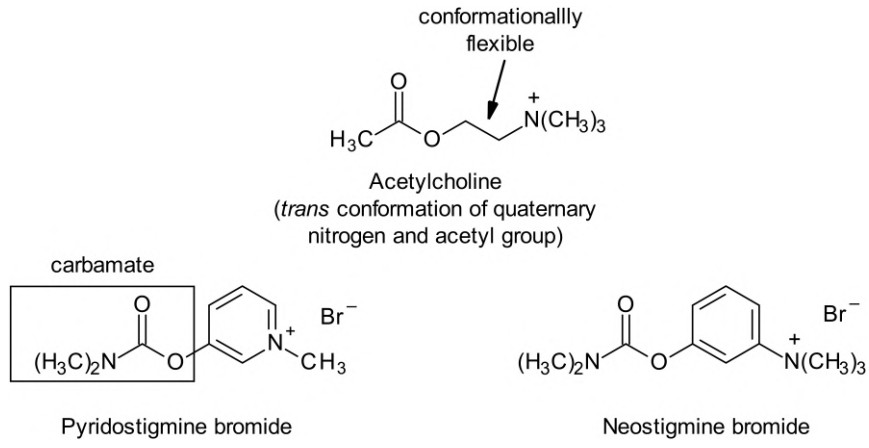


FIGURE 7-19. Pyridostigmine bromide and neostigmine bromide: conformationally restricted carbamate analogs of *trans* acetylcholine.

Another example of the benefits of conformational restriction can be seen with certain NSAIDs, a class of drug molecules previously discussed in this chapter. These drug molecules inhibit cyclooxygenase enzymes, resulting in decreased production of inflammatory prostaglandins. As part of their mechanism of action, these drug molecules must bind to the active site of cyclooxygenase and mimic arachidonic acid, the natural substrate of the enzyme. The binding of fenoprofen and diclofenac to cyclooxygenase involves three key interactions, shown in **Figure 7-20**. The acidic carboxylic acid forms an ionic bond with a positively charged arginine residue. The adjacent (or "top") aromatic ring forms van der Waals interactions with hydrophobic side chains of various amino acids. The second (or "bottom") aromatic ring binds in a hydrophobic trough that is under the "top" ring and somewhat perpendicular to the "top" ring. Both fenoprofen and diclofenac are able to bind to the active site of cyclooxygenase; however, the highlighted *ortho* chloro groups present on diclofenac confer steric hindrance that locks the "bottom" ring perpendicular to the "top" ring. As a result, diclofenac is already locked into the active conformation and does not need to expend as much binding energy to fit into the active site. This is reflected in the relevant affinities or potencies of these two drugs. Diclofenac is dosed at 50 to 75 mg two or three times daily (BID or TID) whereas fenoprofen is dosed at 400 to 600 mg three or four times daily (TID or QID).

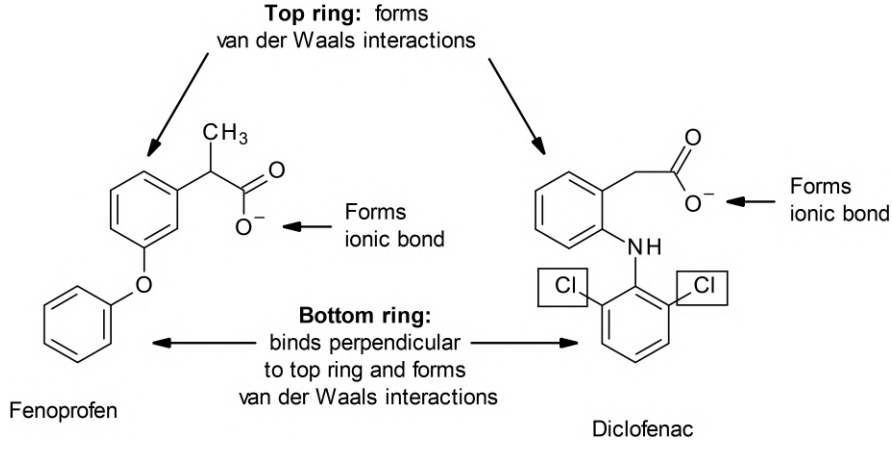


FIGURE 7-20. A comparison of fenoprofen and diclofenac binding to cyclooxygenase.

In summary, conformational restriction can potentially result in a number of pharmacological and therapeutic benefits. Specifically, conformational restriction can increase the specificity of a drug molecule for a single biological target, it can increase the duration of action of a drug molecule, and it can decrease adverse effects. As the NSAIDs illustrate above, although a flexible drug can assume an unfavorable conformation, this requires energy. A conformationally rigid analog with all the necessary groups in the proper orientation would not require this energy and should have a higher affinity for the receptor.

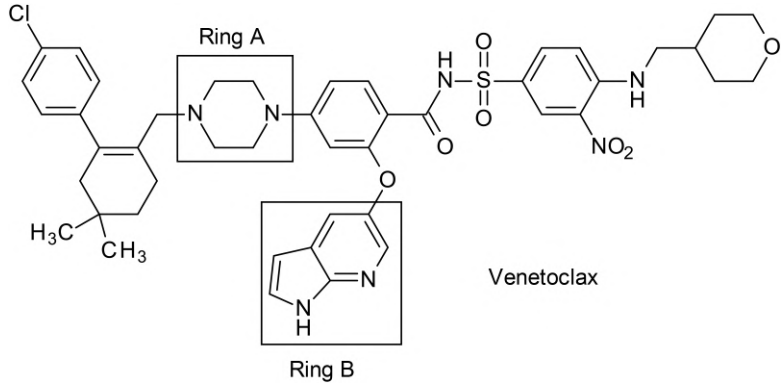
It should also be noted that there are some situations in which conformational flexibility, rather than conformational restriction, bestows a beneficial effect. An example of this can be seen with tamsulosin and prazosin, two drugs previously discussed that can be used to treat benign prostatic hyperplasia (Figures 7-15 and 7-16). These drugs relax the bladder neck and the prostate by blocking the α_1-adrenergic receptor. This causes less pressure on the urethra and increases urine flow. Three subtypes of α_1 receptors have been identified: α_{1a}, α_{1b}, and $\alpha_{1d.}$ The α_{1a} receptors mediate human prostatic smooth muscle contraction whereas α_{1b} and α_{1d} receptors are involved in vascular smooth muscle contraction. Both α_{1a} and α_{1b}, are present in the prostate, with approximately 70% being the α_{1a} receptors. As compared with prazosin, tamsulosin is much more selective for the α_{1a} subtype. This selectivity has been proposed to be due to the increased conformational flexibility of tamsulosin. As a result, tamsulosin interacts with vascular smooth muscle much less than prazosin and therefore causes much less orthostatic hypotension.

Summary of Key Points Regarding Conformational Isomers

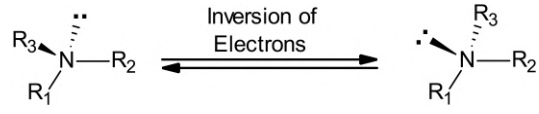
- Unlike configurational isomers (i.e., enantiomers, diastereomers, and geometric isomers), conformational isomers *are not distinct molecules* but rather different orientations of the same molecule.
- The number and location/position of rotatable bonds determine whether a drug molecule is conformationally flexible or conformationally rigid.
- The preferred conformation(s) of a drug molecule are established primarily by electronic and steric effects. Conformations that maximize attractive electronic interactions and minimize steric repulsive interactions are preferred.
- The active conformation(s) of a drug molecule are established by its biological target. To bind to its biological target, the drug molecule must be able to assume a conformation that has *a complementary orientation of key functional groups*.
- The energy required to convert a drug molecule from its preferred conformation to its active conformation *comes from the energy that is released when the drug molecule binds to its biological target*.
- Conformational restriction of a drug molecule can potentially provide several pharmacological/therapeutic benefits, including enhanced specificity for a given biological target, decreased metabolism and increased duration of action, and decreased adverse effects.
- Conformational flexibility can also provide beneficial pharmacological/therapeutic benefits depending on the specific drug or drug class.

STRUCTURAL ANALYSIS CHECKPOINT

Checkpoint Drug 1: Venetoclax



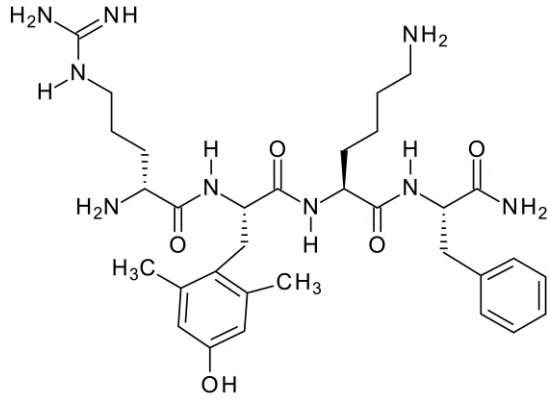
1. Identify all chiral centers and potentially prochiral centers that are present within the structure of venetoclax.
2. Is it possible for venetoclax to have the following:
   A. Enantiomers?
   B. Diastereomers?
   C. Geometric isomers?
   D. Conformational isomers?
3. If you answered YES to any of the questions in Question 2, draw an appropriate isomer.
4. Nitrogen atoms that have sp^3 hybridization can readily undergo inversion of their lone pair of electrons, as shown below. Due to this inversion, a nitrogen atom can readily convert the orientations of its lone pair of electrons. Given this piece of information, predict the preferred conformation of the piperazine ring (ring A) seen in venetoclax.



5. Identify any steric factors that would help to dictate the preferred conformation of the pyrrolopyridine ring (ring B) relative to its surrounding functional groups.

Checkpoint Drug 2: Elamipretide

As a peptide-based drug, elamipretide is derived from naturally occurring and modified amino acids. In Chapter 2 you identified that there are four amino acids present in this drug molecule. Every amino acid (with the exception of glycine) has a chiral center.

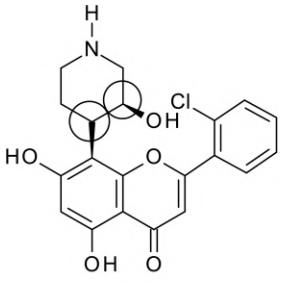


Elamipretide

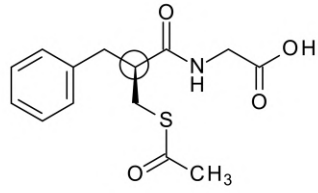
1. How many chiral centers are present in elamipretide? Circle each chiral carbon.
2. For each of the four amino acid/amino acid derivatives, determine how many potential prochiral carbons are present.
3. Consider each of the following types of isomers. Describe what would need to change structurally to produce each type of isomer.
   A. Enantiomer
   B. Diastereomer
4. Provide an explanation as to why geometric isomers are not possible for elamipretide.
5. Based on your evaluation of elamipretide, is it conformationally flexible or conformationally rigid? Provide a brief rationale for your answer.

REVIEW QUESTIONS

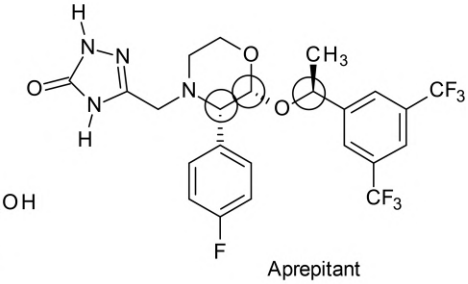
1. Consider each of the structures below and do the following:
   A. Determine whether a chiral carbon is present.
   B. Place an asterisk next to the chiral carbon(s) that is present.
   C. For each of the structures below, determine whether each of the circled atoms is prochiral or not.



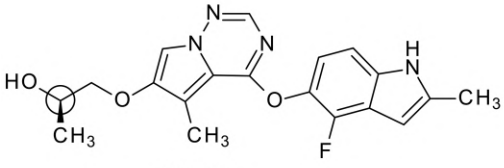
Alvocidib



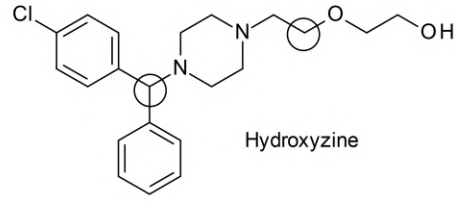
Ecadotril



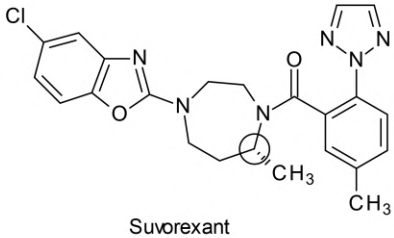
Aprepitant



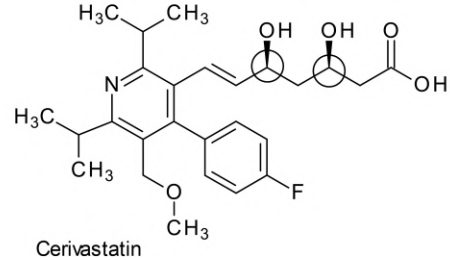
Brivanib



Hydroxyzine

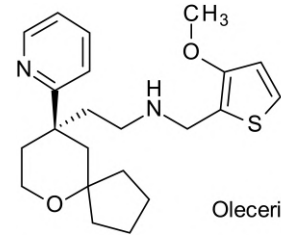


Suvorexant



Cerivastatin

2. Determine which enantiomer (*R*/*S*) is drawn below. List the four unique substituents attached to the chiral carbon and their priorities based on the CIP system.



Oleceridine

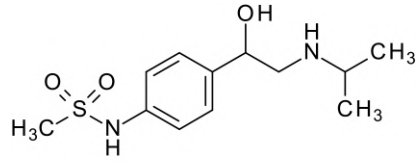
3. List three properties that are identical between *R* and *S* enantiomers and one property that may be very different.

4. Match the enantiomer designations with the correct definition(s). Each definition may be used more than once, and each designation may be matched with more than one definition.

(+)/(–)	1. Absolute configuration
d/*l*	2. Steric arrangement of atoms about a chiral carbon
D/L	3. Direction that enantiomer rotates plane polarized light
R/*S*	4. Dextrorotatory/levorotatory

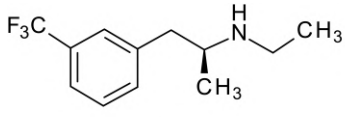
5. For a molecule that has three chiral centers (*R*,*S*,*S*), answer the following questions:
   A. How many (+)/(–) designations are used to describe how the molecule rotates plane polarized light?
   B. Determine which of the following statements is/are true related to this molecule:
      1. It can have an enantiomer.
      2. It can have a diastereomer.
      3. It can have a geometric isomer.
      4. It can have conformational isomer.

6. Sotalol is indicated for the management of arrhythmias and is marketed as a racemate. The *S* isomer is a potassium channel blocker, and the *R* isomer is both a potassium channel blocker and nonselective β antagonist. Provide a brief rationale for why it is possible that these enantiomers have different pharmacological properties.

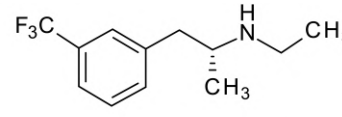


Sotalol

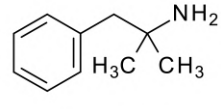
7. Fenfluramine was a drug used as an appetite suppressant in the late 1980s and early 1990s and was sold as a racemate. D-Fenfluramine was an effective appetite suppressant; however, L-Fenfluramine caused significant drowsiness. To combat the fatigue caused by the L isomer, this drug was combined with phenteramine, which has both stimulant and moderate weight loss activity. Evaluate the enantiomers of fenfluramine and phentermine, circle any chiral carbon atoms, and box any prochiral carbon atoms. Provide a brief rationale for why the enantiomers of fenfluramine have different pharmacological properties.



D-Fenfluramine

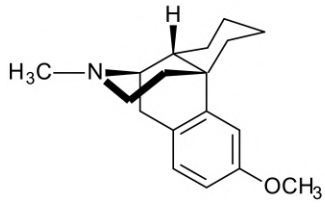


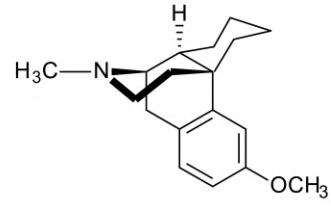
L-Fenfluramine



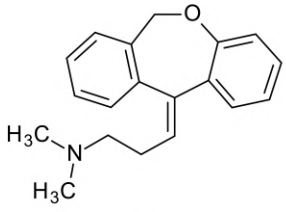
Phentermine

8. Describe how diastereomers are the same and how they are different from enantiomers. Circle all chiral centers in both of the isomers of dextromethorphan drawn below. Determine if the two structures drawn below represent a pair of diastereomers or enantiomers.

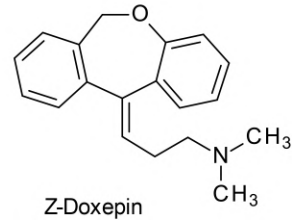




9. Doxepin is administered as an 85 (*E*):15 (*Z*) mixture of stereoisomers. The *E*-stereoisomer is a norepinephrine reuptake inhibitor. The *Z*-stereoisomer is a serotonin selective reuptake inhibitor used in the treatment of depression. Determine the priority of the three double bond substituents using the CIP system.



E-Doxepin



Z-Doxepin

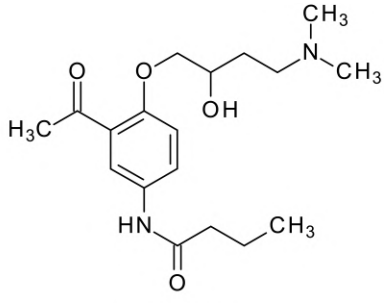
10. Shown below are the structures of acebutolol, estradiol, cefamandole, and nifedipine. For each of these drugs:

    A. Identify all chiral centers.

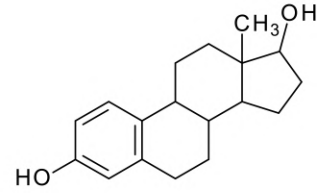
    B. Identify if it can have an enantiomer. Provide an explanation for your response.

    C. Identify if it can have a diastereomer. Provide an explanation for your response.

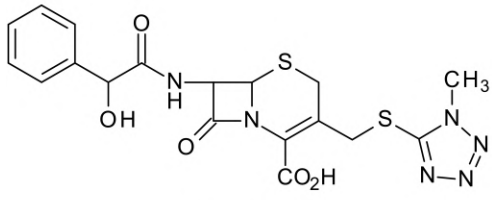
    D. Identify if it can have a geometric isomer. Provide an explanation for your response.



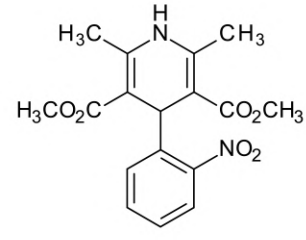
Acebutolol



Estradiol



Cefamandole



Nifedipine

11. Shown below are the structures of fluvastatin and its enantiomer. Which of the following properties/actions would be expected to be identical for fluvastatin and its enantiomer and which would be expected to be different?

    A. Hepatic metabolism

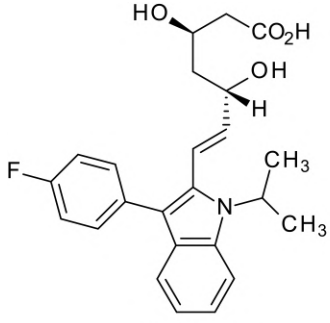
    B. Water solubility

    C. Adverse effect profile

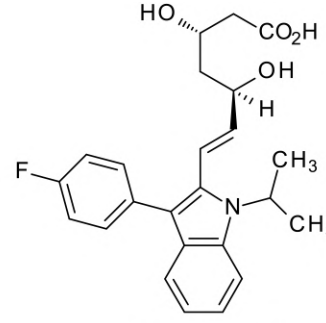
    D. Active renal reabsorption by transport proteins

    E. Potency (dosage given)

    F. Percent ionization at a pH of 7.4

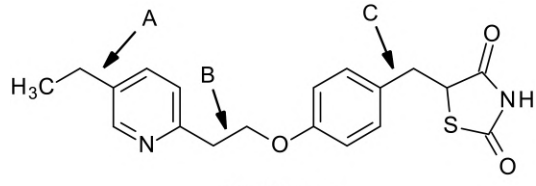


Fluvastatin



Enantiomer of Fluvastatin

12. Shown below is the structure of pioglitazone with three single bonds highlighted (A-C). For each of these highlighted bonds, draw a conformational isomer that could result due to a rotation about the bond and indicate how your conformational isomer could change the binding of pioglitazone to its target receptor.



Pioglitazone

DRUG METABOLISM

LEARNING OBJECTIVES

After completing this chapter, students will be able to

- **Discuss the key concepts governing drug metabolism.**
- **Explain the general mechanistic requirements for cytochrome P450 oxidation as well as identify the factors that can alter the effects of these enzymes.**
- **Identify the metabolic transformations that are required to convert a given drug molecule to a given metabolite.**
- **Predict the possible metabolic transformations that could occur for a given drug molecule.**
  - **For any given functional group within a drug molecule, identify the possible types of metabolic transformations it could undergo.**
  - **For each Phase I metabolic transformation, draw key intermediates and/or the final metabolic product.**
  - **For each Phase II metabolic transformation, draw and/or identify the activated intermediate, identify the transferring enzyme, identify the functional groups that can be conjugated by this path, and identify any deconjugating enzymes.**

The primary purpose of drug metabolism is to enhance the removal of drug molecules from the body by altering or adding functional groups. Drug metabolism is also important for activating prodrugs, converting less active drugs to more active metabolites, inactivating drug action, deactivating toxic compounds, and, in some cases, producing toxic metabolites.

Metabolic transformations can be divided into two main categories: Phase I metabolism and Phase II metabolism.

- *Phase I metabolic transformations* alter functional groups that are initially present within a drug molecule or a biomolecule. There are three types of Phase I transformations: oxidation, reduction, and hydrolysis. Oxidation is the most prevalent of these three. In general, these transformations increase the water solubility of the drug molecule; however, this increase is not always sufficient enough to allow the drug to be readily excreted.

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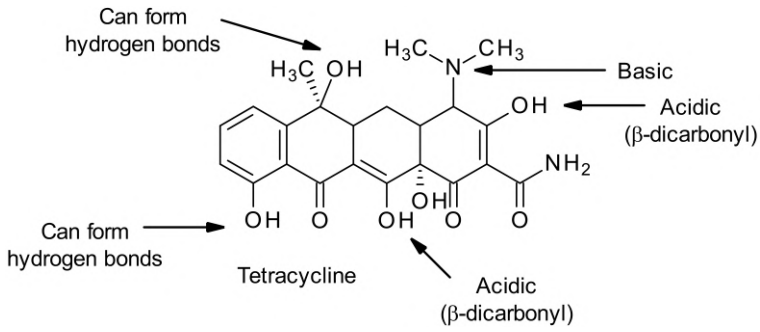
- *Phase II metabolic transformations* all involve the addition of endogenous substances to the drug molecule. This process is known as *conjugation*. The most common Phase II conjugations involve the addition of glucuronic acid, sulfate, or the amino acids glycine or glutamine to functional groups that were either originally present in a drug molecule or added/unmasked by one or more Phase I processes. These conjugated products are highly water soluble, render the drug inactive in most cases, and are readily excreted. Additional Phase II processes involve the addition of glutathione, methyl groups, and acetyl groups to the drug molecule. Glutathione detoxifies highly reactive functional groups, while methylation and acetylation generally terminate biological activity. With the exception of glutathione, there is an initial activation step resulting in the formation of a reactive coenzyme prior to the conjugate being transferred to the drug molecule. Additionally, deconjugating enzymes such as β-glucuronidase and sulfatase can reverse some of these Phase II processes.

This chapter focuses on the common principles of drug metabolism, the mechanisms of Phase I and Phase II metabolic processes, and the functional groups susceptible to each type of transformation. The activation of prodrugs also is discussed when applicable. Further discussions regarding the benefits of prodrugs can be found in Chapters 5 and 9. The primary purpose of this chapter is to illustrate the possible types of metabolic transformation that a drug molecule *could* undergo, thus enhancing a student's ability to predict and understand metabolic pathways. As such, the examples presented in this chapter are not necessarily meant to portray the primary metabolic pathway for a given drug molecule. Instead, they are meant to illustrate the types of transformations that *could* occur based on the functional groups present in the drug molecule. For the sake of illustrating what is possible, a variety of drug molecules are used as examples. The actual metabolic route(s) for any given drug molecule are normally very specific and generally involve both major and minor pathways. Many of these routes are very predictable and others are not. By reviewing the various metabolic transformations and pathways outlined in this chapter, you should be better able to predict the metabolic transformations that *can* occur as well as understand how the actual metabolites are formed. For those who desire a more in-depth discussion of this topic, please consult the drug metabolism chapters found in *Foye's Principles of Medicinal Chemistry* (8th edition) and *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry* (12th edition).[1,2]

GENERAL CONCEPTS OF DRUG METABOLISM

A key to understanding drug metabolism is the simple recognition that most drug molecules are foreign substances (aka xenobiotics). As such, metabolic transformations are used to enhance the removal of these substances from the body. The number of metabolic transformations required to achieve this goal varies from drug molecule to drug molecule. As previously mentioned, the oxidation, reduction, and hydrolysis reactions seen in Phase I transformations alter existing functional groups and enhance their water solubility while the conjugation reactions seen in Phase II transformations add endogenous molecules to further enhance water solubility or deactivate the drug molecule.

Some drug molecules already possess sufficient water solubility and thus do not require any metabolic transformations. These drugs can be eliminated unchanged from the body. An example of this is seen below with tetracycline. This drug has three ionizable functional groups as well as several other functional groups that can form hydrogen bonds and therefore enhance water solubility. *In general, drug molecules that possess high lipid solubility require more metabolic transformations than those that possess low to moderate lipid solubility.*



Metabolism occurs primarily in the liver; however, metabolizing enzymes are also present in the gastrointestinal (GI) tract, kidney, lung, plasma, central nervous system, skin, placenta, and fetus. Within the GI tract, both human and bacterial enzymes can metabolize drug molecules.

There is no absolute requirement that a drug molecule undergo both Phase I and Phase II transformations. In some instances, Phase I transformations are sufficient to provide adequate water solubility for elimination without the need for Phase II metabolism. In other instances, Phase II conjugation is all that is required due to the presence of one or more functional groups that can readily undergo this transformation. Examples of this are seen with lidocaine and temazepam, respectively (**Figure 8-1**). Lidocaine rapidly undergoes two Phase I transformations: oxidative *N*-dealkylation and hydrolysis. The resulting metabolites are inactive and readily excreted without the need for

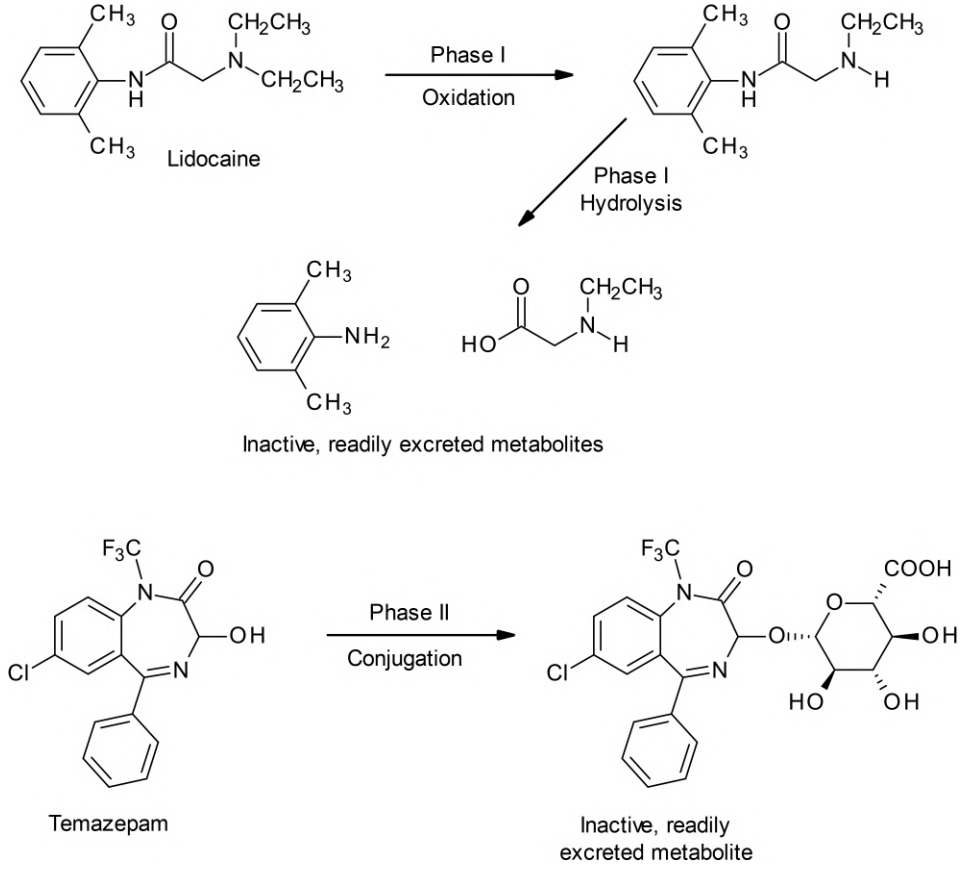


FIGURE 8-1. Lidocaine and temazepam: examples of drug molecules that require only Phase I or Phase II metabolism.

additional Phase II metabolism. The C_3 hydroxyl group present on temazepam allows it to be directly conjugated without the need for any prior Phase I metabolism.

The above examples illustrate a key concept. The liver and other metabolic sites use the *minimum number of transformations* necessary to alter a drug molecule such that it can be eliminated, inactivated, or detoxified. Additionally, metabolic enzymes generally seek to accomplish this goal in the easiest way possible. *Functional groups that are sterically hindered or otherwise difficult to access are much less likely to undergo metabolic transformations than those that are easily accessible.* In this regard, metabolic processes are extremely efficient. They do not use multiple steps when only one suffices, nor do they expend additional energy to accomplish a task when a much simpler solution provides the same result.

Metabolism is also important for the activation of prodrugs. The use of prodrugs to enhance either lipid or water solubility was initially discussed in Chapter 5. As a reminder, a *prodrug* is a drug molecule that has been covalently modified to either an inactive or weakly active analog for the purpose of achieving a specific therapeutic benefit. Once the prodrug is administered, it undergoes metabolic activation to release the original active drug molecule. The bioactivation of a prodrug is normally "programmed" and involves Phase I oxidation and/or hydrolysis. Examples of this can be seen with the ester prodrugs discussed in Chapter 5 and the antithrombotic agent clopidogrel discussed in Chapter 6.

PHASE I METABOLISM: OXIDATION

The Cytochrome P450 (CYP450) Family of Enzymes

Oxidation is the most common type of Phase I metabolism, and most oxidative transformations are catalyzed by the CYP450 family of enzymes. These enzymes contain a heme molecule bound to an iron atom as part of their structure. The 450 designation refers to the ability to absorb light at wavelengths that are at or near 450 nm whenever the heme iron atom is bound to carbon monoxide. These enzymes have been described as *mixed-function oxidases*, a designation that denotes their ability to introduce oxygen into a wide range of functional groups, including aromatic rings, aliphatic chains, alicyclic rings, benzylic carbons, allylic carbons, carbons immediately adjacent to carbonyls, double bonds, carbon-nitrogen functional groups, carbon-oxygen functional groups, carbon-sulfur functional groups, and carbon-halogen functional groups.

From a chemical perspective, *oxidation* refers to the loss of electrons or an increase in the oxidation state of an atom or molecule, while *reduction* refers to the gain of electrons or a decrease in the oxidation state of an atom or molecule. When looking at inorganic reactions, such as the conversion of ferrous ion (Fe^{+2}) to ferric iron (Fe^{+3}), it is obvious that the iron atom has been oxidized. It has lost an electron, and both the oxidation state and the charge have increased by one unit. When looking at organic drug molecules, the changes in electrons and oxidation states are not as easy to discern. Fortunately, there are specific chemical changes that allow for easy recognition. The oxidation of a functional group on a drug molecule results in one or more of the following: a gain in oxygen atom, a loss of hydrogen atom, a loss of an alkyl group, and/or a loss of a heteroatom. Examples of each of these are shown in **Figure 8-2**.

There are several CYP450 enzymes. All of these enzymes have the same mechanism of action, catalyze the same types of transformations, and should be viewed as different versions or isoforms of one another. CYP450 enzymes are expressed in a variety of tissues within the human body: however, they are primarily located in the intestine and liver. Given a specific drug molecule, one isoform may produce one metabolic product while another isoform may produce a different metabolic product. The isoforms are subdivided according to families, subfamilies, and individual genes within the subfamily. All CYP450 isoforms use the CYP prefix followed by an Arabic numeral designating the family, a capital letter designating the subfamily, and a second Arabic numeral designating the individual gene. Hence, the CYP2D6 isoform mentioned above is form 6 of CYP family 2, subfamily D.

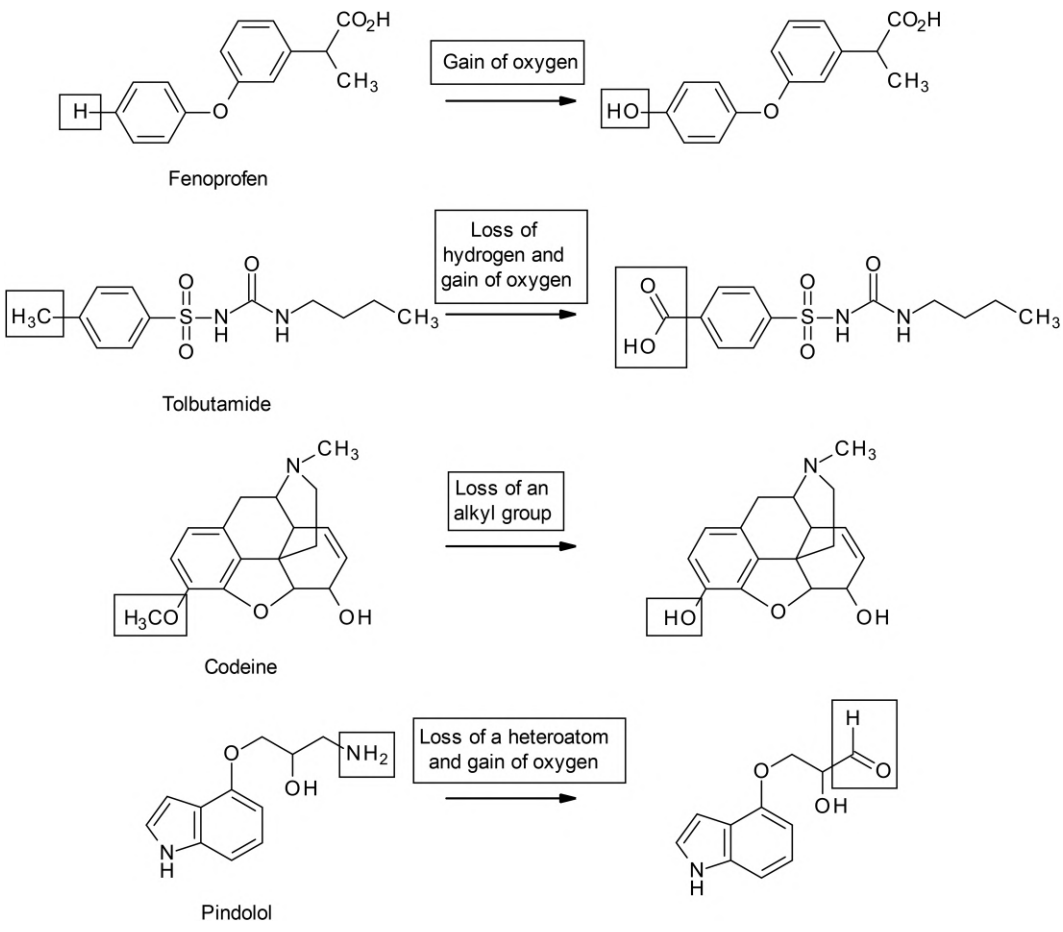
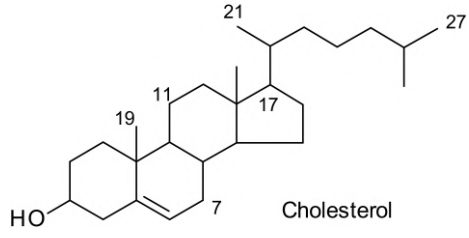


FIGURE 8-2. Examples of drug molecules undergoing oxidation reactions.

Six major mammalian families are required for the metabolism of endogenous substances: CYP7, CYP11, CYP17, CYP19, CYP21, and CYP27. The numbers here refer to the specific carbon atoms found on cholesterol, as shown below. Oxidation of cholesterol at one or more of these locations is required for the biosynthesis of estrogens, progesterone, androgens, bile acids, glucocorticoids, and mineralocorticoids.



There are three major mammalian families involved in drug, or xenobiotic, metabolism: CYP1, CYP2, and CYP3. The various subfamilies and specific isoforms are summarized in **Table 8-1** along with one or two examples of drug molecules that can be metabolized by each subfamily. The CYP2 family is responsible for metabolizing approximately 50% of all clinically important drugs whereas the CYP3A4 isoform is responsible for metabolizing slightly more than one-third of all clinically important drugs. In many cases, a single drug molecule can be metabolized by multiple isoforms. As an example, ondansetron, a serotonin 5-HT_3 receptor antagonist used to treat nausea and

TABLE 8-1. Cytochrome P450 Families for Drug or Xenobiotic Metabolism and Examples of Specific Drugs Metabolized by Each Enzyme

CYP1 Family	CYP2 Family	CYP3 Family
CYP1A1 (e.g., polycyclic aromatic hydrocarbons)	CYP2A6 (e.g., clozapine, nicotine)	CYP3A4 (e.g., erythromycin, nelfinavir)
CYP1A2 (e.g., haloperidol, theophylline)	CYP2B6 (e.g., amitriptyline, diclofenac)	CYP3A5 (e.g., clopidogrel, nifedipine)
	CYP2C8 (e.g., paclitaxel, verapamil)	CYP3A7 (e.g., retinoic acid)
	CYP2C9 (e.g., diazepam, meloxicam)	
	CYP2C19 (e.g., esomeprazole, phenytoin)	
	CYP2D6 (e.g., codeine, propranolol)	
	CYP2E1 (e.g., disulfiram, ethanol)	

vomiting associated with cancer chemotherapy, can be metabolized by CYP1A2, CYP2D6, CYP2E1, and CYP3A4. *Foye's* has extensive tables of substrates for all isoforms listed in Table 8-1.[1]

A simplified mechanism of action of CYP450 enzymes is shown in **Figure 8-3**. The mechanism consists of eight steps, and there are a number of key points to note when evaluating this mechanism. First, an iron atom is an important part of the overall mechanism. This atom changes oxidation states numerous times over the course of several specific steps. Although not shown in the diagram, the iron atom is bound to a heme molecule that is present within the active site of CYP450 enzymes. Second, molecular oxygen (O_2) is required for steps 3 through 8. One of the oxygen atoms becomes part of a water molecule in step 6 while the other is transferred to the drug molecule in step 8. Third, ***the carbon atom that is oxidized must contain a hydrogen atom that can be abstracted***. This is required for the step 7 in the process. Without the hydrogen atom, the carbon radical could not be formed and therefore could not combine with the hydroxyl radical in the last step of the mechanism. As we progress through the different types of oxidative transformations, please note the requirement of a hydrogen atom on the carbon that is to undergo oxidation. Finally, the mechanism requires that the drug molecule is bound to the CYP450 enzyme throughout the entire mechanistic process. Readers who desire a more extensive discussion of this mechanism should consult the *Foye's* or *Wilson and Gisvold's* text previously mentioned.[1,2]

Other Enzymes Involved in Oxidative Transformations

Flavin monooxygenase (FMO) enzymes are much more specific in the transformations that they catalyze than CYP450 enzymes and are primarily involved in the direct oxidation of nitrogen and sulfur atoms located within the structure of a drug molecule. There are five different FMOs expressed within the human body; however, only three of these (FMO1, FMO2, and FMO3) are involved in drug metabolism. FMO3 is located in the liver and is responsible for most of the metabolic transformations. Similar to CYP450, FMOs require nicotinamide adenine dinucleotide phosphate (NADPH) and O_2 as components of their metabolic mechanism. An overview of the FMO catalytic cycle is shown in **Figure 8-4**. In the first two steps, Flavin adenine dinucleotide (FAD) is reduced by NADPH and a molecule of water is added to produce hydroperoxyflavin. In step 3, a nucleophilic attack by the enzyme (i.e., the oxidizing species) causes the drug molecule to be oxidized in a single step compared with the CYP450 enzyme mechanism, which requires that the drug molecule remain bound to the enzyme throughout all eight mechanistic steps. Steps 4 and 5 serve to regenerate FAD.

Alcohol dehydrogenase (ADH) enzymes catalyze the oxidation of primary and secondary hydroxyl groups to aldehydes and ketones whereas aldehyde dehydrogenase (ALDH) enzymes

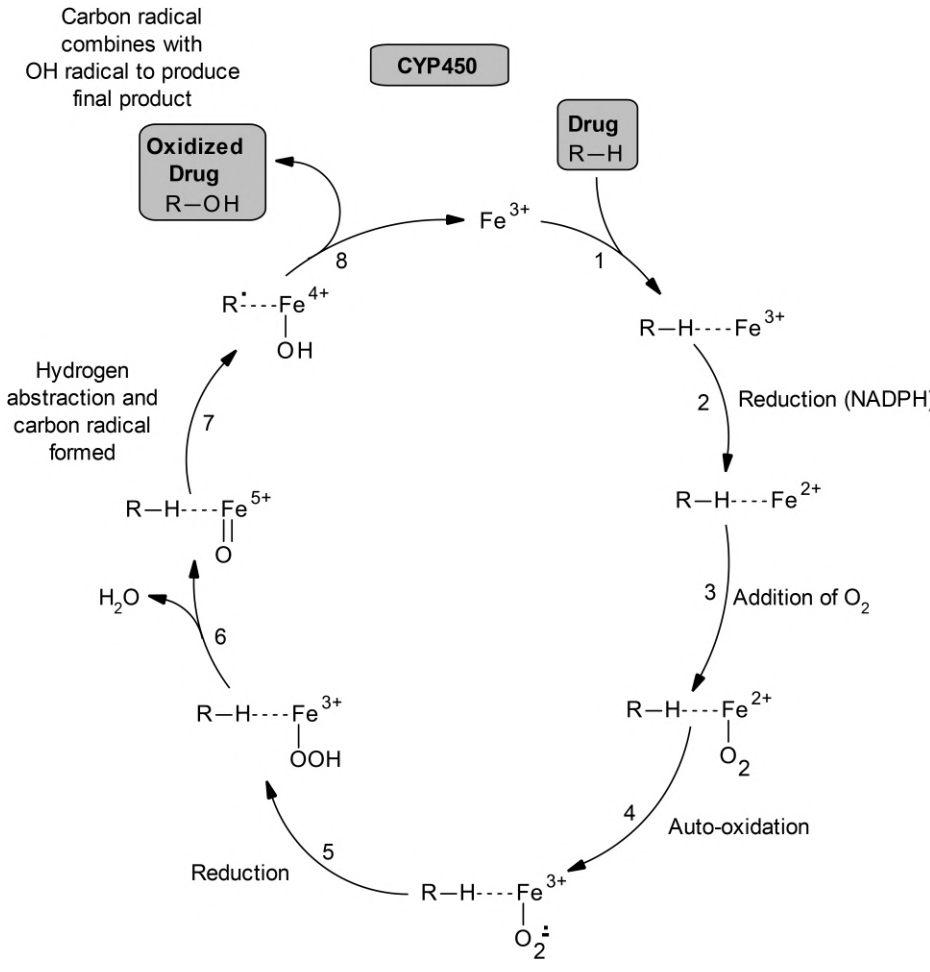
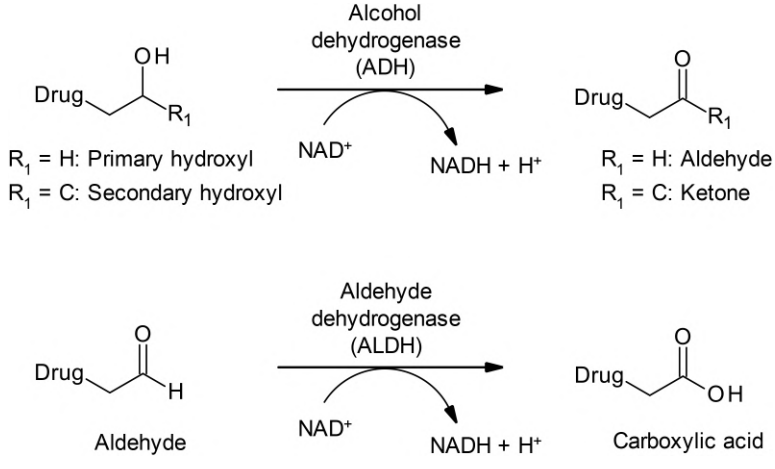


FIGURE 8-3. Mechanism of CYP450 enzymatic oxidation.

catalyze the oxidation of aldehydes to carboxylic acids. These enzymes are different from CYP450 and FMO enzymes and require nicotinamide adenine dinucleotide (NAD^+) as a cofactor. In each of these enzyme catalyzed reactions, the functional group is oxidized and NAD^+ is reduced to NADH + H^+. Examples are shown below.



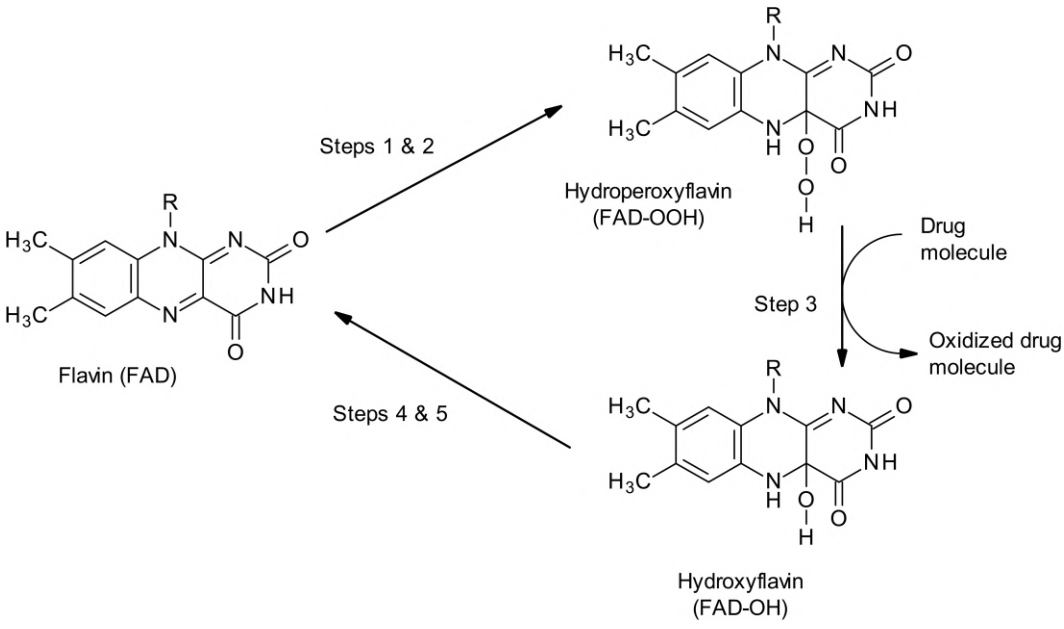


FIGURE 8-4. Mechanism of FMO enzymatic oxidation.

Oxidation of Hydrocarbon Rings and Chains

Oxidation of Aromatic Rings/Aromatic Hydroxylation

In general, unsubstituted phenyl rings are primarily hydroxylated at the *para* position due to a lack of steric hindrance. Hydroxylation can also occur at the *ortho* and *meta* positions; however, these oxidations are generally not seen as much due to steric and electronic factors, respectively. Examples of aromatic hydroxylation are shown in **Figure 8-5**. The examples illustrate two key points. First, whenever more than one aromatic ring can be oxidized, aromatic hydroxylation usually occurs at the least sterically hindered ring and position. This is seen with both paroxetine and fenoprofen. The *para* position of the unsubstituted rings present in both drug molecules is preferred over the more sterically hindered substituted phenyl rings. Second, phenyl rings with electron withdrawing substituents are less likely to undergo aromatic hydroxylation than are unsubstituted rings or those with electron donating substituents. This is seen with both diazepam and ketoprofen. The electron withdrawing chloro group deactivates the upper aromatic ring of diazepam from undergoing aromatic hydroxylation, while the unsubstituted ring can undergo this metabolic transformation. Steric hindrance may also play a role with the upper aromatic ring of diazepam. The ether oxygen present in the structure of fenoprofen is electron donating and enhances *para* hydroxylation. In contrast, the ketone present in ketoprofen is electron withdrawing and deactivates the phenyl ring from aromatic hydroxylation. Approximately 50% of a dose of fenoprofen undergoes aromatic hydroxylation, while this pathway is virtually absent for ketoprofen.

Most aromatic oxidations involve the initial formation of an arene oxide, as shown in **Figure 8-6**. Arene oxides are highly reactive and can form a number of different metabolic products, with the formation of a *para* phenol being the most common. Depending on the electronic nature of the "R" group, a *meta* phenol may also be formed (but only under specific electronic conditions). In addition to aromatic hydroxylation, the arene oxide intermediate can form three other metabolic products. In general, these are minor metabolic pathways. Because arene oxides are highly reactive, they

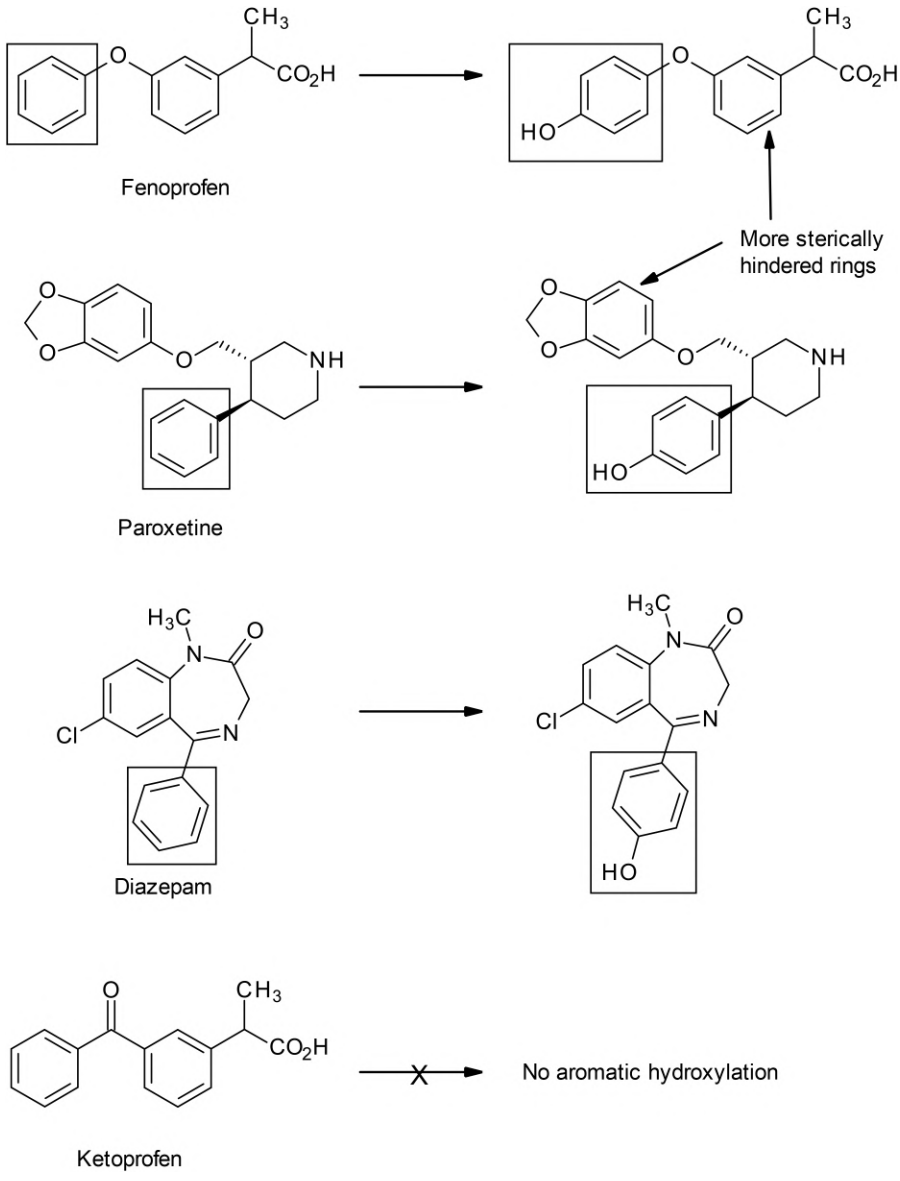


FIGURE 8-5. Examples of aromatic hydroxylation catalyzed by CYP450 enzymes.

may interact with nucleophiles present on DNA, RNA, proteins, enzymes, and/or other macromolecules. These metabolites can contribute to toxic and carcinogenic effects of some drug molecules. Fortunately, glutathione, a naturally occurring endogenous molecule, can also readily react with arene oxides to produce inactive and nontoxic metabolites. This action helps to prevent the formation of toxic and carcinogenic metabolites. Glutathione conjugation is a Phase II metabolic process and is discussed in more detail later in this chapter. Finally, arene oxides can react with water to form a *trans*-dihydrodiol. Dehydrogenation of the diol results in the formation of a catechol.

Aromatic hydroxylation can also occur *ortho* to existing phenolic groups or those groups added by previous metabolic transformations. The resulting catechol ring has enhanced water

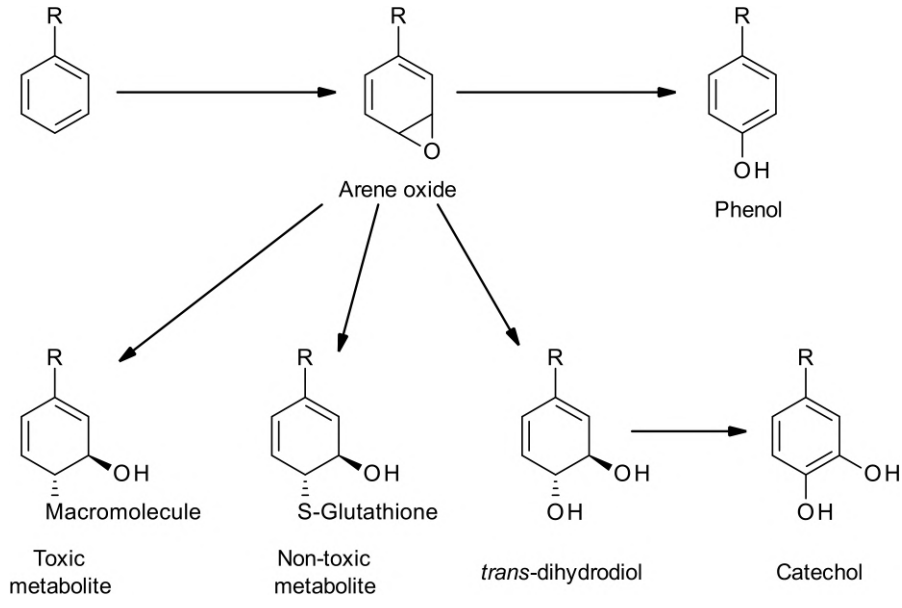
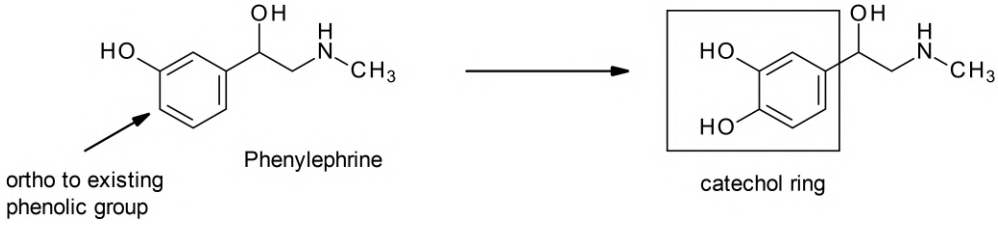


FIGURE 8-6. Possible products of CYP450 catalyzed aromatic oxidation.

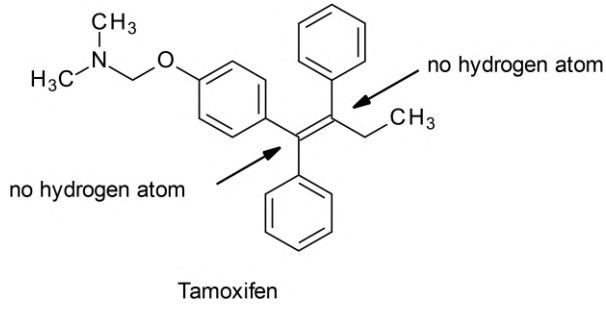
solubility and can undergo further oxidation or conjugation. An example of this is shown below with phenylephrine.



Oxidation of Alkenes

Nonaromatic carbon-carbon double bonds can undergo oxidation similar to that seen with aromatic rings. This can produce epoxides, diols, and peroxides, as shown with protriptyline in **Figure 8-7**.

For this type of transformation to occur, there must be at least one hydrogen atom on one of the two alkene carbon atoms. As such, the alkene bond present within the structure of tamoxifen cannot undergo oxidation.



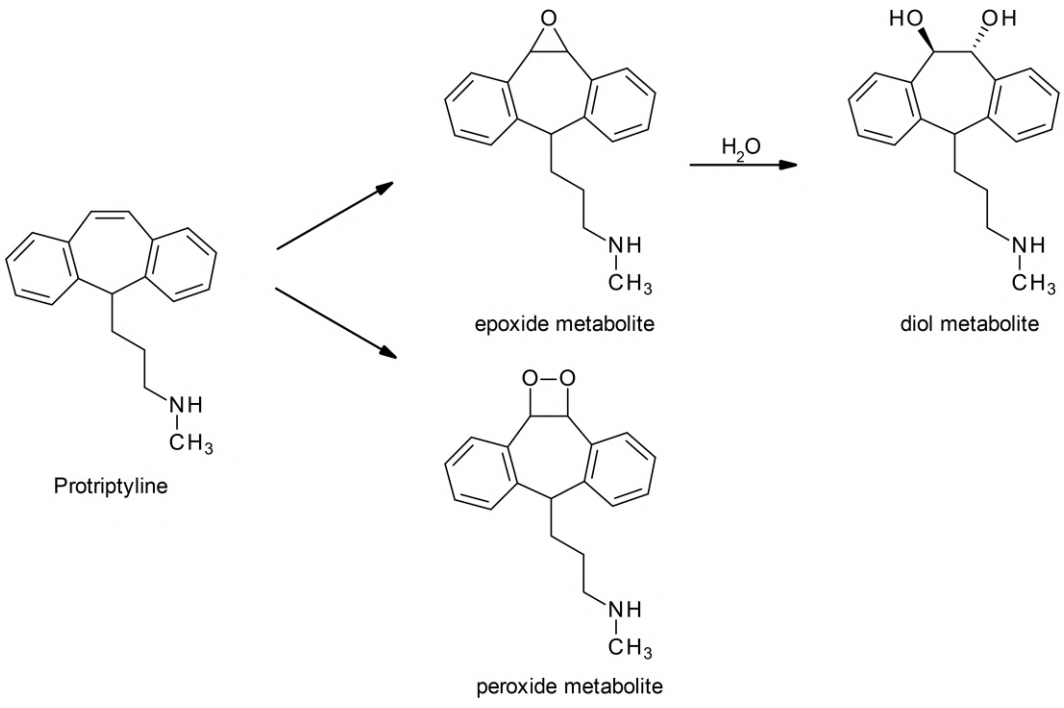


FIGURE 8-7. Examples of alkene oxidation catalyzed by CYP450 enzymes.

Oxidation of Carbon Atoms Adjacent to sp^2 Hybridized Centers

Carbon atoms directly adjacent to a functional group with sp^2 hybridization can undergo oxidation. This metabolic transformation adds a hydroxyl group at benzylic carbon atoms, allylic carbon atoms, and carbon atoms adjacent to an imine bond or a carbonyl bond (e.g., ketone, amide, ester). The carbon atom cannot be part of an aromatic ring system, and it must have at least one hydrogen atom attached to it. Additionally, if the carbon atom has a heteroatom attached to it (e.g., hydroxyl group, ether, substituted or unsubstituted amines), oxidation can still occur; however, the resulting intermediate is normally unstable and results in the formation of a metabolite other than a hydroxylated carbon atom. Examples that include additional heteroatoms are discussed later. We focus here on examples that contain only hydrocarbon.

A *benzylic carbon atom* is an aliphatic carbon atom that is directly attached to an aromatic ring. Examples of benzylic oxidation can be seen in **Figure 8-8** with glyburide and ethinyl estradiol. Please note that ethinyl estradiol has two benzylic carbon atoms. Although each of these carbon atoms has a hydrogen atom attached to it, benzylic oxidation only occurs at the least sterically hindered position. This reinforces what was previously mentioned: metabolic enzymes generally seek to enhance water solubility in the easiest way possible.

An allylic carbon atom is an aliphatic carbon atom that is directly attached to an alkene or, as otherwise stated, directly adjacent to a nonaromatic double bond. Examples of allylic oxidation can be seen in **Figure 8-9** with spironolactone and tamoxifen. Please note that spironolactone has two allylic carbon atoms; however, only one of these can undergo allylic oxidation because the other does not have a hydrogen atom attached to it. Additionally, and as discussed earlier, tamoxifen cannot undergo alkene oxidation due to the lack of a hydrogen atom on the alkene carbon atoms; however, it can undergo allylic oxidation (as well as other metabolic transformations).

Aliphatic or alicyclic carbon atoms that are directly adjacent to either an imine (i.e., a carbon-nitrogen double bond) or a carbonyl-containing functional group can also undergo oxidation and

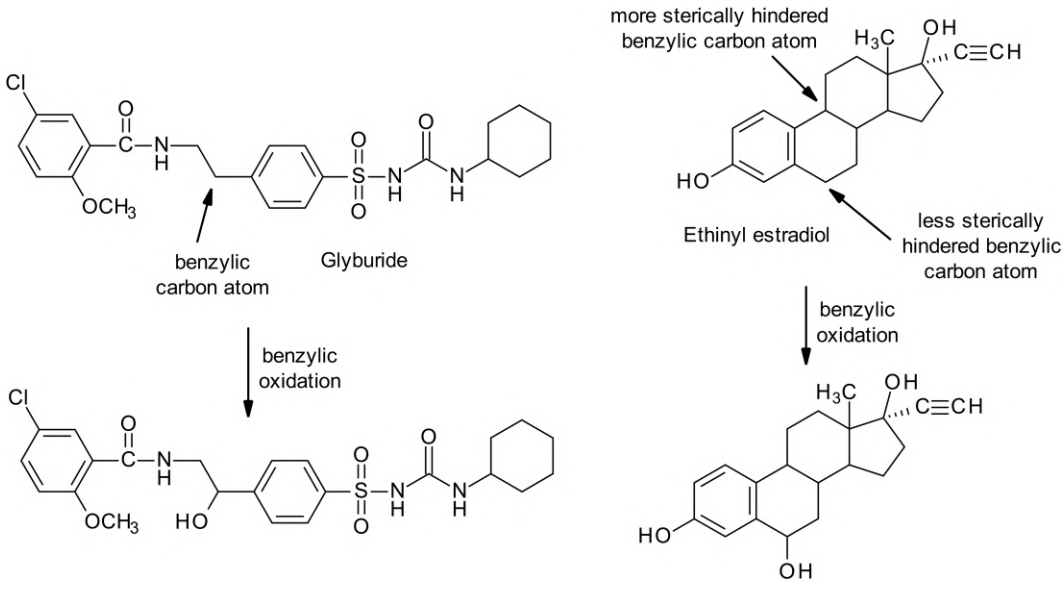


FIGURE 8-8. Examples of benzylic oxidation catalyzed by CYP450 enzymes.

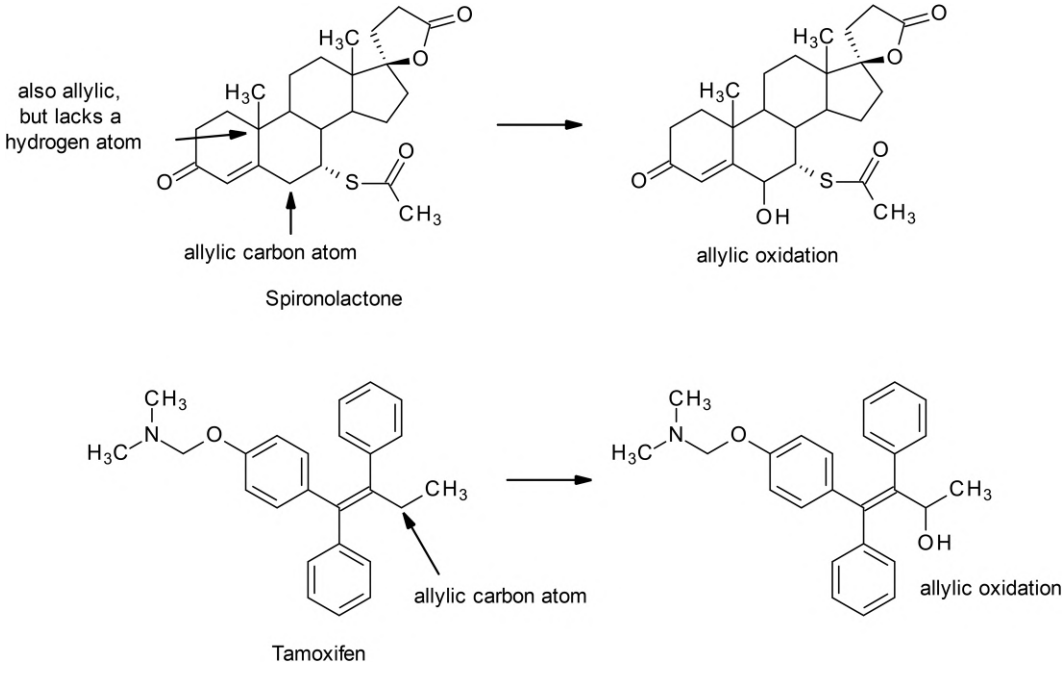


FIGURE 8-9. Examples of allylic oxidation catalyzed by CYP450 enzymes.

introduce a hydroxyl group into the structure of the drug molecule. Examples of these are shown in **Figure 8-10**. Oxidation of flurazepam occurs at the C_3 position of the benzodiazepine ring system. Although this C_3 carbon atom is adjacent to the imine nitrogen (a heteroatom), this is one exception in which the resulting hydroxyl group is stable. Additionally, this carbon atom is adjacent to both an imine and a carbonyl group and is highly activated for oxidation. Oxidation of benzodiazepines at this position represents a major route of metabolism for this class of drug molecules. In general, the

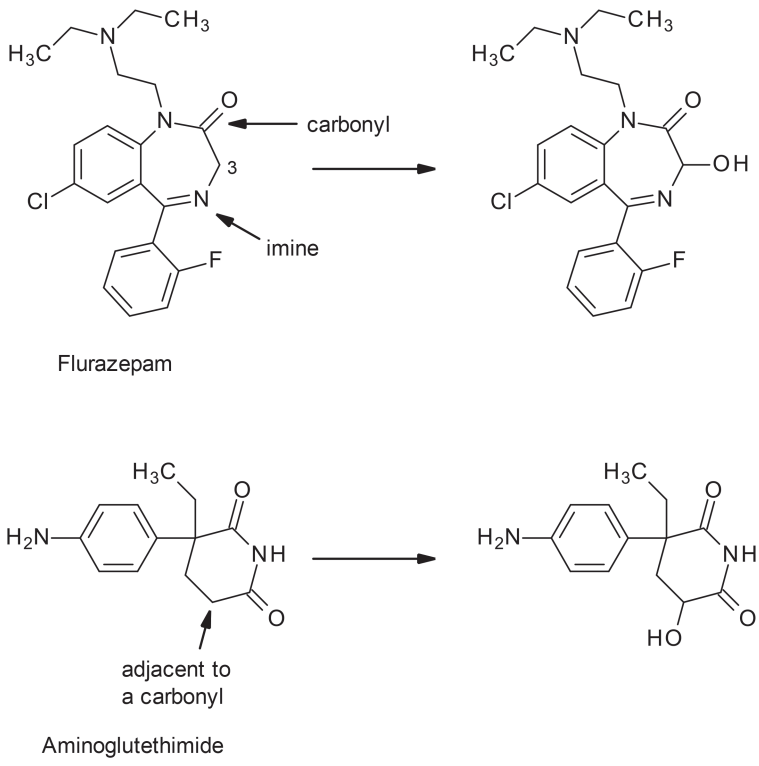


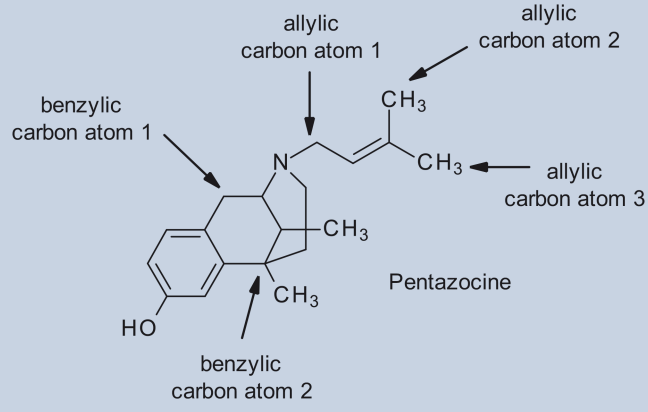
FIGURE 8-10. Examples of CYP450 catalyzed oxidation of carbon atoms adjacent to imines and carbonyl groups.

hydroxylation of a carbon atom directly adjacent to a single carbonyl group is not a major route of drug metabolism; however, an example of this is seen with aminoglutethimide.

Please note that in all examples shown in Figures 8-8 to 8-10, the carbon atoms were prochiral; thus, the oxidation of these atoms generated a new chiral center. Although this does not always occur, it is quite common. It many cases, only one stereoisomer is formed.

Application Question

Shown below is the structure of pentazocine. This drug molecule contains five allylic and benzylic carbon atoms. Evaluate these five potential oxidation sites and identify the carbon atoms that are most likely to be oxidized.



Answer: Allylic carbon atoms 2 and 3 are the least sterically hindered and, thus, are the most likely to undergo oxidation. Furthermore, these two carbon atoms are not identical. Allylic carbon atom 2 is located on the same side of the double bond as a methylene carbon, while allylic carbon atom 3 is located on the same side of the double bond as a hydrogen atom. As such, the oxidation of allylic carbon atom 2 is more sterically hindered than the oxidation of allylic carbon atom 3. The oxidation of allylic carbon atom 3 to a primary hydroxyl group is the major metabolic pathway for pentazocine. Allylic carbon atom 1 and benzylic carbon atom 1 are much more sterically hindered than allylic carbon atoms 2 and 3 and are much less likely to undergo oxidation. Additionally, allylic carbon atom 1 is attached to a heteroatom. If oxidation did occur at this site, the initial metabolite would be unstable and not result in the formation of a hydroxyl group. Benzylic carbon 2 lacks a hydrogen atom and thus does not meet the criteria for CYP450 oxidation.

Oxidation of Aliphatic and Alicyclic Carbon Atoms

Aliphatic carbon chains can undergo oxidation at either the terminal methyl group in the chain or at the penultimate (i.e., next to last) carbon atom in the chain. The locations of these carbon atoms are also known as the omega (ω) and omega-1 (ω-1) positions, and these oxidations are commonly referred to as ω oxidation and ω-1 oxidation. Examples are shown in **Figure 8-11**. Please note that

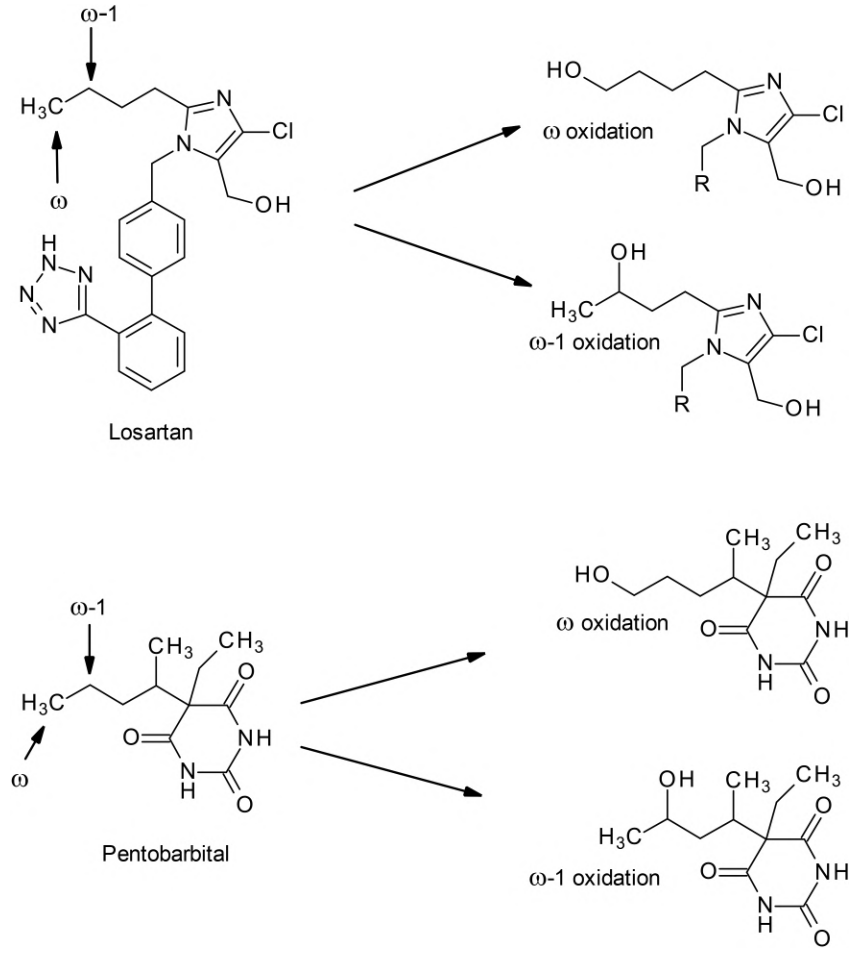


FIGURE 8-11. Examples of ω oxidation and ω-1 oxidation catalyzed by CYP450 enzymes.

while both ω and ω-1 oxidations are often available for drug molecules with alkyl chains, in most cases oxidation would only need to occur at one of these positions to appropriately enhance water solubility and/or provide a functional group that could undergo Phase II conjugation. This is another reminder that drug metabolism occurs in an efficient manner and uses the least number of steps necessary to eliminate and/or deactivate the drug molecule.

Monosubstituted alicyclic rings and nonaromatic heterocyclic rings can also undergo oxidation. Similar to aromatic oxidation, these rings tend to be oxidized at the least sterically hindered positions. For cyclohexane rings, oxidation generally occurs at the C_3 or C_4 position, as seen with dicyclomine in **Figure 8-12**. Please note that due to the electronic nature of an aromatic ring, the C_3 position of a cyclohexane is not the same as the *meta* position of a phenyl ring. As such, oxidation at the C_3 position of cyclohexane ring is more likely to undergo oxidation than is the *meta* position of a phenyl ring. Five- and seven-membered nonaromatic rings can also undergo this type of oxidation, as seen with the cyclopentane ring of penbutolol and the hexahydroazepine ring of tolazamide. As shown in Figure 8-12, the oxidations occur at either the C_3 or C_4 positions of these rings because these carbon atoms are the least sterically hindered. With one exception, all of these oxidations occur at prochiral carbon atoms. Due to its symmetrical nature, the C_4 position of dicyclomine is not a prochiral center. Due to their alicyclic or nonaromatic heterocyclic nature, oxidation at the C_3 or C_4 positions can produce *cis* and *trans* geometric isomers.

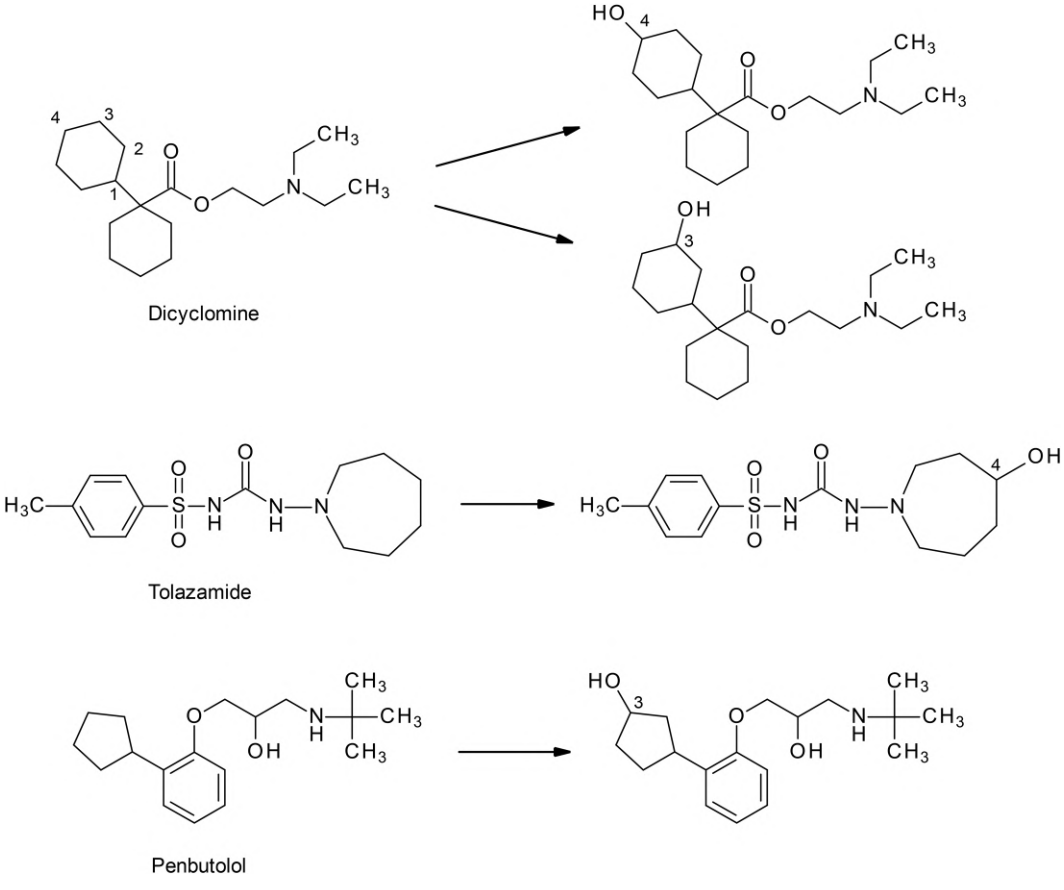


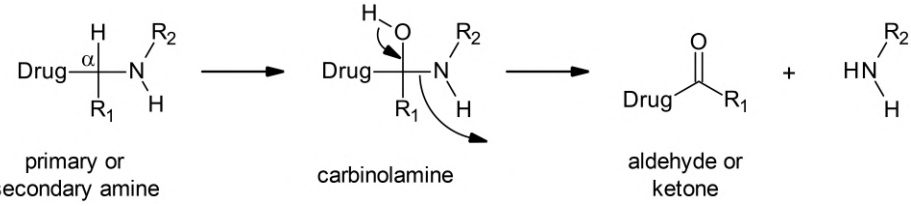
FIGURE 8-12. Examples of CYP450 catalyzed hydroxylation of nonaromatic rings.

Oxidation of Amines, Amides, and Aromatic Nitrogen Atoms

Three major oxidative transformations are available for amines and amides: oxidative deamination, oxidative N-dealkylation, and N-oxidation. The specific transformation often depends on the nature of other functional groups attached to the nitrogen atom. Primary, secondary, and tertiary amines are often metabolized differently, as shown in the examples below. The primary route of oxidative metabolism for aromatic nitrogen atoms is *N*-oxidation because they cannot undergo oxidative deamination or oxidative *N*-dealkylation. Quaternary heterocyclic nitrogen atoms can undergo *N*-dealkylation but not oxidative deamination or *N*-oxidation.

Oxidative Deamination

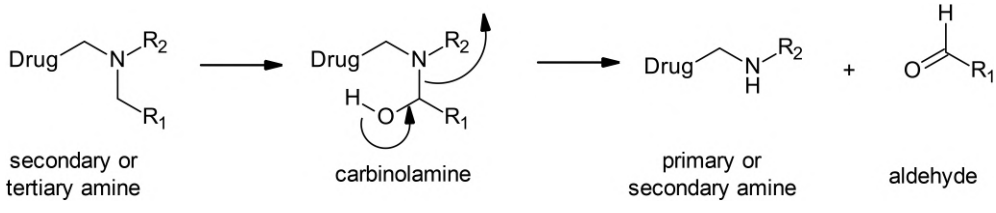
This metabolic transformation primarily occurs with primary amines; however, there is some evidence that it can also occur with secondary amines. For oxidative deamination to occur, the α-carbon (i.e., the carbon atom directly adjacent to the nitrogen atom) must be attached to at least one hydrogen atom. The general mechanism of oxidative deamination is shown below.



In this mechanism, the carbon atom that is α to the amine undergoes oxidation. The resulting intermediate is known as a carbinolamine because the α-carbon atom is now attached to both a hydroxyl (i.e., alcohol) group and an amine. Carbinolamines are unstable intermediates and undergo a further reaction to generate either an aldehyde or ketone, depending on whether the R_1 group is a hydrogen atom or a carbon atom, respectively. This reaction causes the release of ammonia or a primary amine, depending on whether the R_2 group is a hydrogen atom or a carbon atom, respectively. Examples of drugs that can undergo oxidative deamination are shown in **Figure 8-13**. In looking at these examples, please note that the primary or secondary amine is removed and that the resulting metabolite retains the remaining portion of the drug molecule.

Oxidative N-Dealkylation

This metabolic transformation can occur with secondary or tertiary amines or amides. The mechanism of oxidative *N*-dealkylation (henceforth denoted simply as *N*-dealkylation) is very similar to oxidative deamination. As shown below, the alkyl group initially undergoes oxidation to form a carbinolamine. The carbinolamine then undergoes a reaction leading to the formation of an *N*-dealkylated drug molecule and either an aldehyde (shown below) or a ketone, depending on the alkyl group that is removed. Similar to oxidative deamination, the removed alkyl group must contain a hydrogen atom. *N*-Dealkylation of tertiary amines produces secondary amines, and *N*-dealkylation of secondary amines produces primary amines.



Although oxidative deamination and *N*-dealkylation are similar, there is a dis*tinct difference* between these two metabolic transformations. In *oxidative deamination*, a carbon atom is oxidized to an aldehyde or ketone and the nitrogen atom leaves as ammonia or a primary amine.

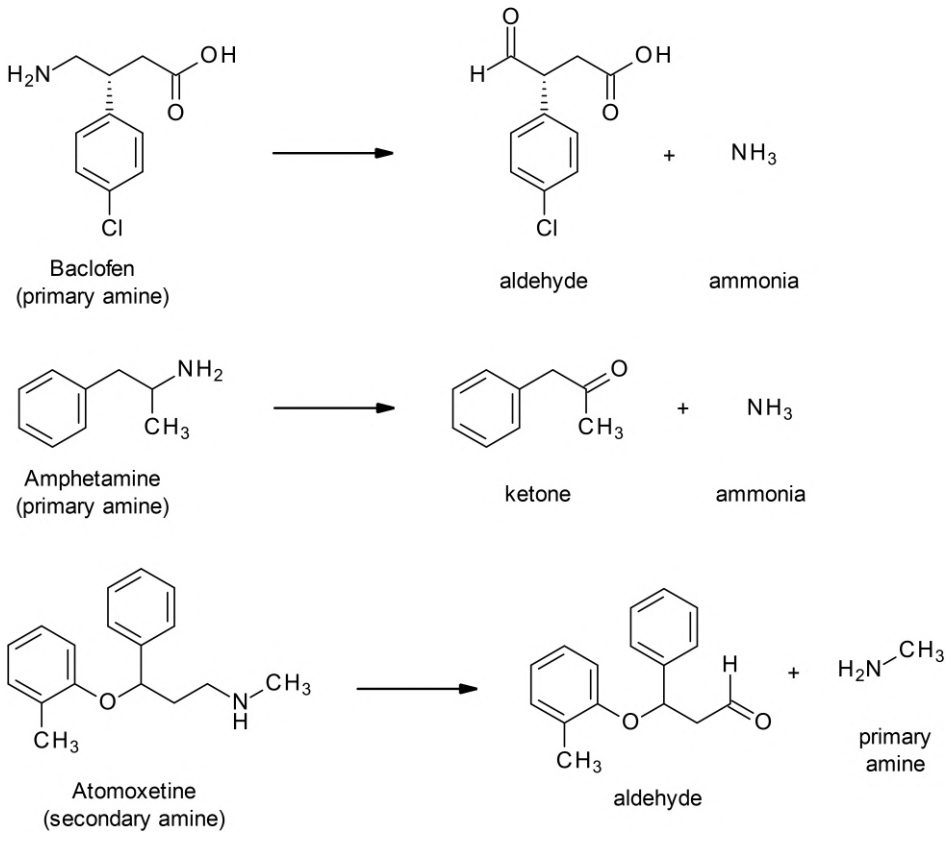
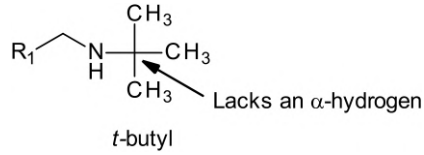


FIGURE 8-13. Examples of oxidative deamination catalyzed by CYP450 enzymes.

As mentioned above, the metabolite no longer contains the nitrogen atom and is composed of the remainder of the drug molecule. In *N-dealkylation*, an alkyl group is oxidized and removed from the drug molecule. The metabolite still contains the nitrogen atom as well as the remainder of the drug molecule. To prevent any confusion between these metabolic processes, readers are highly encouraged to compare the examples in Figure 8-13 and those shown below and take advantage of the fact that *deamination* literally means that an amine is removed from the drug molecule and that *dealkylation* literally means that an alkyl group is removed from the drug molecule.

In general, smaller alkyl groups are more likely to undergo *N*-dealkylation than are larger groups. Examples of alkyl groups known to undergo this type of metabolic transformation include methyl groups, ethyl groups, propyl groups, isopropyl groups, butyl groups, and benzyl groups. Due to the lack of a hydrogen atom, functional groups such as a *t*-butyl group cannot be removed by *N*-dealkylation.



Examples of two drugs that are known to undergo *N*-dealkylation are shown in **Figure 8-14**. The functional groups on meperidine, a tertiary amine, and diazepam, an amide, that can be dealkylated have been highlighted.

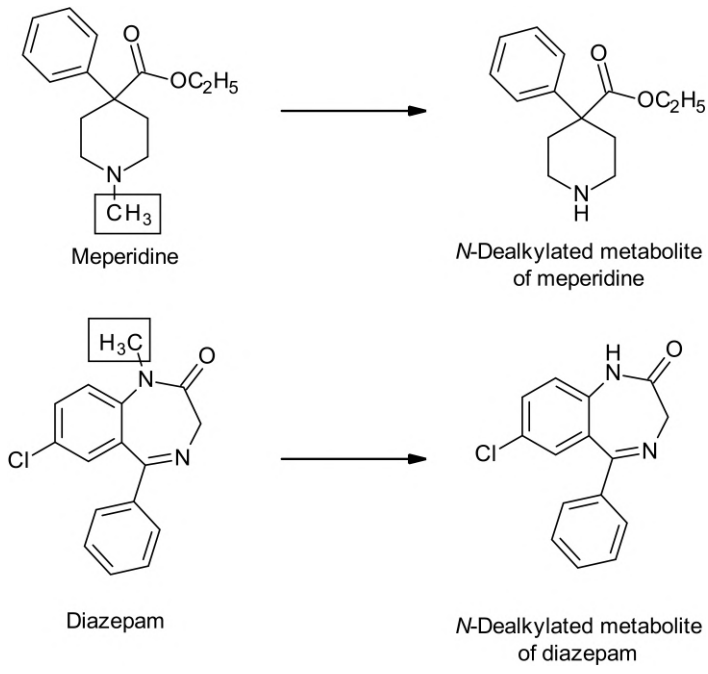
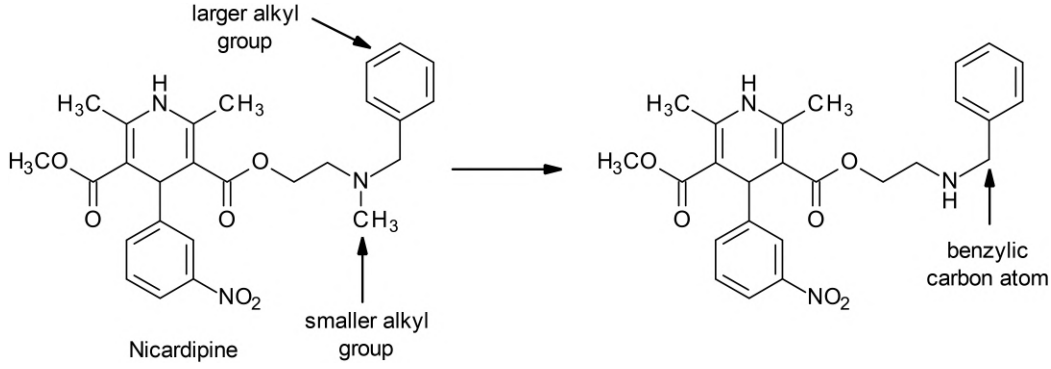


FIGURE 8-14. Examples of drug molecules that can undergo CYP catalyzed oxidative *N*-dealkylation.

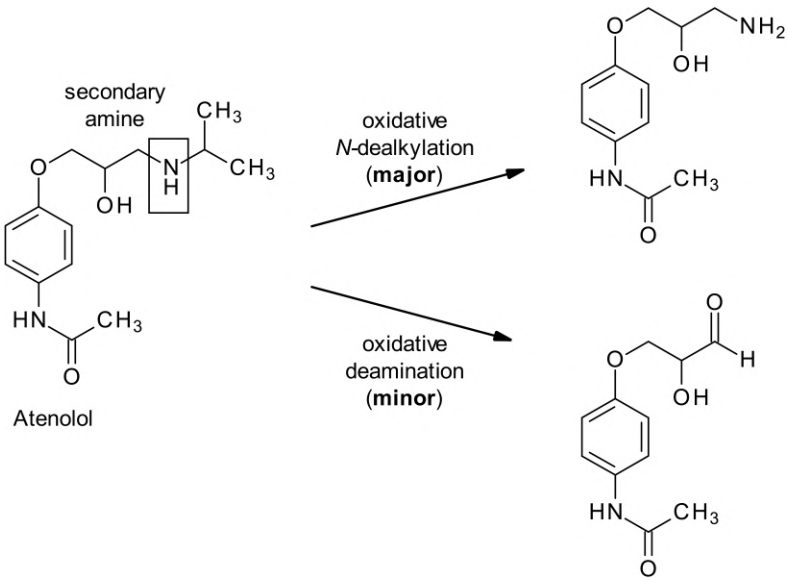
There are four key points regarding drug molecules and N-dealkylation.

1. Whenever a tertiary amine contains two different alkyl groups, as seen with nicardipine, the smaller alkyl group is almost always removed before the larger group.

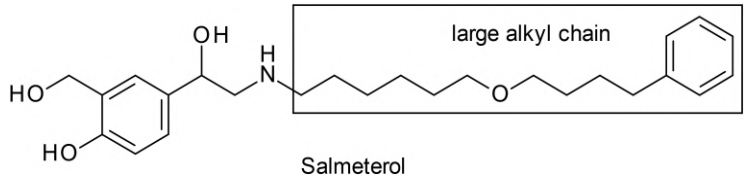


Nicardipine is also useful in reinforcing a previous concept. As shown in its structure, the larger alkyl group contains a benzylic carbon atom that is attached to a heteroatom. As previously discussed, this benzylic carbon atom can undergo oxidation; however, it does not produce a stable secondary hydroxyl group. Oxidation of this benzylic carbon atom forms a carbinolamine that collapses and forms an *N*-dealkylated metabolite. Thus, oxidation at this carbon atom is correctly described as *N*-dealkylation and not benzylic oxidation.

2. As exemplified by atenolol, drug molecules that contain secondary amines can directly undergo oxidative deamination or initially undergo *N*-dealkylation. In these situations, oxidative deamination is normally a minor metabolic pathway, and *N*-dealkylation is normally the major pathway.



3. Secondary and tertiary amines located in the middle of a drug molecule and attached to large alkyl chains are less likely to undergo *N*-dealkylation. This is illustrated with salmeterol and is an example of a previously noted concept. Functional groups that are sterically hindered or otherwise difficult to access are much less likely to be metabolized than those that are easily accessible. In terms of salmeterol, aromatic oxidation and benzylic oxidation are less sterically hindered than is dealkylation of the large alkyl chain.



4. As illustrated in **Figure 8-15**, amitriptyline can undergo two *N*-dealkylations and an oxidative deamination. Similar to atenolol, once the first methyl group is dealkylated, the resulting secondary amine could directly undergo oxidative deamination. Returning to a previously discussed concept, the human body uses only the *minimum number* of these transformations that it needs to enhance the water solubility of the drug and allow for its excretion. For some drug molecules, all of these steps are required; for others, perhaps only one or two.

Secondary and tertiary alicyclic amines can form lactones, as illustrated with niacin (aka nicotinic acid) in **Figure 8-16**. Oxidation of the alicyclic carbon atom adjacent to the tertiary nitrogen produces a carbinolamine. The carbinolamine subsequently breaks down to form a secondary amine and an aldehyde similar to what was seen with oxidative deamination and *N*-dealkylation; however, because this metabolic transformation began with an alicyclic ring, both functional groups remain as part of the initial metabolite. As discussed later in this chapter, aldehydes can be further oxidized to carboxylic acids. Because the secondary amine is close to this newly formed carboxylic acid, the functional groups can combine using a condensation reaction to form a lactone. The methyl group can be removed via *N*-dealkylation either prior to or after lactone formation. Lactone formation does not always occur with alicyclic amines; however, it should be considered as a possible metabolic pathway.

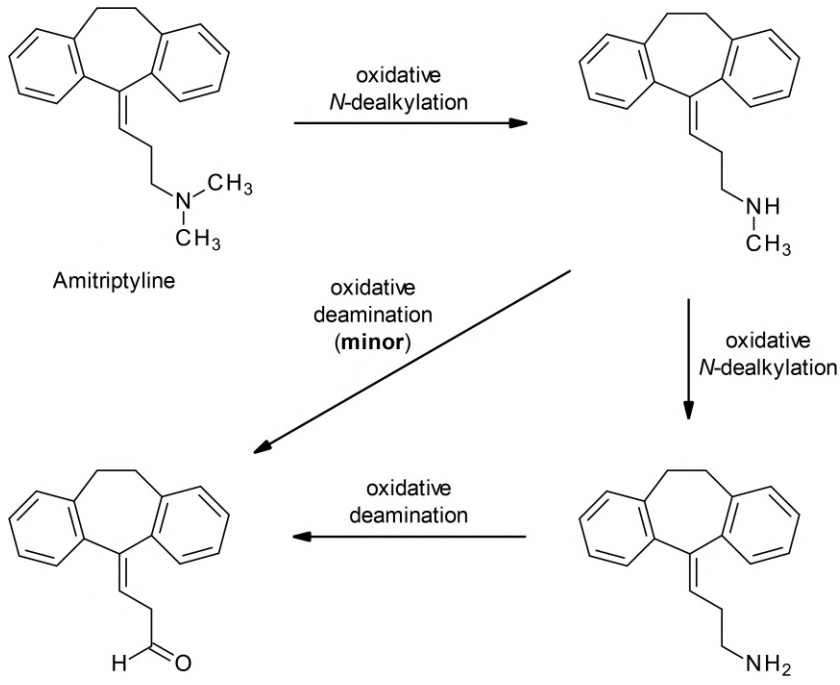


FIGURE 8-15. Possible metabolic pathways for amitriptyline.

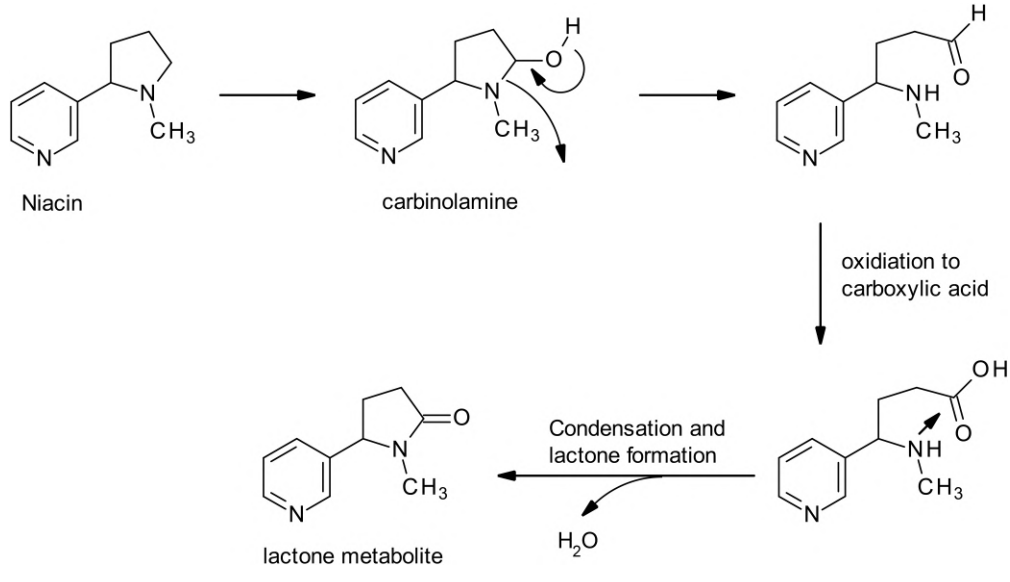


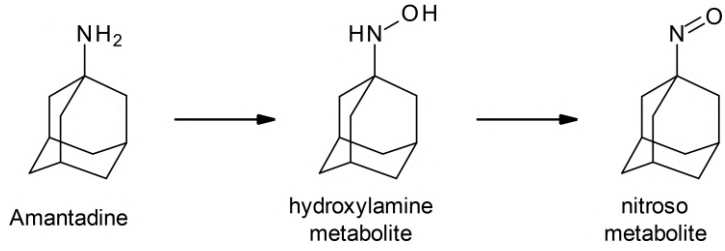
FIGURE 8-16. Formation of a lactone metabolite of niacin.

N-Oxidation

Unlike oxidative deamination and *N*-dealkylation, *N*-oxidation involves a direct oxidation of the nitrogen atom as opposed to an adjacent carbon atom. Although it is not necessary for an adjacent carbon atom to be attached to a hydrogen atom, the products of *N*-oxidation vary based on the presence or absence of a hydrogen atom. *N*-Oxidation of secondary and tertiary amines can be

catalyzed by either CYP450 or FMO enzymes whereas *N*-oxidation of primary amines is almost always catalyzed by CYP450 enzymes.

Primary amines that are attached to a carbon atom that lacks a hydrogen atom can be sequentially oxidized to hydroxylamines and nitroso groups. Overall, only a few drugs have this structural feature. An example is seen below with the antiviral agent amantadine. Oxidative deamination is not possible for amantadine; however, *N*-oxidation can occur.



The carbon atom adjacent to most primary amines is normally attached to at least one hydrogen atom. Although oxidative deamination is the most likely metabolic transformation for a primary amine, it can also be directly oxidized to sequentially form hydroxylamines, imines, oximes, and aldehydes. An example is shown in **Figure 8-17** with primaquine. Please note that the aldehyde metabolite resulting from hydrolysis of the imine is the exact same metabolite that would be formed if primaquine were directly metabolized by oxidative deamination. The oxime metabolite is a tautomeric form of the nitroso group seen with amantadine.

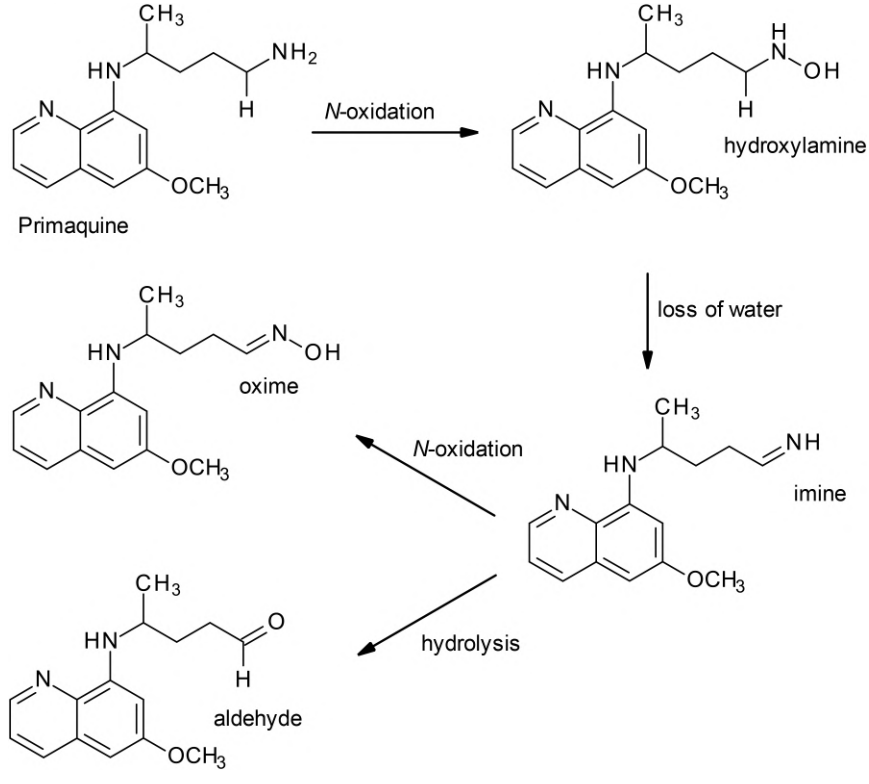


FIGURE 8-17. CYP450 catalyzed *N*-oxidation of the primary amine of primaquine.

N-Oxidation of secondary amines can sequentially form hydroxylamines, imines, nitrones, and aldehydes. On the rare occasion that both carbon atoms attached to the secondary amine lack a hydrogen atom, then only a hydroxylamine can be formed. An example of what could occur with the *N*-oxidation of a secondary amine is shown in **Figure 8-18** with encainide. Similar to primaquine, the original hydroxylamine can undergo a dehydration reaction to yield an imine. Hydrolysis of the imine opens the ring and produces the same metabolic product that can be directly formed via *N*-dealkylation. Further oxidation of the imine produces a nitrone.

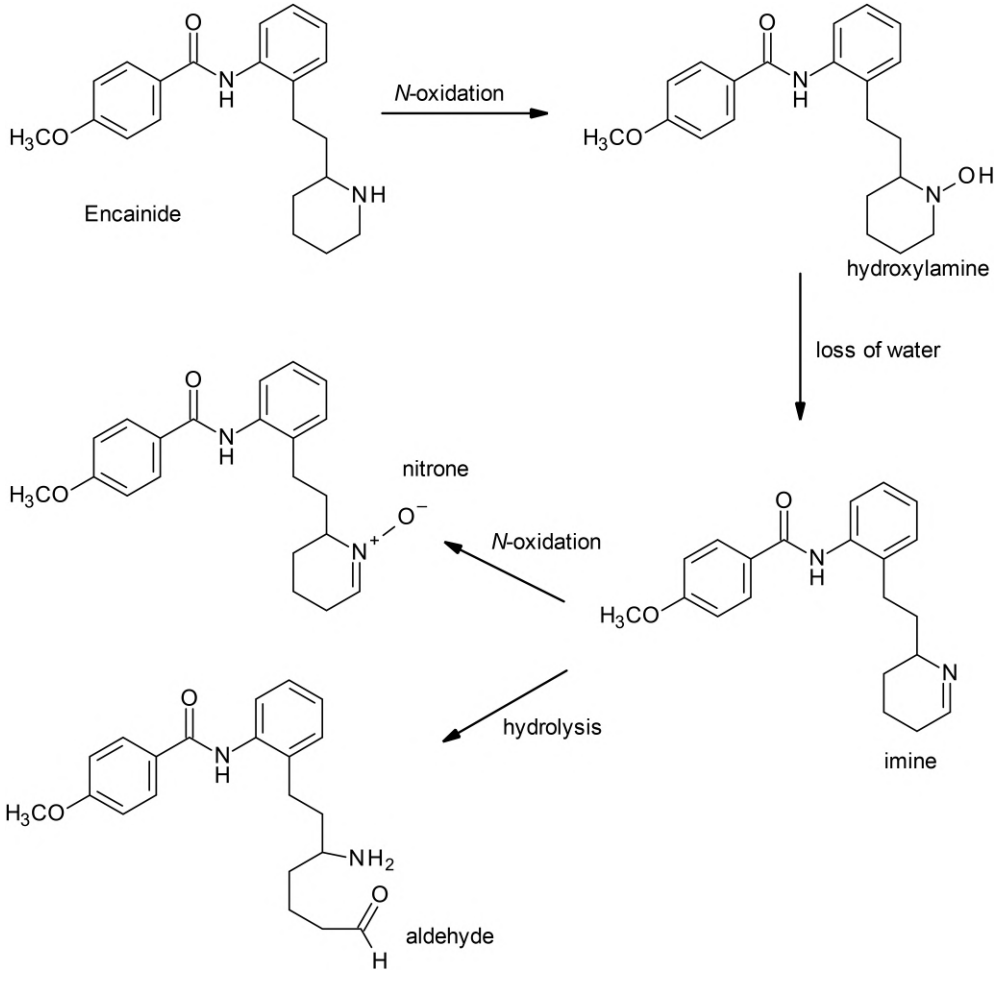


FIGURE 8-18. CYP450 or FMO catalyzed *N*-oxidation of the secondary amine of encainide.

Tertiary amines as well as heterocyclic amines can be directly oxidized to form *N*-oxides. Shown in **Figure 8-19** are the structures of prochlorperazine, a drug with multiple tertiary amines, and zaleplon, a drug with multiple heterocyclic amines. A single nitrogen atom has been chosen to illustrate *N*-oxidation for both of these drugs. Please note that this metabolic transformation could also occur at the other nitrogen atoms on these drugs. As with other examples of *N*-oxidation, there are other metabolic transformations that could occur, some of which may be more prevalent. In the case of prochlorperazine, *N*-dealkylation and aromatic hydroxylation followed by Phase II conjugation are much more prevalent than *N*-oxidation. The examples here are provided to illustrate metabolic pathways that could possibly occur.

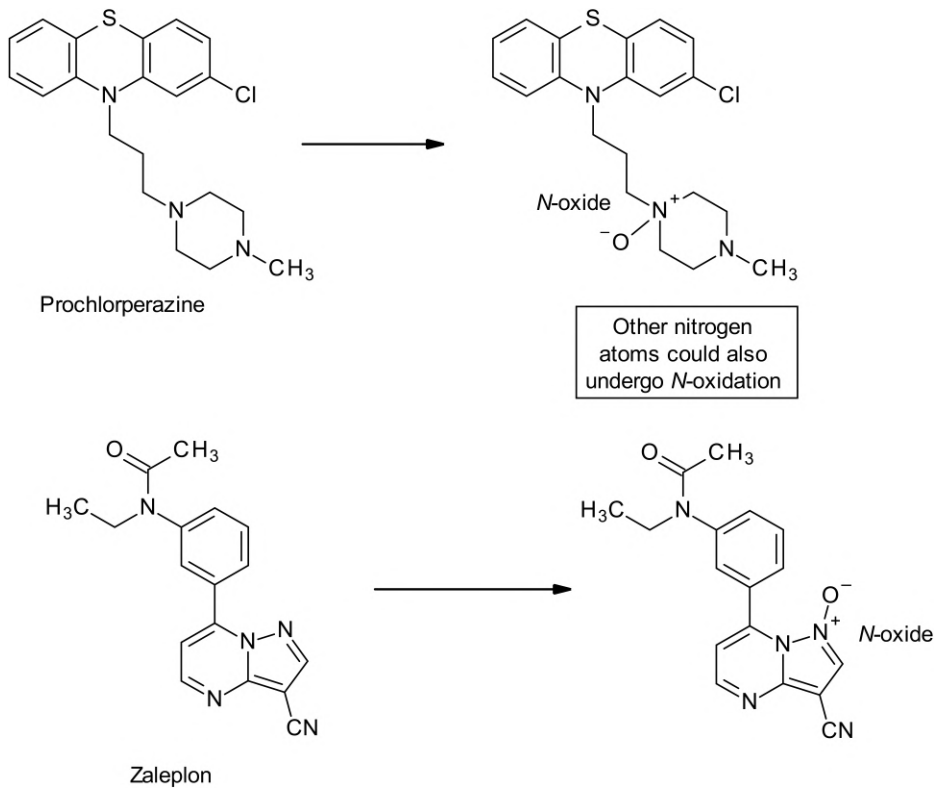


FIGURE 8-19. Examples of *N*-oxidation of tertiary and heterocyclic amines catalyzed by CYP450 or FMO enzymes.

In some instances, *N*-oxidation can lead to the formation of potentially toxic metabolites. This is especially true if adjacent functional groups help to catalyze the formation of a highly electrophilic intermediate. A good example of this is seen with acetaminophen (**Figure 8-20**). When taken in normal doses, approximately 1% to 2% of acetaminophen undergoes *N*-oxidation. Due to the presence of the *para* phenol group, there is a subsequent dehydration and the formation of a highly reactive electrophile. At normal doses, the concentration of this reactive intermediate is low enough that it is readily deactivated by glutathione conjugation; however, in acetaminophen overdoses, saturation of conjugation pathways can lead to liver damage and death.

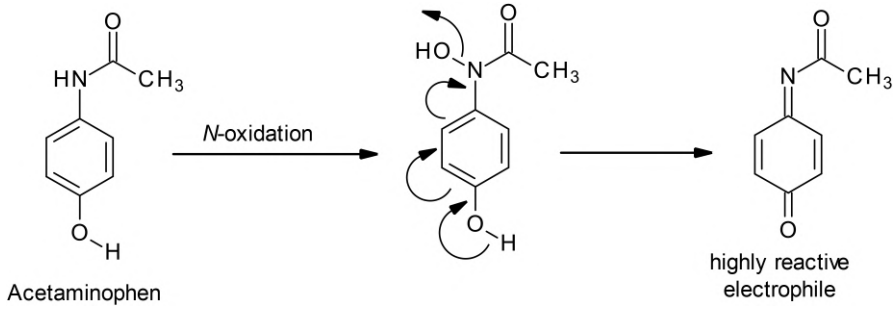


FIGURE 8-20. *N*-Oxidation of acetaminophen and the generation of a potentially toxic intermediate.

Oxidation of Functional Groups with Carbon-Oxygen Bonds

Hydroxyl groups that were initially present within the structure of a drug molecule or were added as a result of the oxidation of hydrocarbon rings and chains can undergo oxidation to produce aldehydes, ketones, and carboxylic acids. As previously mentioned, ADH enzymes catalyze the oxidation of primary and secondary hydroxyl groups to aldehydes and ketones, respectively, while ALDH enzymes catalyze the oxidation of aldehydes to carboxylic acids. These dehydrogenase enzymes are distinct from CYP450 enzymes and are not affected by drugs that induce or inhibit CYP450 isozymes.

Primary hydroxyl groups are initially oxidized to an aldehyde and then to a carboxylic acid. Additionally, aldehydes generated via *N*-dealkylation or oxidative deamination can also undergo additional oxidation to produce carboxylic acids. It is rare that oxidation stops at the aldehyde. Two examples are shown in **Figure 8-21**. The structure of albuterol already contains three hydroxyl

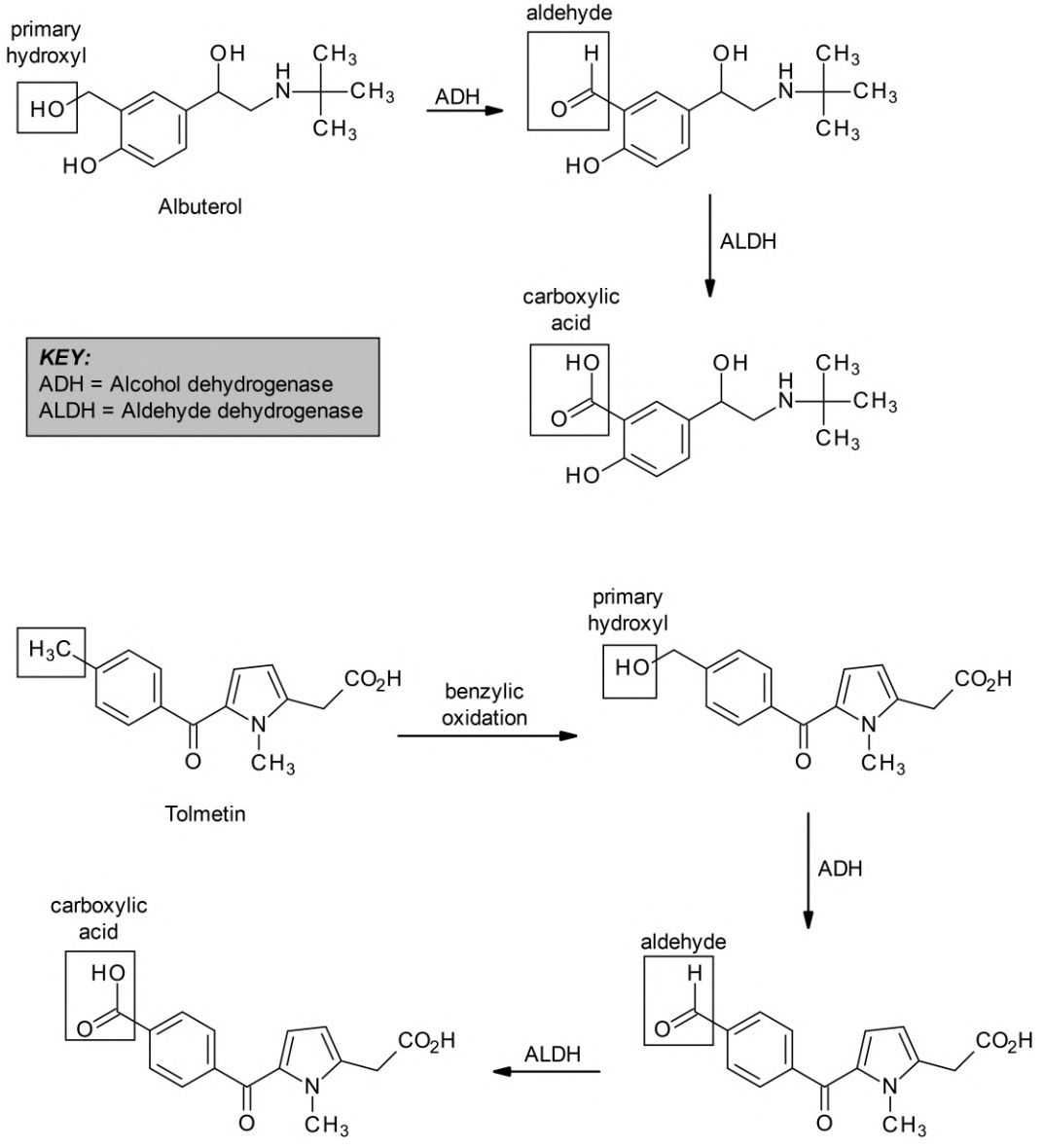


FIGURE 8-21. Oxidation of primary hydroxyl groups to aldehydes and carboxylic acids (see boxes).

groups. The primary hydroxyl group can be initially oxidized to an aldehyde and then to a carboxylic acid. The structure of tolmetin does not initially contain a hydroxyl group; however, benzylic oxidation of the *para* methyl group results in the formation of a primary hydroxyl group. Similar to albuterol, this primary hydroxyl group can be further oxidized to an aldehyde and then to a carboxylic acid.

Secondary hydroxyl groups can be oxidized to ketones. Two examples are shown in **Figure 8-22**. The secondary hydroxyl group originally present within the structure of albuterol can be directly oxidized to a ketone whereas a ketone metabolite of pentobarbital can occur following an initial ω-1 oxidation. Tertiary hydroxyl groups cannot be further oxidized.

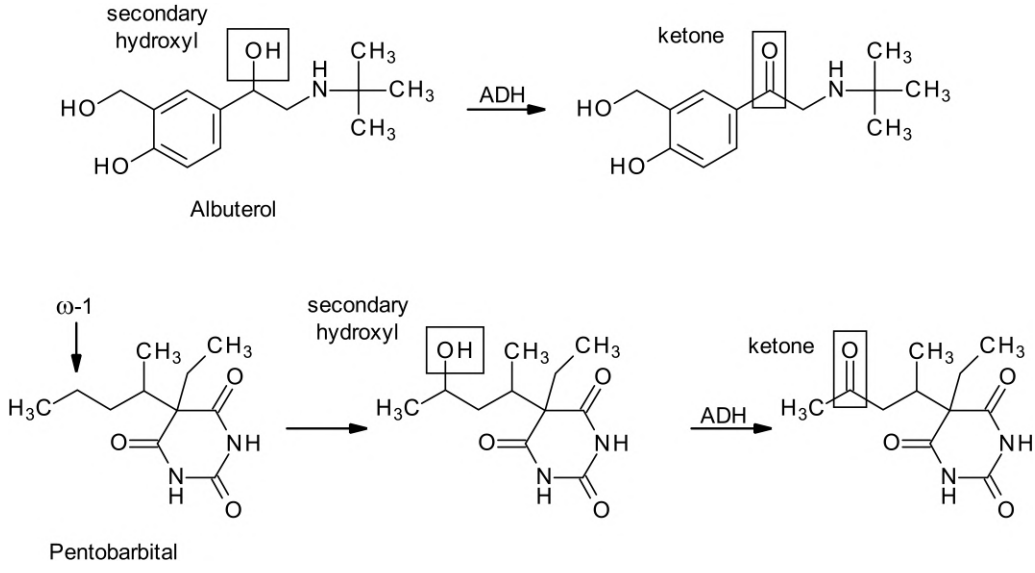
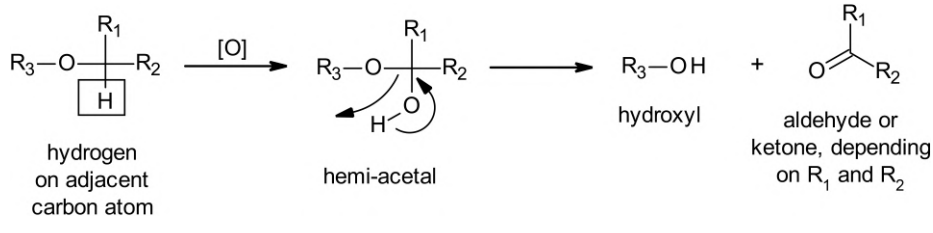


FIGURE 8-22. Oxidation of secondary hydroxyl groups to ketones by ADH (see boxes).

Similar to *N*-dealkylation, ethers can be metabolized via oxidative *O*-dealkylation (henceforth denoted as *O*-dealkylation) to more water-soluble hydroxyl groups. The mechanism of this metabolic transformation is shown below. Please note that this mechanism is very similar to that discussed for *N*-dealkylation and that it requires a hydrogen atom to be attached to at least one of the carbon atoms that are adjacent to the ether oxygen atom. The only mechanistic difference is that the initial intermediate is a hemiacetal instead of a carbinolamine; however, similar to a carbinolamine, a hemiacetal is unstable and subsequently reacts to form a hydroxyl group and either an aldehyde or a ketone.



Examples of drugs that can undergo *O*-dealkylation are shown in **Figure 8-23**. Similar to *N*-dealkylation, smaller alkyl groups are more likely to undergo *O*-dealkylation than are larger groups. Methyl ethers (i.e., methoxy groups), as exemplified by trimethoprim, often undergo *O*-dealkylation. Oxidation of fluoxetine at the benzylic position produces a hemiacetal that can lead

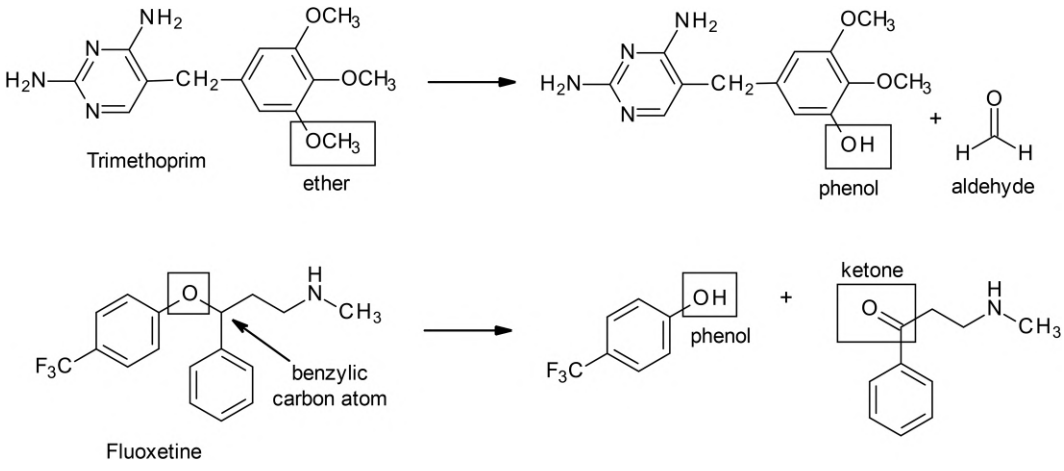


FIGURE 8-23. Examples of oxidative *O*-dealkylation catalyzed by CYP450 enzymes (see boxes).

to *O*-dealkylation. Due to steric hindrance, this *O*-dealkylation represents only a minor metabolic pathway for fluoxetine, with aromatic hydroxylation and *N*-demethylation being its two most common metabolic transformations. Again, the primary objective of these examples is to expose the reader to all of the possible metabolic routes.

The *O*-dealkylation of fluoxetine also helps to reemphasize a key point that was previously discussed with both benzylic oxidation and the *N*-dealkylation of nicardipine. The benzylic carbon atom of fluoxetine is already attached to a heteroatom; therefore, oxidation at this site produces a hemiacetal, not a stable hydroxyl group. Thus, oxidation at this carbon atom is correctly described as *O*-dealkylation and not benzylic oxidation.

Oxidation of Functional Groups Containing Sulfur Atoms

Four main types of oxidation can occur with functional groups that contain a sulfur atom: S-dealkylation, S-oxidation, dimerization, and desulfuration. The mechanism of *S*-dealkylation is identical to *N*- and *O*-dealkylation, requires that the adjacent carbon atom is attached to a hydrogen atom, and is more likely to occur with smaller alkyl groups than larger alkyl groups. This metabolic route is not seen very often due to the limited number of drug molecules that contain a thioether. Examples are shown in **Figure 8-24** with thiethylperazine and ranitidine. This metabolic transformation produces a thiol (or sulfhydryl group) and either an aldehyde or ketone.

Thioethers can also undergo direct *S*-oxidation to produce sulfoxides and sulfones. These reactions are normally catalyzed by FMO enzymes rather than CYP450 enzymes. Thus, thiethylperazine and ranitidine can alternatively be converted to sulfoxides or sulfones (**Figure 8-25**). Please note that the formation of sulfoxides and sulfones occurs in a sequential manner, with the formation of a sulfoxide occurring first. Further oxidation of the sulfoxide produces a sulfone. Please note that thiethylperazine contains two thioethers. Although *S*-oxidation is only shown at one of these, it could also occur at the other thioether.

Drug molecules that contain sulfhydryl groups can be oxidized to disulfides. Similar to *S*-oxidation, FMO enzymes normally catalyze this metabolic transformation. An example of this is seen below with captopril. This particular oxidation is responsible for the relatively short duration of

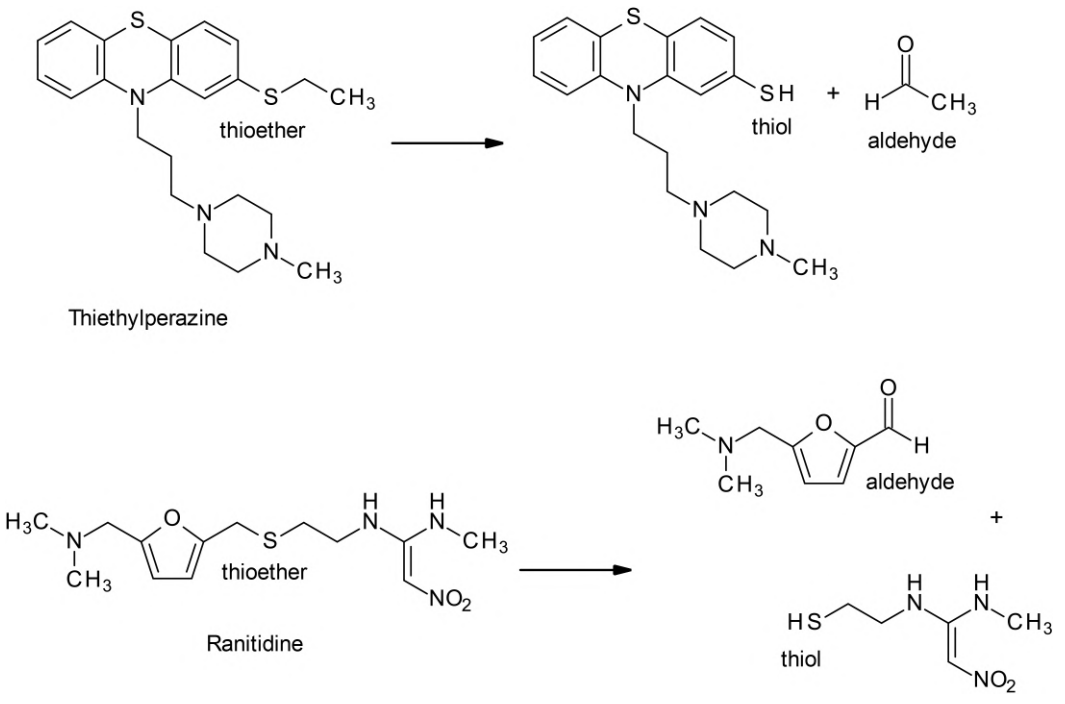


FIGURE 8-24. **Examples of *S*-dealkylation catalyzed by CYP450 enzymes.**

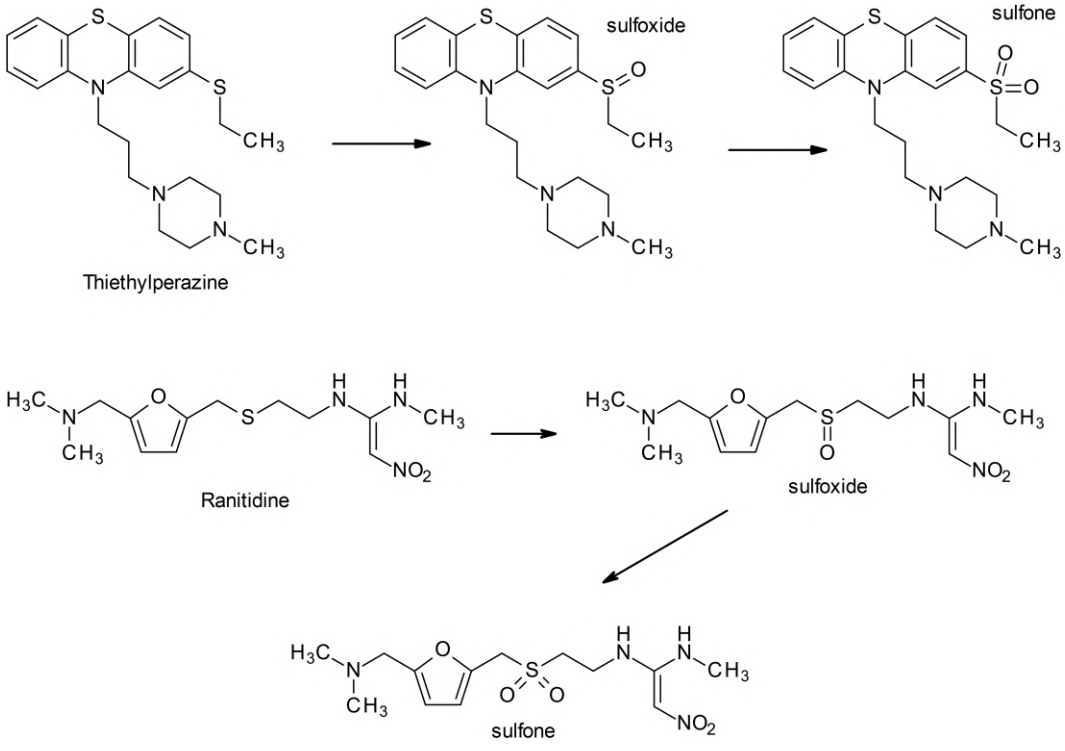
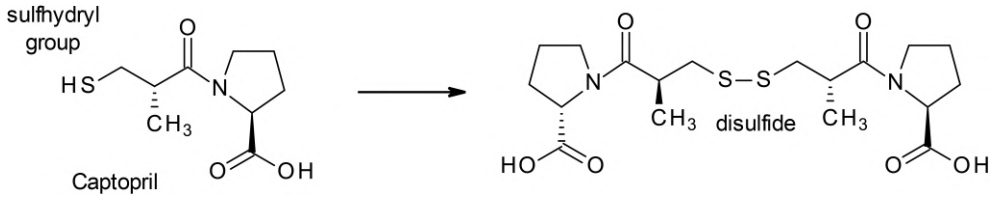


FIGURE 8-25. **Examples of *S*-oxidation catalyzed by FMO enzymes.**

action of captopril. Similar to thioethers, sulfhydryl groups are only seen in a very limited number of drug molecules.



Finally, drug molecules that contain a thiocarbonyl (also known as a thioketone) can undergo a desulfuration reaction in which the thiocarbonyl is converted to a carbonyl. Similar to the above metabolic transformations, only a limited number of functional groups contain a thiocarbonyl. Two examples are shown in **Figure 8-26**.

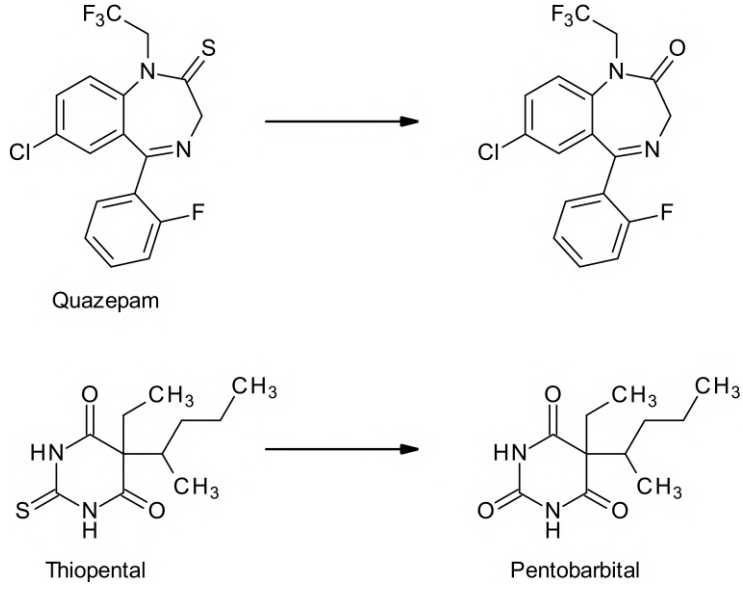
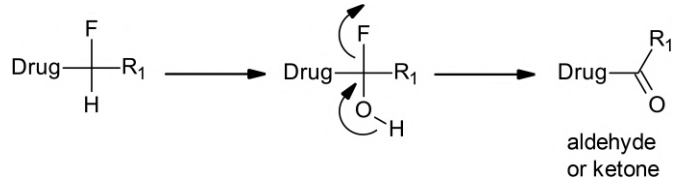


FIGURE 8-26. Examples of desulfuration.

Oxidative Dehalogenation

This type of oxidation can remove halogens from aliphatic chains and aliphatic rings but not from aromatic rings. Similar to all other oxidations, halogens can only be removed from carbon atoms that are also attached to a hydrogen atom. As such, a trifluoromethyl group (CF_3) cannot undergo this metabolic transformation. The general mechanism of this oxidation is shown below. The final product depends on adjacent atoms and functional groups.



If the R_1 group is not a halogen, then the product is generally either an aldehyde or a ketone; however, if the R_1 group is a halogen, a highly reactive acyl halide is formed as an intermediate. Nucleophilic displacement of the halide by a water molecule produces a stable and water-soluble carboxylic acid. An example is shown in **Figure 8-27A** with chloramphenicol. It should be noted that acyl halides are also capable of reacting with cellular biomolecules, typically resulting in hepatotoxicity. This has been observed with fluorinated hydrocarbons (e.g., halothane, enflurane, isoflurane) used to induce general anesthesia. The formation of acyl halides and their reaction with tissue proteins has been shown to be responsible for hepatotoxicity of this class of agents. An example is shown in **Figure 8-27B** with enflurane. Please note that the first two steps are identical to those for chloramphenicol. The key difference is that instead of reacting with a molecule of water, the reactive intermediate reacts with a cellular biomolecule.

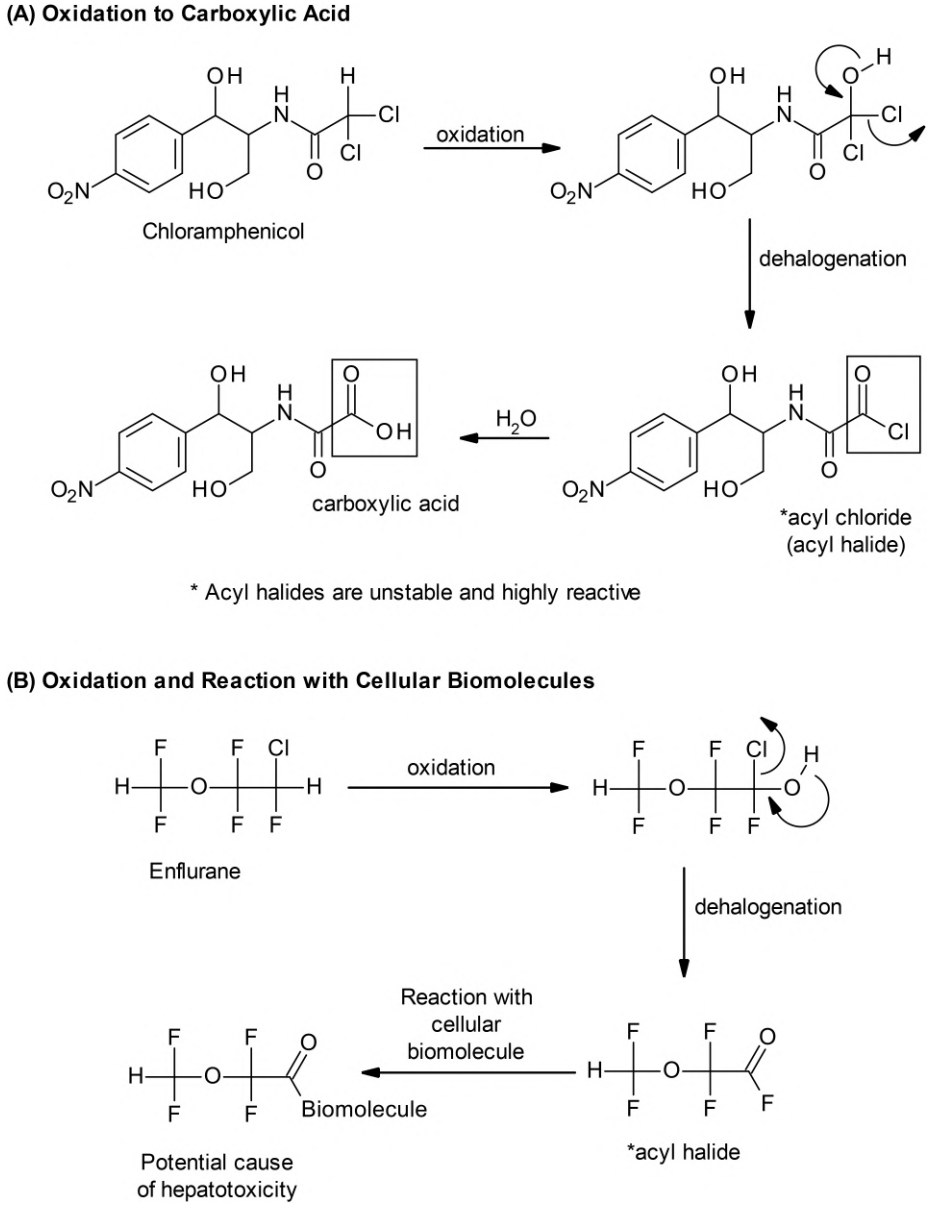


FIGURE 8-27. Examples of oxidative dehalogenation catalyzed by CYP450 enzymes.

PHASE I METABOLISM: REDUCTION

Reduction is the opposite of oxidation and involves the gain of electrons and a decrease in the oxidation state of an atom or molecule. Similar to oxidation, the changes in electrons and oxidation states are not always easy to discern in a drug molecule; however, all of the reductions discussed below involve a gain of hydrogen by the reduced functional group. Reduction is the least common Phase I metabolic pathway because only a few functional groups are susceptible to this metabolic transformation and some of these functional groups, such as azo and nitro groups, are only present on a very limited number of drug molecules. Enzymes that catalyze reduction-based metabolic transformations are found in the liver, kidney, and other tissues. Aldehydes and ketones can be reduced by aldo-keto reductase enzymes or oxidoreductase enzymes whereas azo and nitro groups can be reduced by azoreductase and nitroreductase enzymes, respectively. Additionally, bacteria present within the GI tract are capable of reducing these functional groups. All of these enzymes require NADPH as the reducing species.

Reduction of Aldehydes and Ketones

Aldehydes are generally metabolically unstable and are rarely present on parent drug molecules. As previously discussed, they can be formed via oxidative deamination, *N*-dealkylation, *O*-dealkylation, and *S*-dealkylation. They are primarily oxidized to carboxylic acids; however, in some instances, they can be reduced to a hydroxyl group. An example is shown in **Figure 8-28** with duloxetine. Duloxetine can form an aldehyde intermediate by first undergoing *N*-dealkylation and then oxidative deamination or by directly undergoing oxidative deamination. The resulting aldehyde can be either oxidized to a carboxylic acid or reduced to a primary hydroxyl group.

Ketones that are initially present in a drug molecule or that are introduced by oxidative metabolism can be reduced to secondary hydroxyl groups. Similar to the hydrocarbon oxidations that added a hydroxyl group to a prochiral center, the reduction of ketones often generates only one

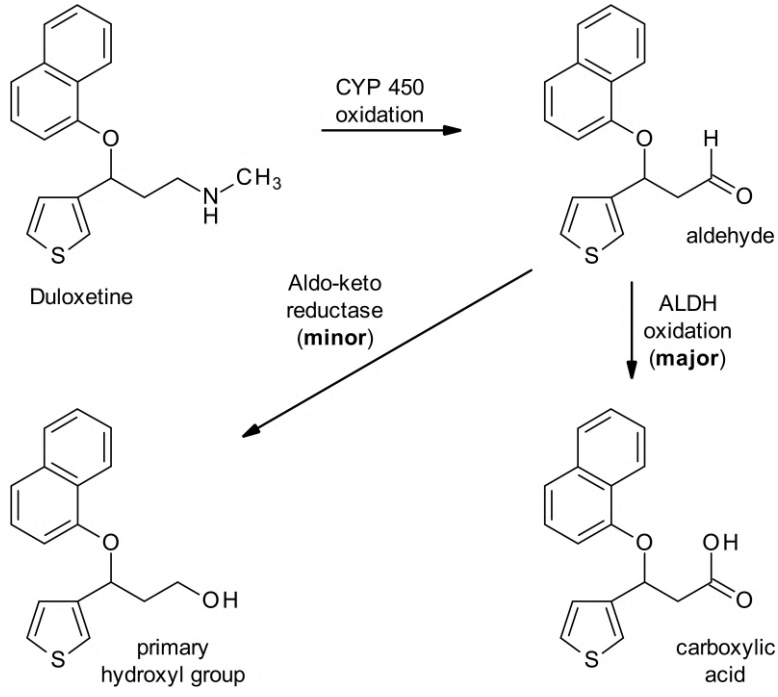


FIGURE 8-28. Metabolic routes for duloxetine.

stereoisomer or generates one stereoisomer as a major metabolite and the other as a minor metabolite. Interestingly, ketones that are formed via the oxidation of enantiomerically pure secondary hydroxyl groups can be reduced to form the opposite enantiomer. This reversible oxidation of a secondary hydroxyl group to a ketone, followed by reduction of the ketone back to a secondary hydroxyl group, is not uncommon, and drug molecules containing these functional groups are often eliminated as Phase II conjugates of the hydroxyl group. Two examples of ketone reduction are shown in **Figure 8-29**. The *R*,*S* isomer is the major metabolic reduction product of warfarin, with the *R*,*R* isomer being a minor metabolite. This is an example of stereoselective drug metabolism. In contrast, the *S*,*S* isomer is the only metabolic reduction product seen in methadone. This is an example of stereospecific drug metabolism.

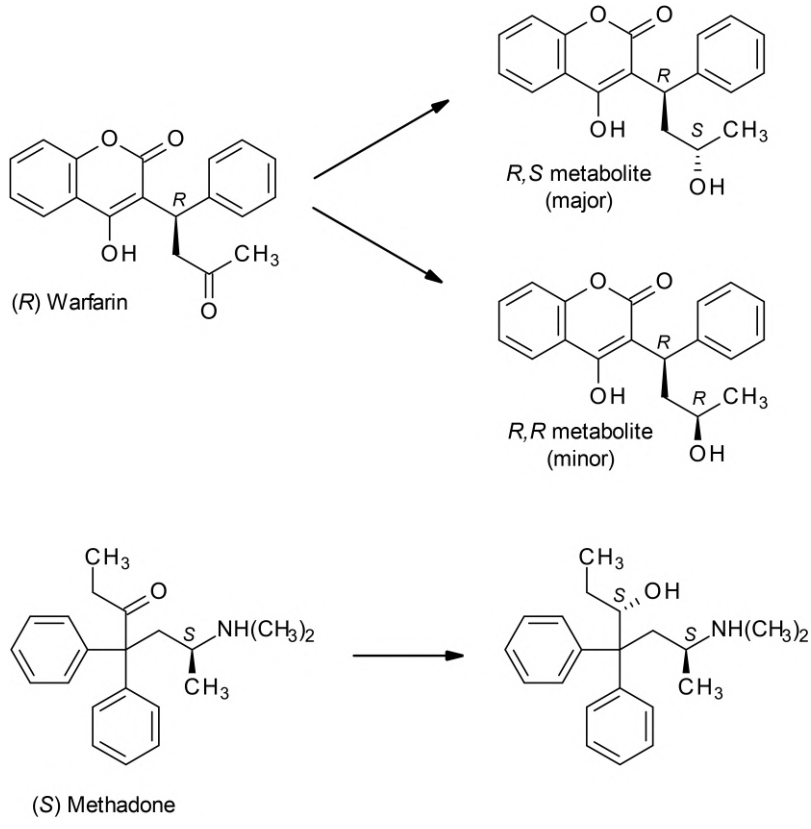
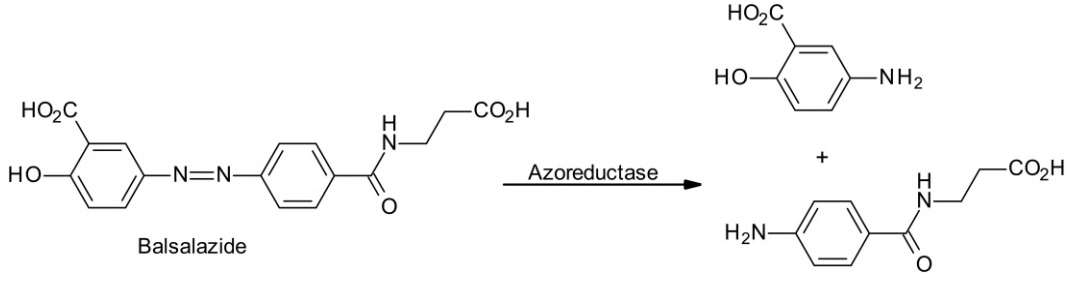


FIGURE 8-29. Examples of ketone reduction by aldo-keto reductase enzymes.

Reduction of Azo and Nitro Groups

Azo groups, like that seen below with balsalazide, can be reduced to produce two aromatic amines. Often, this reduction is catalyzed by bacteria that reside within the GI tract.



Nitro groups can be reduced to primary amines in a series of steps that involve an initial reduction to a nitroso group, followed by a second reduction to a hydroxylamine, and finally end with a third reduction to a primary amine. Please note that the reduction of the nitroso group to a primary amine is the reverse of what was previously discussed for the oxidation of primary amines. Also note that while oxidation adds oxygen atoms to atoms and functional groups, reduction of a nitro group removes oxygen atoms and adds hydrogen. An example of this stepwise reduction is shown in **Figure 8-30** with nitrofurantoin. Examples of other drugs capable of this metabolic transformation are also shown in the figure.

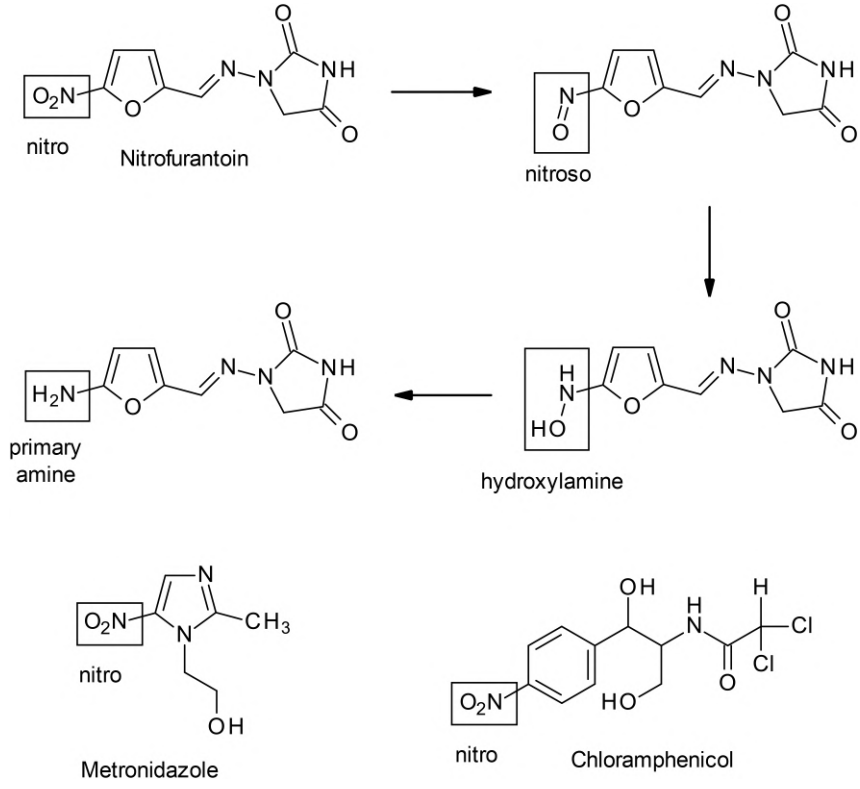
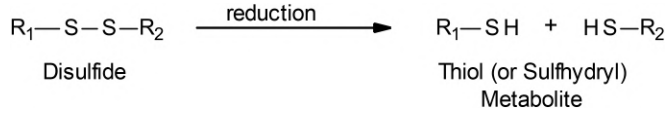


FIGURE 8-30. The reduction of the nitro group of nitrofurantoin by nitroreductase enzymes and examples of other drug molecules that contain a nitro functional group (see boxes).

Miscellaneous Reductions

Drug molecules that contain disulfide bonds as part of their structure can be reduced to thiol (or sulfhydryl) metabolites. The general reaction is shown below. Examples include insulin and eptifibatide. Insulin is an endogenous peptide that contains three disulfide bonds. Two of these are interstrand disulfide bonds that hold the A and B chains together, and the other one is an intrastrand disulfide bond that is necessary for the proper conformation of the two chains. Eptifibatide is a cyclical peptide used to treat acute coronary syndromes. The cyclical nature is due to the presence of a disulfide bond. Similar to azo and nitro groups, not many drug molecules contain disulfide bonds as part of their structure.



Sulfoxides are normally oxidized to sulfones, as shown in Figure 8-25; however, there are some instances in which they are instead reduced to sulfides. One such instance is seen with the non-steroidal anti-inflammatory drug (NSAID) sulindac (**Figure 8-31**). Sulindac is a prodrug. Reduction of its sulfoxide functional group to a sulfide is required for its pharmacological actions.

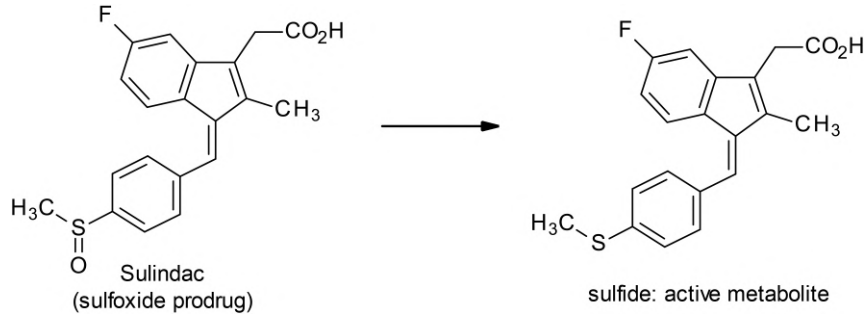
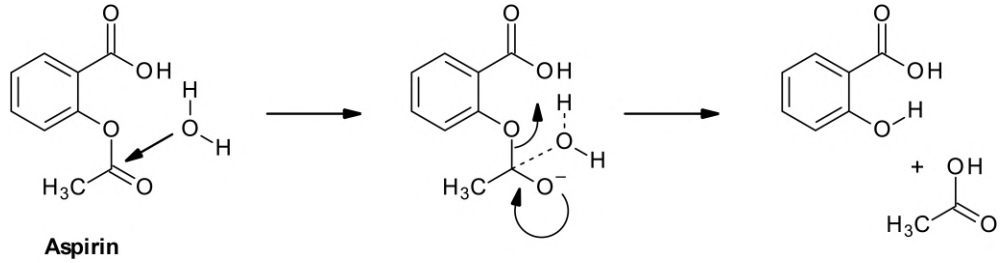


FIGURE 8-31. The reduction of the sulfoxide group of sulindac.

PHASE I METABOLISM: HYDROLYSIS

Taken literally, the term *hydrolysis* means "water" (hydro) to "break" (lysis) a bond. Hydrolysis involves the addition of a molecule of water across a C—X bond and the subsequent cleavage of that bond. In most cases, X is an oxygen or nitrogen atom, and for some functional groups, the carbon atom is replaced by a sulfur or phosphorous atom. Hydrolysis readily occurs with esters, amides, and their cyclic analogs, lactones and lactams.

Hydrolysis is a common Phase I metabolic transformation because esters, amides, lactones, and lactams are present in a significant number of drug molecules. The hydrolysis of these functional groups leads to the formation of carboxylic acids, phenols, hydroxyl groups, and amines. All of these metabolites are much more water soluble than the original drug molecule. The hydrolytic mechanism is illustrated below using the ester bond of aspirin.



Please note that this general mechanism is somewhat simplistic and does not indicate the fact that esterase and amidase enzymes require specific amino acids for enzymatic catalysis. The amino acids serine, threonine, tyrosine, histidine, aspartic acid, and glutamic acid are often involved in enzymatic hydrolysis. Although not shown in the mechanism above, these amino acids are often required to activate the water molecule and enhance its nucleophilicity. Additional examples of drug molecules that undergo hydrolytic metabolism are shown in **Figure 8-32**. The hydrolysis of esmolol, lidocaine, and cephalexin produces inactive metabolites whereas the hydrolysis of simvastatin converts this prodrug to its active metabolite.

Hydrolytic enzymes are ubiquitous in the human body. They are present in the liver but are also widely distributed in other organs and tissues such as the GI tract, plasma, skin, lungs, and kidneys. The lack of the need for hepatic metabolism has led to the development of locally active prodrugs.

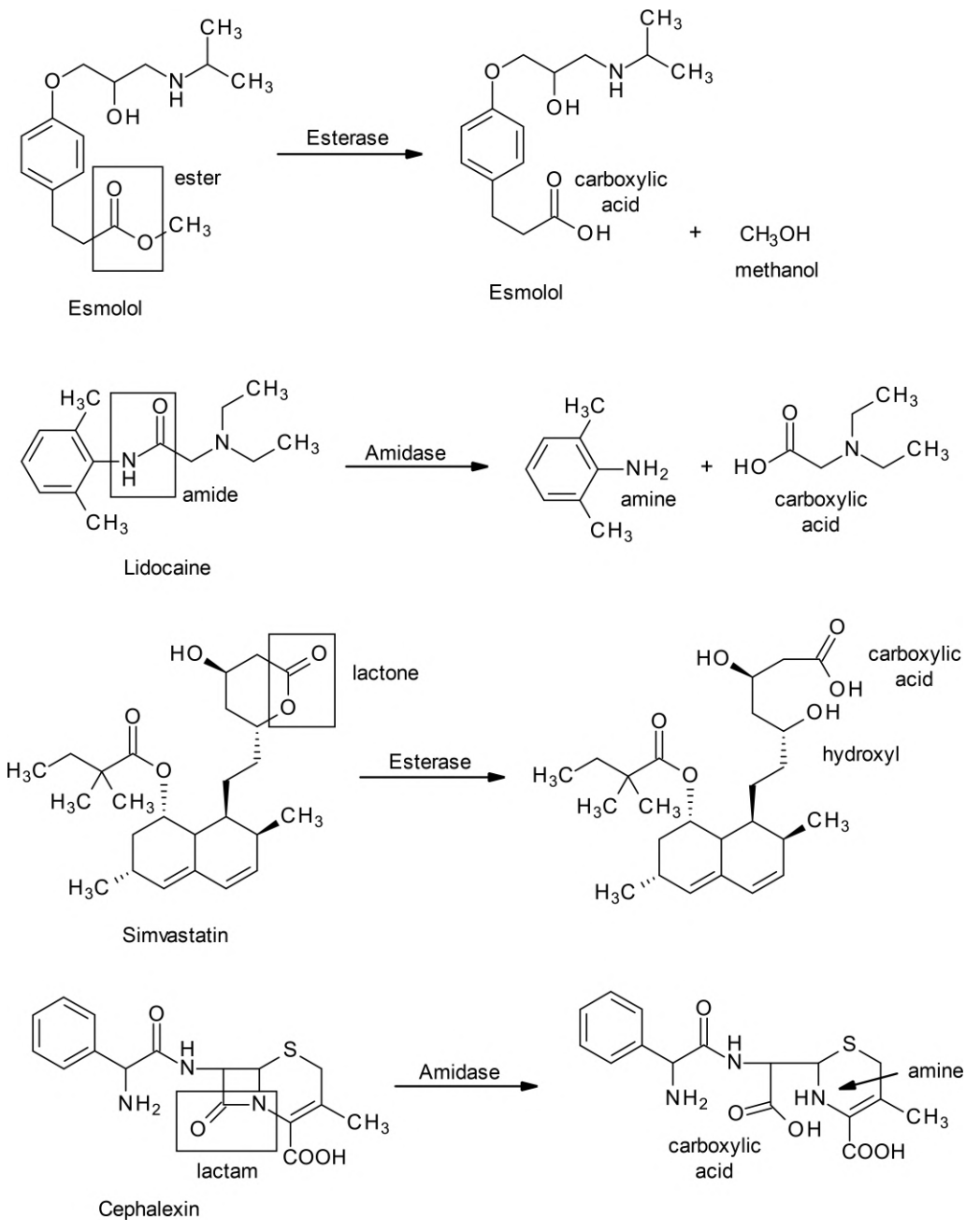


FIGURE 8-32. Examples of ester, amide, lactone, and lactam hydrolysis (see boxes).

As discussed in Chapter 5, lipid-soluble ester prodrugs of glucocorticoids have been developed for topical and pulmonary use. They are hydrolyzed at the site of application and generally do not enter the systemic circulation to any significant extent, nor are they dependent on the liver for activation. Other drug molecules are administered as lipid-soluble esters to enhance their oral absorption. After absorption, these ester prodrugs are hydrolyzed to their active metabolites. This concept was introduced in Chapter 5 and used fenofibrate and candesartan cilexetil as examples. Four additional examples are shown in **Figure 8-33**.

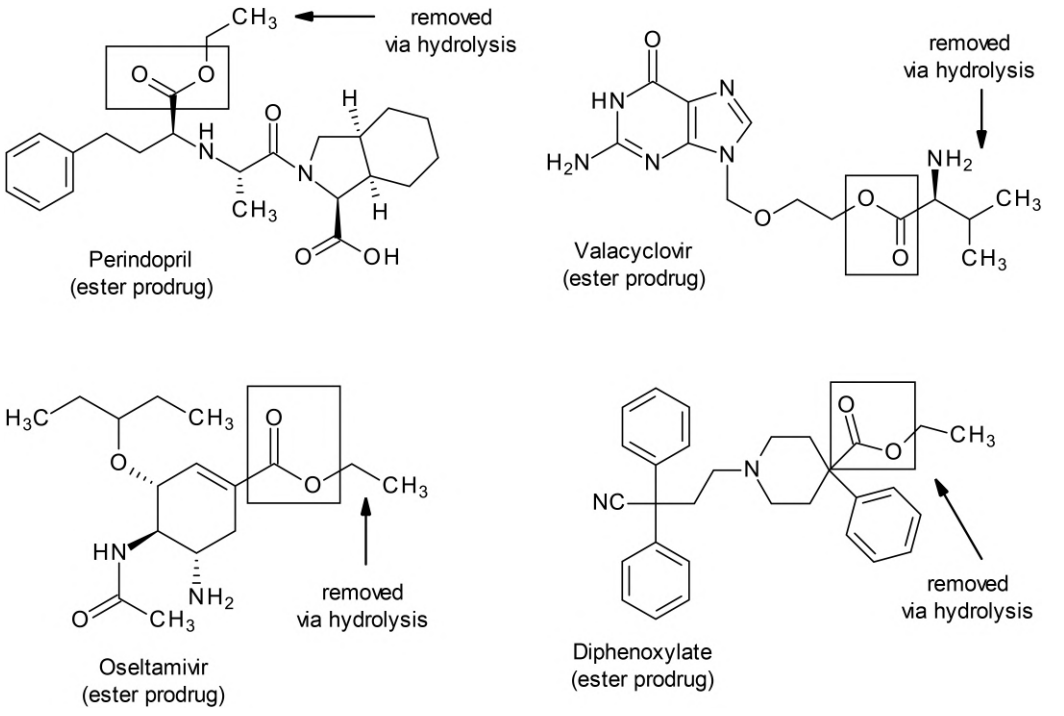


FIGURE 8-33. Examples of ester prodrugs activated by hydrolysis (see boxes).

In some instances, hydrolysis can be mistakenly identified as oxidative *O*-dealkylation. This error most commonly occurs when an ester is hydrolyzed and the carboxylic acid remains on the drug metabolite. An example of this is illustrated in **Figure 8-34** with a closer look at the hydrolysis of diphenoxylate (originally shown in Figure 8-33). The active metabolite of diphenoxylate requires the removal of the ethyl group and the formation of a carboxylic acid. As shown in Figure 8-34, this

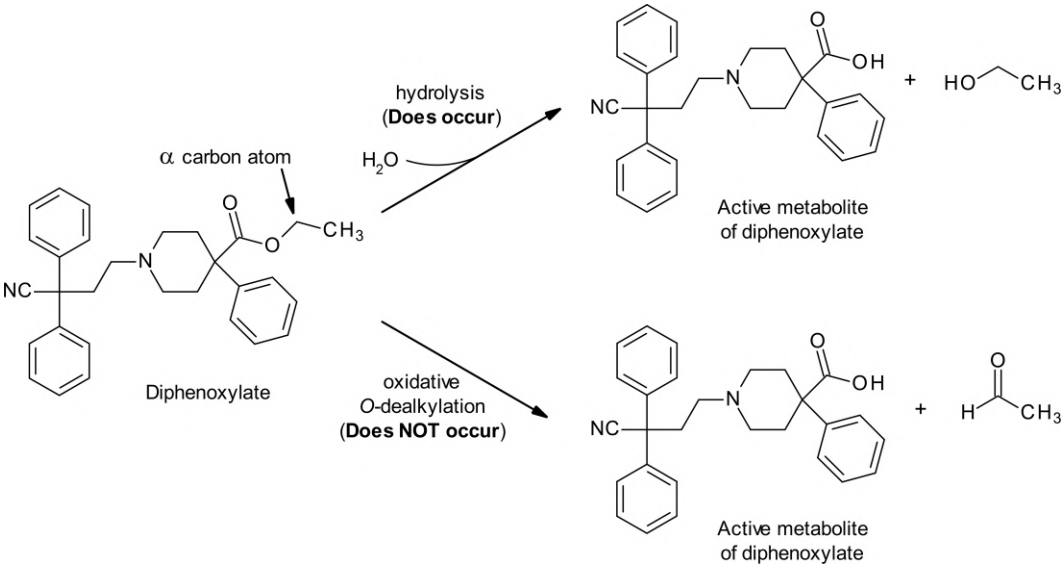
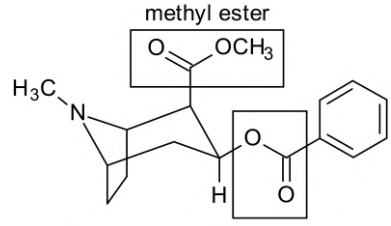


FIGURE 8-34. Comparison of hydrolysis and oxidative *O*-dealkylation.

could *theoretically* occur by either hydrolysis or oxidative *O*-dealkylation. Regardless of the metabolic pathway, the same active metabolite is formed; however, there is a difference in the resulting two-carbon unit. Hydrolysis produces a primary hydroxyl group whereas oxidative *O*-dealkylation produces an aldehyde. These small two-carbon metabolites are often not shown in a metabolic scheme, so how are we able to correctly conclude which metabolic pathway occurred?

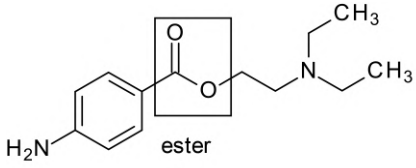
This can be accomplished by comparing the mechanisms of metabolic transformation for these two processes. Oxidative *O*-dealkylation requires CYP450 enzymes, the presence of a hydrogen atom that can be abstracted, and an eight-step metabolic process. In contrast, hydrolysis is a much simpler process requiring two or three steps. Although both CYP450 enzymes and hydrolytic enzymes exist in the liver, the overall distribution and availability of these enzymes is much greater for hydrolytic enzymes. As previously discussed, metabolic processes within the human body are extremely efficient and *will not* use a more complicated pathway when a simpler and more available pathway is present. In conclusion, whenever there is a choice between hydrolysis and oxidative *O*-dealkylation, hydrolysis occurs, not oxidative *O*-dealkylation.

Steric hindrance can determine the site and rate of hydrolysis. Easily accessible esters and amides are hydrolyzed to a greater extent than those located in the middle of the molecule and/or are surrounded by other functional groups. A classic example of this is seen with cocaine. It contains an easily accessible methyl ester as well as a more sterically hindered benzylic ester. While studies show that the hydrolysis of both esters occurs, the hydrolysis of the methyl ester is a major metabolic pathway and the hydrolysis of the benzylic ester is a minor metabolic pathway. An additional example is provided in Figure 8-32 with simvastatin. The lactone is easily accessible, lacks any significant steric hindrance, and is easily hydrolyzed. In contrast, the 2,2-dimethylbutyl ester is less accessible and is somewhat sterically hindered. Although it can be hydrolyzed, the overall rate and extent is less than that of the lactone.

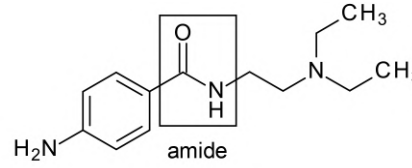


Cocaine benzylic ester

In general, amides and lactams are hydrolyzed at a slower rate than are esters and lactones. A classic example is seen in the comparison of procaine with procainamide. As shown in the structures below, the only chemical difference between these two drugs is that procaine contains an ester and procainamide contains an amide. Due to rapid ester hydrolysis, procaine has a half-life less than one minute. As such, it cannot be administered orally and is used parenterally as a local anesthetic. In contrast, the amide ester of procainamide is hydrolyzed at a much slower rate; in fact, hydrolysis is not the primary metabolic route for procainamide. Procainamide can be given orally for the treatment of certain types of arrhythmias.



Procaine



Procainamide

This is not to say that amides and lactams cannot be rapidly hydrolyzed because there are several examples of this occurring. The amide bond of lidocaine (Figure 8-32) is rapidly hydrolyzed, and its duration of action when given intravenously is only 10 to 20 minutes. The β-lactam class of

antibiotics, exemplified by cephalexin in Figure 8-32, is subject to rapid lactam hydrolysis in basic environments. Additionally, some β-lactams can be rapidly hydrolyzed by the bacterial enzyme β-lactamase. Finally, proteins and peptides can be rapidly destroyed in the GI tract, the plasma, the liver, and other cells by exopeptidases and endopeptidases that break specific peptide (i.e., amide) bonds present within the protein or peptide.

A number of other functional groups can be metabolized via hydrolytic cleavage. These functional groups are not as prevalent in drug molecules and include phosphate esters, sulfonylureas, carbamates, and glycosides. Examples of each of these are shown in **Figure 8-35**. Please note that hydrolysis is a very minor metabolic pathway for some of these functional groups, especially sulfonylureas and carbamates.

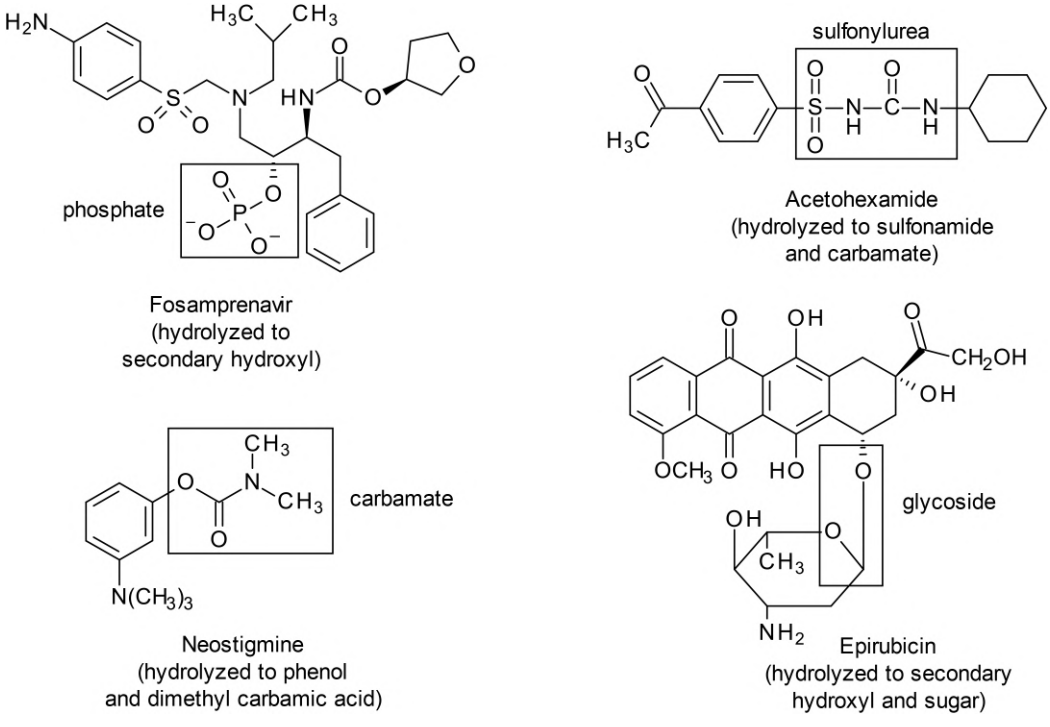
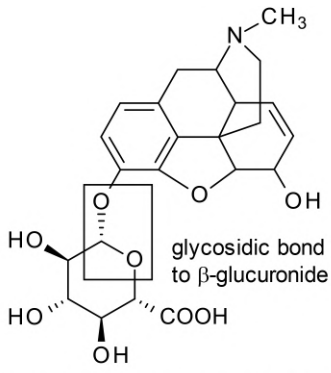
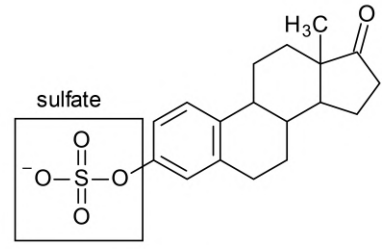


FIGURE 8-35. Examples of other functional groups that can be metabolized by hydrolysis (see boxes).

The hydrolysis of Phase II metabolites is known as *deconjugation* and serves two important roles. First, deconjugation may help to extend the duration of action of a drug molecule. Some glucuronide conjugates are secreted into the small intestine for fecal elimination; however, due to the presence of β-glucuronidases in the intestine, these conjugates can be hydrolyzed and the deconjugated drug molecule can then be reabsorbed. This process is known as *enterohepatic recycling* and allows the drug to reside within the body for a longer period. Second, sulfate conjugates can be used by the body to transport lipid-soluble drugs and biomolecules from one tissue to another. A good example is seen with estrogens. Organ- and tissue-specific sulfatase enzymes serve to liberate the active drug molecules at their site of pharmacological action. Examples of each of these deconjugation pathways are shown in **Figure 8-36**.



Glucuronide conjugate of morphine
(hydrolyzed to phenol
and glucuronic acid)



Estrone sulfate
(hydrolyzed to estrone
and sulfuric acid)

FIGURE 8-36. Examples of Phase II metabolites that can be deconjugated by hydrolysis.

Summary of Phase I Metabolism

- Phase I metabolism uses oxidation, reduction, and hydrolysis to add, alter, or unmask specific functional groups. The minimum number of metabolic transformations required to achieve these goals will occur.
- Functional groups that are easily accessible and not hindered by steric or electronic factors are the most likely to undergo Phase I metabolism.
- Phase I metabolism increases the water solubility of a drug molecule. In some instances, this is sufficient to allow the drug molecule to be eliminated from the body. In other instances, Phase II metabolism is required.
- There is no specific requirement for a drug molecule to undergo Phase I metabolism. Drug molecules that have sufficient water solubility are often excreted unchanged. Drug molecules that already contain functional groups that can directly undergo Phase II conjugation may bypass Phase I metabolism.
- Phase I metabolism may convert an inactive prodrug to an active metabolite.
- Phase I metabolism may convert an active drug molecule into a metabolite that retains similar pharmacological activity.
- Phase I metabolism may convert a drug molecule into a metabolite that has increased toxicity or is responsible for specific side effects.
- Phase I metabolism may convert an active drug molecule into an inactive and nontoxic metabolite.

PHASE II METABOLISM: CONJUGATION

Six Phase II conjugation pathways are available for drug molecules: (1) glucuronic acid conjugation, (2) sulfate conjugation, (3) amino acid conjugation, (4) glutathione conjugation, (5) acetylation, and (6) methylation. Each conjugation pathway uses a specific transferase enzyme that catalyzes the addition of one of these endogenous substances to a functional group that was initially present on a drug molecule or was added or unmasked during one or more Phase I transformations. Conjugation of a functional group with glucuronic acid, sulfate, or an amino acid (e.g., glycine or glutamine) greatly

enhances the water solubility and excretion of a drug molecule. The products of these conjugations are usually inactive; however, there are some instances in which glucuronide and sulfate conjugates retain pharmacological activity. Glutathione reacts with highly electrophilic intermediates and thus serves to detoxify drug molecules and toxic substances. Glutathione conjugates are highly water soluble, readily excreted, and nontoxic. The addition of methyl or acetyl groups does not enhance water solubility but often serves to terminate the pharmacological actions of the drug molecule.

Glutathione conjugation is different from the other five conjugation pathways in two respects. First, all other Phase II conjugation pathways require an initial activation process that enhances the reactivity of the conjugating group, the transferase enzyme, or a specific functional group present on the drug molecule. These activated intermediates serve as cofactors for the transferase enzymes. This initial activation is not necessary for glutathione conjugation because its nucleophilic sulfhydryl group can easily react with a potentially harmful electrophilic functional group. Additionally, because the role of glutathione is to protect cells and tissues from highly reactive electrophiles, it needs to be able to act immediately, without the requirement of an activation process. Second, deconjugating enzymes can reverse the actions of all other Phase II conjugations. As mentioned above, this process is important for the enterohepatic recycling and transport of specific drugs. Because glutathione conjugation protects cells and tissues from potentially harmful electrophilic substances or functional groups, there is no beneficial reason to reverse this process and regenerate toxic substances once they are neutralized.

Table 8-2 provides a list of all six conjugation pathways, the endogenous substance used for conjugation, the names of the activated cofactors, the names of the transferase enzymes used for conjugation, and the names of the deconjugating enzymes.

TABLE 8-2. Summary of Phase II Conjugation Pathways

Conjugation Pathway	Endogenous Substance	Activated Cofactor	Transferase Enzyme	Deconjugating Enzyme
Glucuronic acid conjugation	Glucose-1-phosphate	UDP-glucuronic acid (UDPGA)	UDP-Glucuronyl-transferase (UGT)	*β*-Glucuronidase
Sulfate conjugation	Sulfate	3′-Phosphoadenosine-5′-phosphosulfate (PAPS)	Sulfotransferase (SULT)	Sulfatase
Amino acid conjugation	Glycine and glutamine (major) Aspartic acid, serine, and taurine (minor)	Acyl coenzyme A intermediate of carboxylic acid on the drug molecule	*N*-Acyltransferase (NAT)	Amidase
Glutathione conjugation	Glutathione (GSH)	None	Glutathione *S*-transferase	None
Acetylation	Acetyl CoA	Acetylated *N*-acetyltransferase	*N*-Acetyltransferase	Amidase
Methylation	Methionine	*S*-Adenosylmethionine (SAM)	Methyltransferase	CYP450 (i.e., oxidative dealkylation)

Glucuronic Acid Conjugation

Glucuronic acid conjugation is the most common Phase II transformation pathway for two reasons. First, glucuronic acid is readily available because it is an oxidative metabolite of glucose. Second, there are many functional groups that can be conjugated with glucuronic acid, including hydroxyl groups, phenols, carboxylic acids, tetrazoles, amines, sulfonamides, hydrazines, carbamates, and

sulfhydryl groups. Phenols and hydroxyl groups are the two most common functional groups that undergo glucuronide conjugation. Due to steric hindrance, tertiary hydroxyl groups and tertiary amines are less likely to undergo glucuronic acid conjugation compared with the other functional groups.

The mechanism of glucuronic acid conjugation involves three steps (**Figure 8-37**). The first two steps convert glucose 1-phosphate to UDP-glucuronic acid (UDPGA). The third step is catalyzed by UDP-glucuronyltransferase (UGT) and transfers the activated glucuronic acid to its target functional group. There are two human UGT subfamilies, UGT1 and UGT2, and a large number of isoforms. Each isoform is responsible for the glucuronidation of specific functional groups; however, there is considerable overlap among the isoforms.

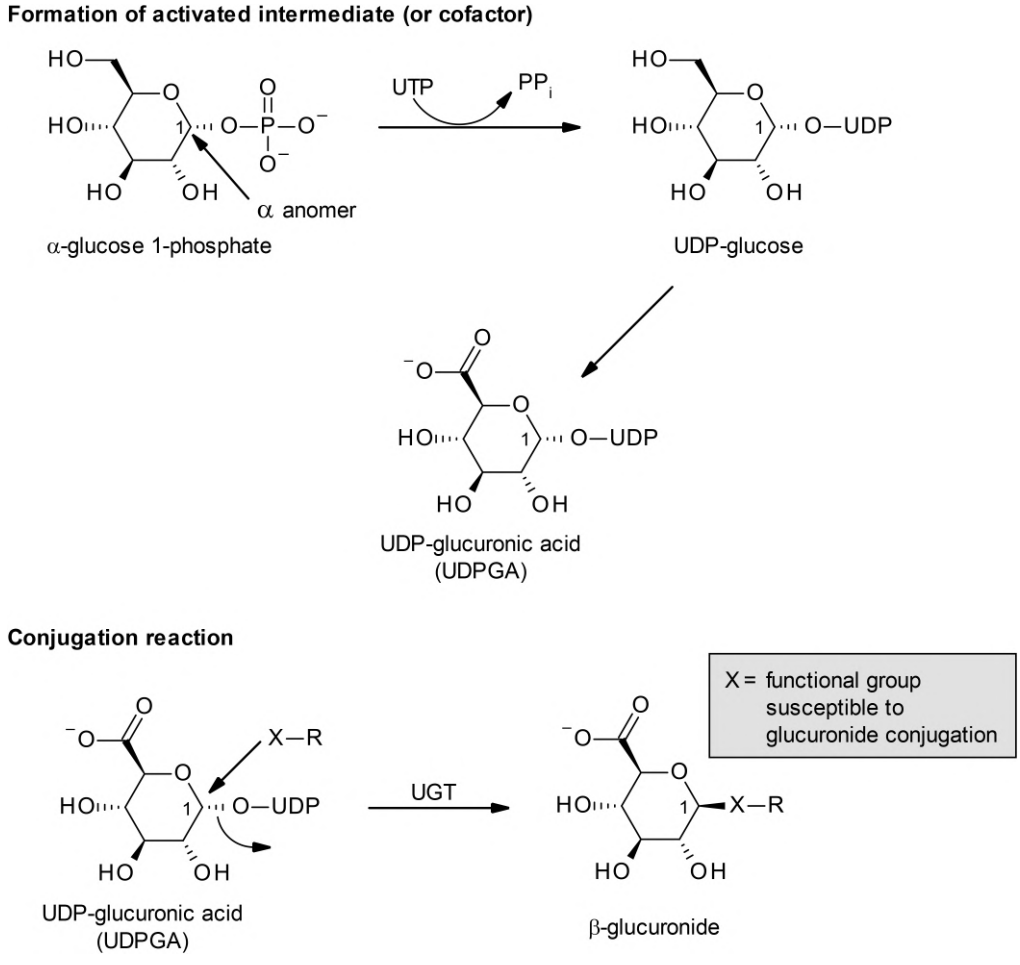


FIGURE 8-37. The mechanism of glucuronide conjugation.

A number of cells are capable of converting glucose 1-phosphate to UDP-glucose because this step is also required for the formation of glycogen. A key point in this initial step is that the α anomer of glucose 1-phosphate is required. Subsequent oxidation of UDP-glucose yields UDP-glucuronic acid. In the conjugation step, a functional group on the drug molecule displaces UDP. This displacement occurs from the opposite side of the UDP molecule. Because this sequence always begins with the α anomer of glucose 1-phosphate, *the conjugation product is always a β-glucuronide*.

Examples of drug molecules capable of undergoing glucuronide conjugation are shown in **Figure 8-38**. Each drug molecule has been chosen to represent a specific functional group that is capable of undergoing this conjugation pathway.

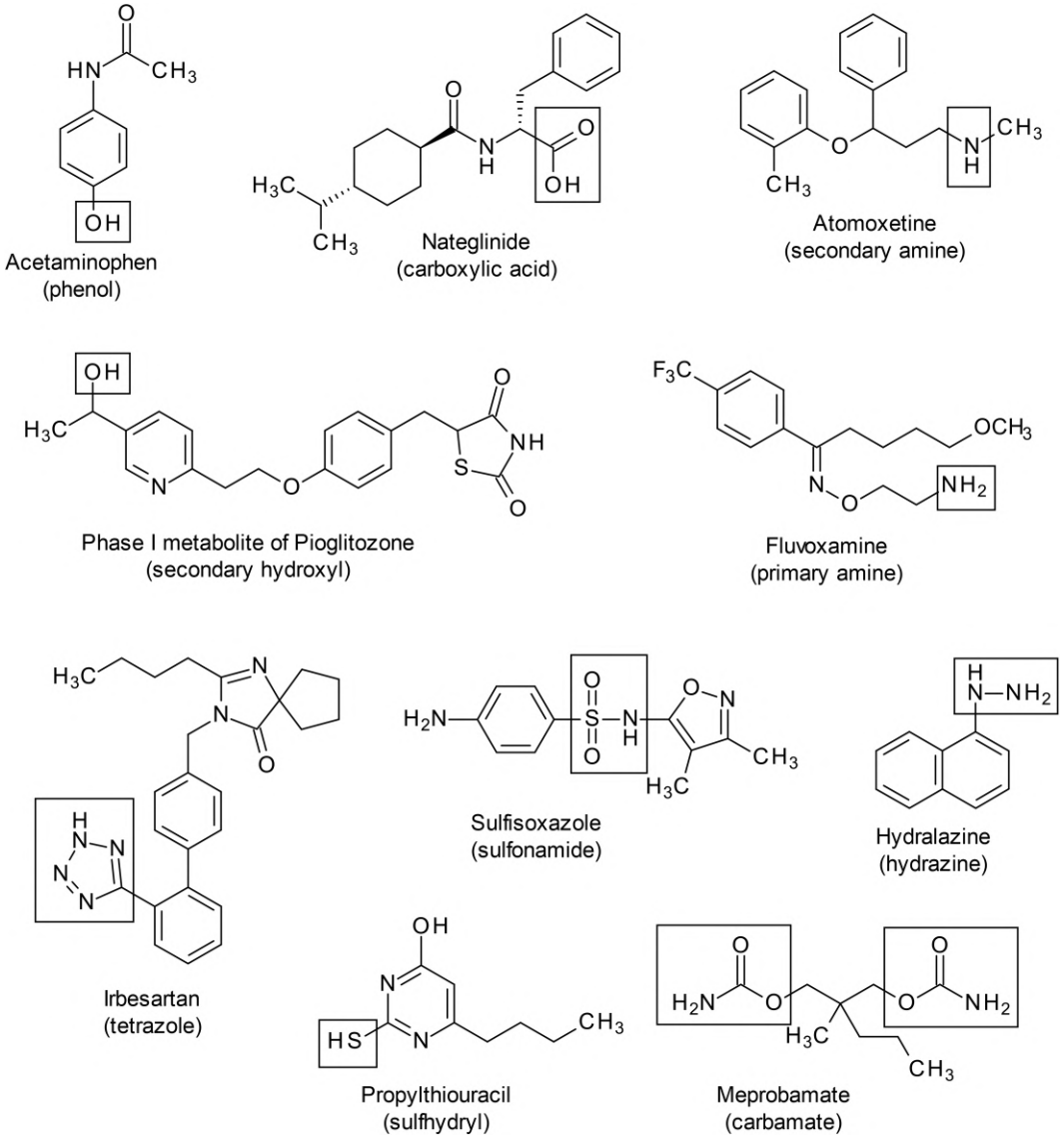


FIGURE 8-38. Examples of drug molecules that can undergo glucuronide conjugation (susceptible functional groups are boxed).

The addition of glucuronic acid to a drug molecule greatly enhances its water solubility due to the negatively charged carboxylic acid and the three secondary hydroxyl groups, all of which are hydrophilic in character. This is shown in **Figure 8-39** with the glucuronide conjugates of acetaminophen and atomoxetine. Please note that the stereochemistry of these metabolites matches the stereochemistry shown in the mechanism of glucuronide conjugation in Figure 8-37.

Some drugs may possess more than one functional group that can undergo glucuronide conjugation. In these situations, multiple glucuronide metabolites may be formed; however, it is uncommon

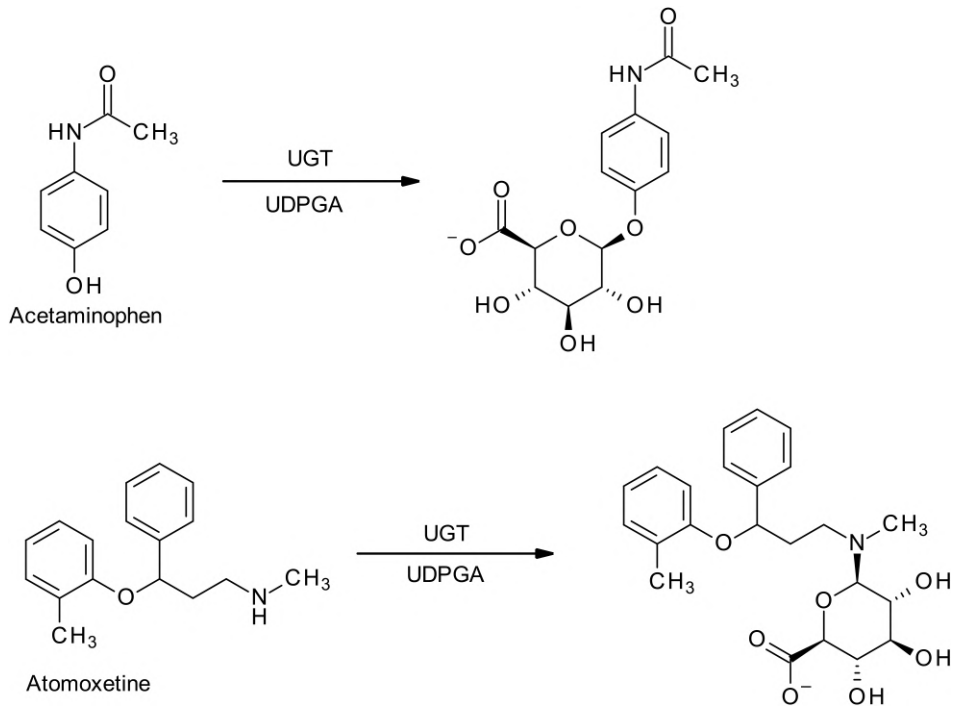
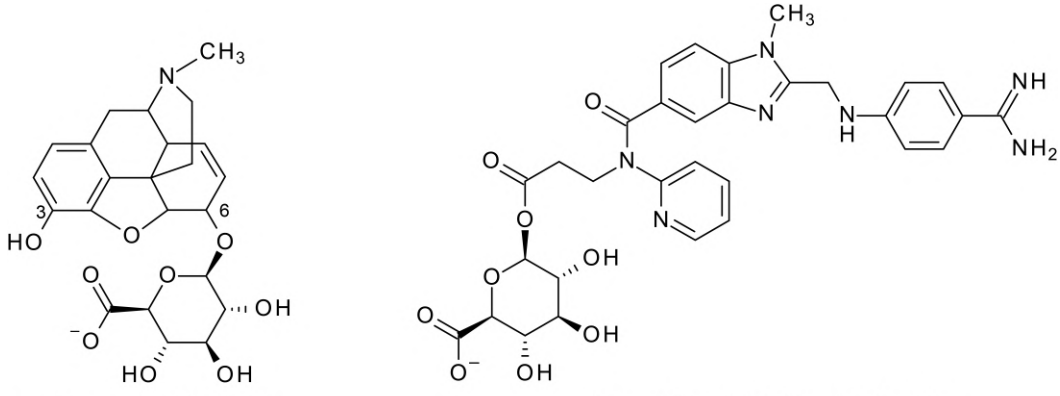


FIGURE 8-39. Glucuronide conjugates of acetaminophen and atomoxetine

that any single metabolite would be conjugated with more than one glucuronic acid. Once adequate water solubility has been added to a drug molecule to ensure its elimination from the body, there simply is no need to metabolize the drug molecule any further. Glucuronide conjugates are easily excreted in the urine. Although most glucuronide conjugates are inactive, there are instances in which activity is retained or enhanced by this conjugation. Morphine and dabigatran provide two examples in which their glucuronide conjugates retain activity or have enhanced activity as compared with the parent drug. The C_6 glucuronide conjugate of morphine is two to three times more active than morphine whereas the glucuronide conjugate of dabigatran retains the same activity as the unconjugated drug. Interestingly, glucuronide conjugation of morphine can produce either inactive or more active metabolites depending on the site of metabolism. Glucuronide conjugation at the C_6 hydroxyl group produces a more active metabolite whereas glucuronide conjugation at the C_3 hydroxyl group (Figure 8-36) produces an inactive metabolite.



C_6 Glucuronide Metabolite of Morphine

Glucuronide Metabolite of Dabigatran

As previously mentioned, some glucuronide conjugates are excreted in the bile and subsequently deconjugated in the GI tract by the enzyme β-glucuronidase. The resulting drugs, metabolites, or endogenous substances can then be reabsorbed via a process known as *enterohepatic circulation* or *enterohepatic recycling*. In general, drug molecules with a molecular weight more than 500 are more likely to undergo this process than those with a lower molecular weight. Oral contraceptives, thyroid hormones, raloxifene (shown in **Figure 8-40**), indomethacin, morphine, pentazocine, and diazepam are examples of drug molecules for which enterohepatic recycling is important to their metabolism, elimination, and duration of action.

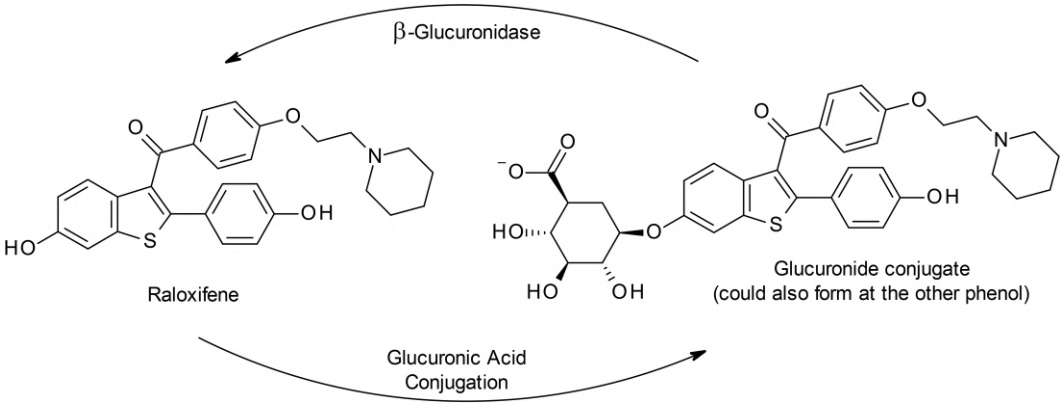


FIGURE 8-40. Enterohepatic circulation of raloxifene.

Sulfate Conjugation

Sulfate conjugation occurs primarily with phenolic hydroxyl groups; however, it can also occur to a minor extent with aliphatic hydroxyl groups, aromatic amines, and *N*-hydroxyl groups. Unlike glucuronic acid, which is readily synthesized from glucose, the body has a much more limited pool of sulfate. Depending on the dose of the drug, it is possible that this pool of sulfate can become depleted, and other metabolic pathways may need to be used. In many cases, when a functional group or drug molecule can be conjugated with either glucuronic acid or sulfate, glucuronide conjugation predominates. Examples of this are seen with both acetaminophen and sulfisoxazole. Although the phenolic hydroxyl group of acetaminophen and the aromatic amine of sulfisoxazole can undergo sulfate conjugation, these are minor metabolic products compared with glucuronide conjugation pathways indicated in Figure 8-38.

The mechanism of sulfate conjugation is shown in **Figure 8-41**. Similar to glucuronide conjugation, the first two steps convert inorganic sulfate to an activated intermediate, 3′-phosphoadenosine-5′-phosphosulfate (PAPS). In the third step, the enzyme sulfotransferase (SULT) catalyzes the transfer of the activated sulfate to its target functional group. There are two human sulfotransferase families, SULT1 and SULT2, and numerous subfamilies and isoforms. Similar to UGT isoforms, each SULT isoform is responsible for the transfer of sulfate to specific functional groups.

Examples of sulfate conjugation are shown in **Figure 8-42**. Given that phenols are the primary substrates for sulfate conjugation, the addition of an ionized sulfate group greatly enhances the water solubility of these drugs and enhances their excretion. As previously mentioned, sulfate conjugation of estrogens increases their ability to be transported in the plasma to target cells and tissues. These target cells contain the enzyme sulfatase that removes the sulfate group and allows for essential estrogen activity. While most sulfated conjugates are pharmacologically inactive, there are a few rare examples in which sulfate conjugation produces an active metabolite. One such example

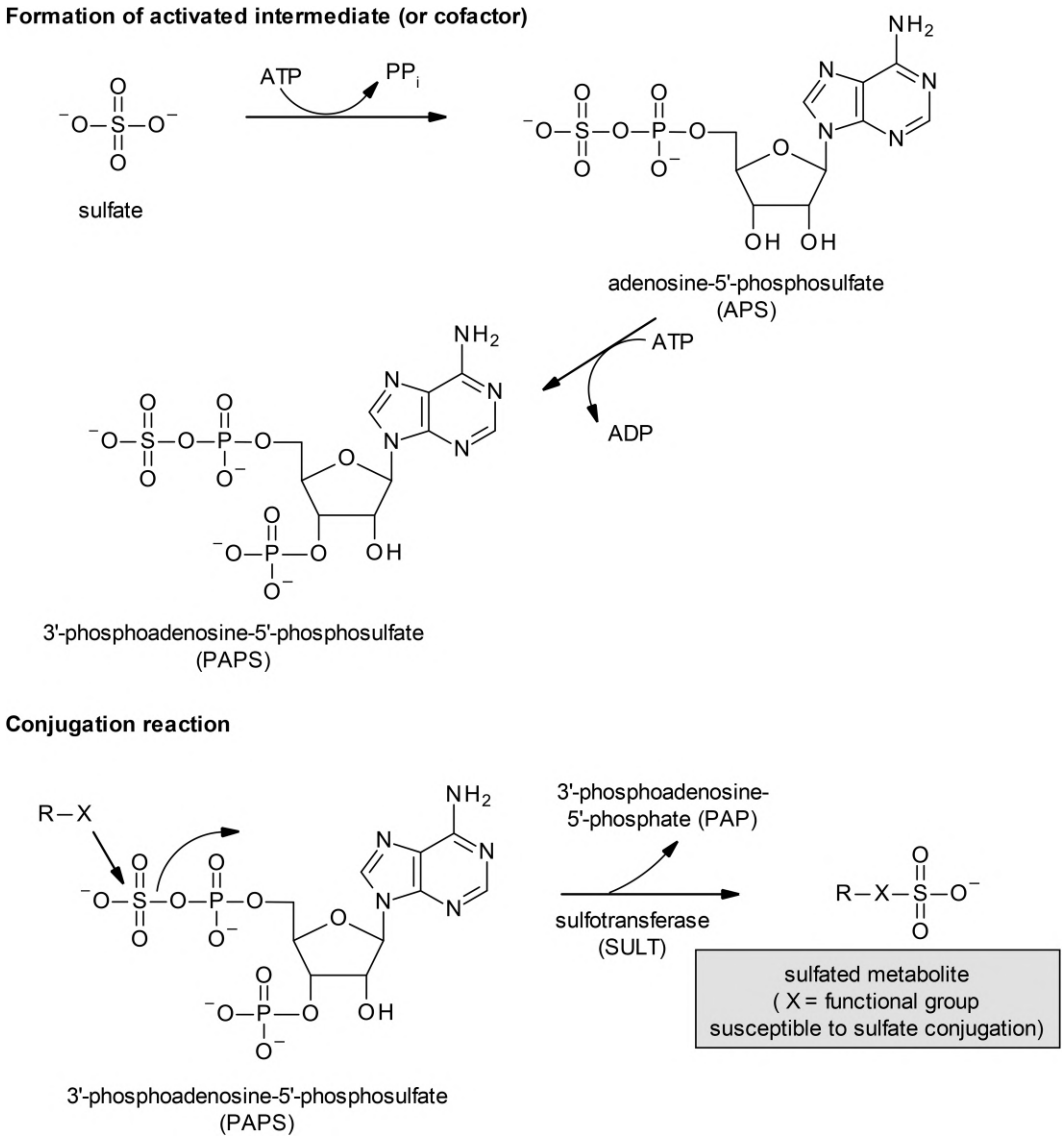


FIGURE 8-41. The mechanism of sulfate conjugation.

is seen with minoxidil. Minoxidil is an antihypertensive agent; however, it does not produce its beneficial therapeutic effects until it is sulfated. Interestingly, conjugation of minoxidil to its glucuronic acid metabolite produces an inactive drug.

Sulfate conjugation can also lead to the production of highly reactive and cytotoxic intermediates. This can occur with aromatic amines that have been oxidized to hydroxylamines via Phase I metabolism, as illustrated in **Figure 8-43**. Sulfate conjugation of these hydroxylamines can lead to a subsequent reaction in which the sulfate is eliminated and an electrophilic intermediate capable of reacting with proteins, enzymes, and/or DNA is formed. This mechanism has been proposed to be responsible for the carcinogenic effects of some polycyclic aromatic amines. Fortunately, this sequence of reactions is a minor metabolic pathway for drug molecules, and depending on the dose of the drug, glutathione can potentially completely neutralize these toxic metabolites.

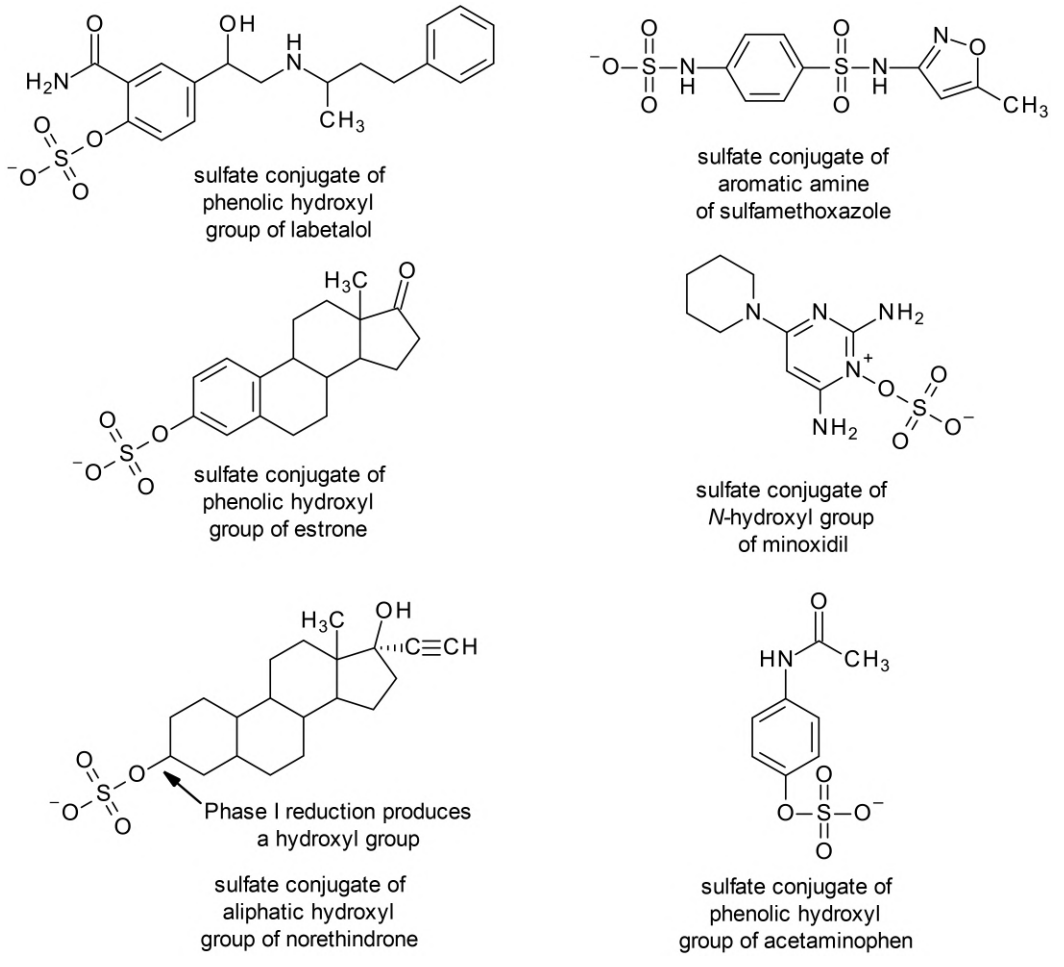


FIGURE 8-42. Examples of sulfate conjugation.

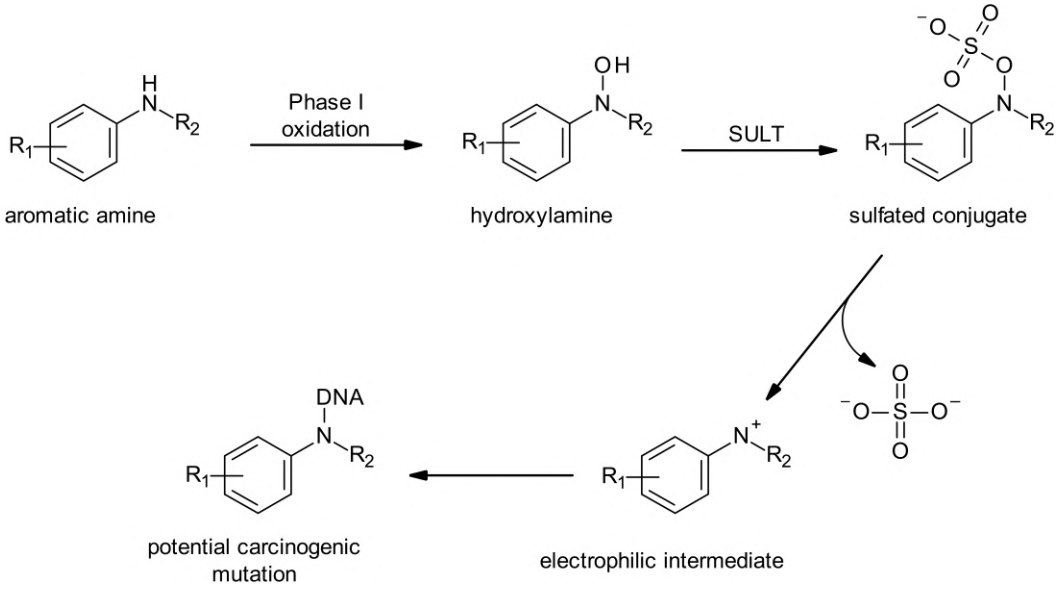


FIGURE 8-43. The formation of electrophilic intermediates from sulfate conjugates.

Amino Acid Conjugation

This conjugation pathway adds amino acids to carboxylic acids that are either initially present within the structure of a drug molecule or introduced by Phase I metabolism. The two most commonly used amino acids in this conjugation process are glycine and glutamine. Aspartic acid, serine, and taurine ($H_2NCH_2CH_2SO_3H$) are also used to a minor extent. Because excess amino acids are not stored in the human body, only a limited supply is available for conjugation. Glucuronic acid conjugation often competes with amino acid conjugation, so amino acid conjugation is primarily limited to aromatic carboxylic acids and aryl acetic carboxylic acids. The presence of an adjacent α-carbon on an aryl acetic carboxylic acid tends to sterically hinder amino acid conjugation and favor glucuronic acid conjugation.

Similar to glucuronic acid and sulfate conjugation, there is an initial two-step activation sequence that is required prior to the addition of an amino acid to a carboxylic acid. The main difference in this transformation is that the carboxylic acid present within the structure of the drug molecule is activated instead of the amino acid. The mechanism of amino acid conjugation is shown in **Figure 8-44** using salicylic acid as an example. In the first step, the carboxylic acid reacts with adenosine triphosphate (ATP) to form a reactive acid anhydride. In the second step, coenzyme A displaces adenosine monophosphate (AMP) to form the activated acyl coenzyme A intermediate. It

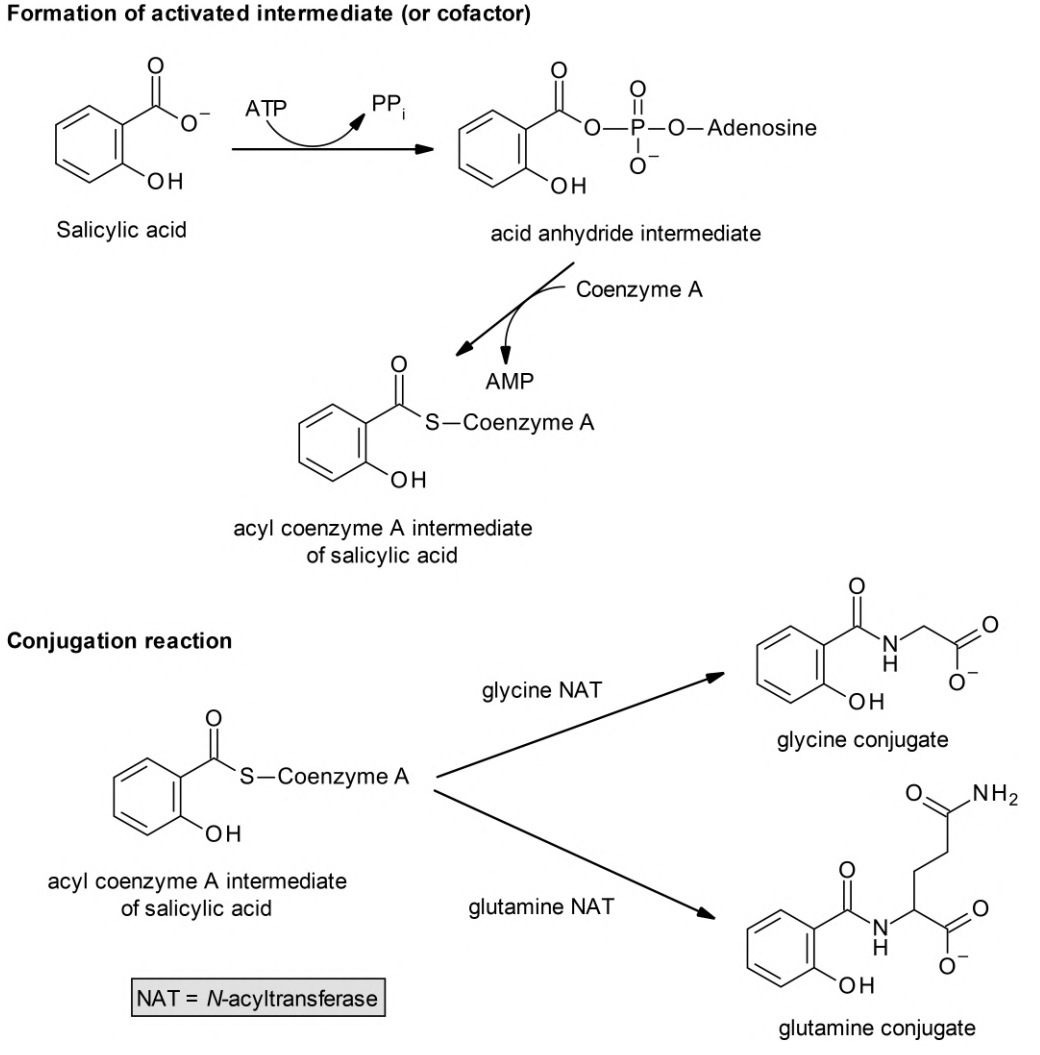


FIGURE 8-44. The mechanism of amino acid conjugation.

should be noted that the major function of coenzyme A in biochemical pathways is to activate and transfer acyl groups. In the citric acid cycle and other biochemical pathways, it serves as the activated carrier of acetyl groups, a specific two-carbon acyl group. In this pathway, coenzyme A serves as the activated carrier of acyl groups of drug molecules. The final conjugation step is catalyzed by either glycine or glutamine *N*-acyltransferase.

When comparing the water solubility of salicylic acid to that of its glycine conjugate, both molecules contain a carboxylic acid; however, the amino acid conjugate provides additional hydrogen binding due to the presence of the amide bond. When glutamine is the amino acid conjugate, it has the ability to participate in additional hydrogen bonding interactions not possible with glycine. Some additional examples of amino acid conjugation are seen in **Figure 8-45**. Rizatriptan provides an example of a coupled Phase I and Phase II metabolic transformation. Initially, the tertiary amine would need to undergo a minimum of three Phase I metabolic reactions: an *N*-dealkylation of one

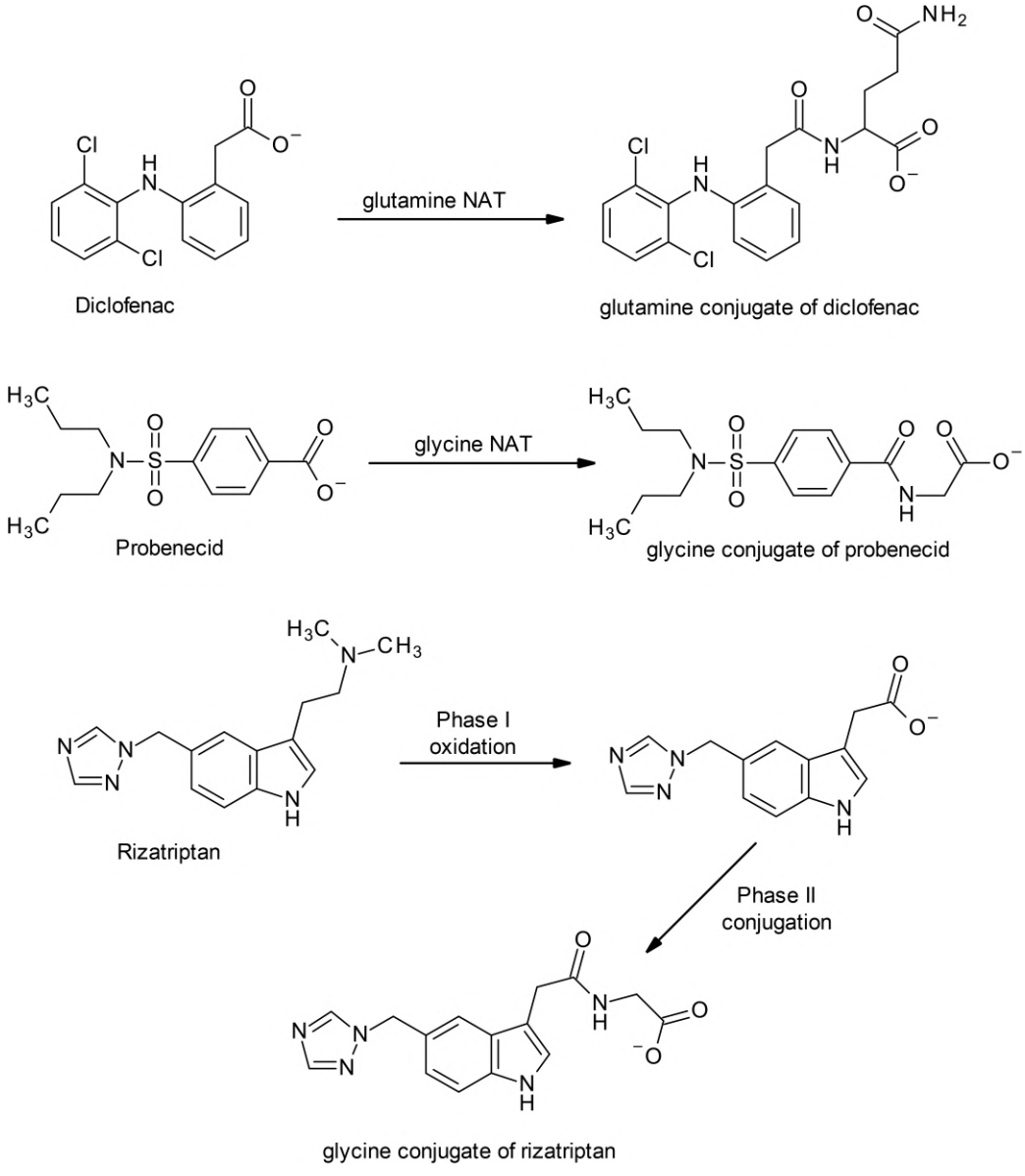


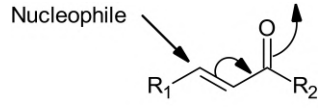
FIGURE 8-45. Examples of amino acid conjugation.

of the methyl groups, oxidative deamination of remaining secondary amine, and oxidation of the resulting aldehyde to a carboxylic acid. The resulting carboxylic acid could then undergo Phase II amino acid conjugation to further enhance its water solubility and aid in its elimination. Amino acid conjugation can be reversed, or deconjugated, by amidases; however, this is much less common than the deconjugation reactions seen with glucuronic acid and sulfate conjugates.

Glutathione Conjugation

Glutathione is what has been called "the true detoxifying agent." It is present in virtually all mammalian tissues and protects the body from potentially harmful electrophiles that are either initially present within the structure of an endogenous or exogenous compound or arise as a product of metabolic processes. Unlike all of the other conjugation pathways, glutathione can form conjugates without the need for activation. In this manner it can promptly inactivate any potentially harmful threats to cells and tissues. As previously mentioned, glutathione conjugation is the only conjugation pathway that cannot be reversed by a deconjugating enzyme.

Similar to glucuronic acid conjugation, glutathione can react with a large number of functional groups. The main criterion is that the functional group must be sufficiently electrophilic. In terms of general reaction mechanisms, functional groups susceptible to glutathione conjugation fall into two main categories: *those that undergo nucleophilic substitution at either a carbon atom or a heteroatom and those that act as a Michael acceptor (e.g., double bond conjugated to a carbonyl), as shown below.*



Michael acceptor
(electrophilic)

Glutathione is a tripeptide consisting of γ-glutamate, cysteine, and glycine. The general mechanism of glutathione conjugation is shown in **Figure 8-46**. The sulfhydryl group of cysteine is

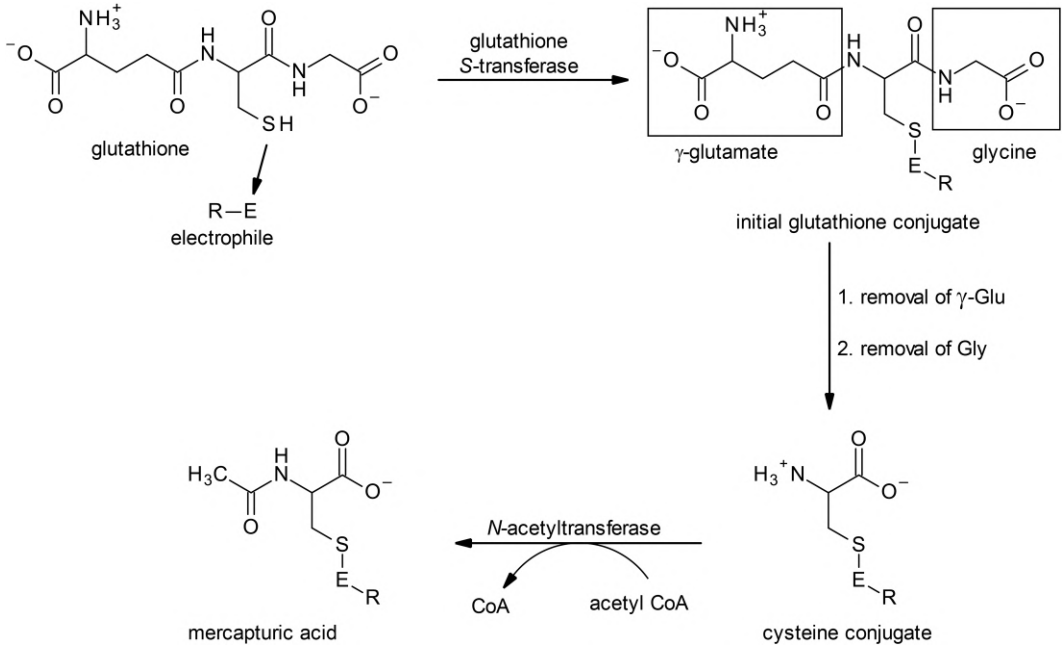


FIGURE 8-46. The mechanism of glutathione conjugation.

nucleophilic and readily reacts with an electrophilic functional group present within a drug molecule (designated as R—E). Because the reactivity is due to the sulfhydryl group, glutathione is commonly abbreviated as GSH. The enzyme responsible for this initial reaction is glutathione *S*-transferase. The resulting glutathione conjugate is generally inactive and nontoxic. This initial conjugate normally undergoes three additional reactions to form what is known as a mercapturic acid, or an *N*-acetylcysteine conjugate. As shown in Figure 8-46, γ-glutamate and glycine are sequentially removed, and the *N*-terminal amine of cysteine is acetylated. The remaining *N*-acetylcysteine conjugate provides adequate water solubility for elimination by virtue of its ionized carboxylic acid and the hydrogen bonding ability of the amide. Although glutathione conjugation does not require activation and cannot be reversed by a deconjugating enzyme, it is the only conjugating pathway that undergoes further metabolism after the initial conjugation has occurred.

Three examples of glutathione conjugation are shown in **Figure 8-47**. As previously discussed, *N*-oxidation of approximately 1% to 2% of a normal dose of acetaminophen produces a highly

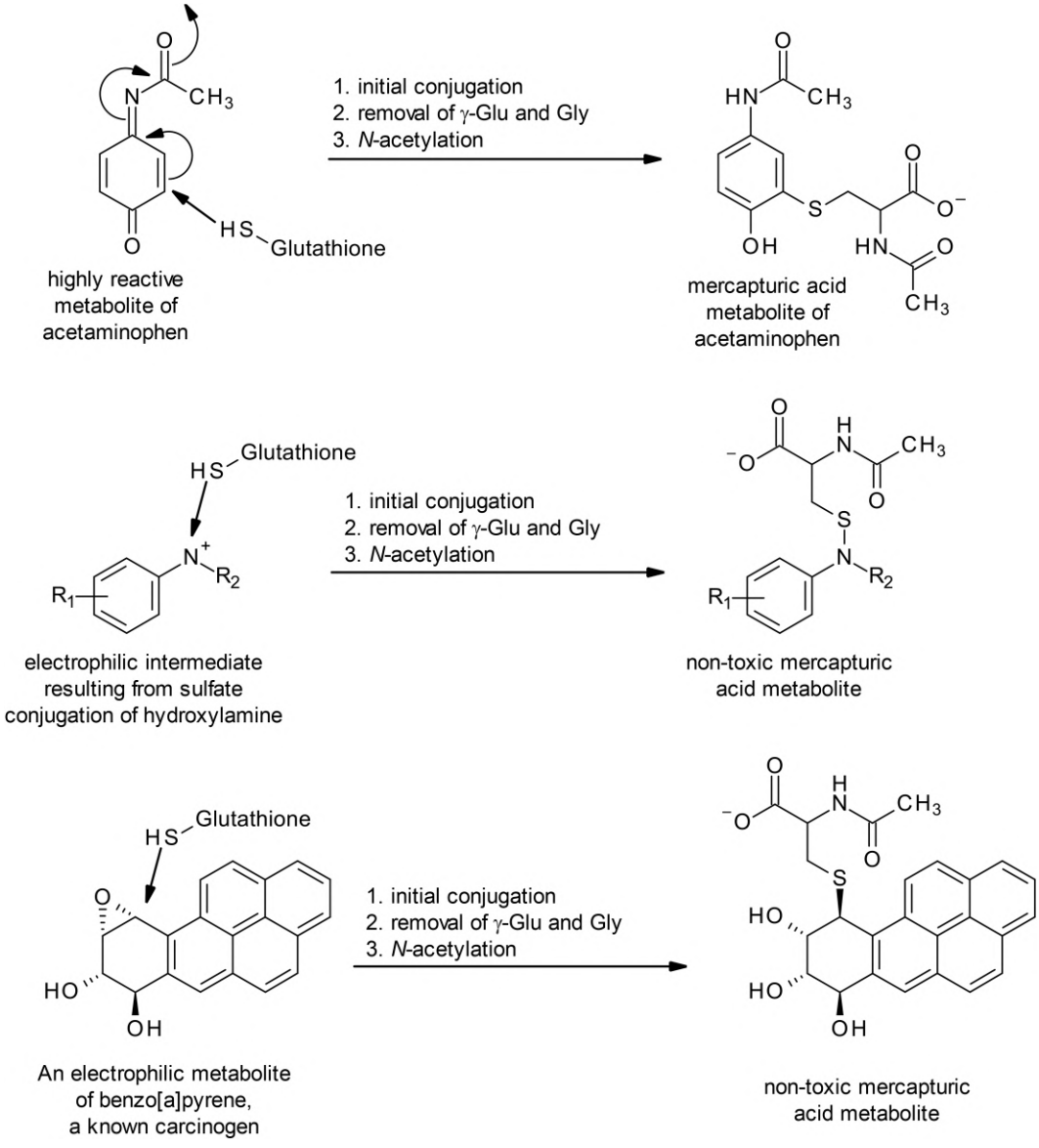


FIGURE 8-47. Examples of glutathione conjugation.

reactive electrophilic intermediate (Figure 8-20). Glutathione swiftly reacts with this intermediate to produce an inactive and nontoxic metabolite. The second example uses the electrophilic intermediate that can be generated by sulfate conjugation of hydroxylamines (Figure 8-43). Once again, glutathione can quickly react with this intermediate and remove any threat of toxicity. Finally, the oxidation of aromatic hydrocarbons, such as benzo[a]pyrene, to epoxide intermediates produces potential carcinogenic metabolites. Glutathione acts to detoxify these reactive intermediates.

As a final consideration, please note that while glutathione is readily available, the amount present in any given cell or tissue is limited. Overdoses of acetaminophen and overexposure to carcinogenic compounds deplete glutathione stores, thereby allowing the electrophilic intermediates to react with cellular nucleophiles, including proteins, enzymes, DNA, and other macromolecules.

Acetylation

This conjugation pathway uses acetyl CoA to acetylate drug molecules that contain primary aliphatic or aromatic amines, hydrazines, hydrazides, or unsubstituted sulfonamides. Although acetyl CoA is readily abundant, acetylation is a relatively minor conjugation pathway because the required functional groups are present only in a limited number of drug molecules. The most commonly encountered functional group, a primary aliphatic amine, is normally metabolized by oxidative deamination rather than acetylation. Unlike the previous four types of conjugation, acetylation does not produce a more water-soluble metabolite. Acetylated metabolites tend to be more lipid soluble than the original drug molecule and often must undergo additional metabolic transformations to be sufficiently water soluble to be eliminated from the body.

The mechanism of acetylation is a two-step process as shown in **Figure 8-48** using hydralazine as an example. In the first step, the acetyl group of acetyl CoA is transferred to the enzyme *N*-acetyltransferase (NAT). In the second step, NAT transfers the acetyl group to the hydrazine group of hydralazine. The transferase enzyme is regenerated in this process and is able to acetylate additional drug molecules.

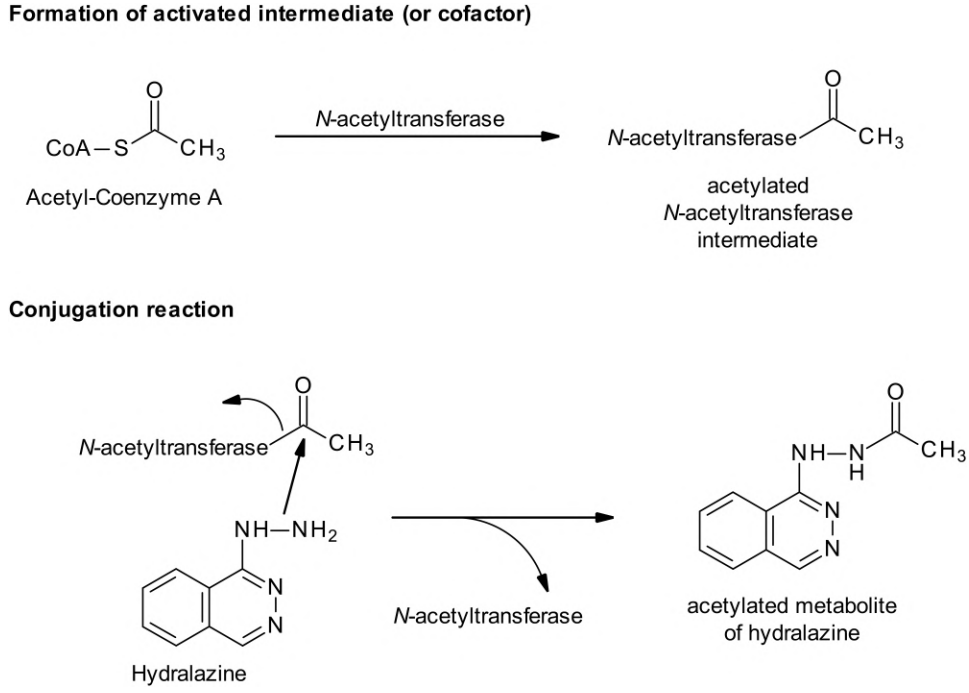


FIGURE 8-48. The mechanism of acetylation.

Similar to other conjugation pathways, there are two major isoforms of *N*-acetyltransferase, NAT1 and NAT2. Genetic polymorphism affects the rate of acetylation of drug molecules and can affect their dosing and toxicity. The human population can be roughly divided into two groups: those who are fast acetylators and those who are slow acetylators. Slow acetylators have a decreased capacity to inactivate drug molecules for which acetylation is the primary metabolic route. Shown in **Figure 8-49** is the acetylation of procainamide, isoniazid, and sulfamethoxazole. This metabolic pathway is the primary route of metabolism for these three drugs. Patients who are slow acetylators are more prone to experience higher blood concentrations and potential toxicity with these drugs than those who are fast acetylators. Conversely, fast acetylators may require higher doses or more frequent administration of drug molecules that are metabolized by this route. In most instances, acetylation produces an inactive metabolite; however, this is not always the case. The acetylation of procainamide produces *N*-acetylprocainamide, an active antiarrhythmic drug. Acetylation can be reversed through hydrolysis catalyzed by an amidase enzyme; however, this is not often observed.

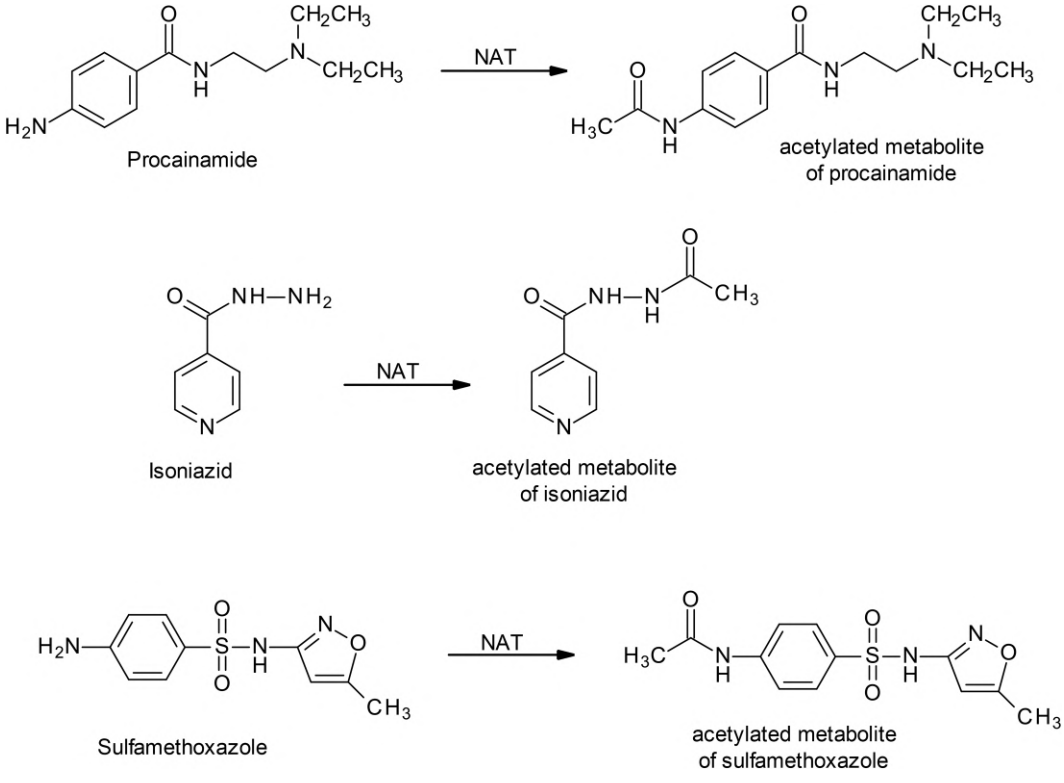


FIGURE 8-49. Examples of drugs known to undergo acetylation as a major metabolic pathway.

Similar to the coupled Phase I and Phase II metabolism of rizatriptan shown in Figure 8-45, **Figure 8-50** provides two additional examples of this concept. Neither tetracaine nor nimodipine contains a functional group that can undergo acetylation; however, Phase I metabolic pathways can provide a primary aromatic amine for both drugs. Tetracaine requires an initial *N*-dealkylation, and nimodipine requires an initial reduction. Once these Phase I metabolic processes have occurred, Phase II acetylation can provide the final product.

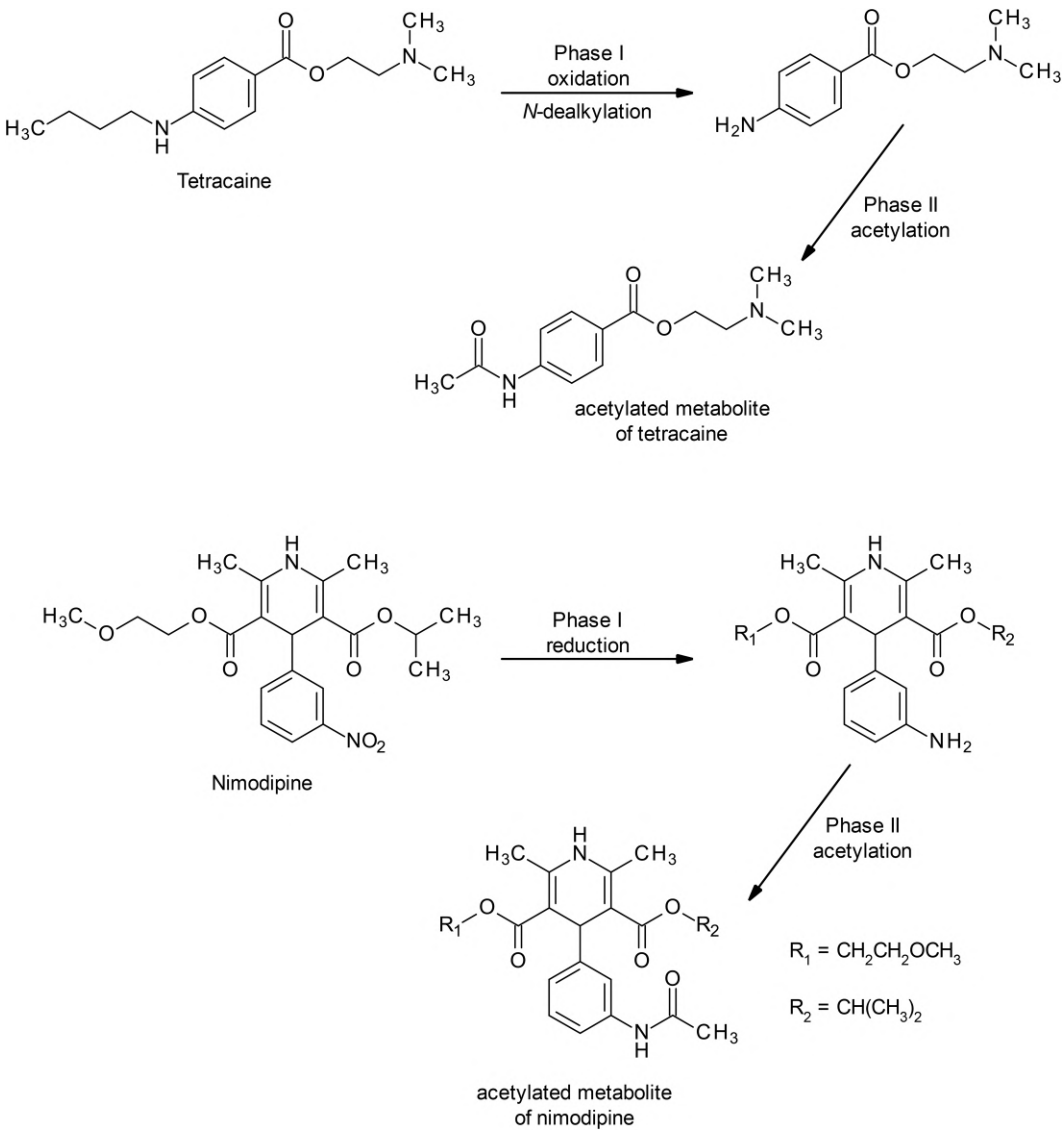


FIGURE 8-50. Examples of coupled Phase I metabolism and Phase II acetylation.

Methylation

Methylation is extremely important in the biosynthesis of nucleic acids, proteins, phospholipids, neurotransmitters, and other amines. In contrast, methylation is a minor metabolic pathway that is seen only with catechols, phenolic hydroxyl groups, amines, and sulfhydryl groups. Each of these functional groups is methylated by one of four different methyltransferase enzymes: catechol-*O*-methyltransferase (COMT), phenol-*O*-methyltransferase (POMT), *N*-methyltransferase, or *S*-methyltransferase. Drug molecules that contain a catechol ring (i.e., an aromatic ring with *ortho* hydroxyl groups) are the most likely to undergo this Phase II conjugation. This includes endogenous amines, such as epinephrine and dopamine, as well as a number of adrenergic agonists, such as methyldopa and isoproterenol. Due to the availability of other metabolic pathways, phenolic

hydroxyl groups are only methylated to a minor extent. The methylation of amines is essential for biosynthetic pathways, but of very limited importance as it relates to drug metabolism. As previously discussed, amines are primarily dealkylated and/or deaminated. Sulfhydryl groups can also be methylated; however, due to their low prevalence in drug molecules, this is not often observed.

The methylation of functional groups occurs via a two-step process, shown in **Figure 8-51**. The methyl donor is methionine; however, it must first be activated to transfer its methyl group. The enzyme methionine adenosyl transferase uses methionine and ATP to synthesize *S*-adenosylmethionine, commonly abbreviated as SAM. The positively charged sulfur atom is electrophilic and allows for the easy transfer of the methyl group to a catechol, a phenolic hydroxyl group, an amine, or a sulfhydryl group. *S*-Adenosylhomocysteine can then be converted back to methionine in two steps to allow additional methylations to occur.

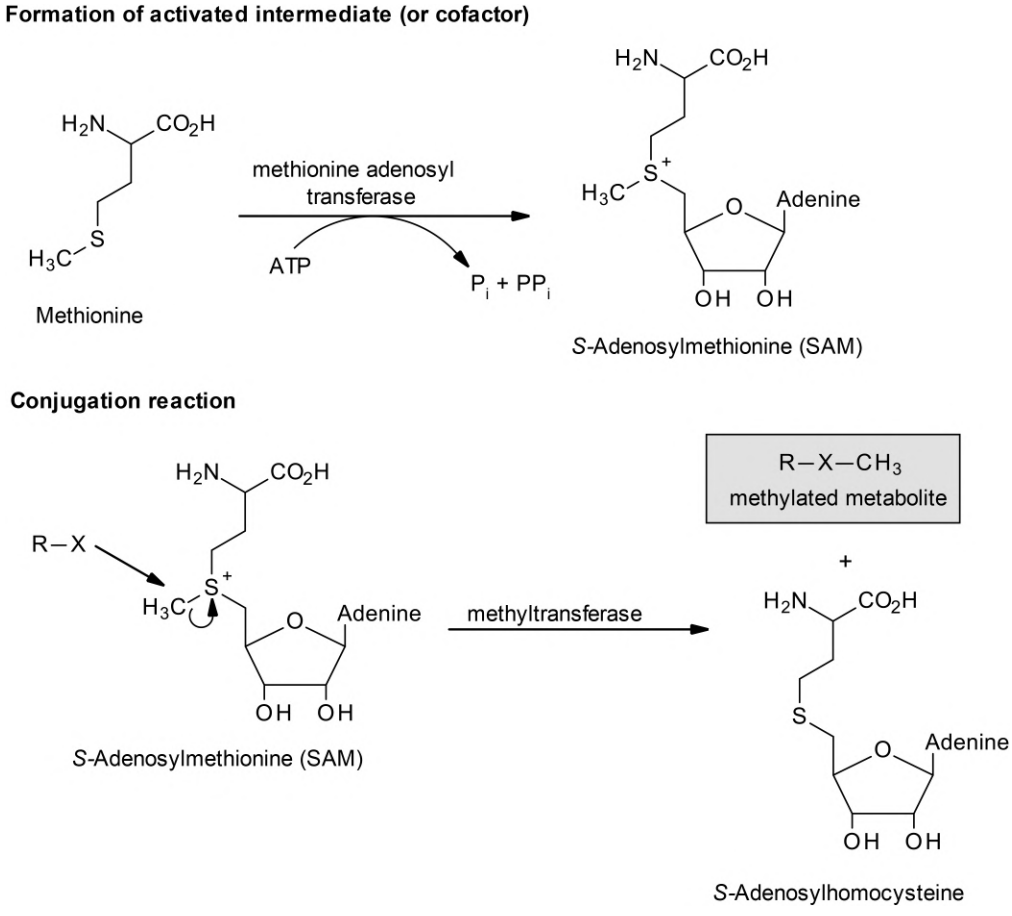


FIGURE 8-51. The mechanism of methylation.

Examples of drugs that can undergo methylation are shown in **Figure 8-52**. Please note that the methylation of catechols occurs preferentially at the *meta* position and that the *N*-methylation of phenylpropanolamine is a minor metabolic pathway for this drug molecule. Similar to acetylation, methylation does not enhance water solubility and does not enhance the elimination of drug molecules. Methylation instead often serves to inactivate drug molecules. Methylation can be reversed through the process of oxidative dealkylation; however, this does not occur very often.

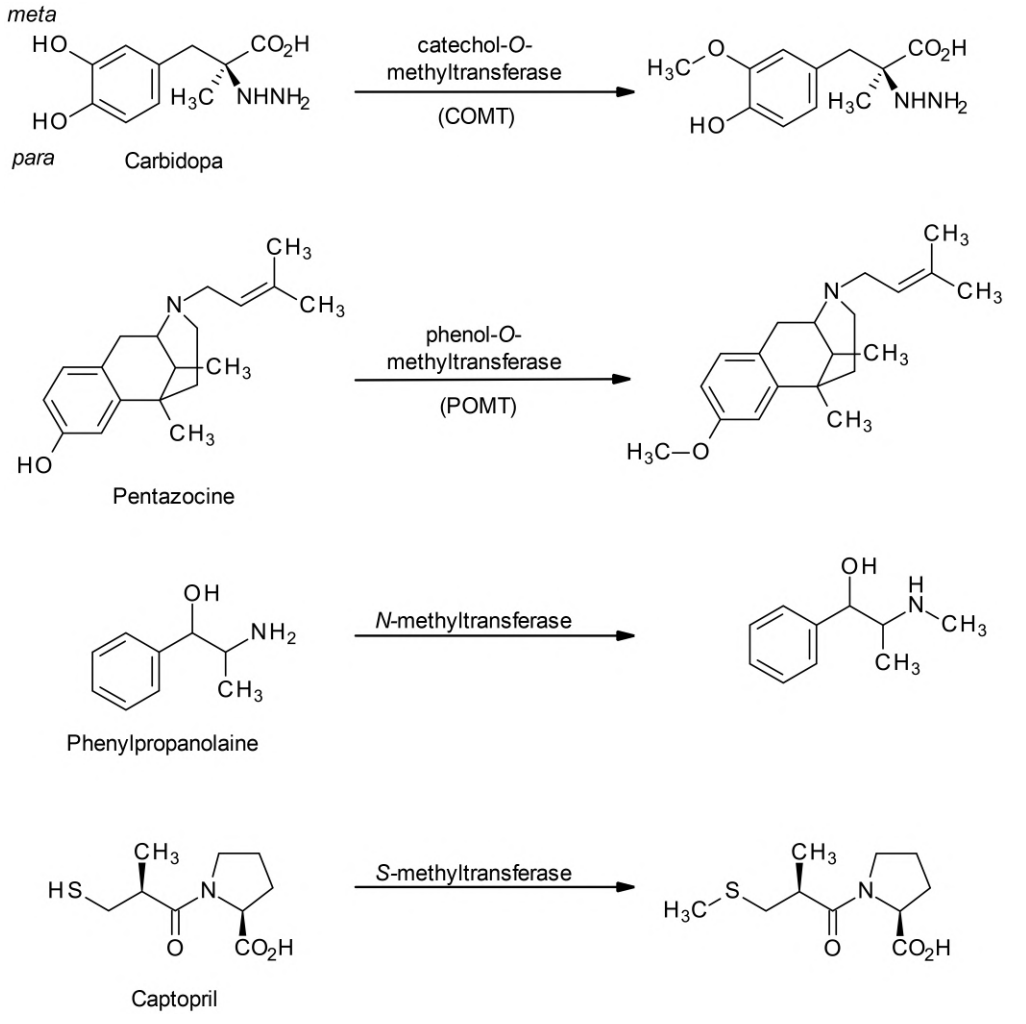
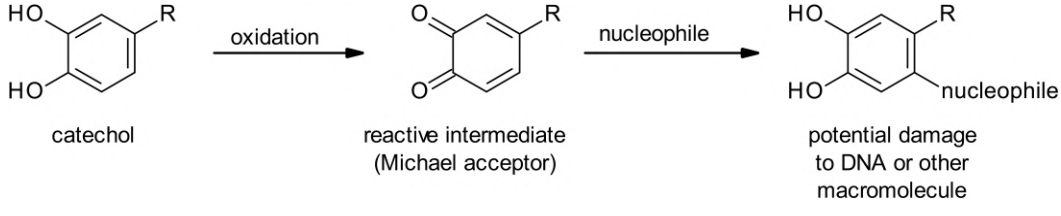


FIGURE 8-52. Examples of methylation.

The methylation of catechol rings serves a second purpose because it prevents their oxidation to highly reactive intermediates, as shown below. As previously discussed, glutathione can help neutralize or inactivate these types of reactive intermediates; however, without COMT, high doses of catechol-containing drugs could deplete glutathione stores.



Summary of Phase II Metabolism

- Phase II metabolism involves the conjugation of endogenous substances to functional groups that were either initially present on the drug molecule or were introduced by Phase I metabolic transformations.
- Conjugation with glucuronic acid, sulfate, or amino acids greatly enhances the water solubility and, therefore, elimination of a drug molecule.
- Glutathione conjugation serves to neutralize or detoxify highly reactive electrophilic intermediates.
- Acetylation and methylation do not enhance water solubility but usually act to terminate the pharmacological actions of a drug molecule. These conjugates may require additional metabolism to be water soluble enough for elimination.
- Although there are some exceptions, most Phase II conjugates are inactive and nontoxic.
- Due to the readily available supply of glucuronic acid and the large number of functional groups that are substrates for UGT, glucuronic acid conjugation is the most common Phase II pathway.
- With the exception of glutathione conjugation, all other Phase II conjugations require an initial activation of either the conjugating group, the transferase enzyme, or the target functional group.
- With the exception of glutathione conjugation, all other Phase II conjugations can be reversed by deconjugating enzymes.
  - Glucuronic acid conjugation and sulfate conjugation are the two most common pathways to undergo a deconjugation step.
  - Through the process of enterohepatic cycling, deconjugation enzymes can help to extend the duration of action of a drug molecule.
  - Deconjugation enzymes can liberate the active drug molecule at its site of pharmacological action.

Factors That Affect Drug Metabolism

Prior to summarizing the key concepts that govern metabolism, it should be noted that genetic differences, physiologic conditions, dosing regimen, dietary influences, and coadministration of other drug molecules can affect the metabolic pathways that have been discussed in this chapter.

The field of study known as pharmacogenomics, or pharmacogenetics, seeks to evaluate the effects of how an individual's genes affect the response to medications. It is well known that the genetic profile of an individual can alter the ability of that person to metabolize a given drug. Individuals with specific genetic mutations, also known as genetic polymorphisms, may possess metabolizing enzymes that are either less active or more active than those found in the general population. Additionally, certain patient populations have been shown to express different enzymatic isoforms that are more or less active than the normal isoform (also known as the wild-type form). This is most commonly seen with the cytochrome P450 monooxygenase enzymes, otherwise known as CYP450 enzymes. Finally, genetic variations among patients or patient populations may alter the number of copies of a given enzyme available for a specific type of metabolic transformation.

Three examples of known polymorphisms that affect metabolic pathways are discussed here. The first example examines the metabolism of the *S* isomer of warfarin, the more active enantiomer. This isomer is primarily metabolized by CYP2C9 to inactive 6- or 7-hydroxy metabolites, with the 7-hydroxywarfarin representing the major metabolite (**Figure 8-53)**. More than 60 different alleles have been identified for the CYP2C9 gene, with CYP2C9*2 and CYP2C9*3 being the most common.

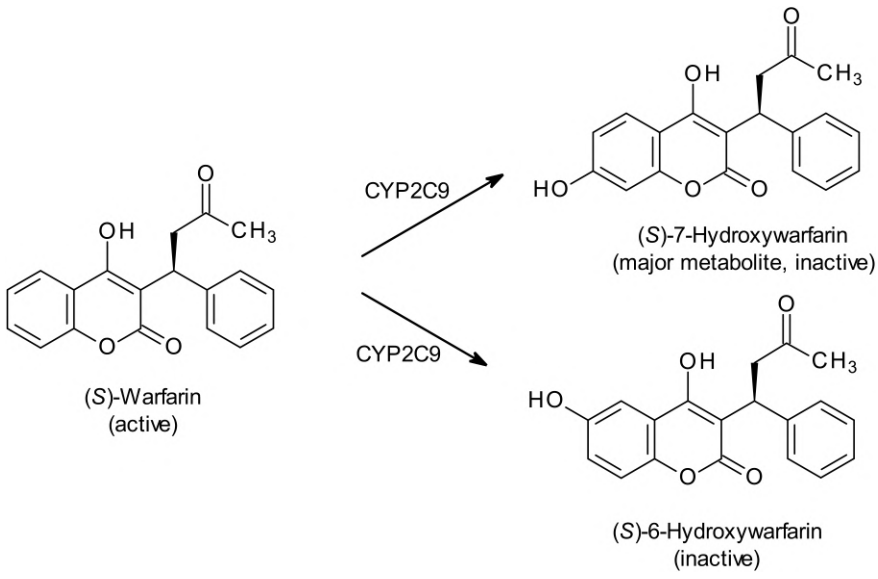


FIGURE 8-53. Metabolism of warfarin by CYP2C9.

Patients who have the normal, or wild-type, CYP2C9 isozyme metabolize warfarin in a normal manner. In contrast, those who have the CYP2C9*2 and CYP2C9*3 alleles have impaired metabolism and require lower doses to avoid adverse effects, specifically increased bleeding tendencies.

A second example is seen with clopidogrel. Clopidogrel is a prodrug that requires CYP2C19 oxidation of its thienopyridine ring to be converted to its active metabolite. This activation was discussed in Chapter 6 and can be found in Figure 6-7. Similar to CYP2C9, a large number of alleles have been identified for CYP2C19. Individuals who possess specific variants of CYP2C19 are poor metabolizers of clopidogrel and fail to adequately convert the prodrug to its active form. Although routine genetic testing for CYP2C19 polymorphisms in patients taking clopidogrel is not currently done, there are published recommendations for poor and intermediate metabolizers for this isozyme.

The third and final example of polymorphisms and drug metabolism involves the metabolism of metoprolol. While metoprolol can be oxidized by a number of CYP isozymes, its primary metabolizing enzyme is CYP2D6. As shown in **Figure 8-54**, metoprolol can undergo *O*-dealkylation (~65%), benzylic oxidation (~10%), and *N*-dealkylation (~10%). The *O*-dealkylated metabolite is rapidly further oxidized to an inactive carboxylic acid. Patients who are poor CYP2D6 metabolizers experience several-fold higher concentrations of metoprolol compared with normal CYP2D6 metabolizers. Although there are no current guidelines for the dosing of metoprolol based on a patient's CYP2D6 status, numerous studies have shown that dosing reductions should be considered in patients who are either intermediate CYP2D6 metabolizers or poor CYP2D6 metabolizers. Additionally, dosage increases of metoprolol have been suggested for patients who are ultrarapid CYP2D6 metabolizers.

Age, existing disease states, and the nutritional status of a patient can also affect drug metabolism. In general, as an individual ages, his or her ability to metabolize drug molecules decreases. Disease states that alter liver function have a pronounced effect on the metabolism of a number of drugs. In these situations, specific dosing guidelines are listed in the drug product literature. Protein and lipid deficiencies can also decrease drug metabolism.

The dose, route of administration, and frequency of administration also play a role in drug metabolism. Let us look at a couple of general examples. Phase II conjugation pathways require the addition of endogenous substances to the drug molecule. The liver, as well as any other organ involved in this type of metabolism, has a finite amount of these endogenous substances

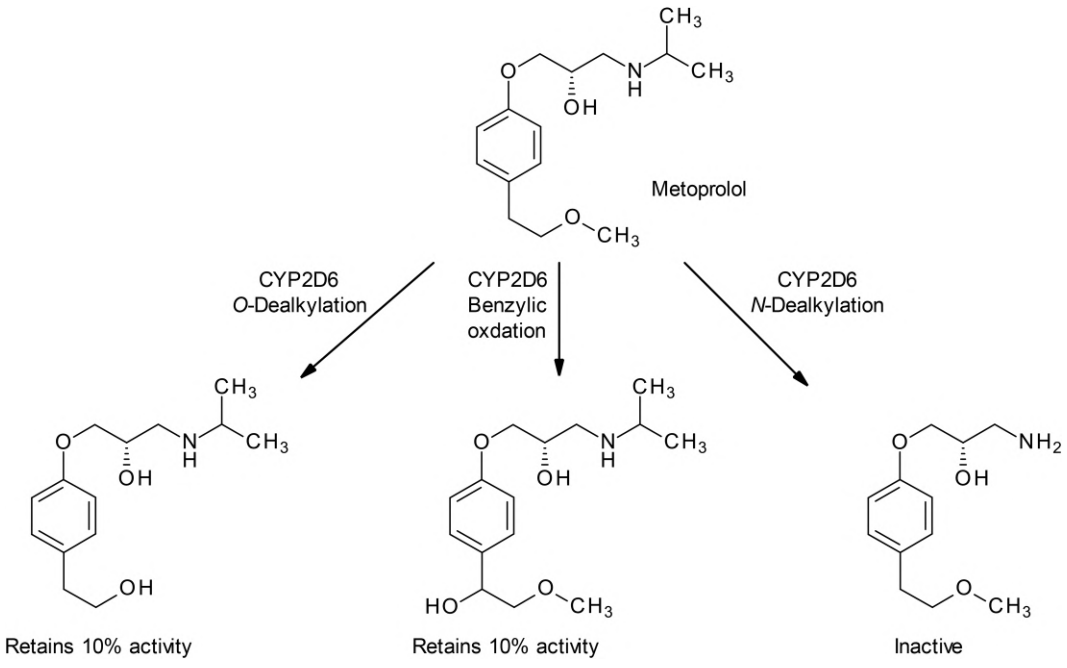


FIGURE 8-54. Metabolism of metoprolol by CYP2D6.

(e.g., glucuronic acid, glutathione). As the dose or frequency of administration of a drug increases, these pathways can become saturated and lead to alternative metabolic pathways. Additionally, the liver often significantly metabolizes lipid-soluble drugs before they reach the systemic circulation. This process, known as *first-pass* or *presystemic metabolism,* often significantly reduces the amount of orally administered drug that is bioavailable. In some instances, this can be avoided by using a different method of administration, such as a parenteral injection or a topical cream.

The coadministration of other drugs can affect drug metabolism in a number of ways. *The most common mechanism is through either the induction or inhibition of specific metabolizing enzymes.* Similar to the genetic deficiencies discussed above, this is most commonly seen with the CYP450 family of enzymes. Induction of CYP450 enzymes enhances their activity, increases the rate of drug metabolism, and in most instances decreases the plasma level of the active drug molecule. Inhibition of CYP450 enzymes decreases their activity, decreases the rate of drug metabolism, and in most instances increases the plasma level of the active drug molecule. There are some instances in which CYP450 metabolism is required to convert an inactive prodrug to its active metabolite. In these instances, induction of the specific CYP450 enzyme would enhance this conversion and potentially increase the desired plasma level of the active metabolite. In contrast, inhibition of the specific CYP450 enzyme would decrease the desired conversion and subsequently decrease the desired plasma levels. These types of drug interactions depend on the specific CYP450 isoform needed for the drug's metabolism as well as the CYP450 isoforms induced or inhibited by a coadministered drug. Let's look at a few examples of the above concepts.

In general, inhibitors or inducers of metabolizing enzymes are classified as strong, moderate, or weak. Itraconazole, an antifungal drug, is both a substrate and a strong inhibitor of CYP3A4. Atorvastatin, an HMG CoA reductase inhibitor used to treat dyslipidemia, is primarily metabolized by CYP3A4. If these two drugs are given together, itraconazole inhibits the metabolism of atorvastatin and increases its plasma levels. Recommendations to avoid serious adverse effects from this drug interaction include reducing the dose of atorvastatin or replacing atorvastatin with another HMG CoA reductase inhibitor that does not require CYP3A4 for metabolism. In contrast to itraconazole,

phenytoin and its prodrug, fosphenytoin, are strong inducers of CYP3A4 and increase the metabolism and decrease the plasma levels of atorvastatin and other drugs requiring this isozyme.

As discussed above, clopidogrel requires CYP2C19 oxidation to be converted to its active metabolite. The proton pump inhibitors omeprazole, lansoprazole, and pantoprazole are CYP2C19 inhibitors. If these drugs are used in combination with clopidogrel, the serum concentrations of the active metabolite of clopidogrel may be decreased. Clopidogrel prevents platelet aggregation and is used to treat acute coronary syndrome and other cardiovascular disorders. Coadministration of clopidogrel with any of these proton pump inhibitors could potentially impair the effectiveness of clopidogrel and not provide the needed cardiovascular effects. A final example of CYP450 drug interactions is seen with tamoxifen. Tamoxifen is used as adjuvant treatment of adult patients with early stage estrogen receptor-positive breast cancer. While tamoxifen is active, its 4-hydroxy-*N*-desmethyl metabolite (endoxifen) is 30 to 100 times more potent than tamoxifen and provides the major therapeutic actions of tamoxifen. The addition of the 4-hydroxyl group is the key metabolic step and requires CYP2D6 metabolism (**Figure 8-55**). Tamoxifen can cause debilitating hot flashes in women, and this adverse effect can be treated with selective serotonin reuptake inhibitors (SSRIs). Some SSRIs, especially paroxetine and fluoxetine, inhibit CYP2D. Due to this drug interaction, paroxetine and fluoxetine should not be used in combination with tamoxifen because their use decreases the formation of the active metabolite and greatly reduces tamoxifen's efficacy. There are other SSRIs that do not interfere with CYP2D6 and the formation of endoxifen and are better choices for treating hot flashes.

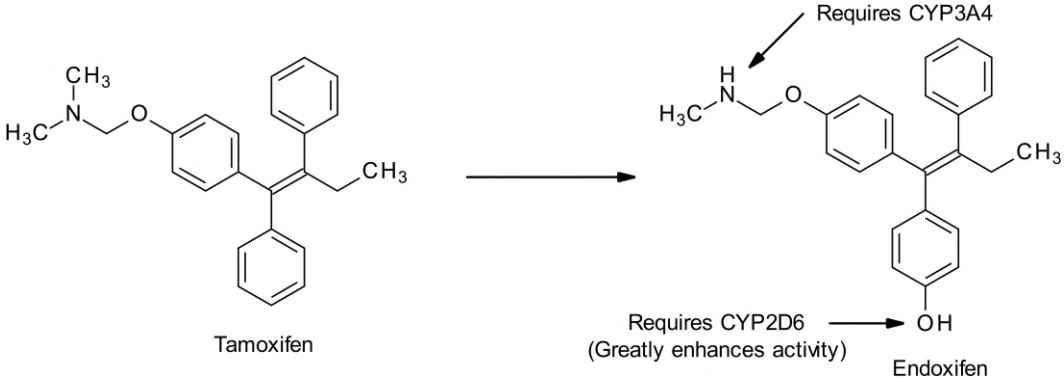


FIGURE 8-55. Metabolic conversion of tamoxifen to endoxifen.

SUMMARY OF THE KEY CONCEPTS GOVERNING METABOLIC TRANSFORMATIONS

The metabolism of drug molecules serves a number of purposes; however, its primary role is to increase the water solubility of drug molecules and thus enhance their removal from the body. Metabolism can alter existing functional groups or add new functional groups through Phase I and/or Phase II metabolic transformations. Oxidation is the most prevalent Phase I metabolic reaction, and most oxidative transformations are catalyzed by the CYP450 family of enzymes. Other oxidative transformations are catalyzed by FMOs, ADHs, and ALDHs. Hydrolysis and reduction represent the other two Phase I processes, with hydrolysis being much more prevalent than reduction. Phase II metabolic transformations involve the addition of endogenous substances to the drug molecule

in a process known as conjugation. Glucuronic acid conjugation is the most common Phase II transformation.

When evaluating known metabolic pathways or predicting possible metabolic routes for a specific drug molecule, the following general concepts should be considered. First, the extent of metabolic transformation for a given drug molecule is generally proportional to its lipid solubility. Drug molecules that have a higher lipid solubility (i.e., increased log *P* values) generally require more extensive metabolism than analogous drug molecules that possess lower lipid solubility. In many instances, drug molecules that possess a low lipid solubility (or high water solubility) can be excreted unchanged (i.e., no metabolic transformations). Second, there is no absolute requirement that a drug molecule undergo both Phase I and Phase II transformations. In some instances, Phase I transformations are sufficient to provide adequate water solubility for elimination without the need for Phase II metabolism. In other instances, Phase II conjugation is sufficient due to the presence of functional groups capable of immediately undergoing conjugation within the parent drug molecule. Third, a functional group or a drug molecule may be capable of undergoing multiple types of transformations. In these situations, the liver and other metabolic sites perform the minimum number of transformations necessary to allow for drug molecule elimination or detoxification. Finally, functional groups are more likely to undergo metabolic transformation if they are easily accessible (i.e., located at the end of a drug molecule instead of the middle), not electronically deactivated, and not sterically hindered.

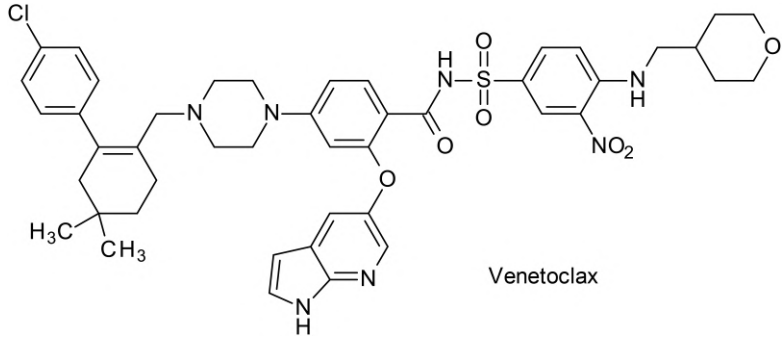
REFERENCES

1. Roche VF, Zito SW, Lemke TL, et al, eds. *Foye's Principles of Medicinal Chemistry*. 8th ed. Philadelphia, PA: Wolters Kluwer; 2020.
2. Beale JM, Block JH, eds. *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*. 12th ed. Philadelphia, PA: Wolters Kluwer/Lippincott Williams & Williams; 2011.

STRUCTURAL ANALYSIS CHECKPOINT

Checkpoint Drug 1: Venetoclax

1. In Chapter 7, you were asked to identify all potential prochiral centers present within the structure of venetoclax prior to a discussion of metabolic pathways. Now that you have reviewed all of the normal metabolic pathways, identify those potential prochiral centers that are valid. Additionally, for each valid prochiral center, identify the metabolic transformation that is required to convert the prochiral center to a chiral center.



Venetoclax

2. Listed below are six metabolic transformations. For each metabolic transformation indicate if it is a Phase I or Phase II transformation and if venetoclax has a functional group

that can participate in the transformation. If you answer YES, then draw the appropriate metabolite; if you answer NO, then provide a brief explanation as to why this metabolic transformation is not possible for venetoclax.

Metabolic Pathways

A. Benzylic oxidation

B. Sulfate conjugation

C. Oxidative *N*-dealkylation

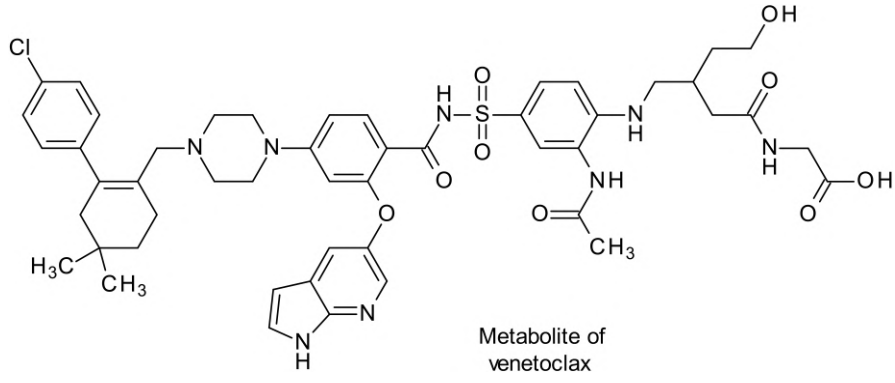
D. Glucuronide conjugation

E. Hydrolysis

F. Alkene oxidation

3. The structure of venetoclax contains three phenyl rings. Although aromatic oxidation could occur at one or more of these phenyl rings, explain why this metabolic transformation has a low probability of occurring.

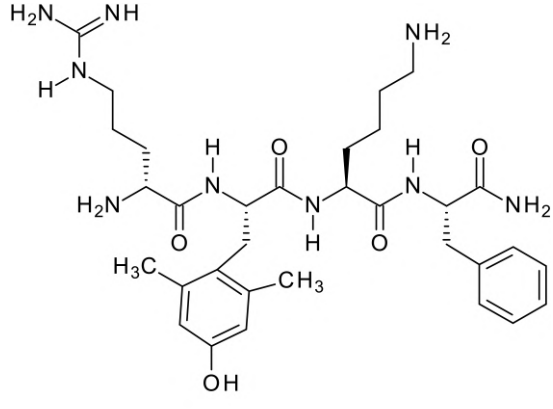
4. Shown below is a potential metabolite of venetoclax. Identify the metabolic transformations required to produce this metabolite. Please note that some steps must occur prior to other steps while other steps are completely independent of one another.



Metabolite of venetoclax

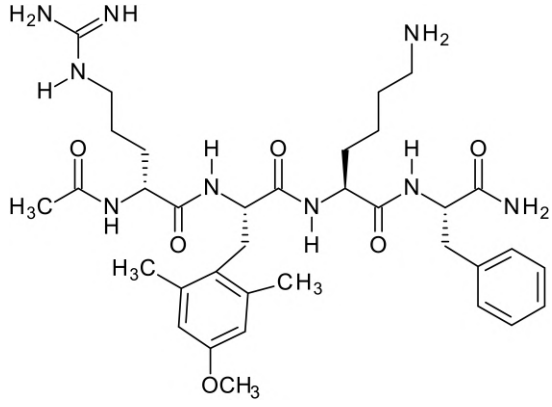
Checkpoint Drug 2: Elamipretide

1. Consider the structure of elamipretide and determine if the drug can be excreted without the need for any metabolic transformation. Provide a brief structural rationale for your answer.



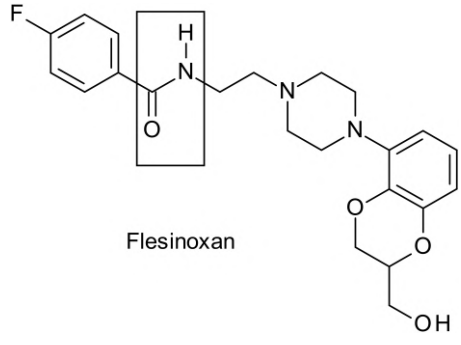
Elamipretide

2. Based on your structural evaluation of elamipretide, does a Phase I metabolic transformation need to occur before a Phase II conjugation reaction occurs? If so, then identify which Phase I transformation must occur and then which Phase II conjugation reaction can occur. If not, then list the name of the functional group(s) that can undergo a Phase II conjugation reaction and the specific Phase II transformation associated with each group.
3. There are a number of Phase I transformations that could occur on elamipretide, including on more than one prochiral center. List ALL possible Phase I transformations and identify which Phase I transformation(s) could convert a prochiral carbon to a chiral carbon.
4. The structure below is one of the prodrug forms of elamipretide that could be considered to improve metabolic stability. Which metabolic transformations must occur to produce the active form of the drug?



REVIEW QUESTIONS

1. Consider the structure of flesinoxan drawn below and do the following:
   A. List all of the Phase I metabolic transformations possible for flesinoxan.
   B. Draw all of the possible products created when the functional group that is boxed undergoes a Phase I metabolic transformation.
   C. Describe how you would modify the structure of flesinoxan to prevent Phase II metabolism.



Flesinoxan

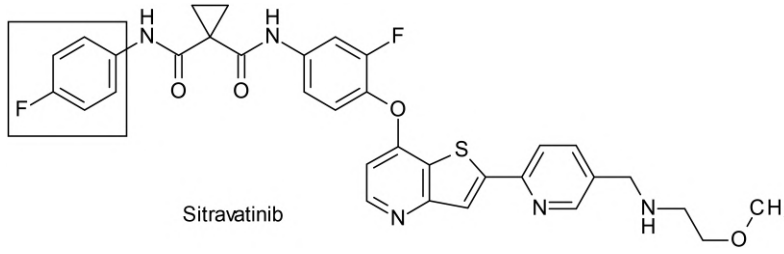
2. Consider the structure below and do the following:

   A. Box the ether that *can* participate in oxidative *O*-dealkylation.

   B. Cross out the ether that *cannot* participate in oxidative *O*-dealkylation. Provide a brief explanation why this transformation isn't possible.

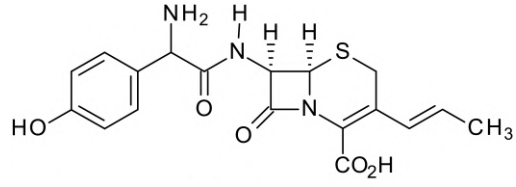
   C. Provide a brief explanation as to why the boxed halogenated aromatic hydrocarbon cannot undergo *para* aromatic hydroxylation.

   D. Circle the functional group(s) that can undergo hydrolysis.



Sitravatinib

3. Cefprozil is a second-generation cephalosporin used in the treatment of bronchitis and infections of the throat, ears, and sinuses. List all of the possible Phase I metabolic transformation(s) in the space below. Directly modify the structure below to show the product of one of the transformations listed.

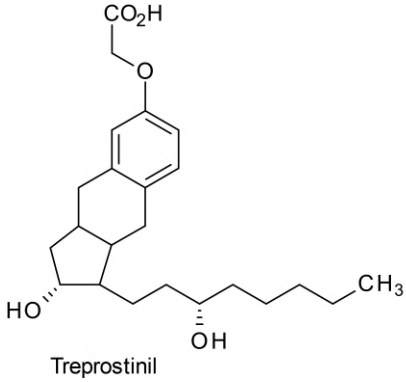


Cefprozil

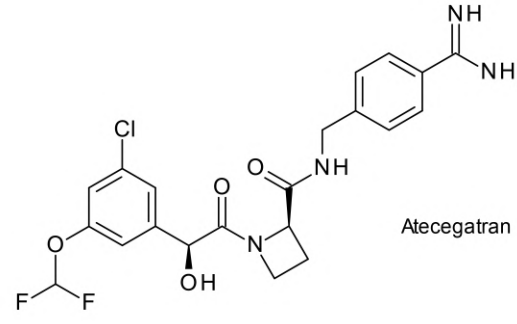
4. Consider the structures of treprostinil and atecegatran drawn below. Can these drug molecules undergo benzylic oxidation? (Circle one) **YES NO**

   A. If you answered YES, then modify the structures below to reflect the product of this Phase I metabolic transformation.

   B. If you answered NO, then briefly explain the structural reason for this.

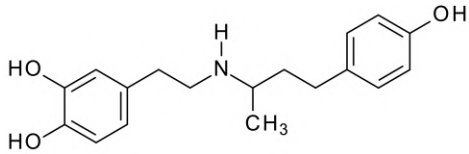


Treprostinil

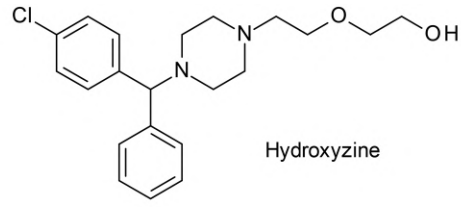


Atecegatran

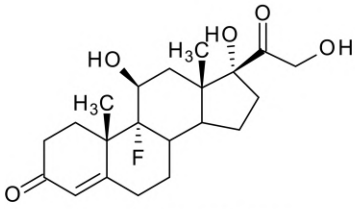
5. For each of the drugs below, list all of the Phase I and any Phase II metabolic transformation(s) possible.



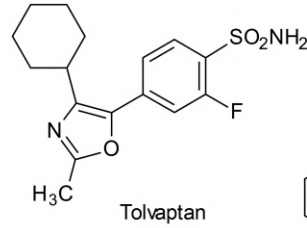
Dobutamine



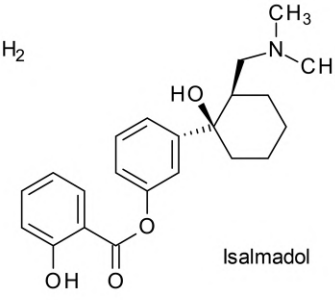
Hydroxyzine



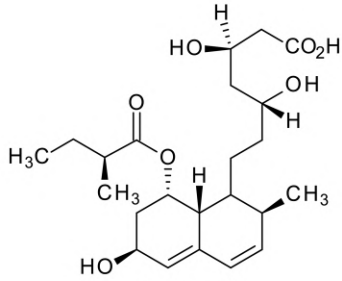
Fludrocortisone



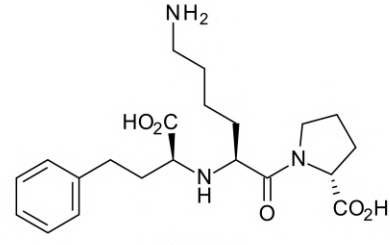
Tolvaptan



Isalmadol



Pravastatin

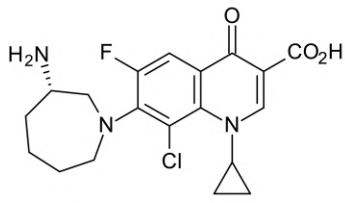


Lisinopril

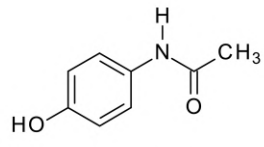
6. For each of the drug molecules drawn below, circle which of the following is TRUE:

   A. The molecule can undergo a Phase II metabolic transformation without having to undergo a Phase I metabolic transformation first.

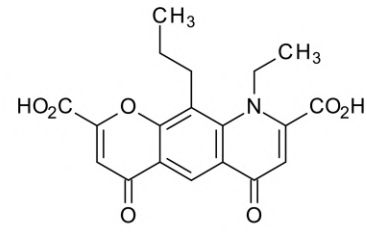
   B. The molecule can only undergo a Phase II metabolic transformation after undergoing a Phase I metabolic transformation.



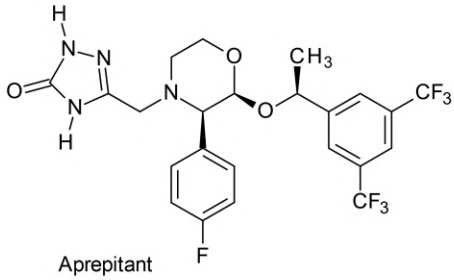
Besafloxacin



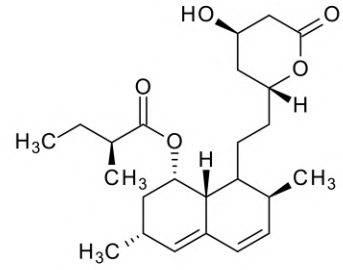
Acetaminophen



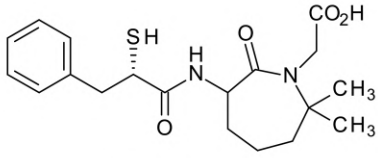
Nedocromil



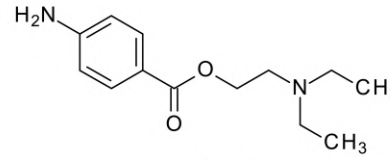
Aprepitant



Lovastatin



Gemopatrilat

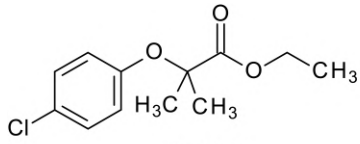


Procaine

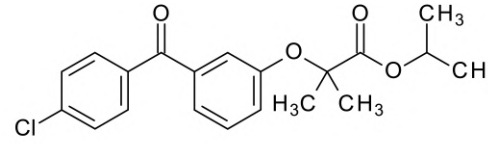
7. Clofibrate, fenofibrate, and gemfibrozil are fibric acid derivatives used in the management of hypertriglyceridemia and dyslipidemia.

   A. Using the chart provided, determine which phase I metabolic transformations are possible for each drug. Answer YES or NO for each metabolic transformation.

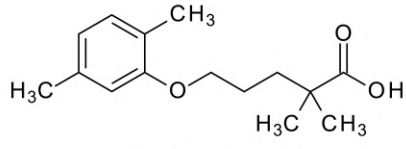
   B. One of the outcomes from Phase I metabolic transformation is drug activation. Given that the fibric acid derivatives participate in a key ionic interaction with their biological target, identify the agent that is drawn in its active form.



Clofibrate



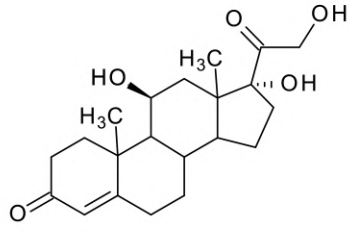
Fenofibrate



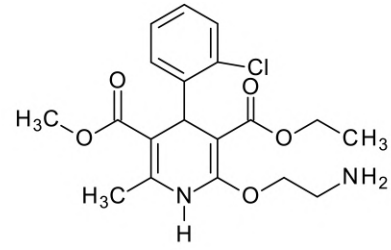
Gemfibrozil

	Oxidative *O*-dealkylation	Benzylic Oxidation	Ester Hydrolysis	*para* Aromatic Hydroxylation	*ortho* Aromatic Hydroxylation
Clofibrate					
Fenofibrate					
Gemfibrozil					

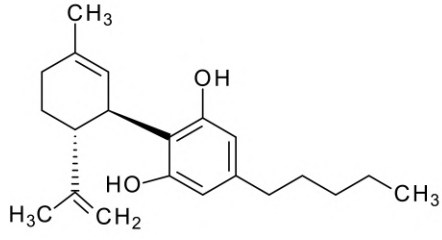
8. Consider each of the four drug molecules and determine if allylic oxidation is possible. For each drug molecule, circle all of the carbon atoms for which allylic oxidation can occur. If allylic oxidation is not possible, then provide a brief structural rationale for why this is the case.



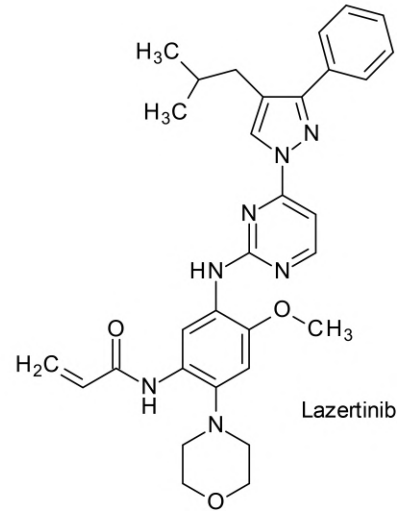
Hydrocortisone



Amlodipine

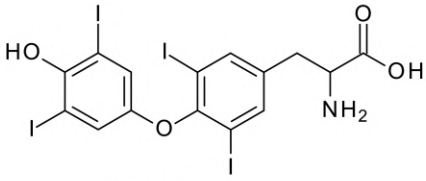


Cannabidiol

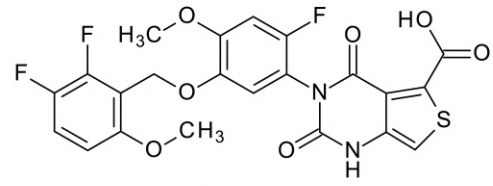


Lazertinib

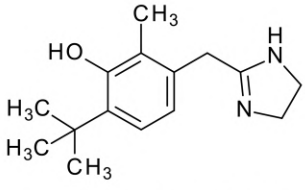
9. For each of the drugs shown below, evaluate their structures and determine which Phase II metabolic transformations can occur without the need for any Phase I metabolic transformations.



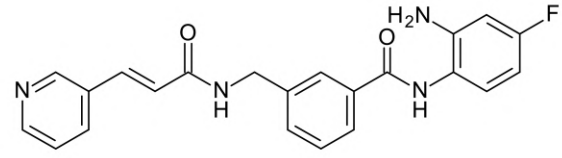
Thyroxine



Linzagolix

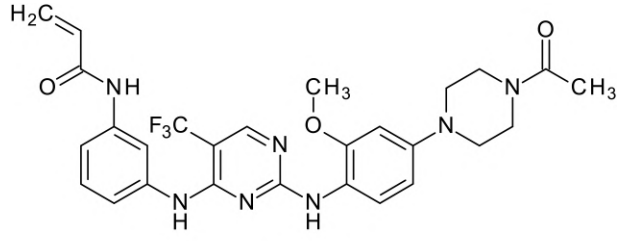


Oxymetazoline



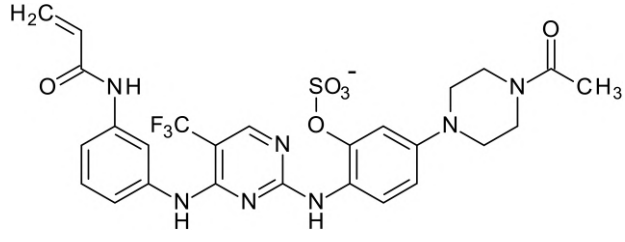
Tucidinostat

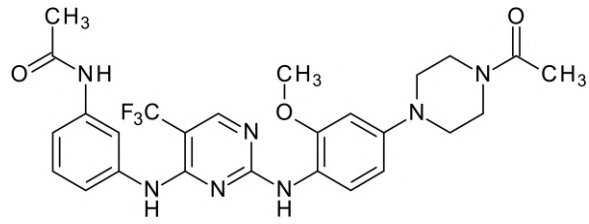
10. The structure of rociletinib and three of its inactive metabolites are drawn below. On the lines below each metabolite, list the Phase I and/or Phase II metabolic transformation(s) that must occur to form each metabolite drawn. Next to the name of each transformation, indicate whether it is a Phase I or Phase II transformation.

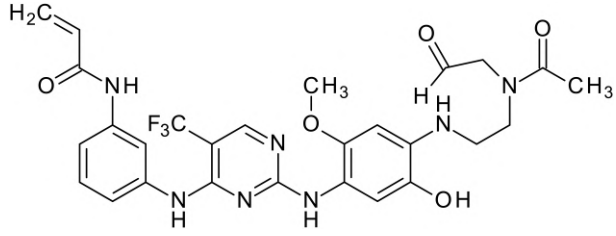


Rociletinib

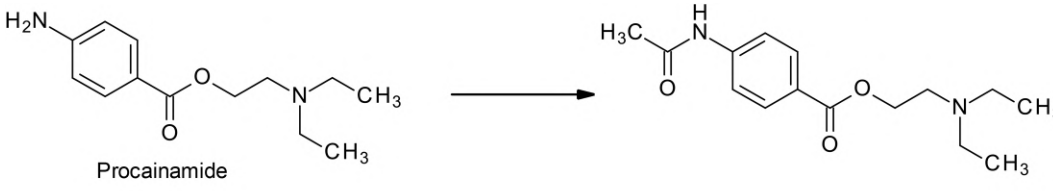
NOTE: The number of lines has nothing to do with the number of transformations required. If you use the same transformation more than once, then indicate with the designation 2X, 3X, etc.



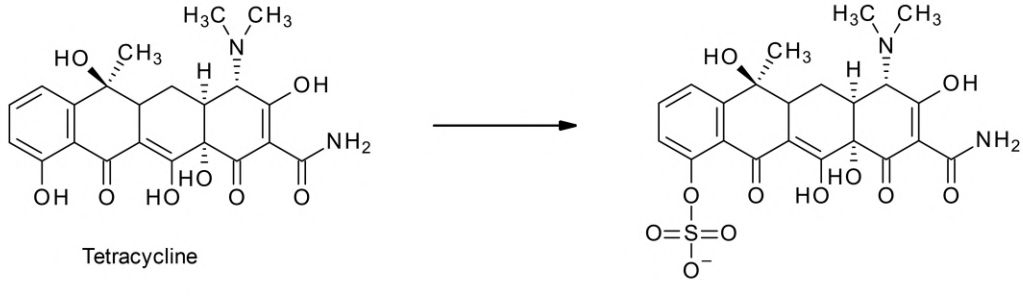




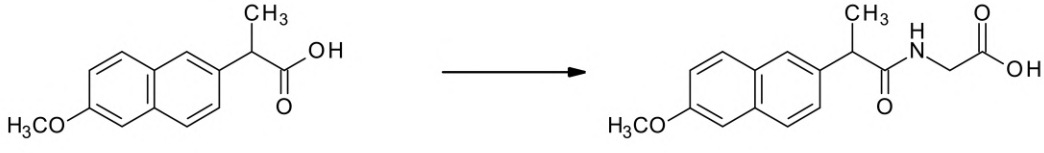
11. Evaluate each of the following metabolic transformations and determine which phase II transformation has occurred. Which metabolic enzyme is responsible for catalyzing each of these transformations?



Procainamide

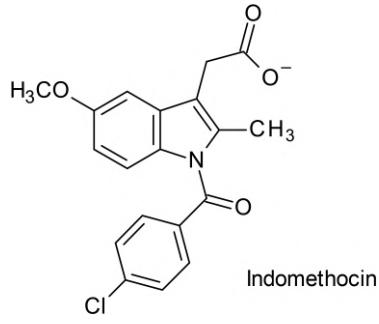


Tetracycline



Naproxen

12. Shown below is the structure of indomethacin in the form that exists at physiologic pH. For each of the listed metabolic transformations, determine whether indomethacin can undergo each transformation. If your answer is "YES," then draw the appropriate metabolite; if your answer is "NO," then provide a brief explanation as to why this transformation cannot occur.



Indomethocin

Pathways:

A. Oxidative deamination

B. Oxidative *O*-dealkylation

C. Aromatic hydroxylation

D. Glucuronide conjugation

E. Amino acid conjugation

F. Hydrolysis

G. Oxidative dehalogenation

STRUCTURE ACTIVITY RELATIONSHIPS AND BASIC CONCEPTS IN DRUG DESIGN

LEARNING OBJECTIVES

After completing this chapter, students will be able to

- **Explain the meaning of the term structure activity relationships and provide examples of this concept.**
- **Compare the structures of two or more similar drug molecules and explain how the differences in their functional groups and/or structures could affect their pharmacological, physicochemical, or therapeutic activity (i.e., predict/understand structure activity relationships).**
- **Explain, in general, the potential advantages of developing analogs of an existing drug molecule.**
- **Provide specific advantages of each of the molecular modification concepts discussed.**
- **Apply the concepts discussed in this chapter to perform rational drug design to create analogs of a given drug molecule.**

The structure of a drug molecule is defined by the relative locations/placements and stereochemical orientations of its functional groups. Each of the previous chapters has focused on selected characteristics of functional groups and has included a variety of examples of how each of these characteristics is important for the overall physiochemical, pharmacological, and therapeutic activity of a drug molecule.

This chapter is designed to be a capstone chapter with two primary objectives. The first objective is to define and provide examples of structure activity relationships (SARs). Many of the examples provided in previous chapters are revisited here with an emphasis on their role in establishing SARs for specific drug classes. Additional examples are also introduced. The second objective is to provide a brief overview of some of the common strategies that are used in rational drug design. In the design of new drug molecules, the primary emphasis is placed on common simple molecular modifications/replacements of existing functional groups. The advantages that each of these types of modifications can provide are also discussed.

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STRUCTURE ACTIVITY RELATIONSHIPS

Overview: What Is Meant by the Term *Structure Activity Relationship*

A *structure activity relationship* (SAR) is a common term used when discussing the medicinal chemistry of a drug molecule. Throughout previous chapters, we examined the overall structure of an individual drug molecule in terms of its component functional groups and the various properties that they possess, which can represent key elements of SARs. In Chapter 2, we learned that each atom within a drug molecule is part of a specific functional group and that some of these functional groups are more important than others. Each functional group has specific electronic, solubility, and steric properties that have an effect on the overall pharmacological and therapeutic actions. In Chapter 3, we reviewed acidic and basic functional groups and the importance of knowing the acid/base character of a drug molecule. This knowledge helped us to examine the proper application of the Henderson-Hasselbalch equation to solve pH/pK_a problems in Chapter 4. In that chapter, the primary goal was to determine if one or more functional groups found within a drug molecule are either predominantly ionized or unionized in a given physiologic environment. From an application standpoint, we learned that functional group ionization affects solubility, duration of action, the ability of a drug molecule to interact with its biological target, and the potential to cause or limit a specific drug interaction. Chapter 5 further explored water and lipid solubility. We examined the advantages of water- and lipid-soluble salts, the need for an overall balance between water and lipid solubility within the structure of a drug molecule, strategies for optimizing solubility to meet a therapeutic need, and the benefits of both water and lipid solubility. In Chapter 6, we explored the types of covalent and noncovalent bonds that can form between the functional groups present on a drug molecule and those present on its biological target(s). In Chapter 7, we considered the stereochemical orientation of functional groups, looked at both configurational and conformational isomers, and identified specific examples of how these isomers can affect drug activity. Finally, in Chapter 8 we reviewed the metabolic transformations that alter specific functional groups to enhance drug elimination, alter the drug's pharmacological activity, or ensure that toxic metabolites are neutralized.

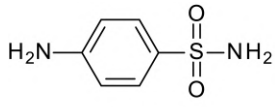
In each of the previous chapters, some of the examples helped to define a concept (i.e., these are water-soluble salts, this is an acidic functional group) whereas others explored how the differences in specific functional groups can affect the overall pharmacological and therapeutic actions of the respective drug molecules. These latter examples fall into the realm of SARs.

An SAR literally refers to the relationship between the chemical structure of a drug molecule and its physicochemical, pharmacological, and therapeutic activities. The term *structure* refers to both the functional groups present within a drug molecule and their stereochemical orientation while the term *activity* is related to the type of pharmacological activity (i.e., agonist or antagonist, enzyme inhibitor), the ability to be absorbed from the site of administration, the ability to interact with a specific biological target and produce a specific pharmacological action, the ability or probability to be metabolized via a specific transformation, the duration of activity, and/or the ability to cause a specific drug interaction or adverse effect.

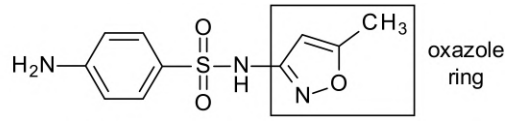
The "Why" or "How" Component of an SAR Statement

A key concept to learn and remember is that each SAR statement must include a "why" or "how" component. Simply stating that "functional group X enhances activity" is of very limited value unless this is followed with a discussion of why or how functional group X enhances the activity. Without the "why" or "how" component, each SAR statement becomes a matter of simple memorization rather than an application of a chemical concept. As an example, let us revisit the sulfonamide class of

antibiotics originally discussed in Chapter 4. The structures of sulfanilamide and sulfamethoxazole are shown below.



Sulfanilamide



Sulfamethoxazole

Both of these drugs mimic *para*-aminobenzoic acid (PABA) and inhibit the bacterial synthesis of folic acid. When these two drugs are compared, it is found that sulfamethoxazole binds tighter to the target enzyme than does sulfanilamide and is less likely to precipitate in the urine and cause crystalluria. In looking at the two drugs, it is visually apparent that the structure of sulfamethoxazole contains an additional oxazole ring whereas sulfanilamide has an unsubstituted sulfonamide. The remaining question then becomes either "Why does this oxazole ring provide these two beneficial effects?" or "How does this structural difference relate to the differences seen in the above activities?" As mentioned in Chapter 4, the oxazole ring is electron-withdrawing in character. This electron-withdrawing nature enhances the ionization and water solubility of the sulfonamide functional group and prevents the drug from precipitating in the urine. Additionally, the enhanced ionization allows sulfamethoxazole to better mimic PABA, hence enhancing its ability to interact with the target enzyme. Using all of this information, we can construct two SAR statements for the sulfonamide class of drugs.

1. Electron-withdrawing functional groups enhance the ionization of the sulfonamide functional group and therefore increase its ability to mimic PABA and inhibit the bacterial biosynthesis of folic acid.
2. Electron-withdrawing functional groups enhance the ionization of the sulfonamide functional group, increase the water solubility of the drug molecule, and decrease the chance of precipitation in the urine.

Both of these SAR statements explain what structural feature is responsible for the beneficial effect and offer an explanation as to why this structural feature is responsible for the beneficial effect. Because of the structural generalities found within these statements, they are applicable to the entire class of sulfonamide antibacterial agents and not just sulfanilamide and sulfamethoxazole. In contrast, if one were to simply identify that these beneficial effects were due to the presence of an oxazole ring, this specific structural information does not allow for the ability to predict the activity associated with other ring systems or functional groups.

Examples of Structure Activity Relationships

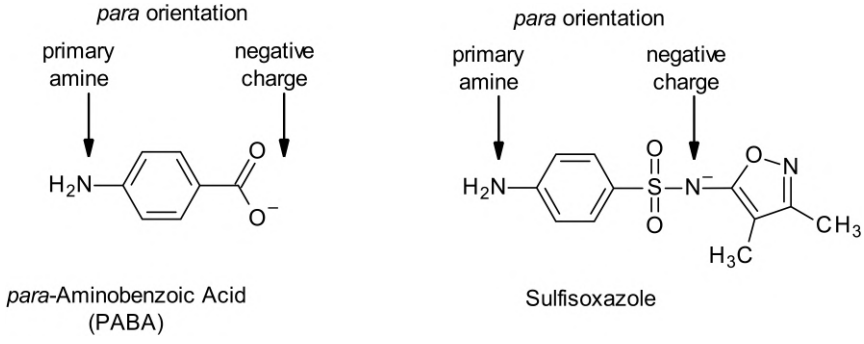
The examples used in this chapter are organized into four main sections. The purpose of these examples is solely to demonstrate specific SARs that exist within drug classes. None of the examples presented represent a complete list of all of the SARs for a specific drug class. This information is readily available in the references cited in Chapter 1. As you review each example, please note that the changes in the component functional groups and/or their stereochemical orientations are responsible for the differences in activity. In other words, the structure dictates the activity.

Structure Activity Relationships and Pharmacological Activity

The mechanism of action of a drug molecule almost always requires specific structural features for it to interact with its biological target. Drug molecules that meet most or all of these structural requirements are generally more active than those that do not. Many classes of drugs have SARs that define both the minimum structural requirements for activity as well as additional structural

features that enhance activity. In this section, we review the SAR required for three different classes of drugs.

The first example revisits the sulfonamide class of antibiotics discussed above. This class of drugs mimics PABA due to the presence of similar functional groups. As shown in **Figure 9-1**, PABA contains a primary aromatic amine and an ionizable carboxylic acid that are located *para* to one another. Thus, sulfonamides, as exemplified by sulfisoxazole, must have a primary aromatic amine and an ionized sulfonamide located *para* to one another. Sulfanilamide and sulfamethoxazole, shown above, also meet these structural requirements, and any alterations to these functional groups eliminate or greatly decrease the antibacterial action.

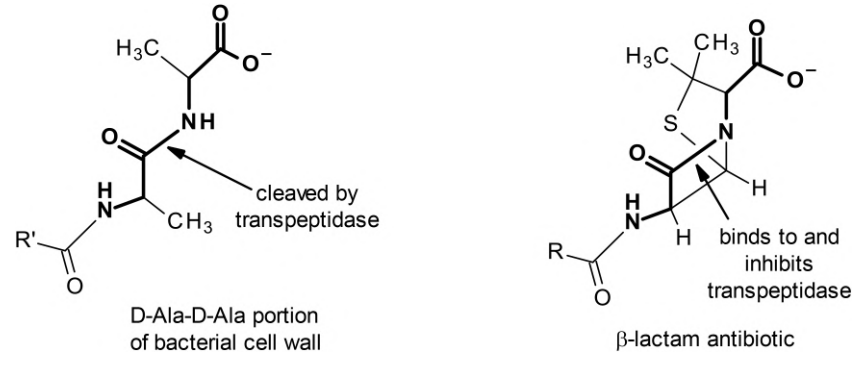


para-Aminobenzoic Acid (PABA)

Sulfisoxazole

FIGURE 9-1. PABA and sulfisoxazole.

A second example revisits the mechanism of the β-lactam class of antibiotics that was introduced in Chapter 6. This class of drugs exerts their antibacterial action via inhibition of the cross-linking of bacterial cell walls. Specifically, they bind to the bacterial enzyme transpeptidase, irreversibly acylate the enzyme, and prevent newly formed peptidoglycan strands from cross-linking. The mechanism catalyzed by transpeptidase involves the initial cleavage of a D-Ala-D-Ala bond that is present on a newly synthesized peptidoglycan strand. As shown in **Figure 9-2**, a β-lactam antibiotic can perfectly mimic the D-Ala-D-Ala sequence found within the peptidoglycan. From a mechanistic perspective as well as an SAR perspective, all active β-lactams must contain an ionized carboxylic acid and an intact β-lactam ring to effectively mimic D-Ala-D-Ala and inhibit transpeptidase.



D-Ala-D-Ala portion of bacterial cell wall

β-lactam antibiotic

FIGURE 9-2. A structural comparison of D-Ala-D-Ala and a β-lactam antibiotic.

Some bacterial organisms produce an enzyme known as β-lactamase. This enzyme catalyzes the cleavage of the β-lactam bond found within the structure of a penicillin or cephalosporin. Because cleavage of this bond destroys the required SAR for transpeptidase inhibition, the presence of this enzyme allows the bacteria to be resistant to certain β-lactam antibiotics. As mentioned in Chapter 2, strategically placed functional groups can provide steric hindrance and prevent β-lactamase from inactivating the β-lactam antibiotic. The steric hindrance found within this class of agents is provided by either a methyl group or a methoxy group that is either directly adjacent to or oriented in the direction of the β-lactam carbonyl group. Examples of specific drugs with these structural features are shown in **Figure 9-3**. Although this steric hindrance confers β-lactamase resistance, it does not interfere with the binding of the β-lactam to transpeptidase. The presence or absence of this structural feature determines whether a β-lactam antibiotic is susceptible or resistant to β-lactamase and thus provides another SAR for this class of drugs.

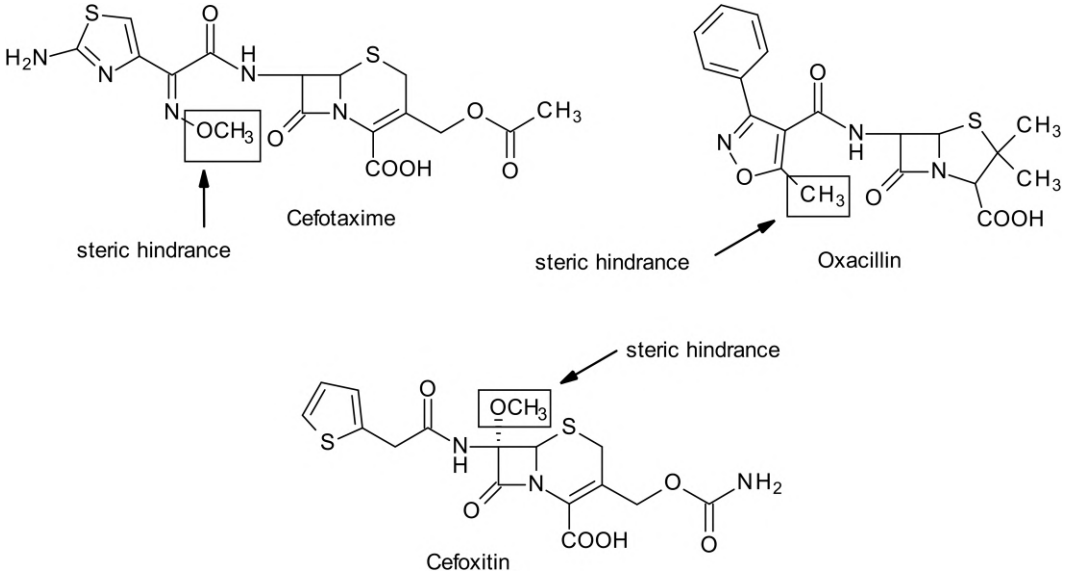


FIGURE 9-3. Examples of β-lactam antibiotics with functional groups that sterically prevent the binding of β-lactamase.

The final example of SARs and pharmacological activity involves inhibitors of the enzyme 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in cholesterol biosynthesis. Inhibitors of this enzyme have been developed for use in the treatment of hypercholesterolemia and other dyslipidemias. A key structural requirement for HMG-CoA reductase inhibitors is the ability to mimic the normal substrate, product, and/or intermediate transition state associated with this reaction, illustrated in **Figure 9-4**. Although significant structural variation in the lower rings is tolerated and, in several cases, enhances activity, the highlighted (boxed) portions of pravastatin and fluvastatin cannot be altered and are present in all HMG-CoA reductase inhibitors. A change in the stereochemistry of either hydroxyl group or the replacement of either hydroxyl groups with another functional group alters the ability to correctly mimic HMG-CoA and greatly decreases or eliminates activity. An ionized carboxylic acid is also required to mimic HMG-CoA. HMG-CoA reductase inhibitors that contain a lactone ring, such as simvastatin, are prodrugs that must first be hydrolyzed to their respective 3,5-dihydroxyacid forms. Finally, the 3-methyl group present within HMG-CoA is not required for inhibitory activity, as evidenced by the lack of this functional group within any of the inhibitors.

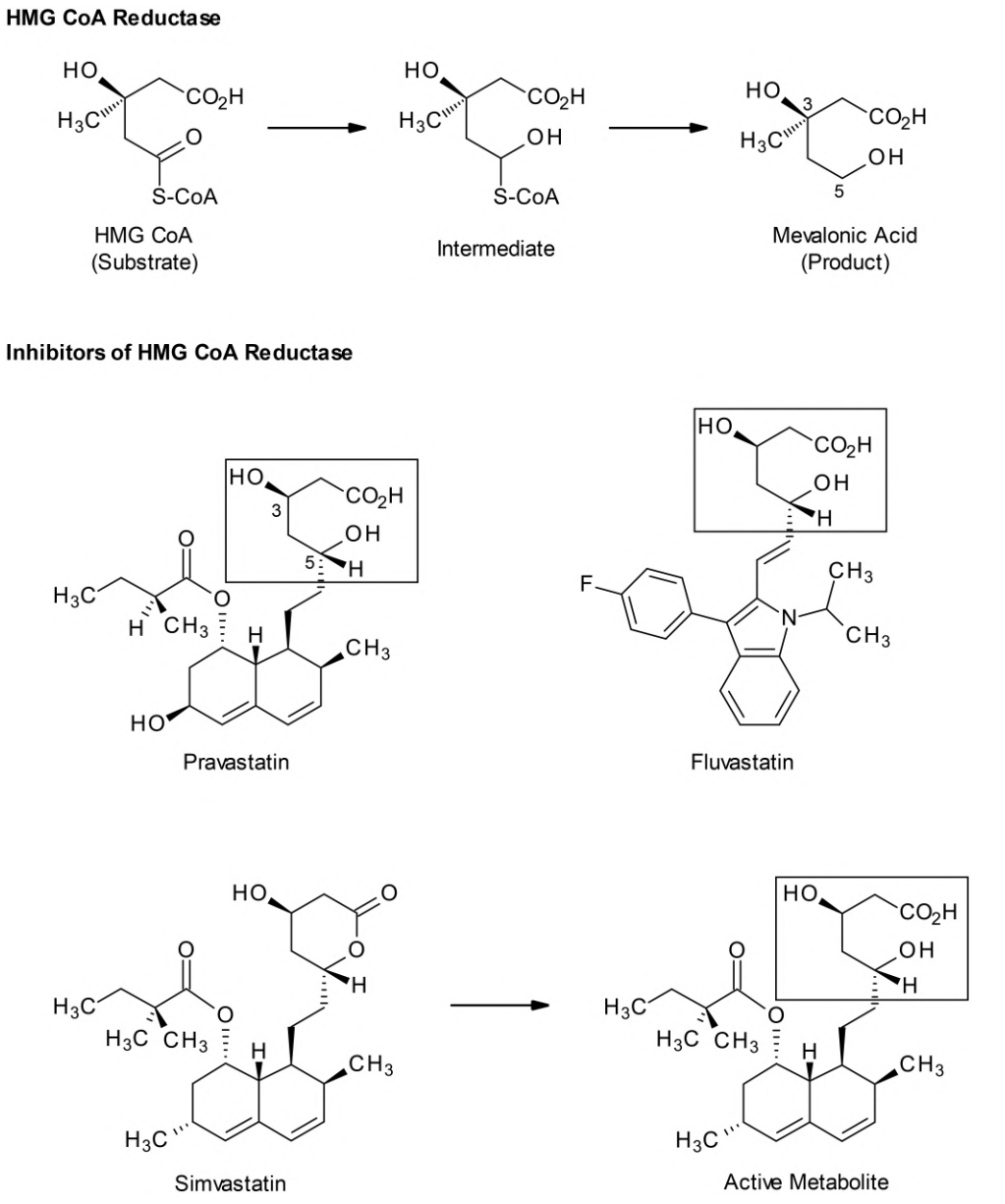


FIGURE 9-4. The conversion of HMG-CoA to mevalonic acid and inhibitors of HMG-CoA reductase.

Structure Activity Relationships and Binding Interactions

Drug binding interactions are critical to the mechanism of drug action and in some instances can bestow selectivity for a specific biological target. Many of the examples discussed below are similar to those listed in the previous section; however, an added emphasis is placed here on binding interactions that lead to target selectivity, alterations that can convert an agonist to an antagonist, the role of conformational restriction, and the challenges associated with alteration of essential functional groups.

Epinephrine and adrenergic agonists (**Figure 9-5**) have been discussed in Chapters 2 and 7 and provide a good example of how structural changes can affect binding interactions and target

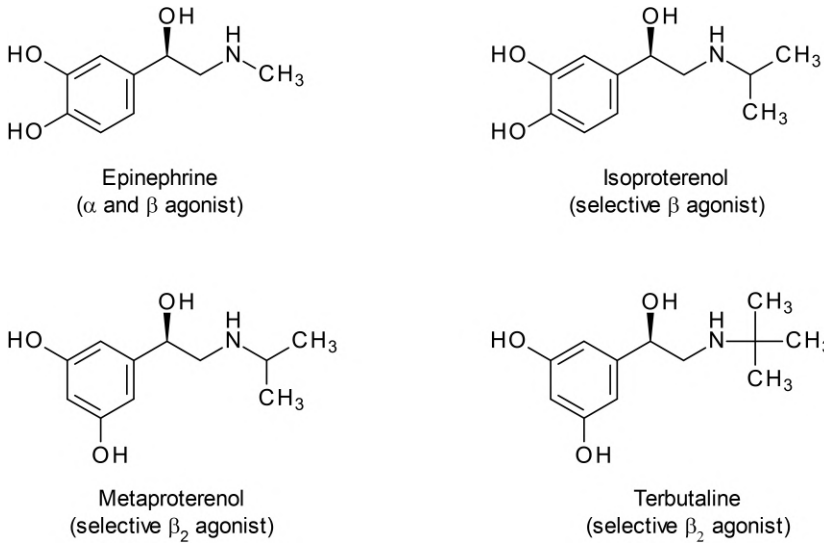


FIGURE 9-5. Epinephrine and selective β-adrenergic agonists.

selectivity. Epinephrine is an endogenous neurotransmitter that binds to both α and β receptors. As discussed in Chapter 7, the *R* configuration of the β-hydroxyl group provides an additional binding interaction compared with the *S* configuration. As a result, adrenergic agonists that have the same stereochemistry seen with *R*-epinephrine are more active than their respective enantiomers. Replacement of the *N*-methyl group of epinephrine with an isopropyl group produces isoproterenol, a drug that is essentially devoid of α-adrenergic receptor activity yet has enhanced agonist activity at the adrenergic β_1 and β_2 receptors. The reason for this selectivity lies in the fact that the β receptors contain a larger hydrophobic binding pocket than the α receptors. Thus, from an SAR perspective, the addition of a larger hydrophobic functional group on the secondary amine provides β receptor selectivity.

Isoproterenol is classified as a nonselective β agonist. The separation of β_1 and β_2 receptor activity can be accomplished either by replacing the isopropyl group with a *t*-butyl group or altering the positions of the hydroxyl groups on the aromatic ring. The reason for this is that the β_1 receptor requires a catechol ring for agonist activity while structural variation of this ring is allowed for the β_2 receptor. Additionally, the β_2 receptor can accommodate a bulkier *N*-substituent (e.g., *t*-butyl group) whereas this structural change decreases the interaction with the β_1 receptor. The use of one or both of these structural changes provides selective β_2 receptor activity, as seen with metaproterenol and terbutaline. Both drugs are used to treat asthma and chronic obstructive pulmonary disease (COPD) as a result of their selective β_2 receptor agonist activity.

While agonist activity at the β_2 receptor is useful in treating asthma and COPD, the ability to block or antagonize the adrenergic β_1 receptor is beneficial in the treatment of hypertension and other cardiovascular disorders. From an SAR point of view, selective β_1 receptor activity was accomplished by retaining the highlighted (boxed) portion of isoproterenol (**Figure 9-6**) and replacing the catechol ring with a *para* substituted phenyl ring. A key SAR that is responsible for the β_1 selectivity is the presence of functional groups on the *para* substitution that are capable of forming hydrogen bonds with the β_1 receptor. The amide group in atenolol provides this structural requirement.

Conformational restriction has been shown to be a key SAR for several classes of drugs. As previously discussed in both Chapters 2 and 7, the presence of *ortho* substituents on the lower ring of diclofenac causes conformational restriction and prevents rotation of this lower ring. This restriction forces the lower ring to align itself perpendicularly to the top ring and therefore is able to bind

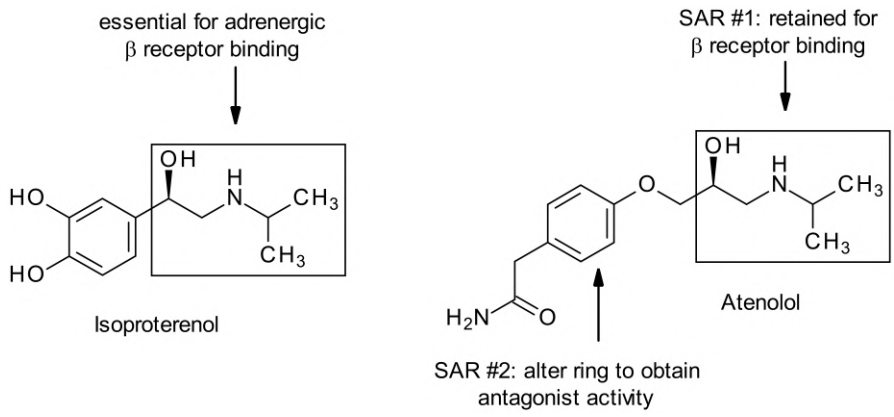
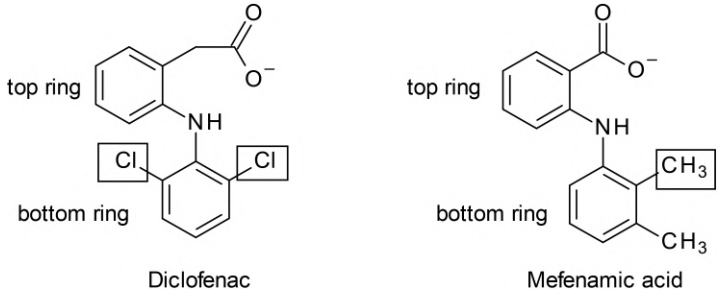


FIGURE 9-6. Structural similarities and differences between isoproterenol (a nonselective β-adrenergic agonist) and atenolol (a selective β_1-adrenergic antagonist).

better to cyclooxygenase enzymes. Other nonsteroidal anti-inflammatory drugs (NSAIDs), such as mefenamic acid, also have this structural feature.



A similar SAR exists for the 1,4-dihydropyridine (1,4-DHP) class of calcium channel blockers. These drugs bind to the L-type of potential dependent (or voltage-gated) calcium channels and block calcium transport into the cell. This action is useful in the treatment of hypertension, ischemic heart disease, and other cardiovascular disorders. For these drugs to interact with their biological target, the phenyl ring must be oriented perpendicular to the 1,4-DHP ring. To "lock in" this perpendicular conformation, all 1,4-DHPs have an *ortho* and/or *meta* substituent located on the phenyl ring. This functional group is generally a methyl group, a nitro group, or a chlorine atom. In all cases, its primary purpose is to provide adequate steric hindrance that restricts rotation and ensures that the required conformation is present. This is illustrated in **Figure 9-7** with nifedipine. The *ortho* nitro group provides steric hindrance and ensures a perpendicular orientation of the two rings.

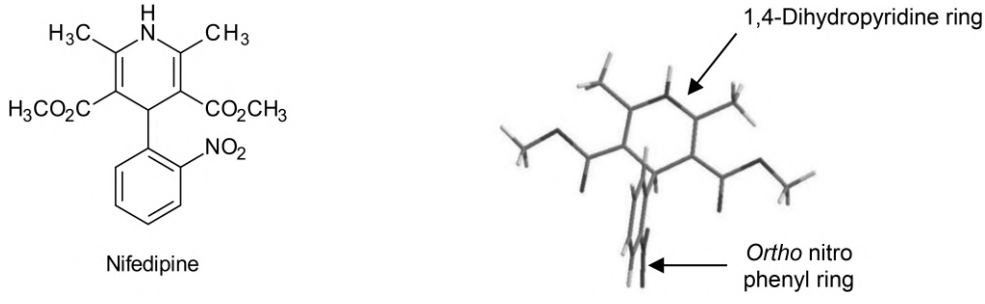
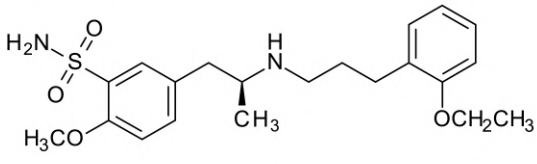


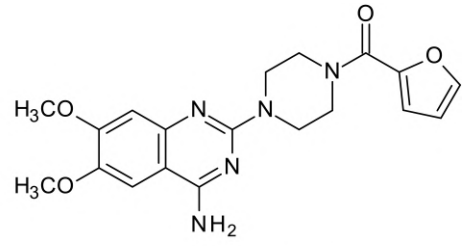
FIGURE 9-7. The structure and a molecular model of nifedipine.

Although the SAR associated with the 1,4-DHPs appears to be the same as that for the NSAIDs, there is a subtle difference. In the case of NSAIDs, adding steric hindrance to lock in a perpendicular conformation enhances their interaction with cyclooxygenase; however, this structural feature is not an absolute requirement for activity. Many NSAIDs lack this conformational restriction and retain anti-inflammatory and antipyretic action. In the case of the 1,4-DHPs, it is essential that the drug molecule contain an *ortho* or *meta* substituent. The lack of this structural requirement greatly decreases or eliminates activity. An important takeaway message is that *some SARs define structural features that are not required yet enhance activity or provide a beneficial effect, while others define structural features that are absolutely required for pharmacological and therapeutic activity*.

It should be noted that there are some instances in which conformational flexibility may be of greater value than conformational restriction. An example of this was previously discussed in Chapter 7 with tamsulosin and prazosin. These drugs are α_1-adrenergic receptor antagonists and are approved to treat benign prostatic hyperplasia (BPH) and hypertension, respectively. The increased conformational flexibility present in tamsulosin allows it to be much more selective for the α_{1a} receptor subtype found in the prostate gland compared with prazosin. This selectivity decreases the side effects observed with prazosin. The explanation or the "why" component of this SAR lies in the increased conformational flexibility that allows tamsulosin to adopt a preferred conformation consistent with that required for binding to the α_{1a} subtype. Additionally, the amount of energy required for tamsulosin to adopt conformations that would allow it to bind to other α_1 subtypes is extremely high. In contrast, prazosin is conformationally rigid, does not need to expend a large amount of energy to adopt multiple conformations, and can easily bind to multiple subtypes of the α_1 receptor. This lack of selectivity allows prazosin to interact with α_{1b} and α_{1d} receptor subtypes that are involved in vascular smooth muscle contraction. Although these actions have some benefit in treating hypertension, they can result in unwanted hypotension in patients treated for BPH.



Tamsulosin: conformationally flexible



Prazosin: conformationally rigid

Angiotensin-converting enzyme (ACE) inhibitors provide a final example of SAR and drug binding interactions. These agents prevent the conversion of angiotensin I, an inactive decapeptide precursor, to angiotensin II, an octapeptide that is a potent vasoconstrictor at the angiotensin II receptor, and are useful in the treatment of hypertension, heart failure, and other cardiovascular disorders. This enzyme is a relatively nonspecific zinc protease that catalyzes the cleavage of a dipeptide from the carboxy terminus of a protein or a peptide. Inhibitors of ACE are either dipeptide or tripeptide analogs that can interact with the enzyme but are not hydrolyzed by the enzyme. A key SAR for this class of drugs is that the molecule must contain a functional group that can interact with the zinc atom in the active site of the enzyme. This relationship was briefly discussed in Chapter 6; however, let us examine this SAR in a little more detail. Captopril is a dipeptide mimic that contains a proline residue and a cysteine analog. As shown in **Figure 9-8**, the sulfhydryl group of captopril can interact with the zinc atom. In the development of captopril, the affinity of this sulfhydryl group for the zinc atom was found to be more than 1000-fold greater than an analogous carboxylic acid. Unfortunately, the sulfhydryl group caused several problems for some patients, including rashes and taste disturbances. Additionally, because the sulfhydryl group is able to form inactive dimers, the drug has a short duration of action and requires multiple daily dosing. Because interaction with the zinc atom is a key SAR, a major challenge in the development of other ACE inhibitors involved replacement of the sulfhydryl group while retaining an affinity similar to that found with

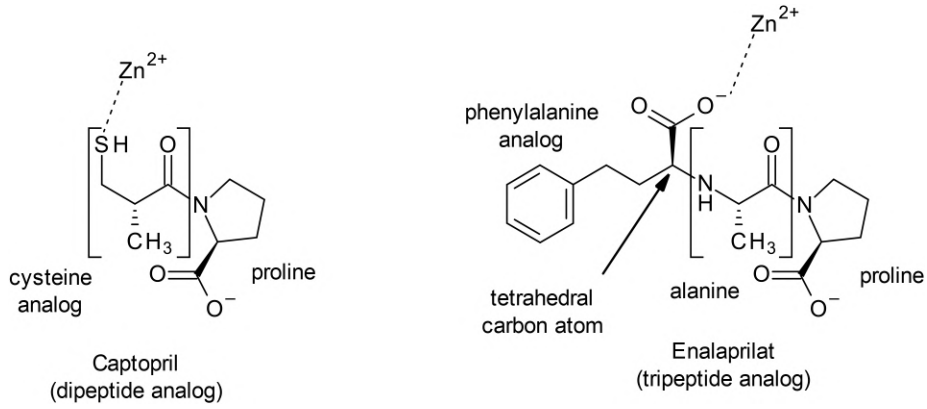


FIGURE 9-8. A comparison of captopril with enalaprilat.

captopril. This was accomplished by three major structural changes, as illustrated with enalaprilat (Figure 9-8). First, the dipeptide analog was replaced by a tripeptide analog. The methyl group of alanine is similar to that seen with the cysteine analog of captopril while the phenylalanine analog provided additional binding interactions not present on captopril. Second, the highlighted tetrahedral carbon is better able to mimic the transition state associated with enzyme hydrolysis than a dipeptide analog. Third, the sulfhydryl group was replaced with a carboxylic acid. The sum of all of these structural changes produced inhibitors of ACE that were as potent or more potent than captopril yet did not contain a sulfhydryl group.

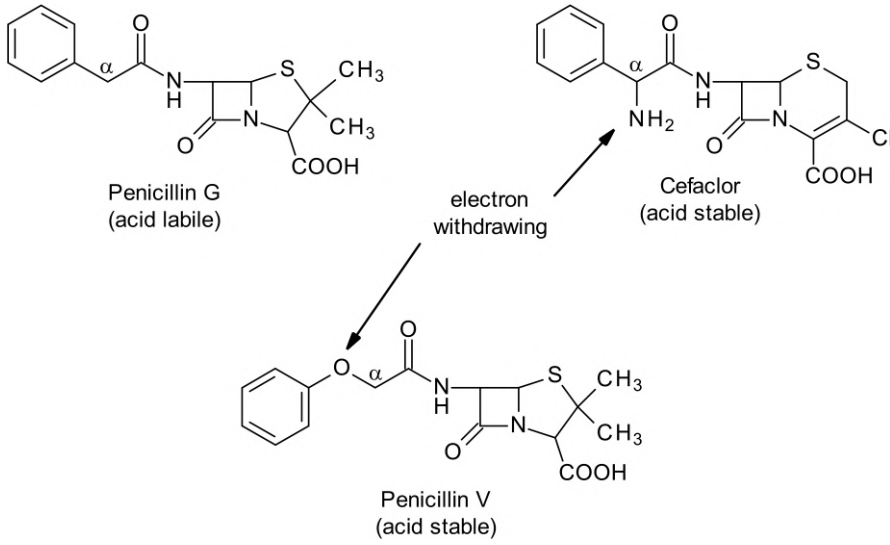
The above examples illustrate several key points. First, some SARs are essential for activity and must be met in one way or another. In the case of ACE inhibitors, one such SAR is the ability to interact with the zinc atom located within the active site of the enzyme. In the case of 1,4-DHPs, the key SAR is the requirement for the two rings to be oriented perpendicular to one another. Second, activity can be retained if a key functional group must be replaced due to an adverse effect, a drug interaction, or another reason. This is seen with captopril and enalaprilat. The loss of potency due to the loss of the sulfhydryl—zinc interaction seen in captopril was balanced by the other structural alterations found in enalaprilat. Finally, the binding of a drug molecule to its biological target is often the result of numerous SARs and not just a single interaction. *As seen with all of these examples, the interactions of key functional groups, the ability to mimic a natural substrate, the presence or absence or steric hindrance, and the size and orientation of specific functional groups can play a key role in SARs and the pharmacological and therapeutic action of a drug molecule.*

Structure Activity Relationships and Absorption, Distribution, Metabolism, and Excretion

The absorption, distribution, metabolism, and excretion (ADME) of a drug molecule depends on its structure and functional groups. As discussed in Chapters 2 and 5, individual functional groups can enhance the overall water or lipid solubility of a drug molecule. This in turn affects the overall absorption of the drug molecule from its site of administration, its ability or tendency to bind to plasma proteins, its ability to cross the blood brain barrier, the extent to which it is metabolized, and its duration of action. Additionally, the metabolic pathways discussed in Chapter 8 were all predicated on the presence of specific functional groups and their location within the structure of the drug molecule. The following examples illustrate how specific structural features can affect the ADME of a drug molecule.

Certain β-lactam antibiotics cannot be administered orally because the acidic environment of the stomach causes an intramolecular reaction that destroys the β-lactam ring. The mechanism of this acid catalyzed degradation was discussed in Chapter 2. Also discussed in Chapter 2 was the fact that this degradation could be significantly decreased if an electron withdrawing group was present

on the α carbon. This is illustrated below with cefaclor and penicillin V. This SAR applies to both penicillins and cephalosporins and in part dictates whether the antibiotic is orally active or must be administered via injection.



A similar SAR is seen with estrogens and androgens. The endogenous compounds estradiol and testosterone cannot be administered orally because their respective C_{17} hydroxyl groups are rapidly oxidized, as shown in **Figure 9-9**. From an SAR perspective, one way to decrease this rapid

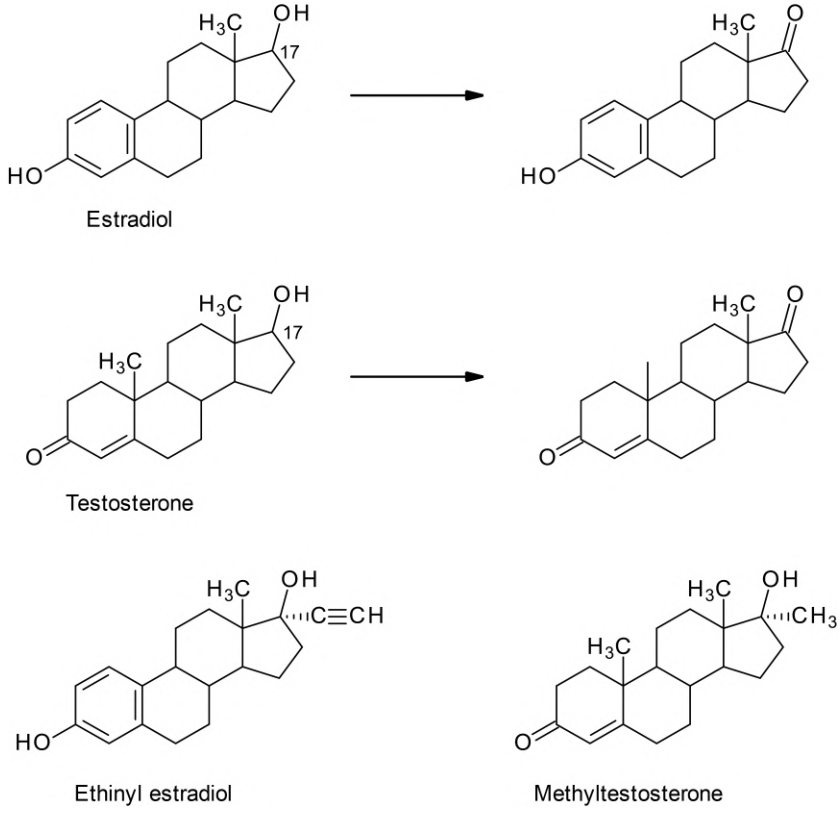


FIGURE 9-9. Estradiol, testosterone, and orally active analogs with 17α substituents.

metabolism is to add a 17α substituent, as shown in methyltestosterone and ethinyl estradiol. This functional group blocks the oxidation of the C_{17} hydroxyl group and allows these drugs to be administered orally.

Within the tetracycline class of antibiotics, a key SAR is the presence or absence of a C_6 hydroxyl group as this functional group affects both oral absorption and the duration of action. Similar to the β-lactam antibiotics, the presence of this functional group leads to an acid-catalyzed dehydration and rearrangement in the stomach, resulting in inactivation of the tetracycline (**Figure 9-10A**). Although this does not prevent the oral use of tetracycline, it does decrease the overall oral absorption of the drug. Analogs that lack a C_6 hydroxyl group (e.g., doxycycline) are not subject to this acid-catalyzed rearrangement and therefore exhibit better oral absorption. The oral absorption of

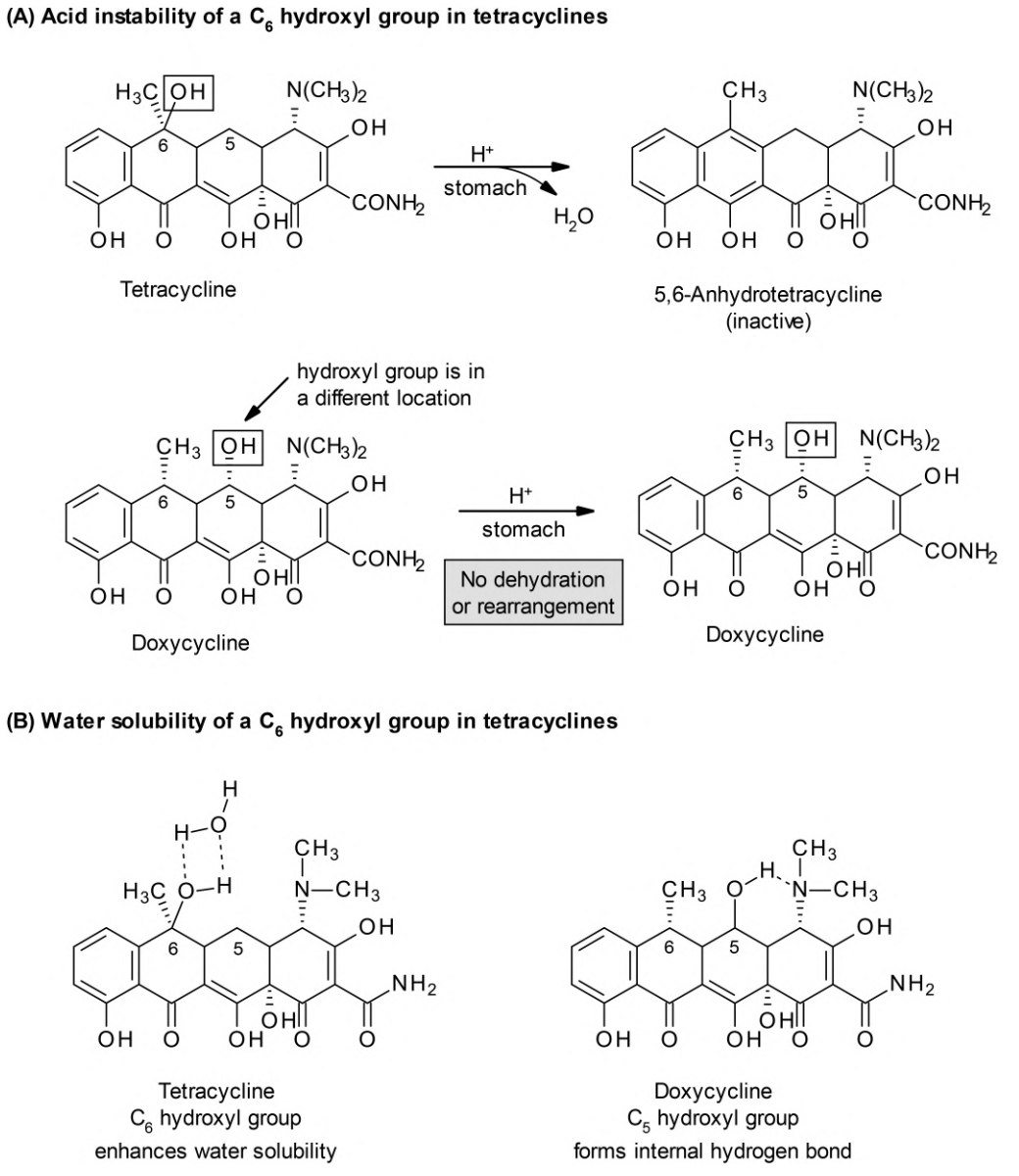


FIGURE 9-10. A comparison of tetracycline and doxycycline and the role of a C_6 hydroxyl group in (A) acid stability and (B) water solubility.

tetracycline is approximately 58% while the oral absorption of doxycycline is approximately 93%. As discussed in Chapter 2, a C_6 hydroxyl group also significantly enhances the water solubility of a tetracycline due to its ability to form hydrogen bonds with water. When the hydroxyl group is moved to the adjacent C_5 atom, internal hydrogen bonding with the tertiary amine decreases the ability to form hydrogen bonds with water. This difference in the binding interactions with water results in significant differences in partition coefficients, with the partition coefficient of doxycycline (0.95) being more than 15 times greater than that of tetracycline (0.056). This is illustrated in **Figure 9-10B**. Because doxycycline lacks a C_6 hydroxyl group, it is more lipophilic and is bound to plasma proteins to a much greater extent than tetracycline. As discussed in Chapter 5, the ability of a drug molecule to bind to plasma proteins decreases its availability to be eliminated and can increase its duration of action. In comparing tetracycline and doxycycline, it is found that their respective half-lives are 10 hours and 15 hours.

In Chapter 5, we discussed the advantages of enhancing water and lipid solubility through the use of salts and ester prodrugs and the addition or alteration of functional groups. Many of the examples discussed in that chapter can be viewed as SARs that can be applied to other drug molecules. A sample listing of these is provided in **Table 9-1**. Additional details associated with these examples can be found in Chapter 5.

As a final example, let us examine a key structural difference between sedating and nonsedating antihistamines. Sedating antihistamines such as diphenhydramine and cyclizine are much more lipophilic than nonsedating antihistamines such as fexofenadine and cetirizine (**Figure 9-11**). In comparing the drug structures, it should be noted that all four molecules contain aromatic rings, aliphatic chains or rings, and at least one tertiary amine. The primary structural difference is that both fexofenadine and cetirizine contain an ionizable carboxylic acid that enhances the overall water solubility of these two drugs. Fexofenadine also has two hydroxyl groups that can form hydrogen bonds with water. The ability to cause sedation relies on the ability of the drug to cross the blood brain barrier and reach specific receptors within the central nervous system (CNS). Diphenhydramine and cyclizine have sufficient lipid solubility to enter the CNS and cause sedation whereas fexofenadine and cetirizine are more water soluble and lack this ability. In terms of SAR, the ability of an antihistamine to produce sedation depends on its lipid solubility, which is determined by the functional groups present within its structure.

Structure Activity Relationships and Drug Interactions and Adverse Drug Reactions

There are several mechanisms by which drug molecules can produce adverse effects and drug interactions with other coadministered drugs. A partial list includes those that are due to normal extensions of a desired pharmacological action (e.g., diuretics can cause excess urination); those that are due to a lack of selectivity and the resulting interaction with an unwanted enzyme, receptor, or other biological target; and those that are due to alterations of CYP metabolic enzyme function (discussed in Chapter 8). Additionally, some drug interactions and adverse drug reactions can be related directly to specific functional groups present on a drug molecule or specific chemical properties of a drug molecule. The following examples illustrate how SARs can impact drug interactions and adverse drug reactions.

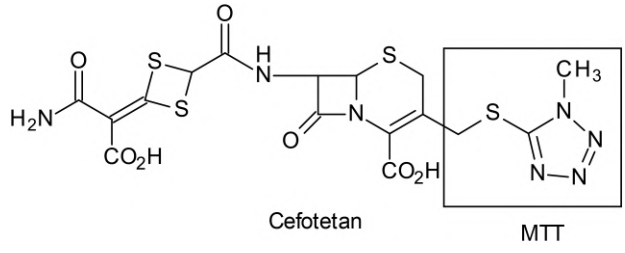
Some adverse drug reactions have been linked to specific functional groups present within drug molecules. As previously mentioned in this chapter, the sulfhydryl functional group present in captopril (Figure 9-8) was responsible for abnormal taste disturbances and rashes. Due to these adverse effects, all subsequently developed ACE inhibitors lack this functional group. An additional example of this concept can be seen with the methyl-tetrazole-thiomethyl (MTT) group present within the structure of cefotetan (shown below). This particular functional group, when present in cephalosporin antibiotics, has been shown to be responsible for acute alcohol intolerance as well as serious bleeding due to platelet dysfunction and/or thrombocytopenia. This particular functional group was

TABLE 9-1. Examples of SARs That Affect the ADME of a Drug Molecule

Specific Example	Structural Change	Effect on ADME	SAR
Naproxen and naproxen sodium	Naproxen was converted to its inorganic sodium salt	Enhances the water solubility and aqueous dissolution of naproxen	Converting drug molecules to their inorganic salt forms can enhance water solubility and aqueous dissolution
Penicillin G and penicillin G benzathine	Penicillin G was converted to a lipid-soluble organic salt	Decreases dissolution and prolongs the release from the injection site	Converting a drug molecule to a lipid-soluble salt can enhance its duration when given as an IM injection
Simvastatin and pravastatin	Structural alterations to increase the water solubility of pravastatin compared with simvastatin	Increased water solubility decreases plasma protein binding	Within a class of drug molecules, those that have a higher lipid solubility tend to have higher plasma protein binding
Quazepam and temazepam	Temazepam contains a hydrophilic hydroxyl group not seen in quazepam	The presence of the hydroxyl group allows it to undergo direct glucuronide conjugation without any oxidative metabolism	Within a class of drug molecules, those that are more water soluble tend to require less metabolic transformations than those that are more water soluble
Fenofibrate and fenofibric acid	Fenofibrate is a lipid-soluble ester prodrug of fenofibric acid	Enhances lipid solubility and allows the drug to pass through the GI mucosal membrane	Converting a drug molecule to its lipid-soluble ester can enhance the absorption of orally administered drug molecules
Prednisolone and prednisolone sodium phosphate	Prednisolone sodium sulfate is a water-soluble ester prodrug of prednisolone	Enhances water solubility and allows the drug to be used as an ophthalmic or IV solution	Converting a drug molecule to its water-soluble ester prodrug form allows it to be formulated as an ophthalmic or IV solution
Beclomethasone and beclomethasone diproprionate	Beclomethasone diproprionate is a lipid-soluble ester prodrug of beclomethasone	Enhances lipid solubility and allows the drug to be better absorbed within the pulmonary tract or nasal cavity	Converting a drug molecule to its lipid-soluble ester prodrug form allows it to be formulated as a pulmonary or nasal inhaler
Triamcinolone and triamcinolone acetonide	Hydroxyl groups are converted to an acetonide functional group	Enhances lipid solubility and allows for better topical absorption	Enhancing the lipid solubility of a drug molecule allows it to better penetrate the skin and to be used as a topical cream or ointment

GI = gastrointestinal; IM = intramuscular; IV = intravenous.

present in a number of cephalosporins; however, once these adverse drug reactions were linked to this functional group, most cephalosporins with the MTT functional group were discontinued.



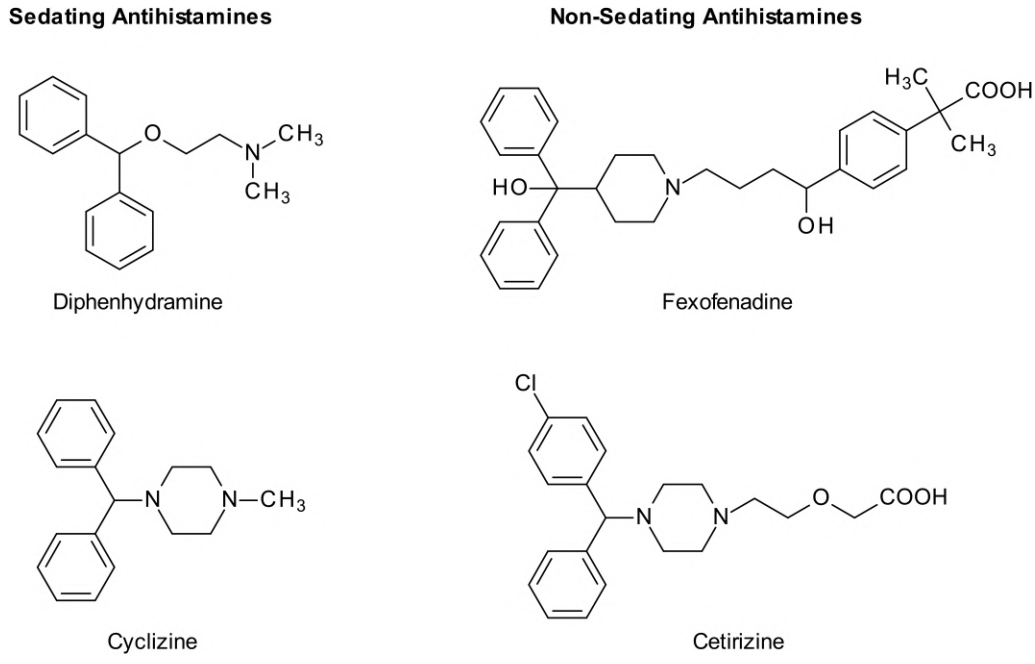


FIGURE 9-11. Sedating and nonsedating antihistamines.

Specific functional groups present on drug molecules within a class of drugs have also been linked to drug interactions. As discussed in Chapter 6 and shown here in **Figure 9-12**, all tetracycline antibiotics contain a β-dicarbonyl group that can form chelates with calcium, magnesium, aluminum, zinc, and iron in the gastrointestinal (GI) tract. These chelates have very poor water solubility and can significantly decrease the absorption of tetracyclines. Preparations that contain these metal ions (e.g., multivitamins, antacids, dairy products) can cause drug interactions if taken concurrently with a tetracycline. This same type of drug interaction occurs with the fluoroquinolone class of antibacterials, although the functional group present within a fluoroquinolone is slightly different than that found in the tetracycline. As shown in Figure 9-12, the quinolone carbonyl oxygen and the carboxylic acid of ofloxacin can form a chelate with a metal ion and can cause a similar drug interaction as observed with the tetracyclines. A key difference between these drug interactions and the adverse drug reactions seen with captopril and MTT-containing cephalosporins is that the functional groups responsible for the drug interactions are also essential for the mechanisms of action of tetracyclines and fluoroquinolones and cannot be replaced.

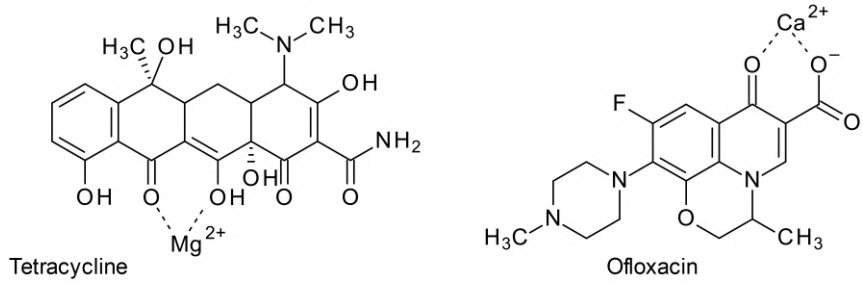


FIGURE 9-12. Chelation of metal ions with tetracyclines and fluoroquinolone antibiotics.

Within a given class of drug molecules, the relative water/lipid solubility of the various drugs may cause or prevent a particular drug interaction. As an example, let us consider the azole class of antifungal agents (**Figure 9-13**). As previously mentioned in Chapter 4, ketoconazole is highly lipid soluble, and its aqueous solubility depends on the ionization of the highlighted (boxed) imidazole ring. Increases in gastric pH decrease the ionization of this basic functional group; therefore, its water solubility, dissolution, and absorption also decrease. As a result, patients requiring ketoconazole therapy cannot take drugs that increase gastric pH such as H_2 antagonists or proton pump inhibitors. This drug interaction is not seen with all azole antifungal agents. Drugs such as fluconazole do not have as much lipid character and do not rely as much on ionization for dissolution and oral absorption to occur. As such, fluconazole does not have a pH dependent absorption and does not exhibit drug interactions with either H_2 antagonists or proton pump inhibitors. Please note that the imidazole ring of ketoconazole is more basic ($pK_a = 6.50$) than the triazole rings of fluconazole ($pK_a = 1.76$).

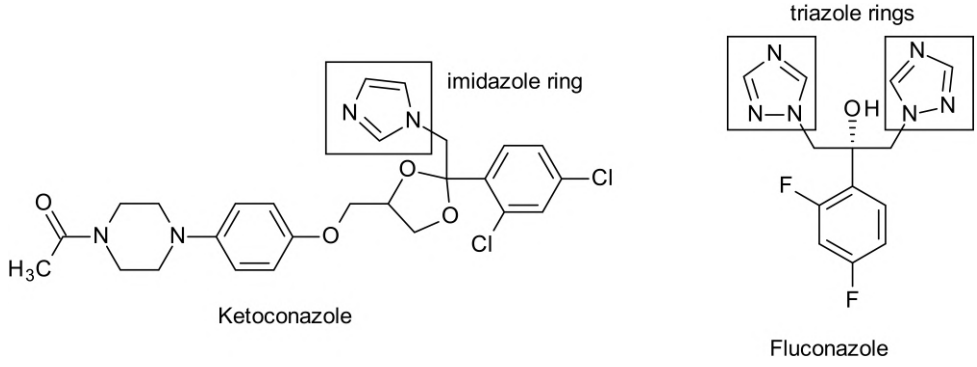


FIGURE 9-13. Ketoconazole and fluconazole.

Finally, the acid/base nature of a drug molecule is important in determining if it will be involved in a plasma protein displacement interaction or not. This is a general SAR that was first discussed in Chapter 3. Acidic drugs can bind to the plasma protein albumin through ionic interactions. Because this binding is somewhat nonspecific, drug interactions can occur when two different acidic drug molecules are competing for the same plasma protein binding site. The displacement of a drug from its plasma protein allows more of the drug to be available to the blood and tissues. This increased availability could affect the incidence of adverse drug reactions or the rate of metabolism/elimination of the drug. These drug interactions are clinically relevant for those acidic drugs that are more than 90% plasma protein bound. Warfarin, NSAIDs, the sulfonylurea class of antidiabetic agents, and phenytoin are examples of drugs and drug classes that are known to have drug interactions due to plasma protein displacement interactions. All of these drugs or classes of drugs are acidic in character. Basic drugs bind to a different plasma protein, α_1-acid glycoprotein, and, similarly to acidic drugs, could cause plasma protein displacement interactions with other basic drugs competing for the same binding sites. In general, plasma protein displacement interactions are seen much more with acidic drug molecules than with basic drug molecules. Because acidic and basic drugs bind to two different plasma proteins, acidic drugs do not cause displacement interactions with basic drugs and vice versa.

BASIC CONCEPTS IN MOLECULAR MODIFICATION

Lead compounds for drug development are often identified through natural product research, chemical alterations of known substrates and products of metabolic pathways, random screening of natural and synthetic molecules, de novo synthesis of compounds designed to interact with a specific biological target, or via structural optimization of a specific adverse effect that is beneficial in the

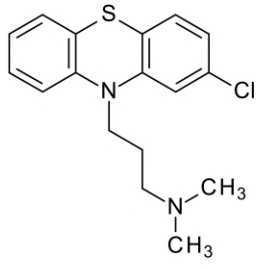
treatment of another disease state. The development of structural analogs can result in drug molecules that possess one or more of the following desired properties: enhanced potency and/or selectivity, a better balance of water and lipid solubility, improved absorption from the site of administration, an optimal duration of action, decreased adverse drug events, and decreased drug interactions.

Molecular modification refers to the process of systematically altering the structure of a lead compound that has a known chemical composition and biological activity. Two primary objectives of this process are to tease out the pharmacophore of the lead compound and develop analogs that could potentially provide more desirable chemical, pharmacological, and therapeutic properties as well as a decrease in the incidence of adverse drug reactions. The term *pharmacophore* refers to the minimum structural features that are required for pharmacological activity. The pharmacophore includes essential functional groups and related chemical properties as well as stereochemical orientations. Inherent in this process is the establishment of SARs for the specific drug or drug class. While an in-depth discussion of molecular modification and drug development is beyond the scope of this text, the remainder of this chapter focuses on some of the most common types of modifications used to enhance the overall activity and physicochemical properties of a lead compound.

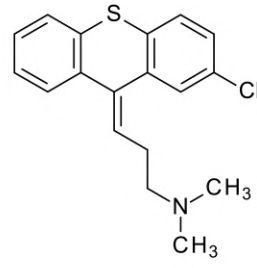
Conformational Restriction

Conformational restriction, discussed in Chapters 2 and 7, is used to lock in a desired conformation to enhance the interaction of a drug molecule with its biological target and decrease interactions with other receptors or biological targets responsible for adverse drug reactions. The SARs for the NSAIDs and the 1,4-DHP class of calcium channel blockers discussed earlier in this chapter involve the use of steric hindrance and provide examples of this concept (see Figure 9-7 and structures of diclofenac and mefenamic acid).

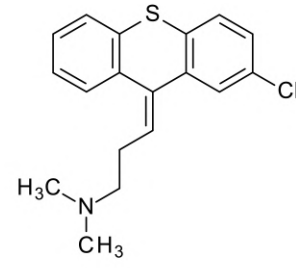
Conformational restriction can also be achieved through the use of double bonds and the formation of geometric isomers. An example of this can be seen with the phenothiazine class of antipsychotic agents (**Figure 9-14**). This class of drugs produces their therapeutic actions by blocking



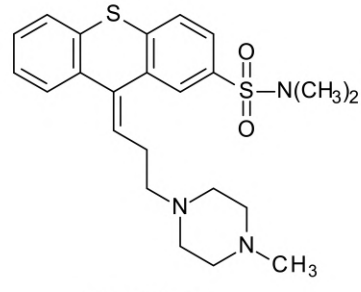
Chlorpromazine



Z-Chlorprothixene



E-Chlorprothixene



Z-Thiothixene

FIGURE 9-14. Chlorpromazine and conformationally restricted analogs.

dopamine action at the D_2 receptor. The active conformation of chlorpromazine requires that the aliphatic amine lies on the same side as the chlorine containing aromatic ring; however, conformational flexibility of this aliphatic chain allows the molecule to adopt a variety of inactive conformations. Evaluation of the structure of chlorprothixene reveals that a double bond has been inserted into this aliphatic chain. This structural modification introduces conformational restriction and produces *Z* and *E* isomers. The *Z* isomer of chlorprothixene, in which the aliphatic chain is positioned on the same side as the chlorine substituted aromatic ring, has much greater potency than the *E* isomer. Further structural modifications yielded thiothixene, a clinically available drug. The *Z* isomer of thiothixene is much more potent than either the corresponding *E* isomer or the saturated analog (i.e., the analog without the double bond).

Variation of Functional Groups

Addition, removal, and/or exchange of one functional group for another can alter the physicochemical characteristics, electronics, and the steric conformation of a drug molecule. This in turn can alter the drug's binding interactions, metabolism, and/or pharmacological effects (both beneficial and detrimental). This type of molecular modification is quite common and is seen in many of the SAR examples already provided (i.e., the addition of a methyl group to testosterone and the alteration of the size of an *N*-alkyl group of adrenergic agonists). Described below are four additional examples of this concept.

All angiotensin II receptor blockers (ARBs) are analogs of the general structure shown in **Figure 9-15**. The highlighted (boxed) acidic functional group participates in an essential binding interaction between the ARB and the angiotensin II AT_1 receptor. This acidic functional group can be

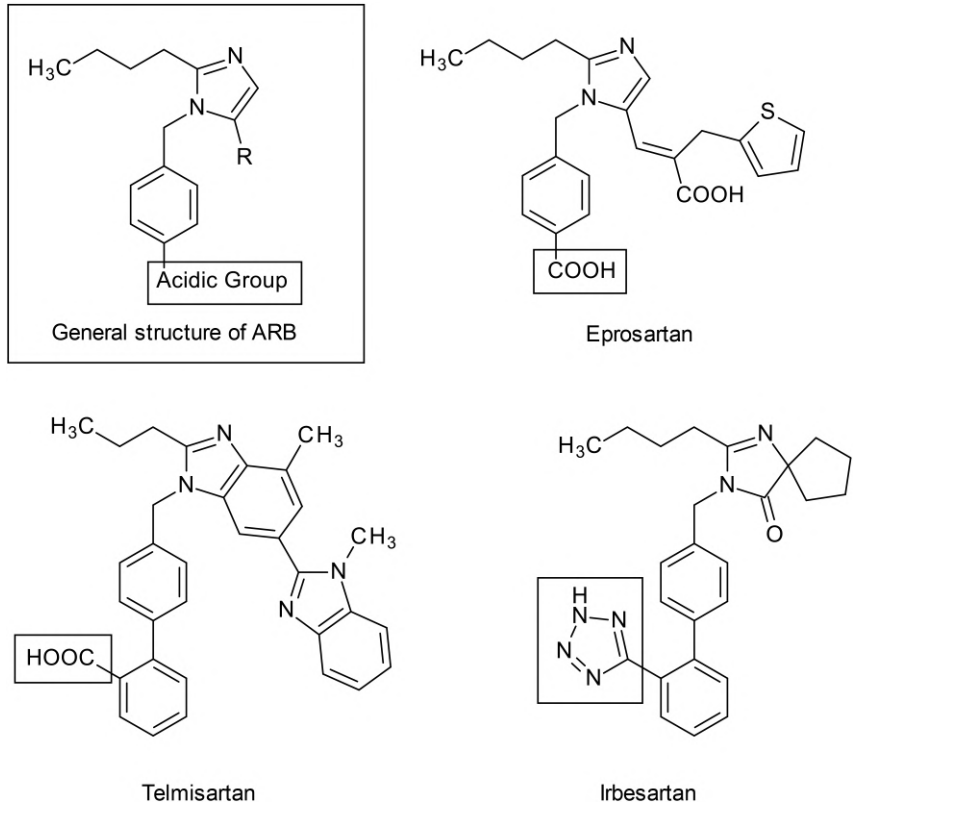


FIGURE 9-15. Angiotensin II receptor blockers and the required acidic functional group.

either a carboxylic acid, as seen in eprosartan and telmisartan, or a tetrazole, as seen in irbesartan. All ARBs, with the exception of eprosartan and telmisartan, contain the tetrazole functional group. Although a carboxylic acid meets the minimal structural requirements for ARB activity, it was found that substitution of the carboxylic acid with a similarly acidic tetrazole provides a number of physicochemical and pharmacological advantages. The tetrazole is less likely to undergo metabolism, is more lipophilic, enhances oral absorption, and allows for a better charge distribution than that observed with analogs that contain a carboxylic acid. As discussed in Chapter 3, the negative charge present at physiologic pH can be equally shared among all five atoms within a tetrazole (as compared with a carboxylic acid, which primarily shares the charge between the two oxygen atoms). This enhanced charge distribution has been proposed to increase/improve the interaction of ARBs with the AT_1 receptor.

The sulfonylurea class of antidiabetic agents provides examples in which different variations lead to different therapeutic advantages. The first generation of sulfonylureas, exemplified by tolbutamide and chlorpropamide in **Figure 9-16**, have small lipophilic functional groups located *para* to the sulfonylurea group. The structure of tolbutamide contains a *para* methyl substituent that undergoes a rapid three-step oxidation to an inactive carboxylic acid metabolite. The half-life of tolbutamide ranges from 4.5 to 6.5 hours and must be administered two or three times daily. Substitution of the *para* methyl substituent with a *para* chloro group, along with a smaller alkyl chain (by one carbon atom), as found in chlorpropamide, extends the half-life of the drug to 36 hours. Unlike the methyl group, the chloro group is electron withdrawing and is not subject to rapid oxidation. This substituent deactivates the phenyl ring, so it limits the possibility of metabolic oxidation. Although the extended duration of action allows for once daily dosing, this can produce more side effects (e.g., hypoglycemia) than observed with tolbutamide. While these substitutions alter the duration of action and the prevalence of an adverse effect, they don't really enhance the binding of the sulfonamides to their target receptor, the ATP-sensitive K^+ channel. Replacement of the small lipophilic functional groups with larger, more diverse functional groups resulted in second generation sulfonylureas, exemplified by glyburide in Figure 9-16. This larger group enhances drug binding interactions with the target receptor and significantly increases the overall activity. Glyburide is between 50 and

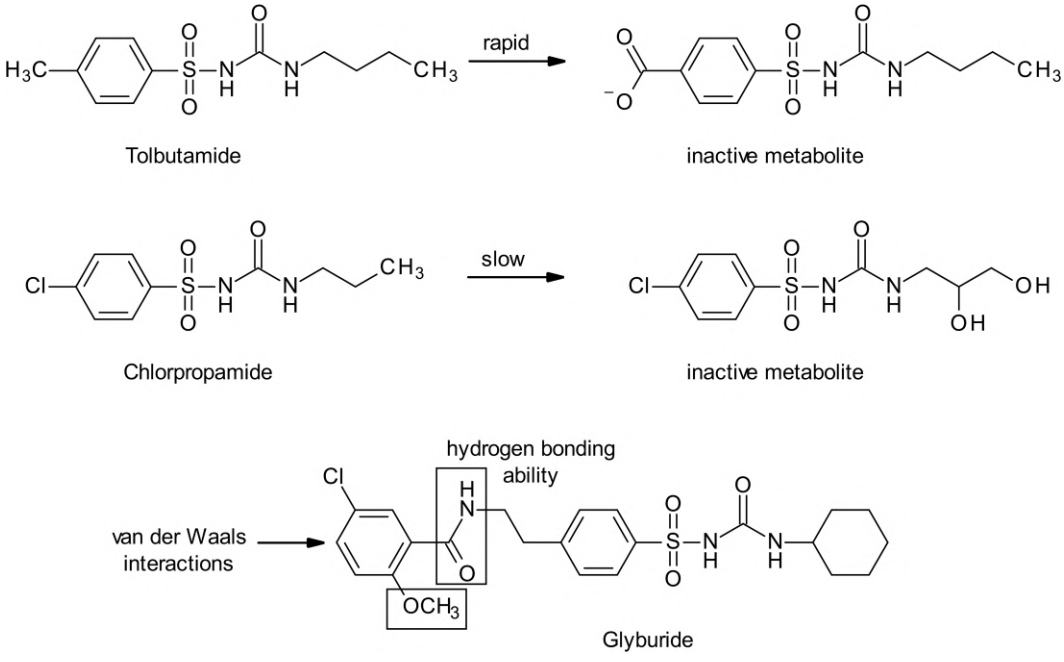


FIGURE 9-16. A comparison of first and second generation sulfonylureas.

100 times more active than either tolbutamide or chlorpropamide. Although glyburide and other second generation sulfonylureas have replaced tolbutamide and chlorpropamide in the treatment of type 2 diabetes, these latter two drugs still serve as good examples of how molecular modification can alter the duration of action of a drug molecule. Due to the rapid three-step oxidation described in this example, *para* methyl groups are not present within the structure of many drug molecules.

Lincomycin (**Figure 9-17**) is a naturally occurring lincosamide antibiotic that inhibits bacterial protein synthesis. Replacement of the highlighted (boxed) hydroxyl group with a chloro group as seen in clindamycin enhances the lipophilic character and imparts two important advantages. Although lincomycin has poor oral absorption and is only administered via intramuscular (IM) injection, clindamycin has an oral absorption of approximately 90%. Additionally, this enhanced lipid solubility allows clindamycin to better penetrate bacterial cell membranes and therefore achieve a higher intracellular concentration than lincomycin.

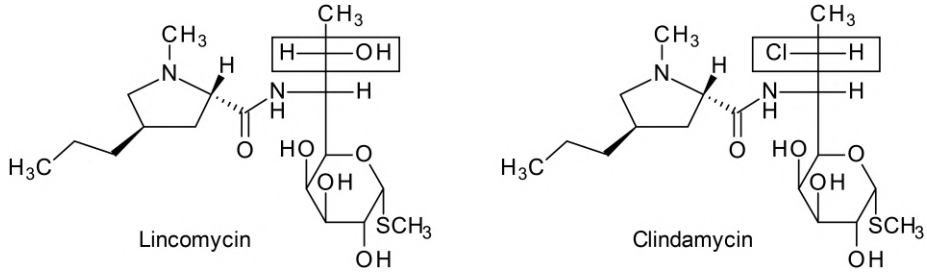


FIGURE 9-17. A comparison of lincomycin and clindamycin.

Variation in functional groups can also convert a drug molecule from being a substrate for an enzyme to an inhibitor of that enzyme. An example of this is seen with the antiviral drugs used to inhibit reverse transcriptase, a viral enzyme required by the human immunodeficiency virus (HIV) for replication. Shown in **Figure 9-18** is the general mechanism by which polymerase enzymes add nucleotide triphosphates to DNA or RNA. This mechanism requires two key structural features: a nucleotide with a triphosphate at the 5′ position and a 3′ hydroxyl group on the growing (or newly synthesized) DNA or RNA chain. The nucleic acid component of the nucleotide can vary but must be able to appropriately pair with the template strand of DNA or RNA. The polymerase reaction

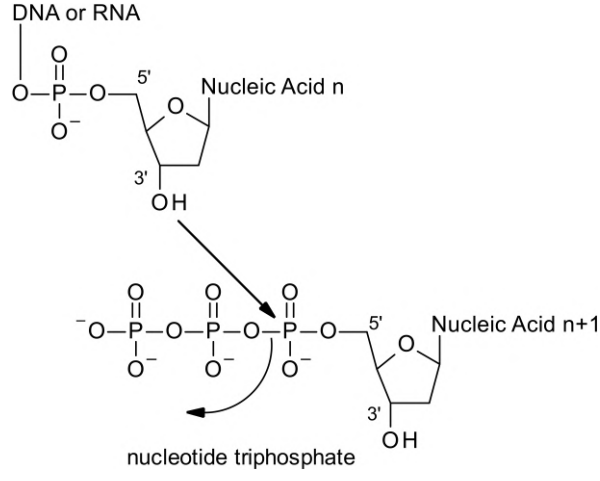


FIGURE 9-18. The mechanism of DNA and RNA polymerase enzymes (DNA sugars are shown).

involves a nucleophilic attack by the 3′ hydroxyl group on the innermost phosphate of the nucleotide that will be added to the polymer. It should be noted that while this schematic depicts DNA synthesis, RNA synthesis occurs via the same mechanism.

Zidovudine, abacavir, and lamivudine are reverse transcriptase inhibitors that are used to treat HIV infections (**Figure 9-19**). These drugs are structural analogs of thymidine, guanidine, and cytidine, respectively. The key structural feature for all of these drugs is that the normal 3′ hydroxyl group has been removed and replaced by a functional group that is not nucleophilic. The 5′ hydroxyl groups have not been altered and can be converted to the active triphosphates. The triphosphate forms of zidovudine, abacavir, and lamivudine can then be incorporated into DNA via the same mechanism as shown in Figure 9-18. HIV synthesizes its DNA from RNA and uses a specific polymerase, reverse transcriptase, to catalyze this reaction. Because zidovudine, abacavir, and lamivudine lack a 3′ hydroxyl group, the reverse transcriptase enzyme cannot add any additional nucleotides to the growing strand of DNA, and the synthesis of HIV DNA is prematurely terminated. This action inhibits reverse transcriptase and the production of new HIV particles. These effects are useful in treating HIV infections.

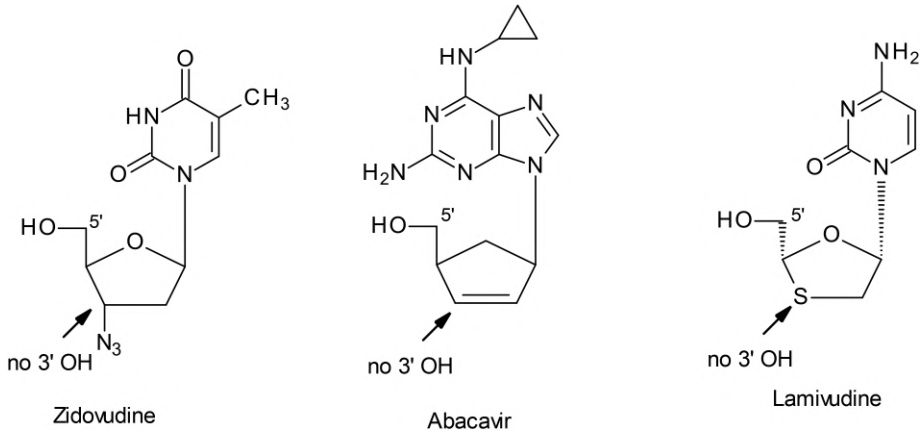
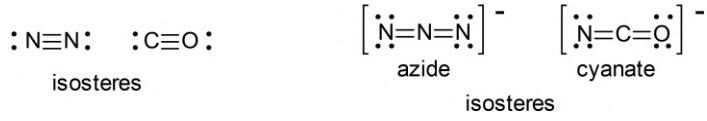


FIGURE 9-19. HIV reverse transcriptase inhibitors. All drugs lack the required 3′ hydroxyl group for HIV reverse transcriptase polymerization.

Zidovudine, abacavir, and lamivudine are examples of an antimetabolite, a drug that is structurally similar to an endogenous cellular metabolite but contains one or more structural modifications that cause it to act as an inhibitor for a specific enzyme rather than a substrate. The sulfonamide class of antibiotics previously discussed in this chapter is another example of an antimetabolite. These drugs are structurally similar to PABA; however, replacement of the carboxylic acid found in PABA with a similarly acidic sulfonamide functional group results in the inhibition of bacterial folic acid biosynthesis.

Isosteres and Bioisosteres

The concept of *isosteres* was first proposed by Irving Langmuir in 1919. He defined isosteres as molecules or groups of atoms that have the same number and arrangement of electrons. According to this definition, carbon monoxide and elemental nitrogen are isosteres, as are the anions azide and cyanate.

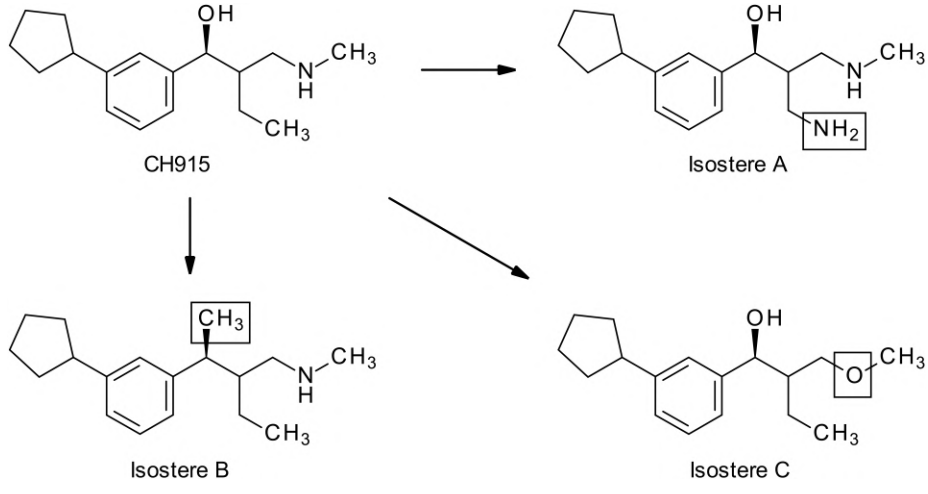


This definition is rather limited and is not very applicable to drug molecules. In 1925, Grimm expanded on Langmuir's work and developed what is known as *Grimm's Hydride Displacement Law*. Grimm organized groups of atoms according to the number of valence electrons and allowed for the fact that these groups may have a different number of atoms. According to Grimm, the addition of a hydride (i.e., a hydrogen atom with its lone electron) to another atom results in a pseudoatom. Both the pseudoatom and the atom having one more electron than the original atom have analogous physical properties. Therefore, the addition of a hydride to a carbon atom would produce a CH pseudoatom. This pseudoatom would be expected to have analogous physical properties with a nitrogen atom (i.e., the atom with one more electron than the original carbon atom). Using this concept, Grimm constructed an initial list of isosteric functional groups (**Table 9-2**).

TABLE 9-2. Isosteres as Defined by Grimm's Hydride Displacement Law

Valence Electrons	4	5	6	7
	C	N	O	F
		CH	NH	OH
			CH_2	NH_2
				CH_3

Examples of isosteric replacements are shown below with CH915, a hypothetical lead compound, and three of its analogs. Each analog contains one isosteric replacement of a single functional group. In Isostere A, an NH_2 group replaces a CH_3 group. In Isostere B, a CH_3 group replaces an OH group, and in Isostere C, an oxygen atom replaces an NH group. Using the list of isosteric functional groups shown in Table 9-2, it is possible to draw other isosteric analogs of CH915.



Erlenmeyer used Grimm's concept and expanded the definition of isosteres to include those atoms, ions, or molecules that are identical in terms of the electrons present in their outer shell. This allowed for the inclusion of several other atoms beyond Grimm's initial work. A truncated list that includes only those isosteres commonly found in drug molecules is shown in **Table 9-3**. In reviewing this table, it should be evident that although there are some similarities, there are also some significant differences among these isosteric groups. Depending on adjacent functional groups, N, NH, and NH_2 may be basic amines capable of undergoing ionization under physiologically relevant

TABLE 9-3. Expanded Table of Isosteres According to Erlenmeyer

Valence Electrons	4	5	6	7
	C	N	O	F
	N^+	CH	NH	OH
		P	CH_2	NH_2
			S	CH_3
				SH
				Cl
				Br
				I

conditions. In contrast, CH, CH_2, CH_3, O, OH, SH, and halogens are neutral functional groups and are not ionized under physiologically relevant conditions. Additionally, N, NH, NH_2, and OH tend to enhance the overall water solubility of a compound while C, CH, CH_2, CH_3, and halogens tend to enhance its overall lipid solubility. The SH group has a limited effect on water solubility. Differences also exist among these isosteres in terms of electronegativity, steric size, and steric shape as well as other physicochemical properties. In terms of molecular modification, it is important that these differences be considered when evaluating the relative activities of isosteric molecules.

Application Question

Question: Evaluate the isosteric substitutions made to the hypothetical lead compound CH915 and provide a summary of the key changes that could result from these structural alterations.

Answer: In Isostere A, a neutral functional group (CH_3) is isosterically replaced with a basic functional group (NH_2). The primary amine would be expected to be ionized in most physiological environments and thus add to the overall water solubility of the isostere. Additionally, because CH915 already has a secondary amine as part of its structure, the addition of a primary amine may affect the pK_a and the ionization of the secondary amine. The presence of this second basic group may also allow an additional ionic bond or ion-dipole bond between Isostere A and its biological targets.

In Isostere B, a polar hydroxyl group is isosterically replaced with a more lipid-soluble methyl group. This substitution enhances the overall lipid solubility of the compound. It also results in the loss of a hydrogen bonding group, which may or may not be required for the interaction of CH915 with its biological targets.

In Isostere C, a basic amine is isosterically replaced with an oxygen atom; hence, the structure of this compound contains a neutral ether functional group instead of a basic functional group. As such, Isostere C would be expected to be more lipid soluble than CH915. Additionally, the binding interactions of these compounds with their biological targets may be significantly different. As discussed in Chapter 6, an ionic bond is often a key recognition between a drug molecule and its biological target. The isosteric replacement of a secondary amine with an ether oxygen atom would prevent this interaction.

Summary: The key point of this initial evaluation is to simply recognize the differences that exist when an isosteric change is made. The ability to make these distinctions becomes much more important whenever you are learning and evaluating the differences in activity of specific drug molecules (or endogenous compounds) and their isosteres.

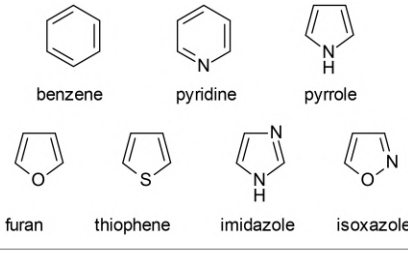
In 1951, Friedman applied the concepts developed by Grimm and Erlenmeyer to biological systems and introduced the term *bioisostere*. According to Friedman, bioisosteres are functional groups or molecules that have similar chemical and physical properties that produce broadly similar biological properties. The latter part of this definition allows for bioisosteres to produce either similar or opposite actions within the same biological system. As such, a bioisosteric substitution can enhance the activity of a given agonist or antagonist or it can result in the conversion of an agonist to an antagonist. Similarly, a bioisosteric substitution can convert a molecule from being an enzyme substrate to an enzyme inhibitor. In terms of the molecular modification of drug molecules, the terms *isosteric replacement* and *bioisosteric replacement* are commonly used interchangeably; however, there is a subtle difference. Because it is not possible to know a priori if a given structural change in a drug molecule will result in the retention of biological activity, the term *isosteric replacement* is appropriate to describe the change in functional groups. Once it is determined that this isosteric change resulted in the retention of biological activity, then the term *bioisosteric replacement* becomes appropriate to use. As an example, it is appropriate to state that *isosteric replacements* of the functional groups present in CH915 resulted in the formation of Isosteres A, B, and C because all of these compounds are hypothetical and have not actually been tested for biological activity. Until biological activity has been determined, it is inappropriate to use the term *bioisosteric replacement* for these compounds.

Over the years, the definition of an isostere or a bioisostere has been expanded to include functional groups that have a similar distribution and location of electron density and that have a similar size and shape. *This led to the establishment of two categories of isosteres: classical isosteres and nonclassical isosteres.*

Classic Isosteres

Classic isosteres are those that follow Grimm and Erlenmeyer's original definitions (**Table 9-4**). The isosteres have been organized according to their valence (i.e., the number of bonds in which they can participate). Within each subclassification, any one functional group can be used in place of another. Also included in Table 9-4 is a list of aromatic ring equivalents, otherwise known as *annular equivalents*. Some of these are simply isosteric replacements of trivalent atoms (e.g., benzene to pyridine) whereas others result from the replacement of the -C=C- group in benzene with either a nitrogen, oxygen, or sulfur atom. Additional isosteric replacements of trivalent atoms result in imidazole, isoxazole, and other five-membered heterocyclic rings.

TABLE 9-4. Classic Isosteres

Monovalent isosteres	H, CH_3, NH_2, OH, F, Cl, SH, Br, I
Divalent isosteres (single bonds)	-CH_2-, -NH-, -O-, -S-
Divalent isosteres (double bonds)	C=O, C=NH, C=S
Trivalent isosteres	-C=, -N =, -P=
Tetra-substituted atoms	=C=, = N^+=, = P^+=
Annular equivalents (aromatic ring substitutions)	 benzene pyridine pyrrole furan thiophene imidazole isoxazole

formation of chlorpropamide, a bioisostere with decreased metabolism and a longer duration of action. Similarly, the isosteric replacement of the hydroxyl group within lincomycin with a chloro group resulted in the formation of clindamycin, a bioisostere with improved oral bioavailability and enhanced bacterial penetration. Procainamide, 6-mercaptopurine, and acetazolamide provide three additional examples of classical bioisosteric replacements.

Procaine (**Figure 9-20**) is a local anesthetic used in a variety of outpatient procedures. Its pharmacological actions are the result of a decrease in sodium permeability in nerve membranes, a mechanism that is also useful in the treatment of arrhythmias. Due to rapid ester hydrolysis, procaine has poor oral bioavailability and a relatively short duration of action. As such, it cannot be used as an antiarrhythmic agent. An isosteric substitution of the ester oxygen atom of procaine with a nitrogen atom results in the formation of procainamide. This bioisostere is less likely to undergo hydrolysis and, as a result, has an oral absorption of 80% to 90% and a longer duration of action. Although procainamide's role in the treatment of arrhythmias has significantly decreased over the last several decades, it is still a good example of how an isosteric replacement can convert a drug that cannot be administered orally into one that can.

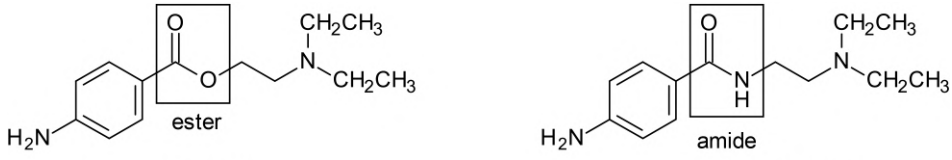


FIGURE 9-20. Procaine and procainamide.

Hypoxanthine is an endogenous compound that is readily converted to inosine monophosphate, a key intermediate in the biosynthesis of both adenosine monophosphate (AMP) and guanosine monophosphate (GMP) (**Figure 9-21**). An isosteric replacement of the OH group with an SH group results in the formation of 6-mercaptopurine, an antimetabolite of hypoxanthine. Similar to hypoxanthine, 6-mercaptopurine can react with 5-phosphoribosyl 1-pyrophosphate (PRPP) to form thio-inosine monophosphate (T-IMP). Once this occurs, T-IMP acts in a competitive fashion to inhibit the conversion of IMP to AMP and GMP. This action is useful in the treatment of acute lymphocytic leukemia.

As shown in **Figure 9-22**, the enzyme carbonic anhydrase catalyzes the hydration of CO_2 to carbonic acid as well as the reverse reaction. This action is important for the reabsorption of sodium bicarbonate from the renal tubules, transport of CO_2 from various tissues to the lung, and formation of aqueous humor in the eye. Inhibitors of this enzyme all contain a sulfonamide functional group in which the nitrogen atom is unsubstituted (e.g., acetazolamide). The unsubstituted sulfonamide group is bioisosteric with carbonic acid. More specifically, the SO_2 group is isosteric with CO_2, and the NH_2 group is isosteric with the OH group. Inhibitors of carbonic anhydrase are therapeutically useful in the treatment of glaucoma and ocular hypertension.

Nonclassical Isosteres

Nonclassical isosteres are those that do not follow Grimm and Erlenmeyer's guidelines. They are often larger in size, rarely have the same number of atoms or pseudoatoms, and can vary substantially in terms of valence electrons. Although this theoretically allows for a more diverse variation of isosteric functional groups, it should be noted that there are many more examples of clinically approved drugs that contain classical bioisosteres than clinically approved drugs that contain nonclassical bioisosteres. A representative list of nonclassical isosteres is shown in **Table 9-5**.

As previously discussed with the ARB class of antihypertensive agents, tetrazoles are bioisosteric replacements for carboxylic acids and provide a number of physicochemical and pharmacological advantages. Histamine H_2 receptor antagonists are another example of the use of nonclassical

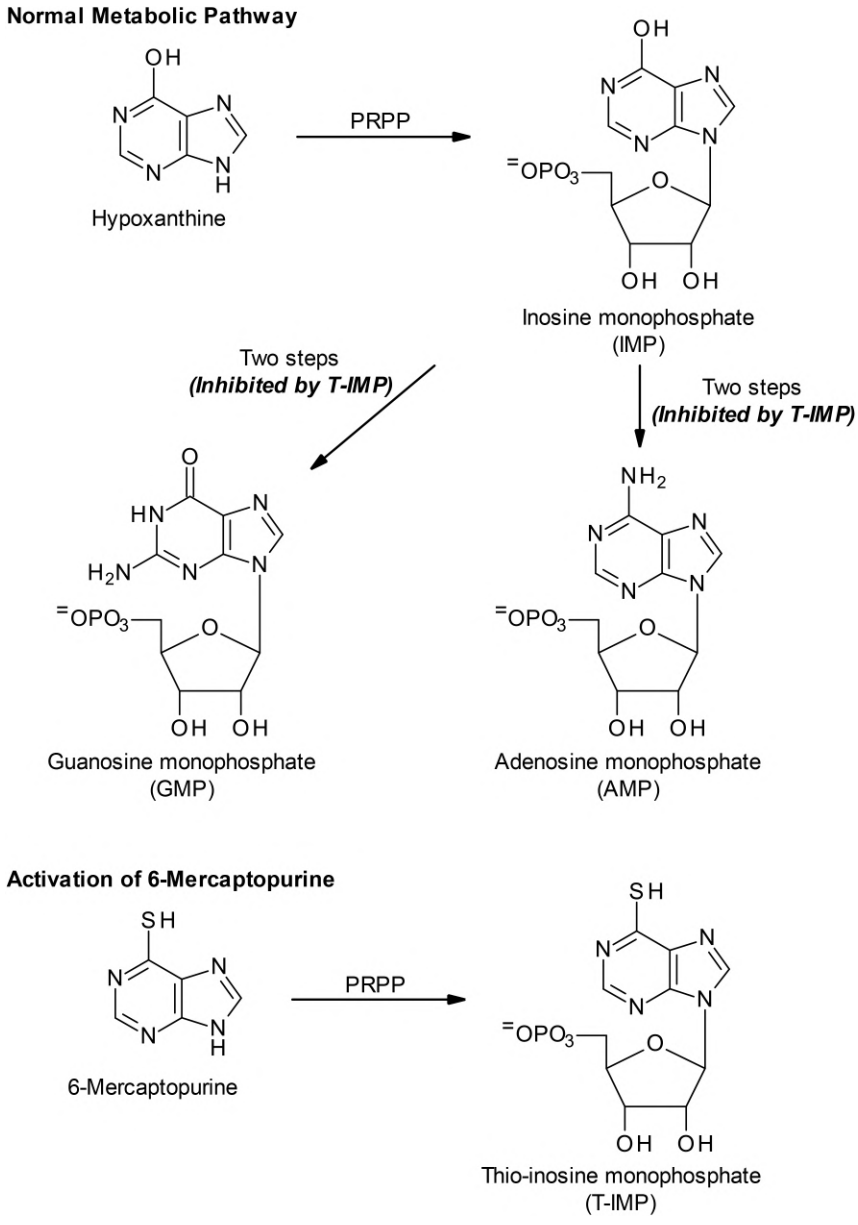


FIGURE 9-21. The formation of GMP and AMP from hypoxanthine and the inhibition by the antimetabolite, 6-mercaptopurine (PRPP = 5-phosphoribosyl 1-pyrophosphate).

bioisosteres. One of the first drug molecules evaluated in clinical studies was metiamide. As seen in **Figure 9-23**, metiamide contains a thiourea functional group as part of its structure. Although this compound was successful in selectively blocking the histamine H_2 receptor, clinical trials revealed that it produced agranulocytosis in a significant number of patients. This adverse effect was subsequently linked to the presence of the thiourea functional group. Cimetidine, the first H_2 receptor antagonist approved by the Food and Drug Administration (FDA), contains a nonclassical bioisosteric replacement of the thiourea group. From a chemical perspective, this nonclassical bioisostere is similar to thiourea in that it is a polar, nonbasic, nitrogen-containing functional group. Development of ranitidine and nizatidine included a different nonclassical bioisostere for the thiourea group as well as a few other structural alterations, including the use of annular equivalents.

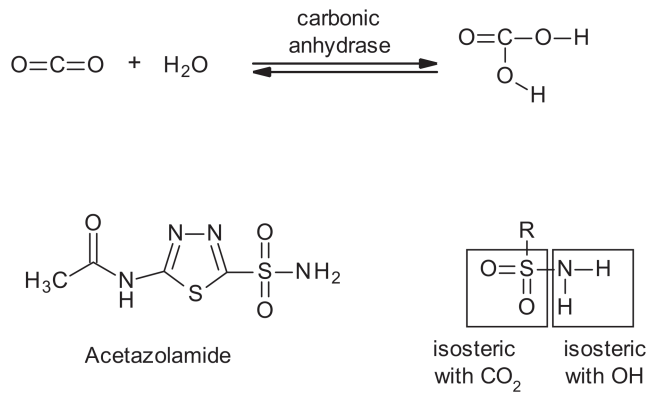
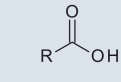
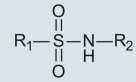
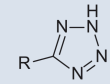
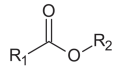
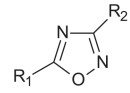
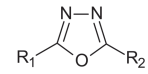
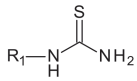
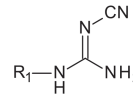
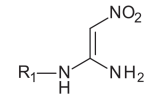
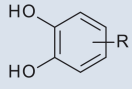
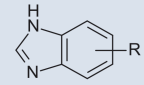
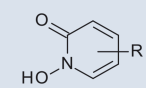


FIGURE 9-22. Carbonic anhydrase and its inhibition by sulfonamide (see boxes).

TABLE 9-5. Nonclassical Isosteres

Original Functional Group	Bioisosteres	
 Carboxylic acid	 Sulfonamide	 Tetrazole
 Ester		
Halogens (F, Cl, Br, I)	CF_3 (Trifluoromethyl)	CN (Nitrile)
 Thiourea		
 Catechol		

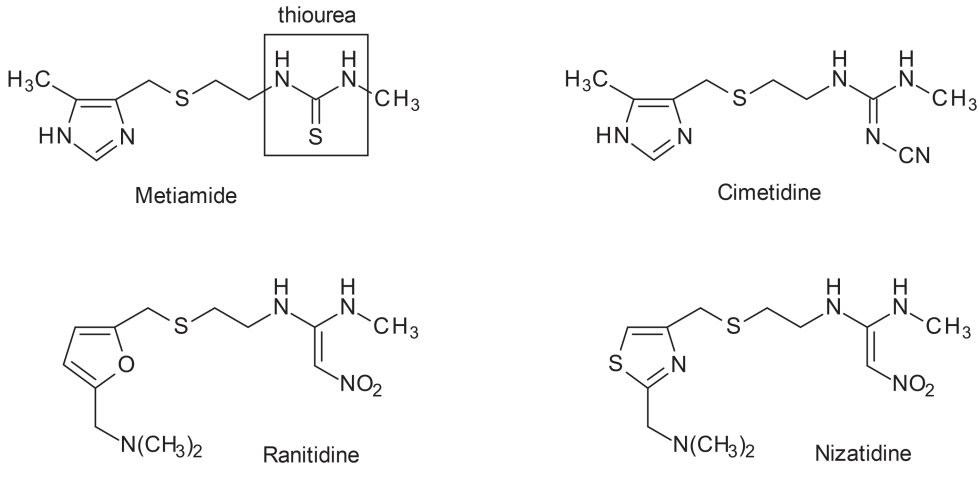
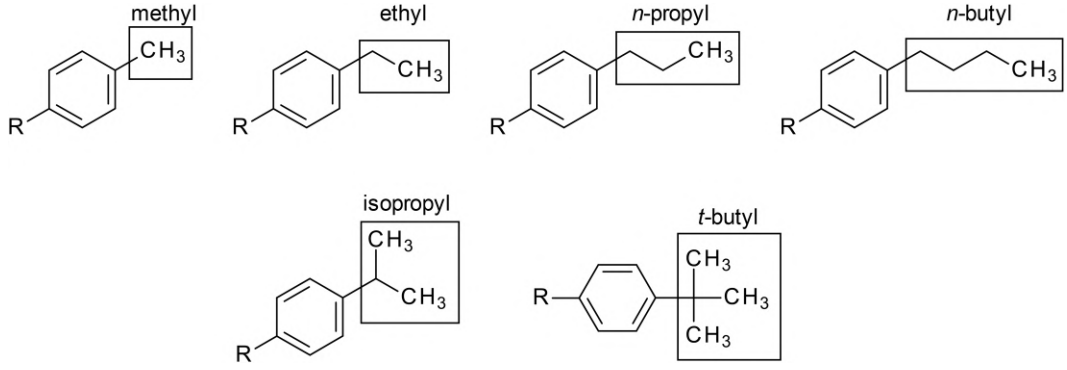


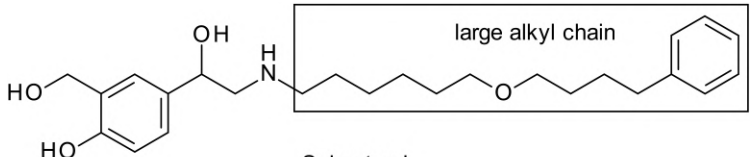
FIGURE 9-23. H_2 receptor antagonists as examples of nonclassical bioisosteres.

Homologation

Homologation refers to the process of successively adding methylene groups (CH_2) to a hydrocarbon chain. A simple example of this concept is shown below (see boxes). The original methyl substituent can be successively lengthened to produce an ethyl homolog, an *n*-propyl homolog, and an *n*-butyl homolog. Methylene groups can also be added to create branch points, as seen with the isopropyl and *t*-butyl homologs. Homologation successively increases the overall size of the functional group as well as its lipophilic character.



This type of molecular modification can be used to investigate the steric dimensions of a drug molecule's biological target(s), enhance the lipid solubility of the drug molecule, and optimize the binding interactions between the drug molecule and its biological target(s). Additionally, homologation can be used to enhance the selectivity of a drug molecule for a specific biological target. An example of this concept was previously discussed with the adrenergic agonists in Figure 9-5. Epinephrine nonselectively binds to both α- and β-adrenergic receptors. Homologation of the methyl group to an isopropyl group produces a selective β-adrenergic agonist. This selectivity is due to the fact that the β-adrenergic receptors contain a larger hydrophobic binding pocket than the α receptors. Further homologation to a *t*-butyl group provides a selective β_2 receptor agonist for the exact same reason. The β_2 receptor has room to accommodate a larger and bulkier *N*-substituent as compared with the β_1 receptor. This last point is further exemplified with salmeterol, a long-acting, selective β_2 receptor agonist used for the treatment of asthma and COPD. The large alkyl chain and phenyl ring represent homologs of the original methyl group seen within the structure of epinephrine.



Salmeterol

Homologation can also be used to convert an agonist at a specific receptor to an antagonist at that same receptor. Some biological targets (primarily protein receptors) contain auxiliary or accessory binding sites that are located directly adjacent to the active/required binding site. When an agonist binds to the receptor, these auxiliary sites are often unoccupied or only partly occupied, and the agonist produces its normal response. This response often requires a specific conformational change that triggers a cascade of events that may include intracellular signal transduction, second messenger production, gene regulation, and/or the opening of an ion channel. Drug homologation allows the resulting analog to bind to both the normal agonist site as well as to an auxiliary site. Occupation of the auxiliary site by the drug analog can cause the receptor to adopt an altered conformation that is not able to trigger this cascade of events. As such, agonist activity is blocked, and the homolog acts as an antagonist. An example is seen with the opioid analgesics (**Figure 9-24**).

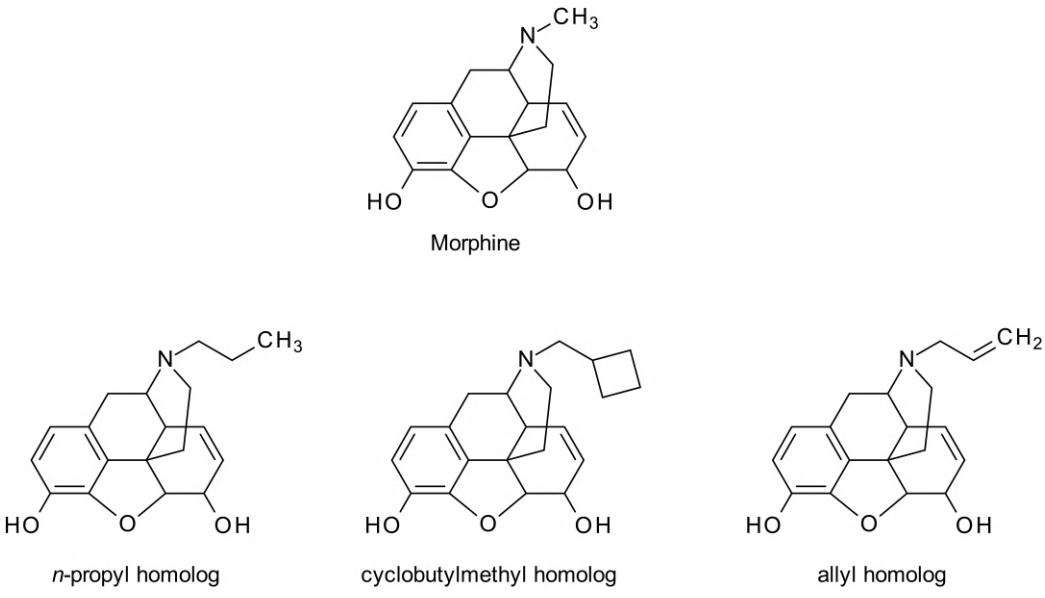


FIGURE 9-24. Morphine and its *n*-propyl, cyclobutylmethyl, and allyl homologs.

Homologation of the methyl group of morphine to a propyl group, a cyclobutylmethyl group, or an allyl group produces a drug that acts as an antagonist at the opioid μ receptor. Although the cyclobutylmethyl group is technically not a homolog due to the presence of a ring structure, it is sufficiently similar to homologs in that it is larger than the methyl group present on morphine. The allyl group is simply an unsaturated analog of the propyl group.

The addition of large, lipophilic functional groups to a drug molecule is a type of molecular modification that is similar to homologation. It is primarily used to convert agonists to antagonists through the addition of hydrocarbon chains and rings that can interact with auxiliary or accessory binding sites present on specific receptors. The primary difference is that the structural additions are not limited to the successive lengthening or branching of a hydrocarbon chain. Muscarinic antagonists, commonly referred to as anticholinergic agents, provide good examples. As illustrated in **Figure 9-25**, the major chemical difference between acetylcholine and dicyclomine and propantheline is the presence of aromatic and/or aliphatic rings that have been added to the terminal methyl group of acetylcholine. The boxed sections of dicyclomine and propantheline can structurally mimic acetylcholine when evaluated on an atom-by-atom basis. Both of these drugs can bind to the muscarinic receptor and inhibit the action of acetylcholine.

Chain Branching

This type of molecular modification is less common than those previously described and often involves either the insertion of a methyl group into an unbranched alkyl chain or the movement of a functional group to change an unbranched alkyl chain into a branched alkyl chain. This type of structural modification can create a new chiral center and therefore can alter both the size of the alkyl chain and the orientation of other functional groups. As with homologation-based modifications, the addition of a hydrocarbon branch to an alkyl chain may enhance the overall lipid solubility of the drug molecule, alter the selectivity of a drug molecule for a specific biological target, and decrease the metabolism of a drug molecule.

Within the phenothiazine class, some drugs can be used as antipsychotic agents to treat schizophrenia and other psychotic disorders while others can be used as antihistamines to treat pruritus and motion sickness. The antipsychotic effects are due to the blockade of dopamine D_2 receptors,

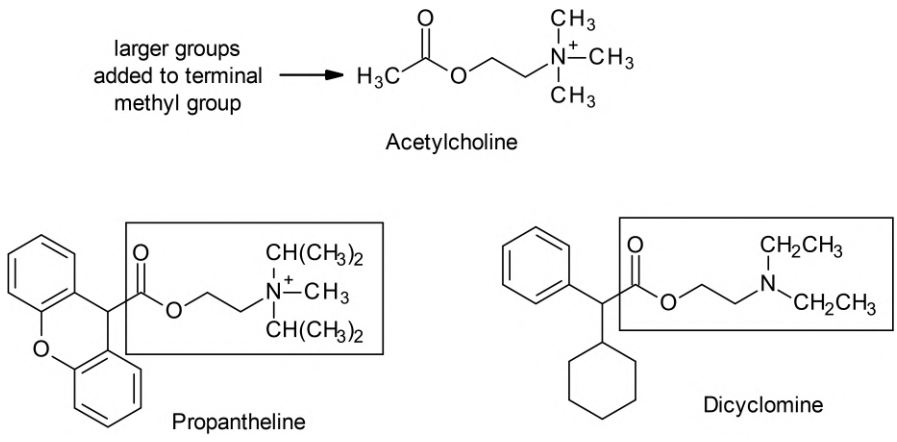


FIGURE 9-25. Acetylcholine and anticholinergic agents (boxed portions of the structures mimic acetylcholine).

whereas the antihistamine effects are due to the blockade of peripheral histamine H_1 receptors. For a phenothiazine to interact with the dopamine D_2 receptor, it must contain an electron withdrawing group attached to the phenothiazine ring, and it must have an unbranched three-carbon chain that separates the phenothiazine nitrogen atom and the side chain amine. Removal of the electron withdrawing group and addition of a branch in the alkyl chain significantly decreases dopamine D_2 receptor binding and enhances interaction with the histamine H_1 receptor binding. Examples are shown in **Figure 9-26**.

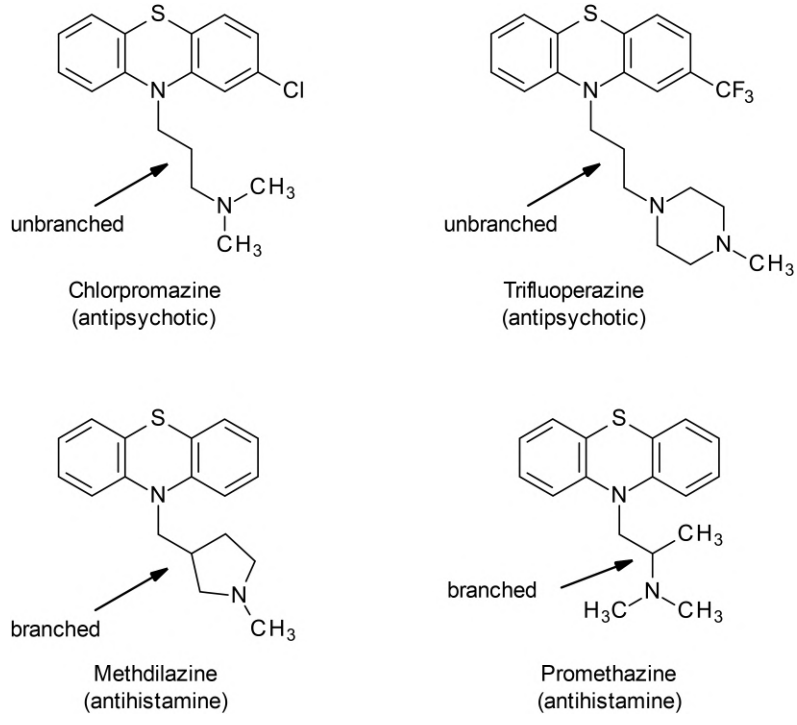


FIGURE 9-26. Phenothiazines with unbranched and branched alkyl chains.

The isosteric conversion of procaine to the orally active antiarrhythmic agent procainamide was discussed earlier in this chapter. Lidocaine is similar to procaine in several respects. It possesses both local anesthetic and antiarrhythmic properties, undergoes rapid metabolism (half-life of 15-30 minutes), has a short duration of action, and cannot be administered orally. When administered intravenously (IV), lidocaine initially undergoes oxidative *N*-dealkylation to yield an active metabolite, monoethylglycinexylidide. Subsequent amide hydrolysis causes this metabolite to be inactive (**Figure 9-27**). Because lidocaine already contains an amide group, it was not possible to impart oral activity by using the same molecular modification as seen with procaine; however, the use of chain branching was successful in meeting this goal. Tocainide is an α-methyl analog of monoethylglycinexylidide. The α-methyl group provides a branch in the alkyl chain between the amide carbonyl group and the primary amine. This α-methyl group provides sufficient steric hindrance to amide hydrolysis and allows tocainide to be used orally. Unlike lidocaine, tocainide is slowly metabolized and has a half-life of 12 hours.

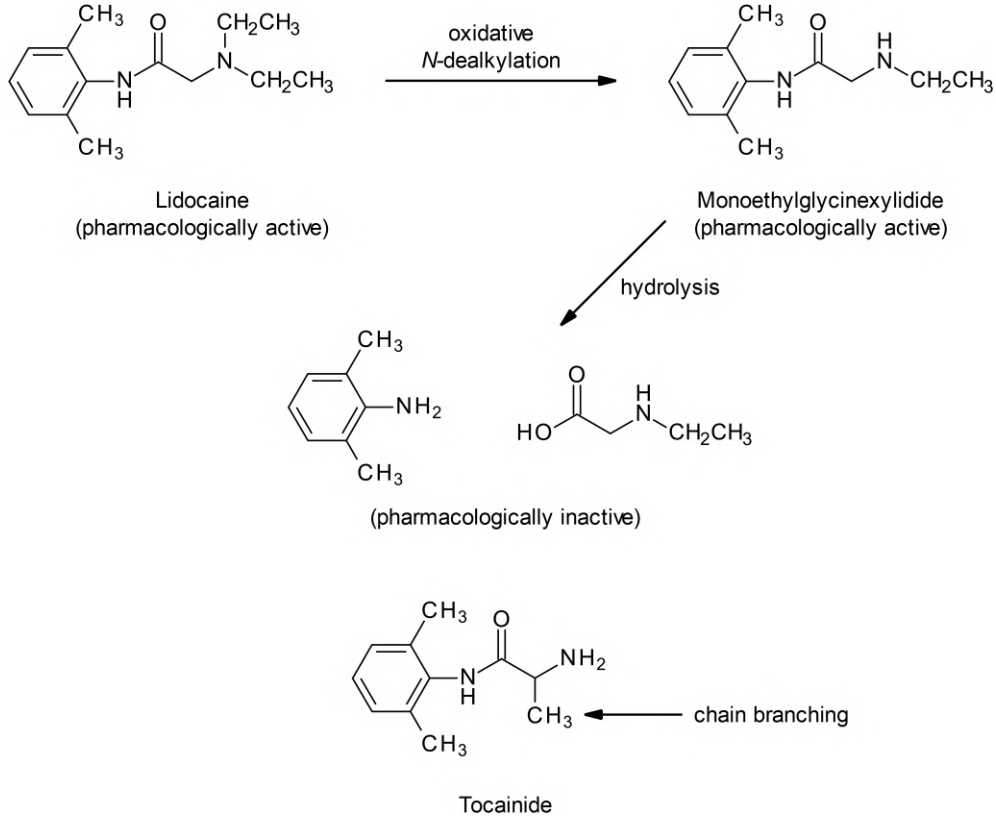


FIGURE 9-27. Lidocaine and tocainide.

The Conversion of an Active Drug to a Prodrug

In this type of molecular modification, an active drug molecule is altered to produce an inactive or significantly less active analog known as a *prodrug*. Upon therapeutic administration, the prodrug is converted in vivo to the active drug molecule by one or more of the metabolic transformations that were discussed in Chapter 8. This metabolic conversion is known as *bioactivation*.

The most common type of prodrug modification is the conversion of hydroxyl groups or carboxylic acids to either water- or lipid-soluble esters. Esterases are ubiquitous within the human body

and can easily convert this type of prodrug to the corresponding active drug molecule. Examples of this concept along with their physicochemical advantages were discussed in Chapters 5 and 8 and have been summarized in **Table 9-6**. Two additional examples of ester prodrugs are presented here.

TABLE 9-6. Advantages of the Use of Water- and Lipid-Prodrug Esters

Water-Soluble Prodrug Esters	
Advantage	**Example**
Allow for the preparation of concentrated ophthalmic solutions	Prednisolone sodium phosphate (Chapter 5)
Allow for the preparation of concentrated IV solutions	Chloramphenicol sodium succinate (Chapter 5); fosphenytoin (Chapter 9)
Lipid-Soluble Prodrug Esters	
Advantage	**Example**
Enhance oral absorption	Fenofibrate, candesartan cilexitil (Chapter 5); perindopril, valacyclovir, oseltamivir, diphenoxylate (Chapter 8)
Allow for the preparation of IM or subcutaneous depot injections	Haloperidol decanoate, estradiol valerate (Chapter 5)
Enhance the palatability of oral suspensions	Clindamycin palmitate (Chapter 9)
Enhance the absorption of drugs that are administered via oral or nasal inhalation	Beclomethasone dipropionate (Chapter 5)
Enhance the absorption of drugs that are administered as topical creams or ointments	Hydrocortisone butyrate (Chapter 5)

Phenytoin is an antiepileptic agent used to treat status epilepticus, partial seizures, and tonic-clonic seizures. Although it can be administered orally or parenterally, there are a number of limitations with its parenteral use. Phenytoin is compatible with normal saline but not 5% dextrose or Lactated Ringer's solution (two standard IV solutions). Additionally, it should not be administered by the IM route due to erratic absorption and well-documented tissue damage. Fosphenytoin (**Figure 9-28**) is a more water-soluble prodrug of phenytoin. It is compatible with all standard IV solutions, can be administered by the IM route, has fewer adverse reactions at the site of administration, and can be given at a faster rate. After administration, fosphenytoin undergoes a two-step bioactivation process. Hydrolysis of the phosphate group produces an unstable carbinolamine similar to that produced during oxidative deamination and oxidative *N*-dealkylation. The carbinolamine then collapses (as discussed in Chapter 8) to release formaldehyde and phenytoin.

Clindamycin is an antibiotic used to treat a variety of infections, including those located in the lower respiratory tract and the intra-abdominal region. Similar to many other antibiotics, clindamycin has a bitter taste that prevents administration as an oral solution for children as well as for patients who have difficulty swallowing tablets or capsules. To increase the palatability of clindamycin, it was modified to a lipid-soluble palmitate ester (**Figure 9-29**) and formulated as a suspension. In this formulation, the palmitate ester does not dissolve in the saliva. The patient tastes the water-soluble flavoring normally added to these suspensions and does not detect the bitter tasting drug. This is similar to the lipid-soluble stearate salt of erythromycin discussed in Chapter 5. The key difference between these two examples is that the palmitate ester prodrug requires in vivo hydrolysis to release clindamycin while salts simply need to dissociate.

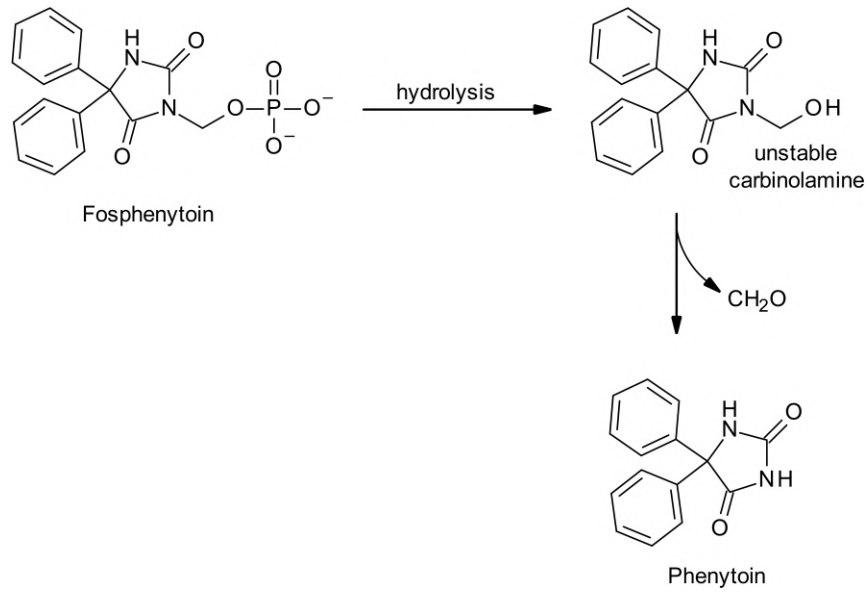


FIGURE 9-28. Metabolic conversion of the prodrug, fosphenytoin, to the active drug, phenytoin.

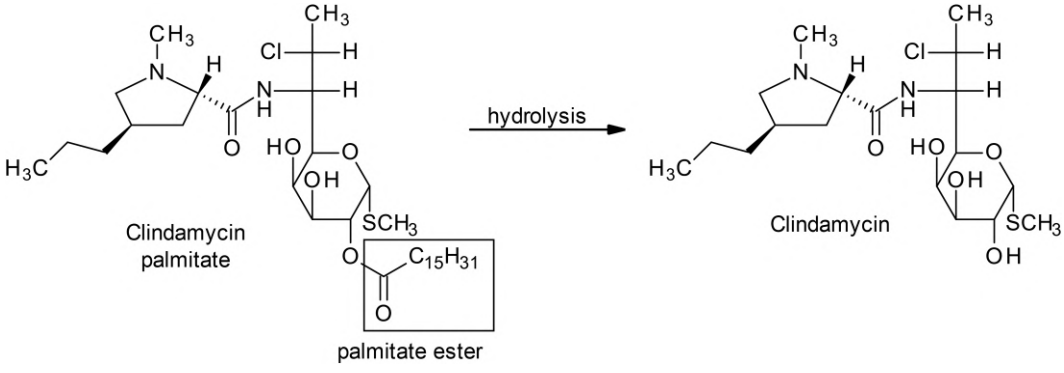
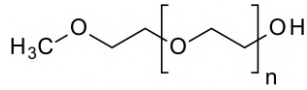


FIGURE 9-29. Clindamycin and its lipid-soluble palmitate prodrug.

Although water- and lipid-soluble esters are the most common types of prodrugs, other hydrolysable functional groups, such as amides and carbamates, have been used to develop prodrugs with distinct therapeutic benefits. Additionally, metabolic activation by mechanisms other than hydrolysis has led to the development of prodrugs with specific advantages beyond those that can be achieved by altering the water or lipid solubility of a drug molecule. Seven examples are provided below. The first three examples illustrate the usefulness of amide and carbamate prodrugs and the other four examples illustrate prodrugs that are activated by mechanisms other than hydrolysis.

Interferon alfa-2a and alfa-2b are drugs used in the treatment of hepatitis B and hepatitis C infections. They are similar to naturally occurring interferon and provide an antiviral effect by enhancing the production and/or release of specific enzymes that inhibit viral replication. To enhance their duration of action and thus require less frequent subcutaneous injections, these drugs can be converted to polyethylene glycol (PEG) prodrugs, or pegylated interferons. The PEG chain is linked to lysine residues present on interferon alfa-2a and alfa-2b. Hydrolysis of the resulting amide releases

interferon alfa-2a and alfa-2b. The general structure of monomethoxy PEG is shown below. It can be linked to proteins in a linear fashion or as a single branch with lysine.



monomethoxy
polyethylene glycol (PEG)

Isavuconazole is an azole antifungal drug indicated for the treatment of invasive aspergillosis and invasive mucormycosis in adults. Similar to other azole antifungals, it exerts its mechanism of action by inhibiting the fungal synthesis of ergosterol. It is marketed as an *N*-(3-acetoxypropyl)-*N*-methylamino-carboxymethyl prodrug known as isavuconazonium sulfate (**Figure 9-30**). The prodrug is highly water soluble and can be given either orally or via IV injection. The enhanced water solubility allows it to be formulated without a cyclodextrin vehicle, thus avoiding the concerns of nephrotoxicity seen with other parenteral azole antifungals (e.g., posaconazole and voriconazole). As shown in Figure 9-29, hydrolysis of the carbamate releases an unstable carbinolamine that spontaneously degrades to generate isavuconazole.

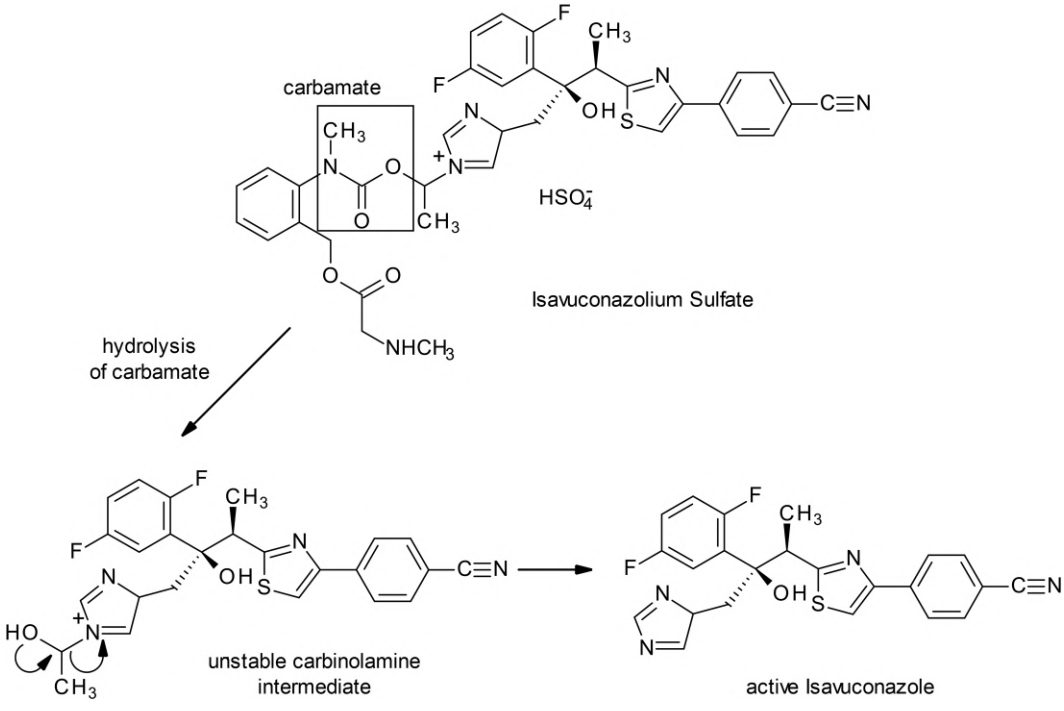


FIGURE 9-30. The bioactivation of isavuconazolium to isavuconazole.

Brentuximab vedotin (**Figure 9-31**) is an antibody-drug conjugate directed against the CD30 antigen expressed in Hodgkin lymphoma and anaplastic large cell lymphoma. The antibody, brentuximab, is linked to a cytotoxic drug, monomethylauristatin E (MMAE), via a valine-citrulline dipeptide and a *para*-aminobenzylcarbamate spacer. This linker ensures that the cytotoxic MMAE is not easily released from the antibody under physiologic conditions, thus helping to prevent toxicity to noncancerous cells. The antibody binds to CD30 found on the surface of malignant cells (such as Hodgkin's lymphoma cells) and the conjugate is internalized. Once this conjugate enters the lysosomal compartment, the acidic environment allows cathespin B to cleave the linker and release MMAE.

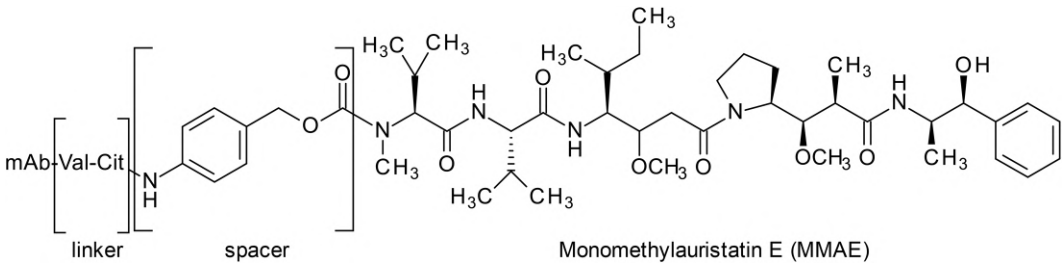


FIGURE 9-31. Brentuximab vedotin (mAb = brentuximab).

Mechlorethamine (**Figure 9-32**) is an alkylating agent used in the treatment of a variety of neoplastic disorders including Hodgkin's disease and chronic lymphocytic leukemia. Mechlorethamine is highly reactive with a half-life of less than 10 minutes. It is available as a powder that must be dissolved in either sterile water or normal saline immediately prior to use. Due to its high reactivity, mechlorethamine must be administered as an IV bolus injection over 2 to 5 minutes, and extreme precaution must be taken with its administration. Similar to mechlorethamine, the structure of cyclophosphamide contains two β-chloro ethyl groups that are required for alkylation. The primary

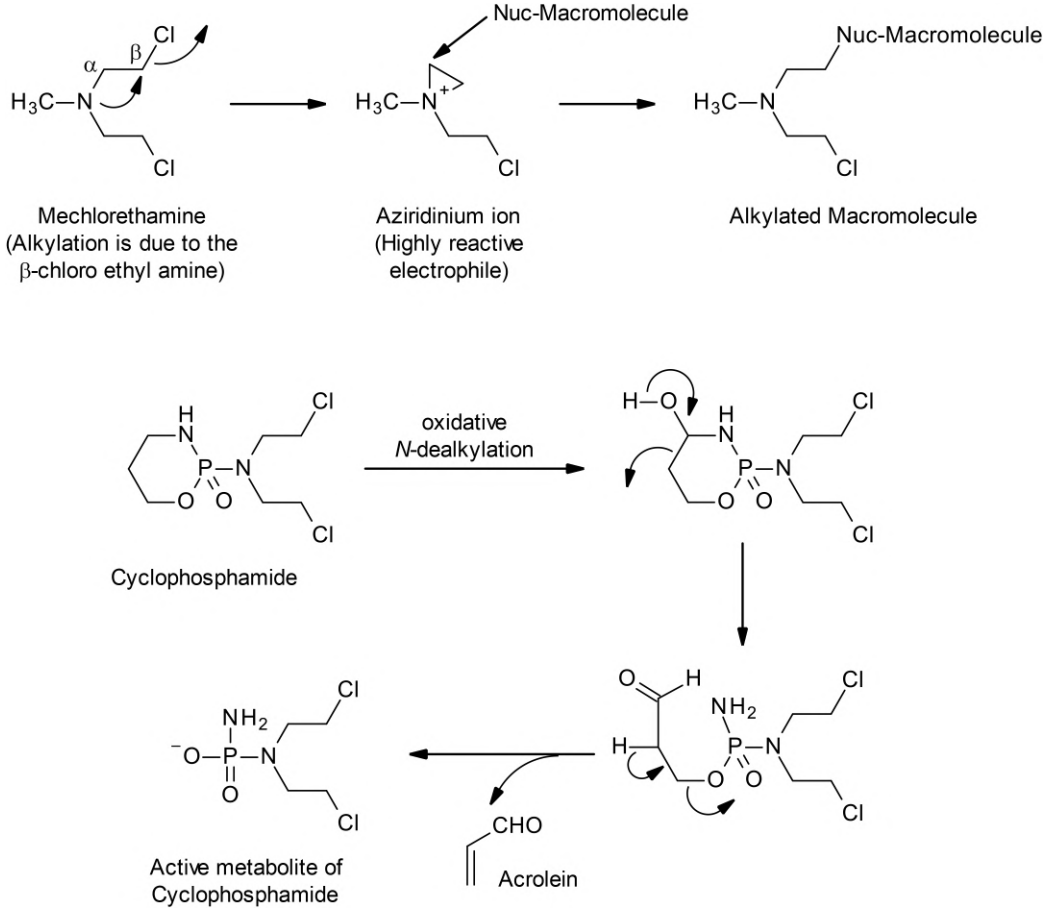


FIGURE 9-32. The mechanism of alkylation of mechlorethamine and the bioactivation of cyclophosphamide (a prodrug analog of mechlorethamine).

difference between these two drugs is that cyclophosphamide is an inactive prodrug. The presence of the unionized phosphoramide withdraws electrons from the adjacent nitrogen atom, thus preventing it from attacking the β-chloro ethyl groups. As such, it does not possess the high reactivity observed with mechlorethamine and has a much longer half-life (8 hours). It can be administered orally and as either an IV bolus injection or as a slow IV infusion. Similar to mechlorethamine, cyclophosphamide must be dissolved in sterile water, normal saline, or 5% dextrose solution; however, these solutions are stable for up to 24 hours. Once administered, it undergoes oxidative *N*-dealkylation. The resulting intermediate is unstable, and the subsequent reaction releases the active metabolite of cyclophosphamide. This active metabolite contains an ionized phosphate amide that alters the electronics of the functional group and allows it to form the same reactive aziridinium ion as seen with mechlorethamine. Due to its prodrug nature, cyclophosphamide is a safer agent and is much easier to administer. This has led to its preferential use and approval for the treatment of a wide variety of neoplastic disorders (i.e., cancers).

Throughout this text, NSAIDs have been discussed and used as examples. Most drugs in this class nonselectively inhibit cyclooxygenase enzymes, COX-1 and COX-2, and as a result decrease the biosynthesis of prostaglandins. Although this action is beneficial in providing analgesic, anti-inflammatory, and antipyretic effects, it can also lead to GI distress, including ulceration and bleeding. There are two reasons for these adverse effects. First, NSAIDs must contain an acidic functional group to interact with cyclooxygenase enzymes (see discussion in Chapter 2). Unfortunately, acidic functional groups represent a primary insult to the GI system and can directly cause irritation, ulceration, and bleeding in some patients. Second, due to their nonselective nature, most NSAIDs inhibit the GI production of PGE_2 and PGI_2. These prostaglandins are responsible for inhibiting gastric acid secretion, increasing mucus secretion, helping to maintain the integrity of the gastric mucosa, and inhibiting gastric damage caused by ulcerogenic compounds. The use of a nonacidic prodrug, such as nabumetone (**Figure 9-33**), eliminates the direct GI effects caused by active, acidic NSAIDs. Additionally, because the prodrug is not active until it is absorbed and undergoes bioactivation, there is not as much local inhibition of PGE_2 and PGI_2 biosynthesis.

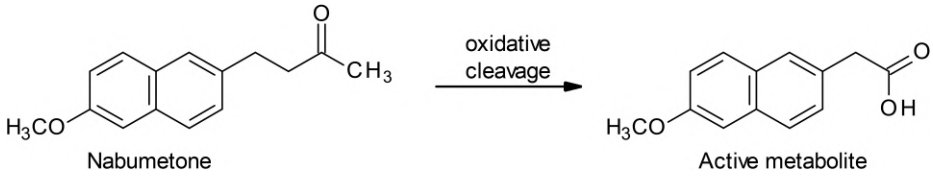


FIGURE 9-33. The bioactivation of nabumetone to its active metabolite.

Methenamine and omeprazole are examples of prodrugs that are selectively activated at their sites of action. Formaldehyde (CH_2O) has been shown to be effective in treating urinary tract infections due to its ability to denature bacterial proteins. The actions of formaldehyde are not specific for microorganisms, so direct administration of formaldehyde, either orally or parenterally, would cause significant toxicity to a patient. To circumvent this problem, the prodrug methenamine was developed. As shown in **Figure 9-34**, methenamine undergoes bioactivation to produce

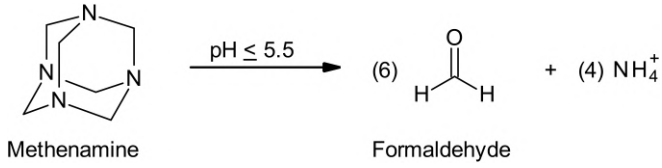


FIGURE 9-34. The bioactivation of methenamine to formaldehyde.

formaldehyde in an acidic environment. To avoid degradation in the stomach, methenamine is administered as an enteric-coated tablet, which prevents its release until it reaches the small intestine, where it undergoes dissolution and absorption. At a physiologic pH of 7.4, methenamine is stable and does not release formaldehyde in the blood or other tissues; however, once methenamine is filtered or secreted into the urine, the normal acidic environment (pH ~5-6) of the urine releases formaldehyde. Once this occurs, formaldehyde can exert its effects on the microorganisms that are responsible for causing the urinary tract infection.

Omeprazole (**Figure 9-35**) is a proton pump inhibitor used in the treatment of gastric and duodenal ulcers, gastroesophageal reflux disease, and other hypersecretory conditions. The proton pump, also known as H^+/K^+-ATPase, is a transport protein located in the parietal cells of the gastric mucosa that is responsible for secreting protons into the stomach (i.e., acid secretion). Omeprazole and other drugs within this chemical/pharmacological class of drugs irreversibly inhibit this process by covalently binding to H^+/K^+-ATPase. Because covalent bond formation requires the drug to be highly reactive, omeprazole and other drugs within this class were designed as site selective prodrugs. The acid catalyzed rearrangement illustrated in Figure 9-35 can occur only in the highly acidic environments found in the stomach and the parietal cells. Similar to methenamine, the use of an enteric-coated formulation allows omeprazole to be released and absorbed in the small intestine. Once it reaches the parietal cells, the acid catalyzed rearrangement occurs and the reactive intermediate is formed in very close proximity to H^+/K^+-ATPase. A cysteine residue present on H^+/K^+-ATPase readily reacts with the active metabolite to irreversibly inhibit the transport protein.

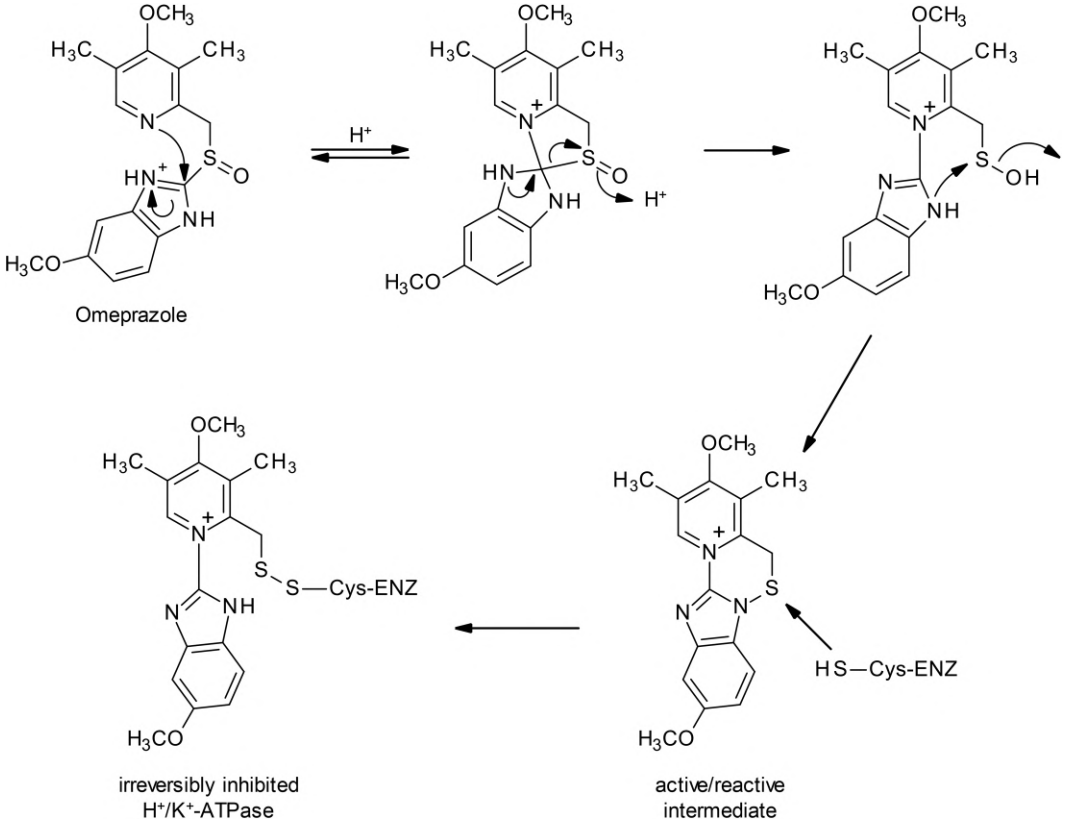


FIGURE 9-35. Bioactivation of omeprazole and the reaction of the active metabolite with H^+/K^+-ATPase.

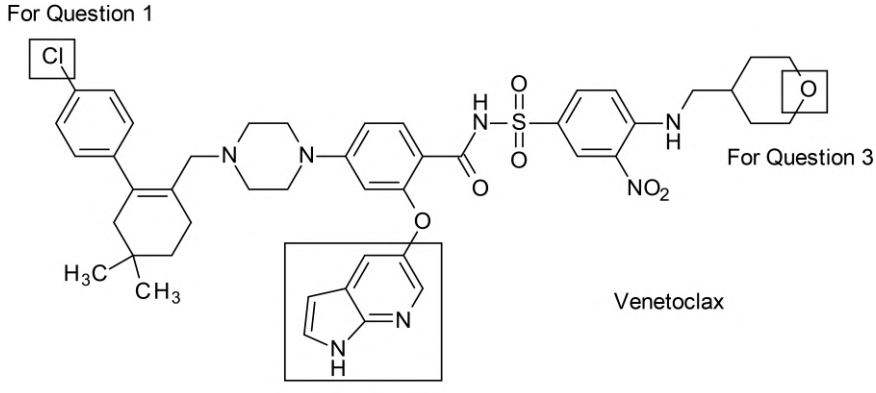
SUMMARY

Structure activity relationships represent a cornerstone for the discipline of medicinal chemistry as they define the chemical rationale for a given physicochemical, pharmacological, and/or therapeutic response. The electronic, physicochemical, and steric contributions of functional groups present within the structure of a drug molecule affect the drug molecule's ability to interact with specific biological targets and produce specific pharmacological effects. Additionally, functional groups and the overall structure of a drug molecule determine the absorption, distribution, metabolism, and excretion of the drug. Finally, certain drug interactions and adverse drug reactions can be directly linked to a specific functional group or structural feature within a drug molecule. As you progress to the study of specific drug molecules and drug classes, remember that each SAR statement that you encounter should include a "why" or "how" component that links a specific functional group that is inherent within the chemical structure to a specific physicochemical, pharmacological, or therapeutic effect.

Molecular modification of a lead compound is a commonly used strategy to develop new drug molecules. This strategy has been successful in enhancing the potency and/or selectivity of a lead compound, altering the water/lipid solubility balance to meet a specific need, optimizing the duration of action, and decreasing drug interactions and adverse drug reactions. Conformational restriction, the variation of functional groups, the use of isosteres, homologation, chain branching, and the conversion of an active drug molecule to a prodrug are the most common types of molecular modification. Reviewing these strategies can help you to understand the rational design of drug analogs and how the SARs for a specific class of compounds were established. Additionally, because these types of modification are common, you are likely to encounter them in the future whenever new drugs are approved by the FDA.

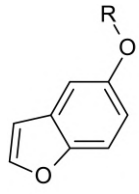
STRUCTURAL ANALYSIS CHECKPOINT

Checkpoint Drug 1: Venetoclax

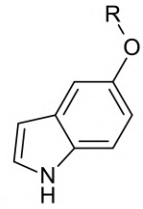


1. For the purpose of this question, assume that a research group designed six analogs of venetoclax by replacing the boxed *para* chloro group with the following functional groups: F, OH, NO_2, OCH_3, CH_3, and CN. In testing these compounds and comparing their activities to venetoclax, it was found that the F group retained activity, the NO_2 and CN analogs had enhanced activity, the CH_3 analog was 100 times less active, and the OH and OCH_3 analogs were essentially inactive. What SAR can be deduced from this information?

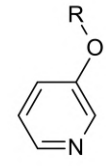
2. For the purpose of this question, assume that a research group designed four analogs of venetoclax by replacing the boxed pyrrolopyridine bicyclic ring with the following four rings.



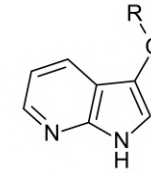
Analog A



Analog B



Analog C



Analog D

   In testing these analogs and comparing their activities to venetoclax, it was found that Analog B had similar activity, Analog D was 10 times less active, and Analogs A and C were 1,000 times less active. What SAR can be deduced from this information?

3. The boxed ether oxygen can be isosterically replaced by a secondary amine (-NH-) or a methylene carbon atom ($-CH_2-$). For each of these isosteric replacements, explain how their chemical differences could affect the actions of venetoclax.

4. Evaluate the structure of venetoclax and determine if it could easily be converted to a prodrug to enhance water solubility.

Checkpoint Drug 2: Elamipretide

Elamipretide is part of a family of tetrapeptides called the Szeto-Schiller (SS) peptides. It was discovered when a library of small molecules was screened for μ-opioid receptor activity. The ability of this agent to restore bioenergetics in aging and in a variety of disease states was a serendipitous discovery.*

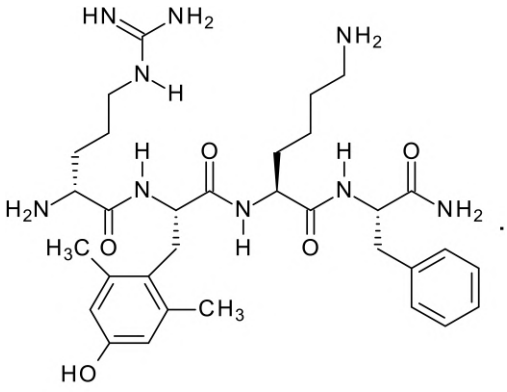
A. SS-02 was found to have potent central analgesic activity (μ agonist). It is taken up by several cell types without the need for transporters or receptors. It crosses lipophilic membranes (including the blood brain barrier) without the need for transporters. SS-02 selectively partitions into the inner mitochondrial membrane.

B. SS-31 (elamipretide) and SS-20 also selectively partition into the inner mitochondrial membrane but do not possess μ agonist activity.

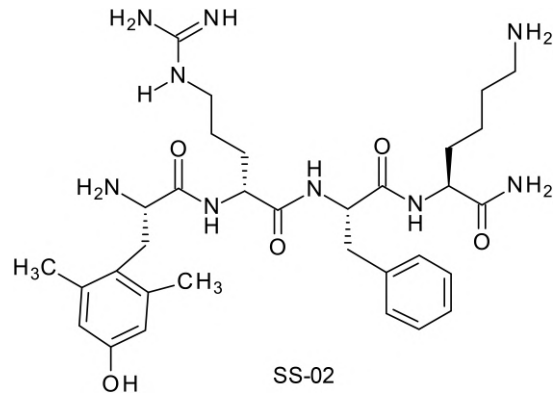
C. Only SS-31 and SS-20 bind to cardiolipin, which is expressed in the inner mitochondrial membrane.

D. An electrostatic interaction occurs between the two basic amino acids found in the tetrapeptides and the phosphate head found within cardiolipin. This causes alignment of the hydrophobic amino acids/amino acid derivatives with a hydrophobic region within cardiolipin.

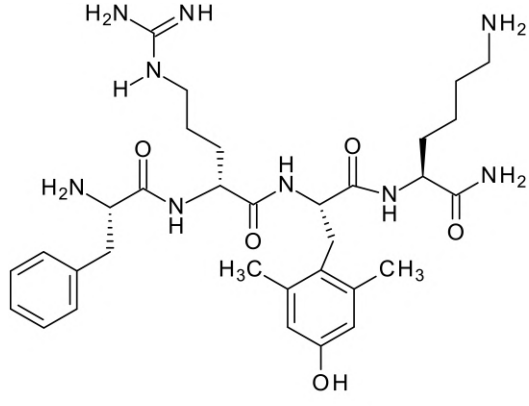
*Szeto HH, Birk AV. Serendipity and the discovery of novel compounds that restore mitochondrial plasticity. *Clin Pharmacol Ther.* 2014;96(6):672-683.



Elamipretide (SS-31)



SS-02



SS-20

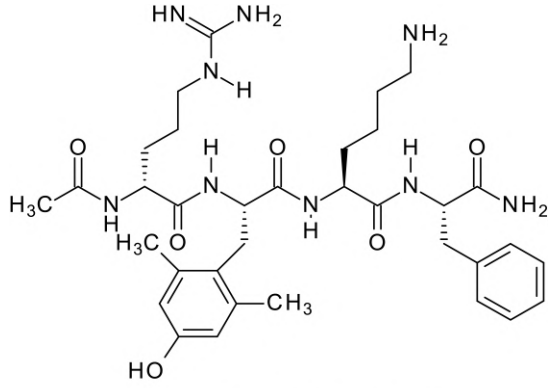
1. Evaluate the amino and carboxy terminal amino acids for each of the three tetrapeptides and list the possible binding interactions in the table provided. Which interactions are unique to SS-02 that might be required for μ receptor activation?

	Amino Terminus	Carboxy Terminus
SS-31 (elamipretide)		
SS-02		
SS-20		

2. Consider the spatial positioning of the two basic amino acids found within each of these tetrapeptides. Given the need to participate in electrostatic interactions with a phosphate head, provide a rationale for why all three peptides should be able to participate in this type of interaction.
3. Consider the locations of the hydrophobic amino acids relative to the two basic amino acids found within each of these tetrapeptides. Provide a rationale for why only SS-31 and SS-20 bind to cardiolipin.
4. In Chapter 8 you evaluated a prodrug form of elamipretide (drawn below).

   A. List two reasons why delivery of a prodrug might be valuable.

B. Provide a structural rationale for why this prodrug is not degraded by amino- and carboxypeptidases.



Prodrug of Elamipretide

REVIEW QUESTIONS

1. Endogenous insulin is a polypeptide composed of two peptide chains (A and B chain). The A chain is 21 amino acids long and the B chain is 30 amino acids long. The two peptide chains are linked by two critical disulfide bonds. A portion of the A chain is cyclic due to the presence of an additional disulfide bond. For insulin to exert its action, it must interact with and activate insulin receptors that are located on target cells. The SARs associated with this endogenous hormone are listed below.

   Insulin A chain SAR:

   1. Shorten chain ∴ virtually no biological activity (lifts termini of A chain off receptor surface)
   2. Remove *C*-terminal asparagine ∴ no biological activity (key receptor binding interaction)
   3. Replace *N*-terminal glycine with L-alanine ∴ retain biological activity (steric requirement allows addition of small hydrophobic substituent)
   4. Replace *N*-terminal glycine with D-alanine ∴ no biological activity (tight steric requirements on receptor binding surface)
   5. Remove *N*-terminal glycine ∴ no biological activity (key receptor binding interaction)

   Insulin B chain SAR:

   1. Remove first 6 amino acids from *N*-terminus ∴ retain biological activity (no interference with key receptor binding interactions)
   2. Remove last 3 amino acids from *C*-terminus ∴ retain biological activity (no interference with key receptor binding interactions)
   3. Single residue deletions B24 to B26 provide analogs twice as potent as insulin (improved receptor binding interactions)

General Insulin A and B chain SAR:

1. Amino acids can be replaced inside the chains; be careful how many are (D) (alter conformation of protein when there are too many D amino acids)
2. Disulfide linkages (3) must be present for biological activity (required for active conformation of hormone)

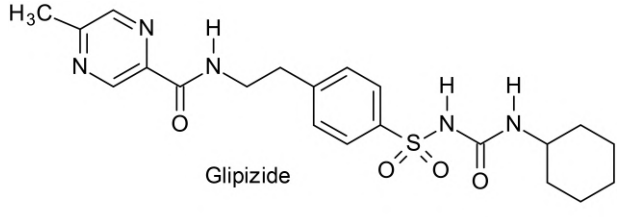
The primary structure of insulin is highly conserved across several animal species. A geneticist recently isolated and characterized several forms of insulin from the Catywhompus, a rain forest dwelling animal. He identified several molecular characteristics found within the insulin mimics and tried to predict whether any of these insulins would be biologically active in humans. Consider the SAR requirements for insulin provided and each set of structural characteristics and determine if the insulin is likely to be ACTIVE or INACTIVE in humans. If the insulin is INACTIVE, then indicate which structural feature(s) is/are likely to be the problem.

A. Insulin #1: Several amino acids are different from those found in human insulin in the A chain. Overall, the number of amino acids in the A chain remains the same. The amino and carboxy terminal residues remain identical to that of human insulin. One disulfide bond is present. Otherwise, the insulin structure is identical to human insulin.

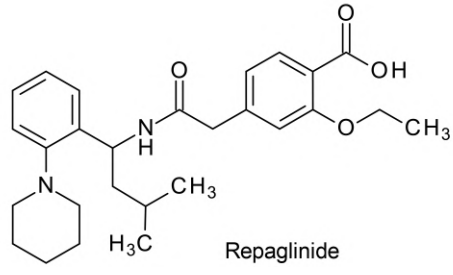
B. Insulin #2: The primary structure of the B chain is nearly identical to that of human insulin; however, it is shorter at the amino terminus by 10 amino acids and at the carboxy terminus by 2 amino acids. Otherwise, the insulin structure is identical to human insulin.

C. Insulin #3: Almost half of the amino acid residues in both the A and B chains are D-amino acids. A total of three disulfide bonds are present in the insulin molecule. The A chain amino terminus has been replaced by D-alanine. Otherwise, the insulin structure is identical to human insulin.

2. The sulfonylurea (e.g., glipizide) and meglitinide (e.g., repaglinide) classes of antidiabetic agents are effective in the treatment of type 2 diabetes because of their ability to increase insulin secretion from the pancreatic β cells. An acidic functional group is a structural requirement for activity. The meglitinide class of agents is derived from the sulfonylureas. Which type of molecular modification has occurred in this evolution?



Glipizide

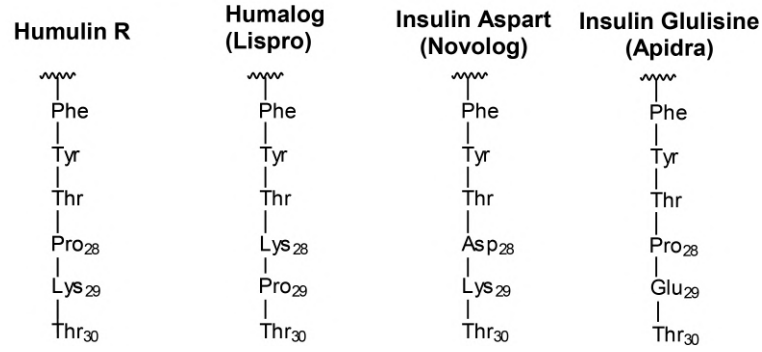


Repaglinide

3. There are several insulin analogs currently marketed that have a shorter onset and duration of action as compared with the endogenous hormone (see partial structures below). The insulin in these formulations is inactive and must undergo at least one dissociation step after administration to the active monomer.

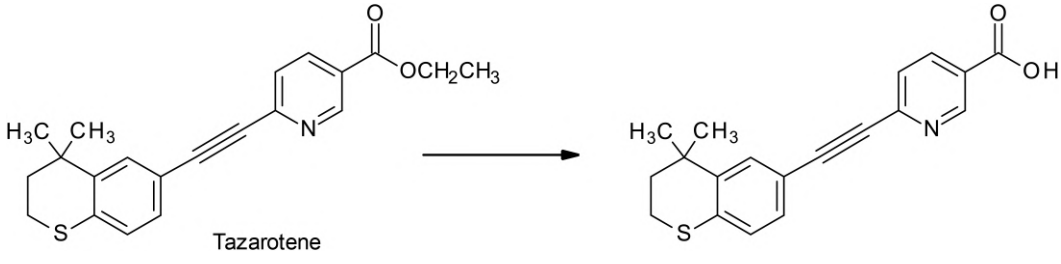
   From a structural perspective these insulin analogs have been altered at the B chain C-terminus, an area of the peptide that is not involved in receptor binding interactions. In Humulin R, the proline residue (position 28) causes a conformational kink in the protein structure and a "hook" is formed. There is some evidence that this "hook" allows for insulin-insulin dimerization. In Humulin R, the insulin dimers must dissociate to the active monomer, a process that takes a small amount of time. Humulin R is considered a short-acting insulin.

Alterations to B chain C-terminus:

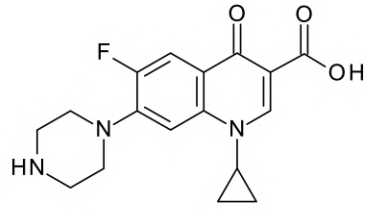


   Provide a structural rationale for why Lispro and Insulin Aspart are considered ultra-short insulins. Be sure to include in your answer a discussion about dimer formation.

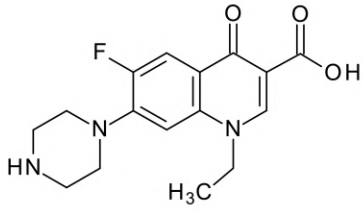
4. Tazarotene is marketed as a prodrug for the topical treatment of psoriasis, acne, and sun damaged skin. Activation of the drug is shown below. Provide a potential rationale for why tazarotene is modified to be an ester. (HINT: Agents that are administered topically must absorb into the hydrophobic components of the skin to be effective. Remember, absorption across lipophilic membranes is improved as hydrophobic character increases.)



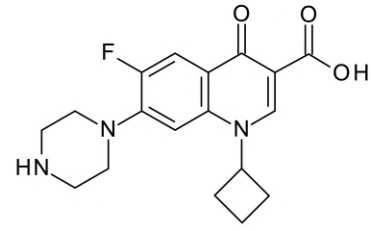
5. Ciprofloxacin is a fluoroquinolone antibiotic effective in the treatment of infections caused by gram-negative pathogens. A series of analogs (A–D) were synthesized in a research laboratory and tested at Abbott Laboratories. The testing results indicated that only analog A was effective in killing gram-negative pathogens, and the rest were completely inactive. Provide a structural rationale for why you think that the other analogs were inactive.



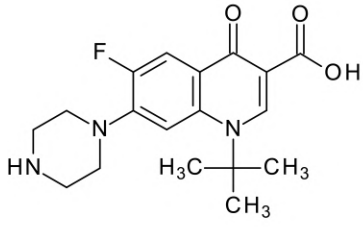
Ciprofloxacin



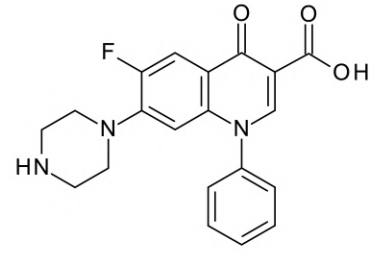
Analog A



Analog B

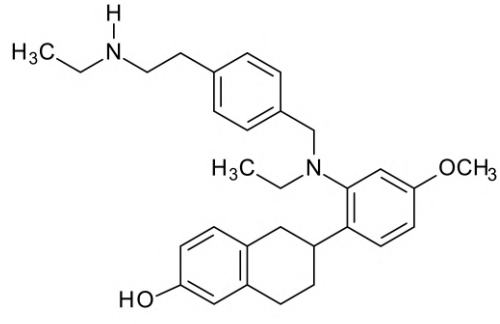


Analog C



Analog D

6. RAD-1901 is an investigational selective estrogen receptor degrader that acts as an estrogen receptor antagonist in breast and uterine tissues and an estrogen receptor agonist in bone. Unfortunately, it undergoes rapid metabolism to a series of inactive metabolites. Propose at least two different isosteric modifications that will limit or prevent this rapid metabolic inactivation and provide a rationale for how each modification will decrease the potential for metabolic inactivation.

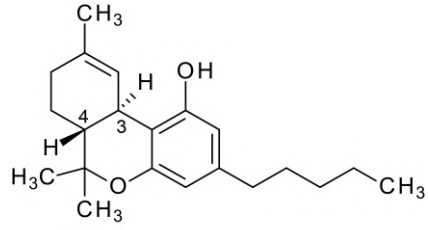


RAD-1901

7. Cannabinoid SARs were first reported in the early 1940s. A molecule possesses cannabinoid activity if it includes the following requirements:

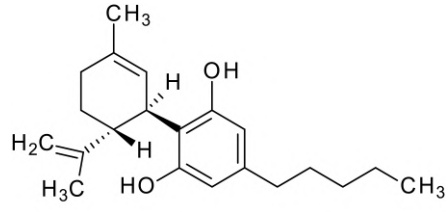
   - Benzopyran ring (oxygen atom can be replaced by nitrogen atom)
   - Nonplanar alicyclic ring (3, 4 position). Planar ring reduces activity. A bulky substituent at the 4 position is acceptable instead of a nonplanar alicyclic ring.
   - Substituents on the alicyclic ring are acceptable without loss of activity.
   - The alicyclic ring attachment to the benzopyran ring can be substituted by a heterocyclic ring.
   - In the aromatic ring, esterification of the phenol is acceptable.
   - The length of the aromatic side chain can vary, but a three-carbon chain is minimally required. Branching of this chain enhances potency. Attachment of the side chain to the aromatic ring can be via an oxygen atom.

   A. The structure of tetrahydrocannabinol is drawn below. Box each of the structural requirements and label A–F.



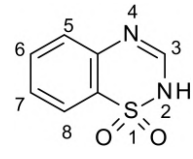
Tetrahydrocannabinol

   B. Evaluate the structure of the tetrahydrocannabinol analog against the SAR required for cannabinoid activity. Determine if this analog possesses cannabinoid activity and provide a brief structural rationale for your answer.



Tetrahydrocannabinol analog

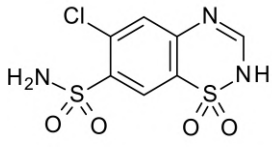
8. The thiazide diuretics bind to Cl^- site of the distal tubular Na^+/Cl^- Cotransporter system and prevent sodium and chloride reabsorption. Structural modification can lead to enhanced diuretic effect and/or duration of action. Using the list of structural modifications below, analyze each pair of thiazide diuretics and answer the questions posed.



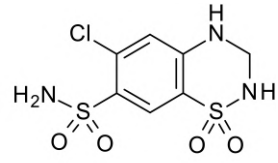
   - *N*-2 Substitution decreases polarity and increases duration of action
   - Lipophilic groups at C-3 enhance diuretic effect and increase duration of action

- Saturation of the 3,4-double bond enhances diuretic effect
- An electron withdrawing group at C-6 enhances diuretic effect (e.g., chloro, CF_3)
- Unsubstituted C-7 sulfamoyl group (SO_2NH_2) enhances diuretic effect

Pair #1 (Chlorothiazide and Hydrochlorothiazide): Which drug has the greater diuretic effect? Provide a brief structural rationale for your answer.

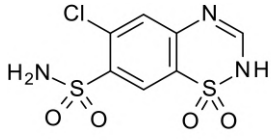


Chlorothiazide

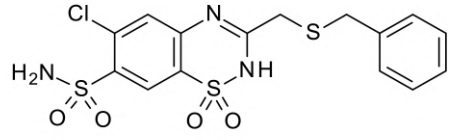


Hydrochlorothiazide

Pair #2 (Chlorothiazide and Benzthiazide): Which drug has a longer duration of action? Provide a brief structural rationale for your answer.

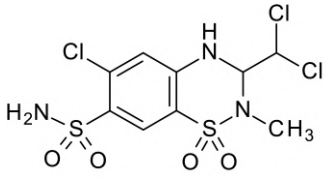


Chlorothiazide

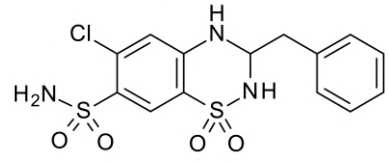


Benzthiazide

Pair #3 (Methylclothiazide and Bendroflumethiazide): Which drug has a longer duration of action? Provide a brief structural rationale for your answer.

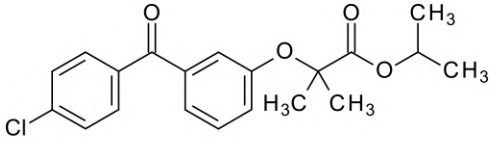


Methylclothiazide

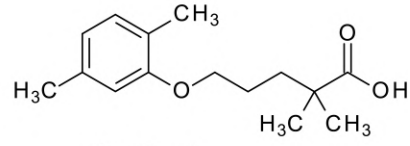


Bendroflumethiazide

9. The fibric acid derivatives stimulate lipoprotein lipase (lowering triglyceride levels) and are PPARα agonists (increasing high-density lipoprotein levels). The fibrates bind to PPARα via an ion–dipole interaction with a tyrosine residue within PPARα. Fibrates with a *para* chloro substituent or chloro containing substituents have significantly longer half-lives. Evaluate the following pair of fibric acid derivatives and answer the questions posed.



Fenofibrate

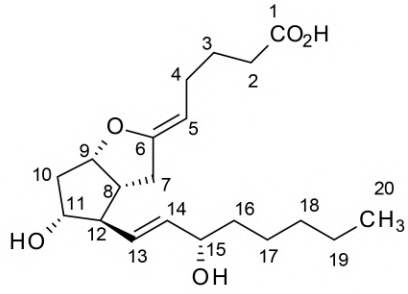


Gemfibrozil

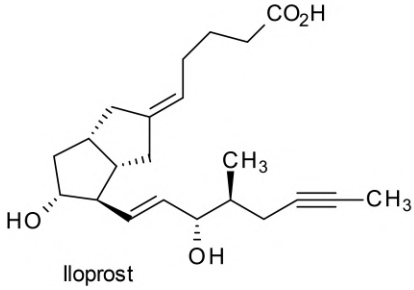
A. Which drug is able to interact with PPARα? Provide a structural rationale for your answer. If a drug is unable to interact with PPARα, then determine which Phase I metabolic transformation(s) must occur for the drug to be in its active form and interact with its biological target?

B. Which drug has a longer duration of action? Provide a structural rationale for your answer.

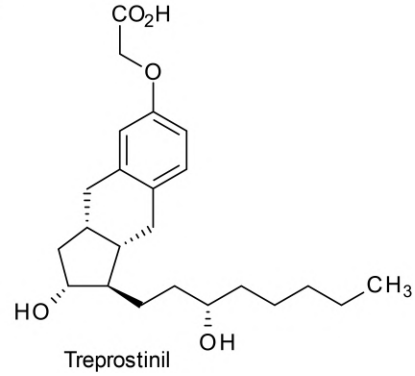
10. In patients diagnosed with pulmonary arterial hypertension (PAH) there is a downregulation of prostacyclin synthetase resulting in less PGI_2 production. This vasoactive prostaglandin binds to and activates the IP receptors leading to important vasodilation and antiproliferative activity. PGI_2 (aka epoprostenol) and two synthetic PGI_2 analogs that are used in the management of PAH are drawn below.



Epoprostenol (PGI_2)



Iloprost

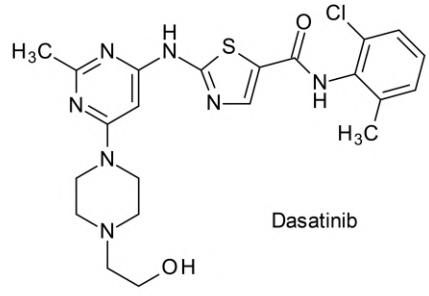


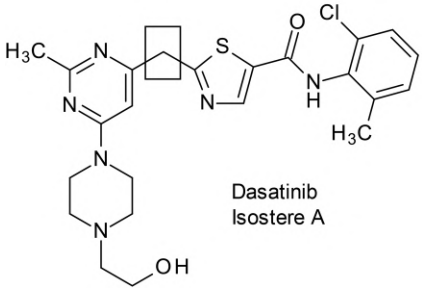
Treprostinil

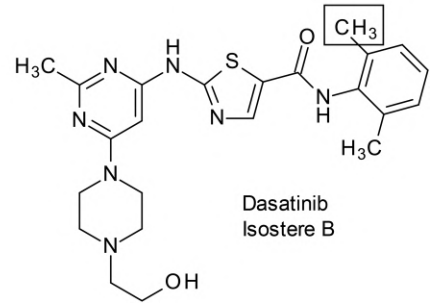
Evaluate each drug molecule and each SAR in the table. Place a check mark or comment in the table if the drug molecule follows the SAR.

	Epoprostenol (PGI_2)	Ilprost	Treprostinil
C-1 carboxylic acid to participate in required ionic or ion/dipole interaction			
C-11 and C-15 hydroxyl groups to participate in H-bonding interactions			
Cyclopentane ring system + C-5 and C-13 double bonds (to maintain geometry)			
C-6 oxygen (increases potency and undergoes rapid metabolism to inactive drug)			
Additional functional groups capable of H-bonding interactions (decreases receptor affinity)			

11. Dasatinib is tyrosine kinase inhibitor that is used to treat Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) in both adults and pediatric patients older than 1 year. The structures of dasatinib and two isosteres of dasatinib are shown below. The isosteric changes are boxed.







A. For Isostere A, indicate if this is a classical or a nonclassical isostere and predict how this isosteric change could alter the ability of dasatinib to bind to its target receptor.

B. For Isostere B, indicate if this is a classical or a nonclassical isostere and predict how this isosteric change could alter the duration of action of dasatinib.

12. As a pharmacy student, your medicinal chemistry processor constantly uses the abbreviation "SAR" in his class.

A. Explain what these initials represent and what this term means.

B. What is the most important component of an SAR?

WHOLE MOLECULE DRUG EVALUATION

LEARNING OBJECTIVES

After completing this chapter, students will be able to

- **Evaluate a drug structure and identify the key structural features required to address a specific structure-based question.**
- **Identify the appropriate concepts applicable to answering specific structure-based questions.**
- **Correctly apply basic medicinal chemistry concepts to drug- and structure-based questions.**

The Structural Analysis Checkpoint questions in Chapters 2 to 9 allowed you to sequentially answer questions pertaining to venetoclax and elamipretide as new concepts were introduced. By the end of Chapter 9, whole molecule drug evaluations had been conducted for these two drugs. In this final chapter, twelve additional drugs, drug pairs, or drug classes have been selected for similar whole molecule drug evaluation. Unlike the previous chapters, in which the questions focused specifically on one or two concepts, the questions here challenge you to conduct a comprehensive evaluation of the selected drugs and select the concepts that are applicable to the scenarios provided.

The selected examples represent three levels of evaluation.

1. Level 1: evaluates the chemical properties of a single drug molecule.
2. Level 2: evaluates a pair of structurally related drugs and requires the reader to compare and contrast their chemical properties.
3. Level 3: evaluates multiple drugs within a single chemical/pharmacological class and requires an evaluation of the structure activity relationships (SARs) of the drug class.

The answers to all of these questions can be found in the Appendix.

ALISKIREN (LEVEL 1)

Aliskiren is an orally active agent used in the treatment of hypertension. This nonpeptide drug acts as an inhibitor of renin, the enzyme that converts angiotensinogen (its endogenous substrate) to angiotensin I. Biologically inactive, angiotensin I is rapidly converted to angiotensin II

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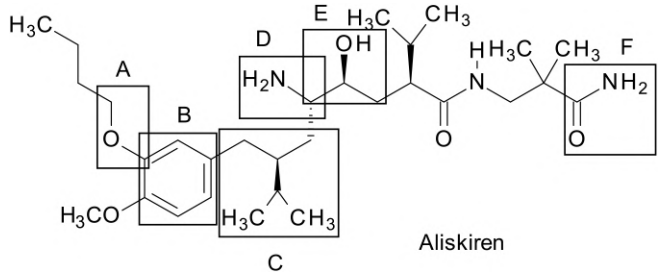
by angiotensin-converting enzyme. Angiotensin II is a potent agonist when bound to its receptor and produces significant vasoconstriction as well as an increase in blood pressure. In the presence of aliskiren, angiotensinogen is not converted to angiotensin I, so less angiotensin II is produced to activate the angiotensin II receptor. Consequently, less vasoconstriction occurs, and a drop in blood pressure results.

1. Conduct a structural evaluation of aliskiren, focusing on the boxed functional groups, and use the information in the grid to inform your answers to the questions that follow.

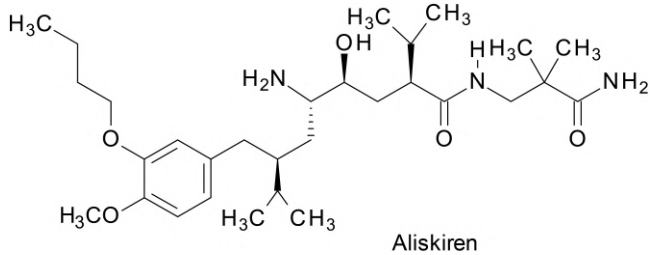


Aliskiren

	Name of Functional Group	Character: Hydrophilic and/or Hydrophobic	Character: Acidic, Basic, or Neutral (Provide pK_a When Relevant)	Function: Contribute to Aqueous Solubility and/or Contribute to Absorption	Function: Interaction(s) Possible with Biological Target at Physiologic pH = 7.4	Function: Amino Acids That Can Interact with the Functional Group via Ion–Dipole Interactions at pH = 7.4[a]
A						
B						
C						
D						
E						
F						

[a]"None" is a possible answer.

2. Aliskiren is marketed as the pure 2*S*, 4*S*, 5*S*, 7*S* enantiomer. Circle all of the chiral carbon atoms and determine if *diastereomeric* or *geometric isomers* are possible.

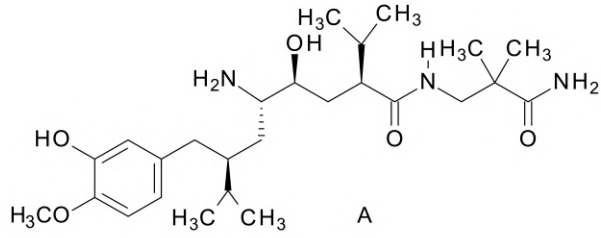


Aliskiren

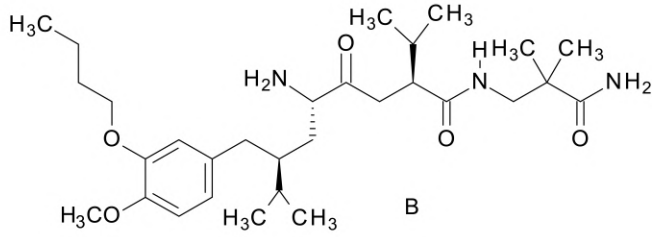
3. Although aliskiren is administered orally, its oral bioavailability is ~2.5% and is very poorly absorbed. Using the information in the structure evaluation grid, provide a structural rational for this unfortunate property.

4. Approximately 25% of the absorbed dose of aliskiren is excreted in the urine unchanged. It is unknown how much of an absorbed dose is metabolized, and several metabolites from

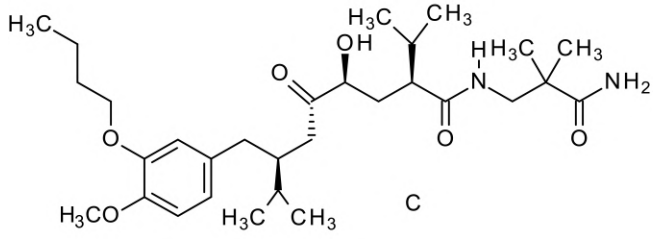
CYP3A4 mediated transformations have been identified. Several possible metabolic products are drawn below. Identify which metabolic transformation has occurred and whether it represents an *oxidative transformation*.



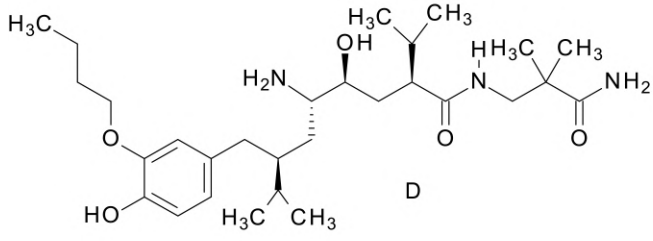
A



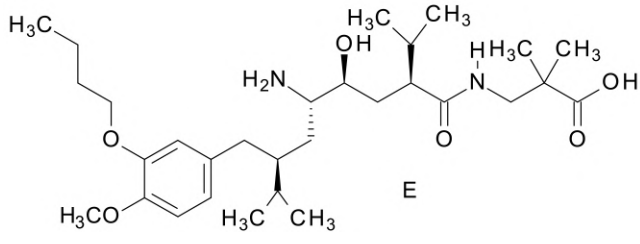
B



C



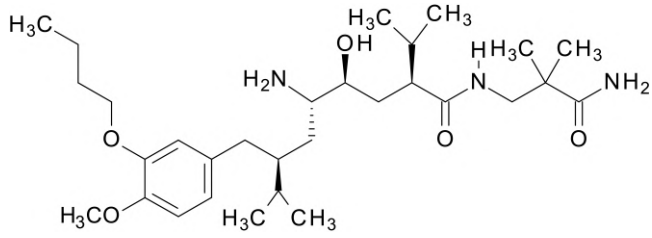
D



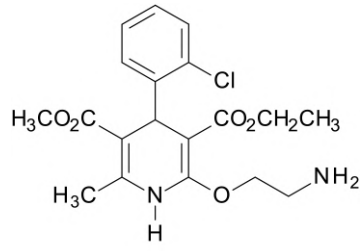
E

	Name of Metabolic Transformation	Oxidative or Nonoxidative
A		
B		
C		
D		
E		

5. Renin catalyzes the cleavage of a specific Leu-Val peptide bond within the structure of angiotensinogen. The structure of aliskiren contains functional groups that mimic the side chains for these two amino acids. The hydrolyzable peptide bond (found between Leu-Val) has been replaced by a nonhydrolyzable hydroxyethylene group in aliskiren. Circle the functional groups that mimic the amino acid side chains of these two amino acids and box the nonhydrolyzable hydroxyethylene group.



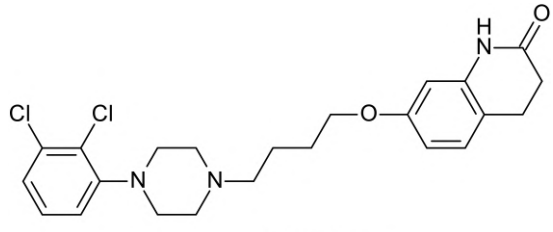
6. Aliskiren can be coadministered with other antihypertensive agents to provide better hypertension management. Amlodipine is a second-generation dihydropyridine calcium channel blocker used in the treatment of hypertension. Aliskiren and amlodipine are both plasma protein bound (47% to 51% and 93% to 97%, respectively). How likely is it that a plasma protein binding interaction will occur if these drugs are coadministered?



Amlodipine

ARIPIPRAZOLE (LEVEL 1)

Shown below is the structure of aripiprazole, a serotonin receptor modulator used for the treatment of depression, schizophrenia, autism, mania, and bipolar disorder.



Aripiprazole

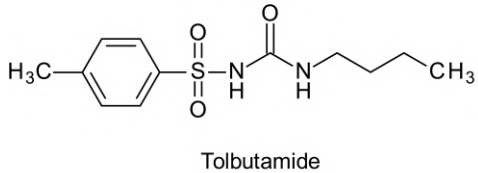
1. Identify all of the *acidic* and *basic* functional groups, provide the normal *pK_a range* for each of the identified functional groups, and identify if each functional group would be primarily *ionized* or *unionized* at a urine pH of 5.6.

2. Identify all of the remaining functional groups and indicate how each group contributes to the overall *water solubility* or the overall *lipid solubility* of aripiprazole.

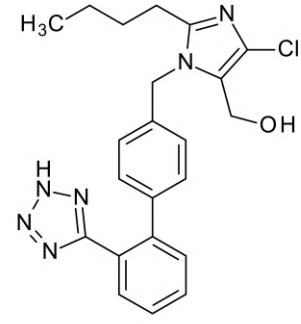
3. Using your answers from the previous two questions, provide an explanation as to why aripiprazole can be administered orally for the indications previously listed.

4. The acidity and basicity of functional groups can vary based on the presence or absence of adjacent functional groups. The normal pK_a range for an aromatic amine is 2 to 5. Conduct a structural evaluation of aripiprazole and predict whether the pK_a of the aromatic amine present would be expected to be at the lower or upper end of this range. Provide a rationale for your prediction.

5. Drug molecules that are highly plasma protein bound may undergo a drug interaction due to the displacement of one drug molecule from a plasma protein by another drug molecule. Shown below are the structures of tolbutamide and losartan. Both of these drugs are highly plasma protein bound. Aripiprazole is also highly plasma protein bound (> 99%). Would you expect there to be a drug interaction between aripiprazole and either tolbutamide or losartan?



Tolbutamide

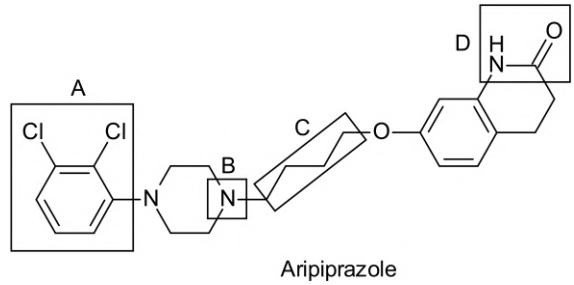


Losartan

6. Draw a water-soluble organic salt of aripiprazole.

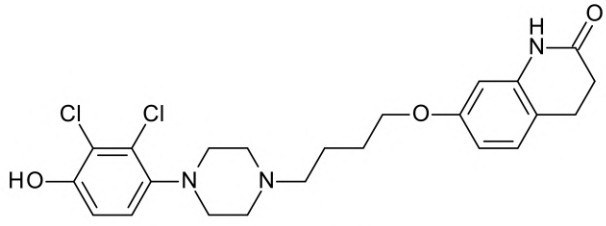
7. The structure of aripiprazole does not contain any chiral carbon atoms and thus does not have enantiomers. Its structure contains 10 prochiral methylene (CH_2) groups that could potentially be converted to chiral centers. Using the known metabolic pathways discussed in Chapter 8, identify which of these 10 prochiral methylene atoms are most likely to generate a chiral center on metabolism.

8. Assume that the boxed functional groups of aripiprazole form four key binding interactions with a serotonin receptor. Further assume that these binding interactions occur with the side chains of Tyr, Asp, Ile, and Gln. Using this information, identify four possible binding interactions between aripiprazole and the given amino acids. Assume that this binding interaction occurs at a physiologic pH of 7.4.

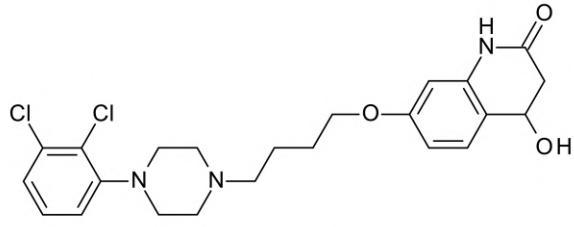


Aripiprazole

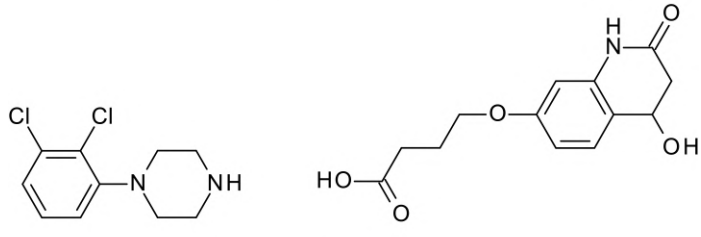
9. Shown below are three known metabolites of aripiprazole. Identify the metabolic transformations that would be required to form each of the metabolites. For each metabolic transformation, indicate if it is a Phase I transformation or a Phase II transformation.



Metabolite A



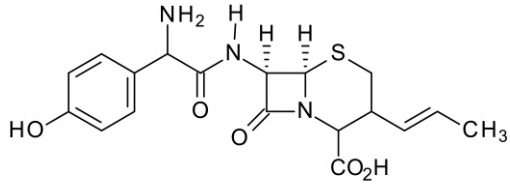
Metabolite B



Metabolite C

CEFPROZIL (LEVEL 1)

Cefprozil is a second-generation cephalosporin that exhibits good gram (+) activity with improved gram (–) activity as compared with the first generation cephalosporins. Effective against most bacteria that cause upper and lower respiratory infections as well as skin infections, cefprozil was a first-line anti-infective agent until an increase in the incidence of resistance and the development of newer agents decreased its "favored" status.



Cefprozil

1. Conduct a complete structural evaluation of cefprozil and use the information in the grid to inform your answers to the questions that follow:

Name of Functional Group	Character: Hydrophilic and/or Hydrophobic	Character: Acidic, Basic, or Neutral (Provide pK_a When Relevant)	Function: ↑ Solubility and/or ↑ Absorption	Function: Interaction(s) Possible with Biological Target at Physiologic pH (7.4)	Function: Amino Acids That Can Interact with Functional Group via H-bonding (at pH = 7.4)[a]

[a]"None" is a possible answer.

2. Based on the information in the structure evaluation grid, determine if cefprozil is an *acidic*, *basic*, or *amphoteric* drug. Provide a brief explanation for your answer.

3. Cefprozil is administered orally as a tablet or liquid suspension. Consider each of the acidic and basic functional groups and determine whether each group will be predominantly ionized or unionized as it moves through the gastrointestinal (GI) tract, into systemic circulation, and then into the urine. (The relevant pK_a values = 10, 1.7, and 7.2.) Complete the grid below.

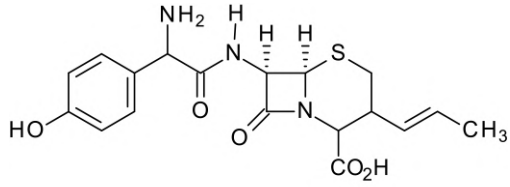
Answer

Name of Functional Group	Acidic or Basic (pK_a)	Ionized or Unionized at pH = 5 (Saliva)	Ionized or Unionized at pH = 1 (Stomach)	Ionized or Unionized at pH = 7.4 (Plasma)	Ionized or Unionized at pH = 8 (Intestine)	Ionized or Unionized at pH = 6 (Urine)

4. Given the predominant ionization state of these acidic and basic functional groups as they traverse the GI tract and the information in the structure evaluation grid, determine in which GI compartment(s) drug absorption could occur.

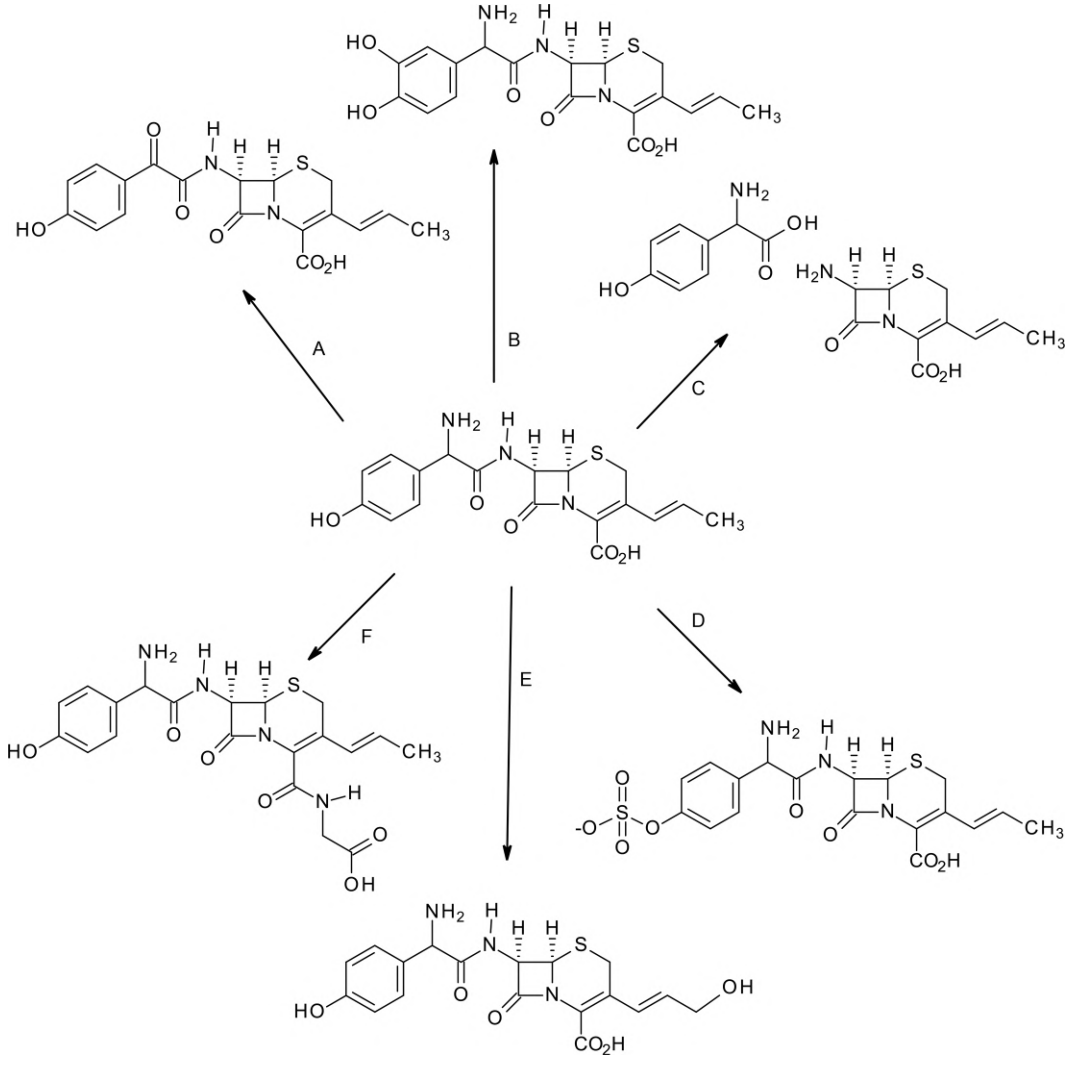
5. There are two geometric isomeric forms of cefprozil, both of which are active as antibacterial agents. Draw the two geometric isomers and identify which is the *Z* isomer and which is the *E* isomer. Would you expect these two isomers to have the same or different physical and chemical properties?

6. Circle all of the chiral carbons found within cefprozil. Determine whether diasteromeric forms of ceprozil are possible. If diastereomers are present, then determine if they would be expected to have similar or different physical and chemical properties.



Cefprozil

7. Approximately 60% of a cefprozil dose is recovered in the urine unchanged. Because impairment in hepatic function increases the half-life of the drug by several hours, it is likely that cefprozil undergoes a variety of metabolic transformations catalyzed by liver enzymes. For each of the metabolic transformations A–F, identify which metabolic transformation has occurred and whether the product formed was the result of a Phase I or Phase II metabolic transformation.

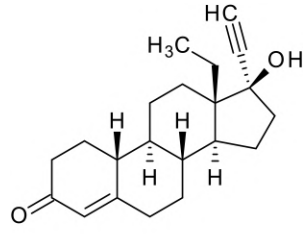


	Name of Transformation	Phase I or Phase II
A		
B		
C		
D		
E		
F		

8. Like the penicillins, the cephalosporins suffer from chemical instability of the β-lactam bond. Chemical hydrolysis of this bond renders the drug inactive. This bond is also subject to cleavage by β-lactamases (due to the presence of a nucleophilic serine side chain [CH_2OH] within the active site of the enzyme). Show how the β-lactam bond can be hydrolyzed by chemical and enzymatic (β-lactamase) mechanisms.

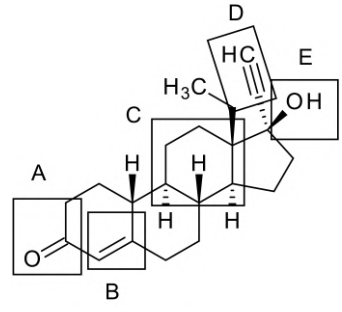
LEVONORGESTREL (LEVEL 3)

Levonorgestrel is a first-generation progestin that is a component of several contraceptive methods. Alone, as well as when paired with estrogen, levonorgestrel is an effective oral contraceptive. This hormone combination is also available as a patch. Levonorgestrel is the active ingredient in an intrauterine device (IUD), in an implantable contraceptive, and as a component of Plan B emergency contraception.



Levonorgestrel

1. Consider only the boxed functional groups found in levonorgestrel and complete the grid below. *Reminder:* When we use the term *solubility*, we think about aqueous solubility whereas when we use the term *absorption*, we think about the ability of a drug to be absorbed across a lipid bilayer.



Levonorgestrel

	Name of Functional Group	Character: Hydrophobic, Hydrophilic, or Both	Function: Contribute to Aqueous Solubility or Absorption
A			
B			
C			
D			
E			

2. In Chapter 2 we discussed that the electronic effect of a functional group is measured by its ability to either donate its electrons to adjacent atoms or functional groups or pull or withdraw electrons away from adjacent atoms or functional groups. Describe the electronic effect of two of the functional groups (A and E) in levonorgestrel. (*HINT:* Remember to consider both inductive and resonance effects!)

3. Consider your answers to Questions 1 and 2 and the information presented in Figures 2-12 and 2-13 and provide a structural rationale for why levonorgestrel can be administered orally as well as via a patch-based formulation.

4. Is levonorgestrel an electrolyte or a nonelectrolyte? Provide a brief structural rationale for your answer.

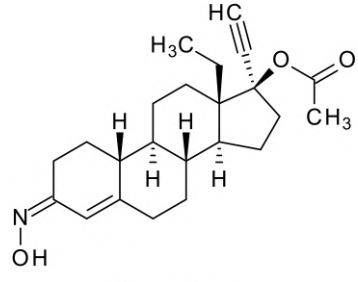
5. Levonorgestrel undergoes extensive protein binding by sex hormone binding globulin (48%) and albumin (50%). Provide a structural rationale for why levonorgestrel is bound to albumin.

6. In Chapter 5, we learned that functional groups have an influence on the partition coefficient of a drug molecule. We know that levonorgestrel can be administered orally (log $P = 3.8$). An orally available prodrug of levonorgestrel (norgestimate) is also available (log $P = 4.11$).

   A. Based on your knowledge of how structure influences log *P*, what type of structural modification to levonorgestrel would cause the log *P* value to decrease?

   B. From a drug solubility perspective, did your proposed structural modification enhance water solubility or lipid solubility?

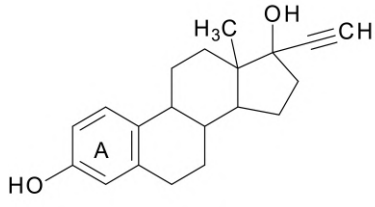
   C. Compared with levonorgestrel, is norgestimate more water soluble or lipid soluble? Provide a structural rationale for this change in log *P*.



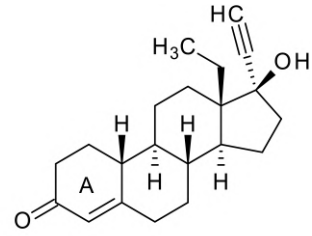
Norgestimate

7. The progesterone receptor contains a hydrophobic cavity in which the receptor agonist interacts. The estrogen receptor contains a similar cavity but also requires the natural hormone to have an aromatic A ring (flat) and to interact via two critical H-bonding interactions that are a specific distance apart. Shown here is ethinyl estradiol, an orally active

estrogen agonist. Provide a structural rationale for why levonorgestrel is an agonist at both the progesterone and estrogen receptors.



Ethinyl estradiol

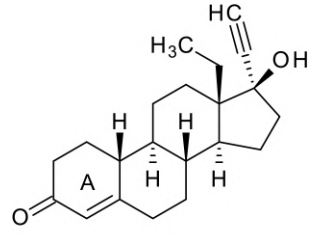


Levonorgestrel

8. In Chapter 7 you learned about different types of isomers. Although marketed as a racemic mixture, levonorgestrel is the only active isomer. For levonorgestrel to bind and activate the progesterone receptor, the steroid ring system must be in an all *trans* configuration to remain relatively flat.

   A. What kind of isomer is the *trans* referring to?

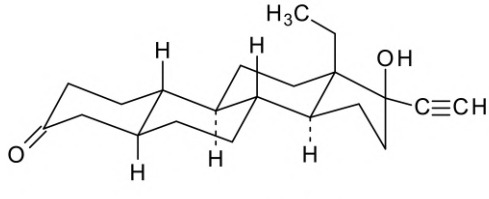
   B. Box all of the *trans* relationships in levonorgestrel.



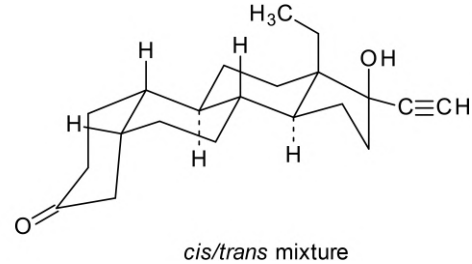
Levonorgestrel

   C. The structures below represent saturated analogs of levonorgestrel (the double bond in the A ring has been reduced). Evaluate the structures below and box the functional groups that are in the *cis* configuration.

   D. Predict whether the steroid skeleton would be able to adopt the shape necessary to allow for interactions with the hydrophobic cavity found in the progesterone receptor and the H-bonding interactions with the estrogen receptor if a mixed *cis/trans* configuration was present.



All *trans* configuration



cis/trans mixture

9. Levonorgestrel does not undergo first pass metabolism and is 100% bioavailable, which you might find somewhat surprising.

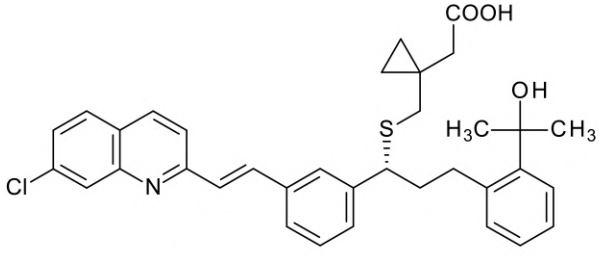
   A. Describe first-pass metabolism and the types of molecules that are typically subject to it.

B. Provide a structural rationale for why you might expect levonorgestrel to exhibit first-pass metabolism.

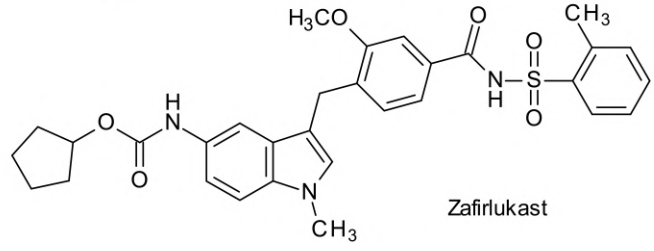
C. Identify which Phase I and II metabolic transformations are possible.

MONTELUKAST AND ZAFIRLUKAST

Shown below are the structures of montelukast and zafirlukast. These drug molecules are administered orally for the treatment of asthma and allergic rhinitis.



Montelukast



Zafirlukast

1. The structure of montelukast contains one acidic functional group ($pK_a = 4.4$) and one basic functional group ($pK_a = 3.1$), whereas the structure of zafirlukast only contains one acidic functional group ($pK_a = 4.3$). Identify these *acidic* and *basic* functional groups and predict whether they will be primarily *ionized* or primarily *unionized* at a stomach pH of 1.9, a urine pH of 5.4, a cellular pH of 6.1, a plasma pH of 7.2, and a solution pH of 8.3.

Functional Group	Acidic or Basic	Primarily Ionized or Unionized				
		1.9	5.4	6.1	7.2	8.3

2. In the previous question, we examined three pK_a values in five different environments for a total of 15 different scenarios. Which of these 15 scenarios allow you to use the Rule of Nines to calculate the percent of ionization of the functional group in the specific environment? Identify the specific scenarios and use the Rule of Nines to calculate the percent of the functional group that would be ionized.

3. The normal pK_a range for sulfonamides is 5 to 10. The pK_a value for the sulfonamide present within the structure of zafirlukast is 4.3. Conduct a structural analysis and provide a reason why this pK_a value is lower than the normal range.

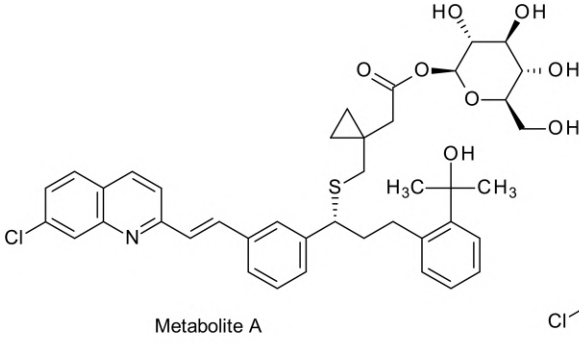
4. The sodium salt of montelukast is required for its oral administration whereas zafirlukast can be administered orally as its unionized free acid form. Conduct a structural analysis of these two drug molecules and provide an explanation for this difference.

5. Montelukast and zafirlukast exert their mechanism of action by interacting with cysteinyl leukotriene receptors and blocking the normal actions of endogenous leukotrienes (LTC_4, LTD_4, and LTE_4). It has been proposed that this interaction requires five key elements: an ionic bond, a hydrogen bond interaction in which the antagonist acts as the acceptor, and the interaction of the antagonist with three separate hydrophobic pockets within the receptor. Using this information and the structures of montelukast and zafirlukast, propose potential binding interactions between these drug molecules and cysteinyl leukotriene receptors. Assume that all binding interactions occur at a physiologic pH of 7.4.

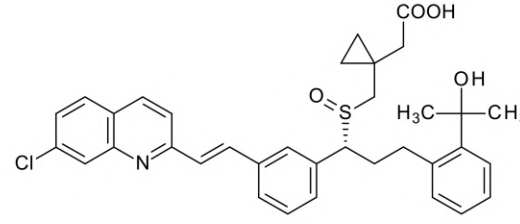
6. Evaluate the structures of montelukast and zafirlukast and determine if they can have *enantiomers*, *diastereomers*, *geometric isomers*, and/or *conformational isomers*.

7. Calculated log *P* values of montelukast and zafirlukast lie in the range of 5.5 to 6.4 depending on the computer program used to predict these values. Given this information, would these drug molecules be predicted to be *highly plasma protein bound* or *minimally plasma protein bound*? Additionally, would you expect these drug molecules to undergo *extensive hepatic metabolism* or be primarily *excreted unchanged*?

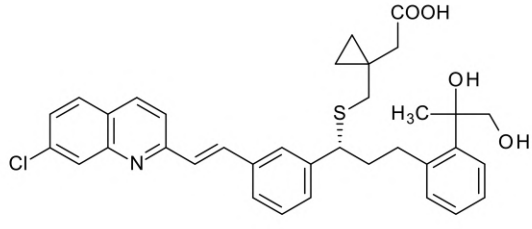
8. Shown below are four known metabolites of montelukast. Identify the metabolic transformations that are required to produce these metabolites. For each transformation, identify if it is a *Phase I* transformation or a *Phase II* transformation.



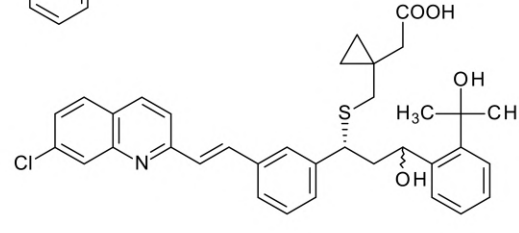
Metabolite A



Metabolite B

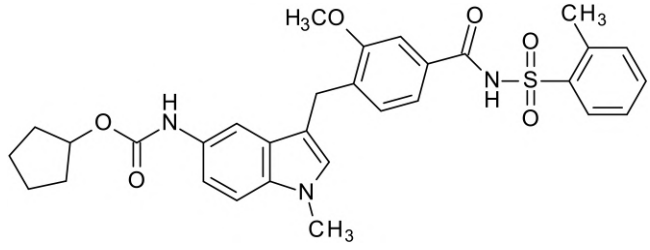


Metabolite C



Metabolite D

9. Shown below is the structure of zafirlukast and a list of five metabolic transformations. For each metabolic transformation, indicate if it is a *Phase I* or a *Phase II* transformation and if zafirlukast has a functional group present to participate in the transformation. If you answer ***YES***, then draw the appropriate metabolite; if you answer ***NO***, then provide a brief explanation as to why this metabolic transformation is not possible to perform with zafirlukast.



Metabolic Pathways

A. Methylation

B. Aromatic oxidation

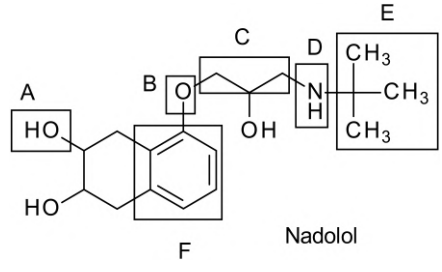
C. Hydrolysis

D. Oxidative *O*-dealkylation

E. Benzylic oxidation

NADOLOL AND OTHER β-ADRENERGIC ANTAGONISTS (LEVEL 3)

Shown below is the structure of nadolol, a β-adrenergic receptor antagonist (aka a β blocker) that is used to treat hypertension and angina. Please note that the stereochemical designations of chiral centers have been purposefully omitted for the first two questions. The SAR of β-adrenergic agonists and antagonists was briefly discussed in Chapter 9, and this information may be helpful in conducting a structural evaluation for some of the questions.

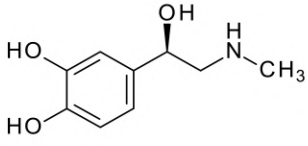


Nadolol

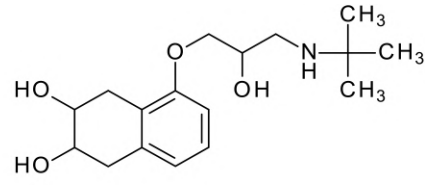
1. Using the table below, identify the six boxed functional groups. For each of the functional groups you identify, indicate if it is primarily *hydrophilic* or *hydrophobic* in character. Also provide a brief explanation for your response.

	Functional Group Name	Hydrophilic or Hydrophobic
A		
B		
C		
D		
E		
F		

2. Epinephrine is a naturally occurring hormone that is secreted by the medulla of the adrenal glands. It plays a role in the "fight or flight" response and acts as an agonist at the β-adrenergic receptors. Compare the structures of nadolol and epinephrine and provide an explanation as to why nadolol is able to act as a β-adrenergic receptor antagonist.

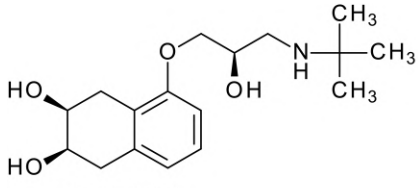


Epinephrine

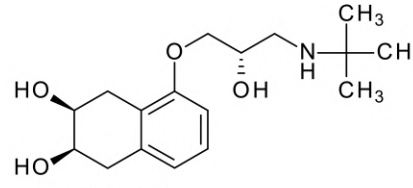


Nadolol

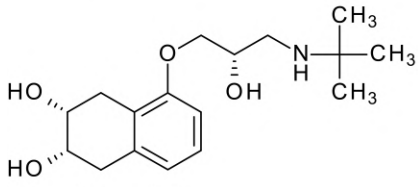
3. The structure of nadolol contains three chiral centers; however, the two hydroxyl groups in the bicyclic tetrahydronaphthylene (aka tetralin) ring are always in a *cis* configuration. Nadolol is marketed as a pair of racemates as shown below. Two of these stereoisomers exhibit superior binding interactions with the β-adrenergic receptor while the other two do not. Using these structures as well as the structure of epinephrine, identify the more active stereoisomers and provide an explanation for your choice.



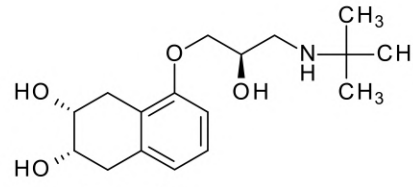
Isomer A-1



Isomer B-1



Isomer A-2



Isomer B-2

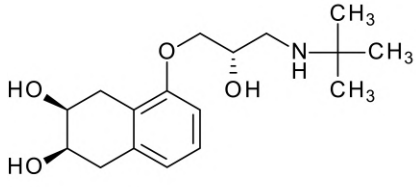
Racemic Pair A

Racemic Pair B

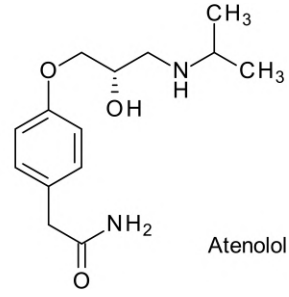
4. In examining the β blocker class of drugs, nadolol has the lowest oral absorption (30%) and the second lowest log *P* value (1.3). Given this information, what chemical strategies could be used to enhance the oral absorption of nadolol?

5. Nadolol is a nonselective β blocker. Its actions at the β_1-adrenergic receptor are responsible for its beneficial effects in hypertension and angina; however, its actions at the β_2-adrenergic receptor are responsible for certain adverse effects, such as bronchoconstriction. Atenolol (shown below) is a selective β_1 blocker and is often preferred over nadolol

and other nonselective β blockers. Compare the structures of nadolol (isomer B-1) and atenolol and offer an explanation why atenolol is selective for the β_1-adrenergic receptor and nadolol is not.

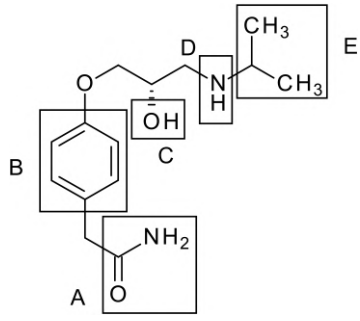


Nadolol



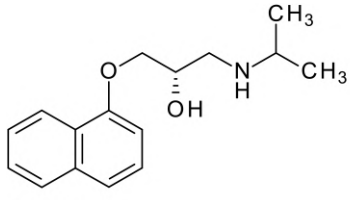
Atenolol

6. Using the table below, identify the types of binding interactions that could be possible between the boxed functional groups on atenolol and the β_1-adrenergic receptor. Also identify amino acids present within a protein receptor whose side chains could participate in the interactions that you identified. Assume a plasma pH = 7.4 for all ionizable functional groups.

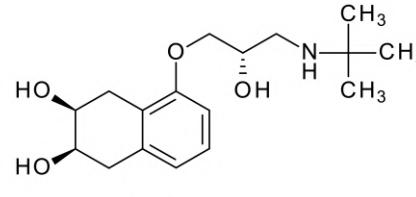


	Functional Group	Types of Binding Interactions	Amino Acids Capable of Forming Specific Binding Interactions
A			
B			
C			
D			
E			

7. Shown below is the structure of propranolol, a nonselective β blocker similar to nadolol. In comparing these two drugs, it is found that one of these undergoes extensive first-pass metabolism while the other is essentially eliminated unchanged. Additionally, one of these drugs can be used to treat central nervous system (CNS) disorders such as anxiety and prophylactic prevention of migraine headaches while the other cannot. Conduct a structural analysis of these two drugs and identify which drug undergoes first-pass metabolism and which drug can be used for CNS disorders.



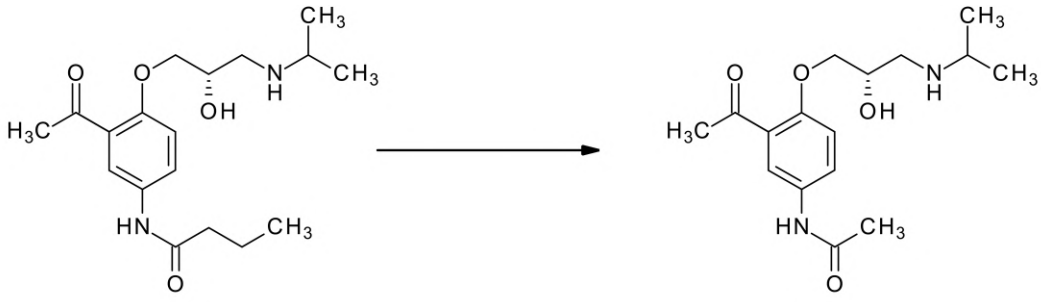
Propranolol



Nadolol

8. The following questions pertain to acebutolol, a selective β_1 blocker similar to atenolol.

   A. Diacetolol is an active metabolite of acebutolol. What metabolic pathways are required to convert acebutolol to diacetolol?



Acebutolol Diacetolol

   B. Shown below is a list of five metabolic transformations. For each metabolic transformation, indicate if it is a Phase I or a Phase II transformation and if the structure of acebutolol has a functional group that can undergo the indicated transformation. For the purposes of this question, only consider the functional groups that are initially present within the structure of acebutolol. If you answer ***YES***, then draw the appropriate metabolite; if you answer ***NO***, then provide a brief explanation as to why this metabolic transformation is not possible for acebutolol.

Metabolic Pathways

   A. Oxidative deamination

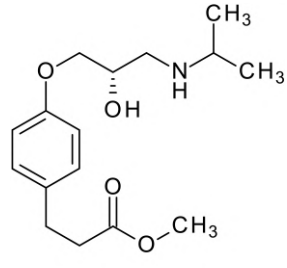
   B. Reduction

   C. Benzylic oxidation

   D. Oxidative *O*-dealkylation

   E. Sulfate conjugation

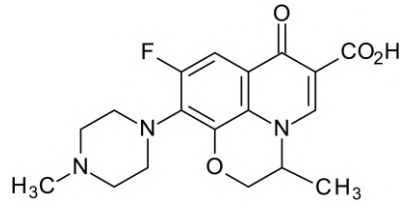
9. Esmolol is similar in structure to both acebutolol and atenolol and is a selective β_1 blocker. It is unique in that it has an extremely short duration of action and must be used as a continuous intravenous (IV) infusion. Esmolol is useful in the acute control of hypertension and in the treatment of certain supraventricular arrhythmias. Identify the metabolic pathway that rapidly inactivates esmolol, draw the product of this reaction, and provide an explanation as to why the metabolite is inactive.



Esmolol

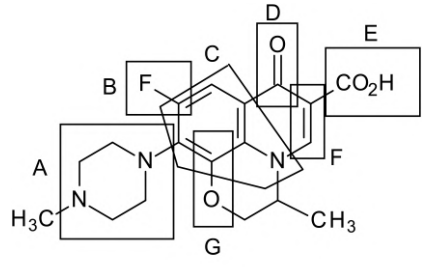
OFLOXACIN (LEVEL 1)

Ofloxacin is a synthetic antibacterial agent and is a member of the second generation of fluoroquinolones. It is formulated as an oral tablet, an eye drop (to treat pink eye), and an ear drop (to treat otitis media) and can be administered by IV route to treat both gram (+) and gram (–) infections. As an oral tablet or IV solution, it is typically used in the treatment of bronchitis; community-acquired pneumonia; infections of the cervix, urethra, and urinary tract; and prostatitis.



Ofloxacin

1. First, we need to conduct a thorough structural evaluation of ofloxacin so that we can explore the different routes of administration. Complete the grid below to start the structure evaluation process. *Reminder:* When we use the term *solubility*, we think about aqueous solubility whereas when we use the term *absorption*, we think about the ability of a drug to be absorbed across a lipid bilayer.



Ofloxacin

	Name of Functional Group	Hydrophobic and/or Hydrophilic	Contributes to Aqueous Solubility and/or Absorption
A			
B			
C			
D			
E			
F			
G			

2. Considering the boxed functional groups found in ofloxacin, which of the functional groups are best described by the following terms. *HINT:* You may need to consider inductive and resonance effects for some of these terms.

   A. Electron withdrawing group

   B. Electrophilic functional group

C. Nucleophilic functional group

D. Electron donating group

3. Ofloxacin has several pK_a values. Complete the table below to provide structural evidence for this. Determine if ofloxacin is an electrolyte, a nonelectrolyte, or is amphoteric.

	Name of Functional Group	Acidic, Basic, or Neutral	pK_a Value/Range
A			
B			
C			
D			
E			
F			
G			

4. As an oral tablet, ofloxacin has 98% oral bioavailability, is 65% to 80% excreted unchanged in the urine, and is 32% protein bound. Consider only the acidic and basic functional groups in each pH environment and, using a qualitative approach, determine if each functional group is predominantly ionized or unionized in each pH environment.

Name of Functional Group	pH = 2 (Stomach)	pH = 7.4 (Plasma)	pH = 5 (Urine)	pH = 8.5 (Large Intestine)

A. Provide a brief structural rationale for why ofloxacin is largely excreted unchanged in the urine.

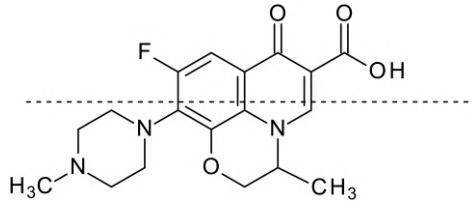
B. Provide a brief structural rationale for why ofloxacin can be formulated as an aqueous solution (pH = 6.5), as an eye drop, and as an ear drop.

5. Drugs are often formulated in a modified form to improve aqueous or lipid solubility.

A. Determine if ofloxacin can be formulated as a sodium salt and/or as a hydrochloride salt. Modify the structure to show the possible inorganic salt form(s) of the drug and determine whether this modification will improve the aqueous or lipid solubility of the drug.

B. What kind of structural modification could be made to ofloxacin to improve its lipid solubility?

6. Four molecules of the fluoroquinolone antibacterial agents interact with bacterial DNA via interactions with functional groups along the top of the molecule. There are also drug-drug self-association interactions along the lower half of the molecule. The molecules themselves are oriented in two pairs.



A. Consider the functional groups on the top half of the molecule. What kind of interactions could those functional groups participate in with the bacterial DNA?

B. Consider the functional groups on the bottom half of the molecule. What kind of interactions could those functional groups have between each pair of molecules as part of the drug-drug self-association interactions?

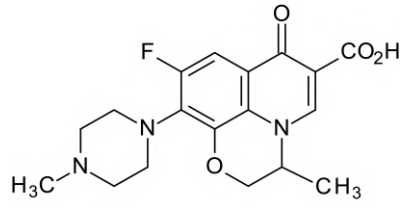
C. Consider the core of the molecule (functional groups that the line is drawn through. What type of interaction is likely to be occurring to keep two molecules paired together?

7. Ofloxacin is marketed as a racemate. Levofloxacin is the active isomer. There has been no effort to market only the active isomer.

   A. Circle the chiral center(s) in ofloxacin.

   B. Which of the following isomers are possible: enantiomers, diastereomers, geometric isomers, conformational isomers?

   C. Given the location of the chiral carbon(s), determine which interactions (drug–target or drug–drug) are impacted by chirality.



Ofloxacin

8. Ofloxacin has 98% oral bioavailability and is primary eliminated unchanged via a renal route. A small portion of each does undergoes metabolism in the liver and is eliminated via a fecal route.

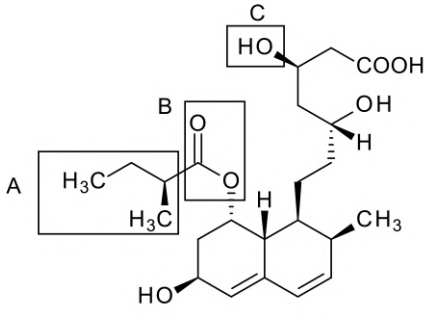
   A. Does ofloxacin undergo first-pass metabolism?

   B. List all possible Phase I and Phase II transformations.

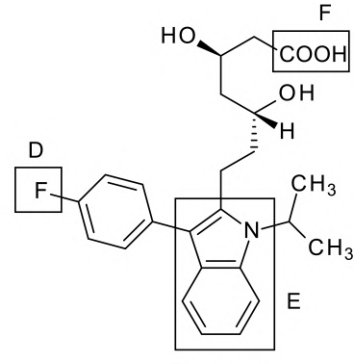
   C. Based on your knowledge about ofloxacin excretion, in which patients is ofloxacin likely to be contraindicated?

PRAVASTATIN AND FLUVASTATIN (LEVEL 2)

Shown below are the structures of pravastatin and fluvastatin. These drug molecules are used in the treatment of various types of hyperlipidemia/dyslipidemia. A total of six functional groups have been boxed.



Pravastatin



Fluvastatin

1. Using the table below, identify the six boxed functional groups. For each of the functional groups you identify, indicate if it is *hydrophilic* or *hydrophobic* in character. Also provide a brief explanation for your response.

	Functional Group Name	Hydrophilic or Hydrophobic
A		
B		
C		
D		
E		
F		

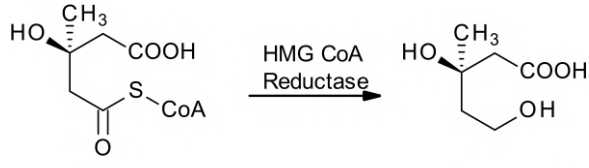
2. The log *P* values of pravastatin and fluvastatin are 1.44 and 3.62, respectively. Conduct a structural analysis of these drug molecules and provide a structural explanation for the difference in these log *P* values.

3. The normal pK_a range for carboxylic acids is 2.5 to 5. The pK_a values for the carboxylic acids present within the structures of pravastatin and fluvastatin are 4.21 and 4.56, respectively. Conduct a structural analysis of these drugs and provide a plausible reason why these pK_a values are at the high end of the normal range.

4. Using the pK_a value of fluvastatin (4.56), calculate the percent of fluvastatin that would be unionized in a urine pH of 5.20.

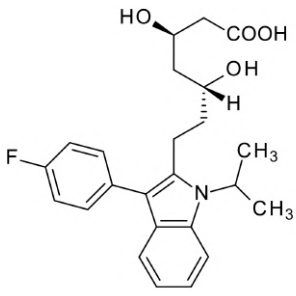
5. Although the Rule of Nines cannot be used to solve Question 4, it can be used to help verify that the answer obtained using the Henderson-Hasselbalch equation is correct. How would you use the Rule of Nines to help verify your answer to question 5?

6. Pravastatin and fluvastatin exert their hyperlipidemic effects by inhibiting the enzyme HMG CoA reductase. As shown below, HMG CoA reductase converts 3-hydroxy-3-methylglutaryl CoA (HMG CoA) to mevalonic acid. This conversion is required for the synthesis of cholesterol and acts as a primary control site for production of this endogenous steroid. Using the structures of HMG CoA, mevalonic acid, pravastatin, and fluvastatin, provide a structural explanation as to how pravastatin and fluvastatin inhibit HMG CoA reductase.

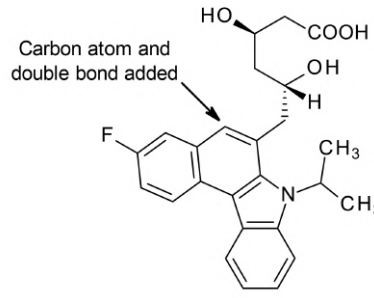


HMG CoA Mevalonic Acid

7. Shown below are the structures of fluvastatin and a conformationally restricted analog. The conformational restriction results from the addition of a carbon atom and a double bond and essentially abolishes the therapeutic activity of fluvastatin. Using these structures, postulate a reason why this structural change results in a loss in activity.

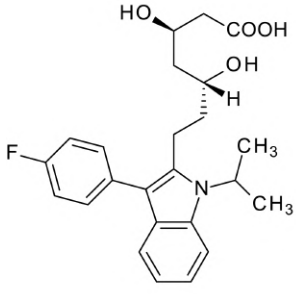


Fluvastatin

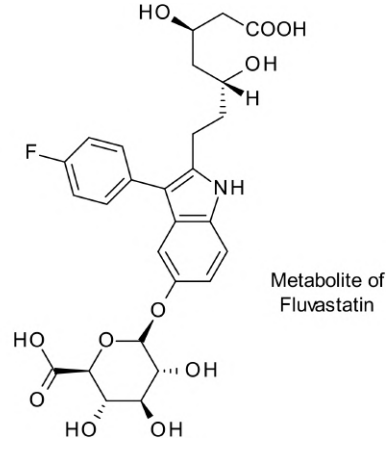


Conformationally Restricted Analog of Fluvastatin

8. Shown below is a known metabolite of fluvastatin. Identify the metabolic transformations required to produce this metabolite.

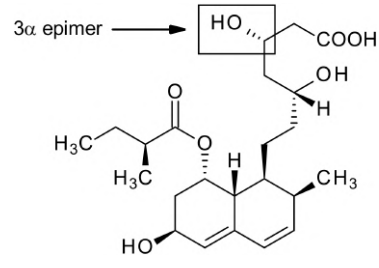


Fluvastatin



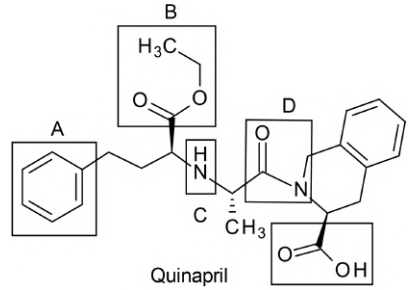
Metabolite of Fluvastatin

9. Pravastatin is primarily metabolized to its 3α epimer. This metabolite is completely inactive as an HMG CoA reductase inhibitor. Identify the metabolic transformations required to produce this metabolite and provide an explanation as to why this metabolite is inactive.



QUINAPRIL (LEVEL 1)

Shown below is the structure of quinapril. It is an angiotensin-converting enzyme (ACE) inhibitor that is used in the treatment of hypertension and heart failure. Five functional groups are identified.

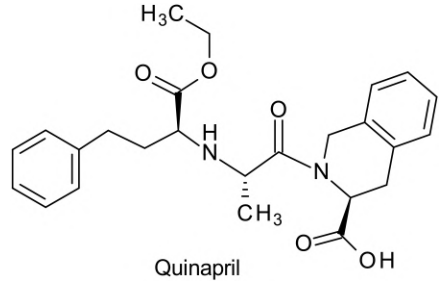


Quinapril

1. Using the table below, identify the five boxed functional groups. For each of the functional groups you identified, indicate if it is *hydrophilic* or *hydrophobic* in character. Also provide a brief explanation for your response.

	Functional Group Name	Solubility Effect of Functional Group
A		
B		
C		
D		
E		

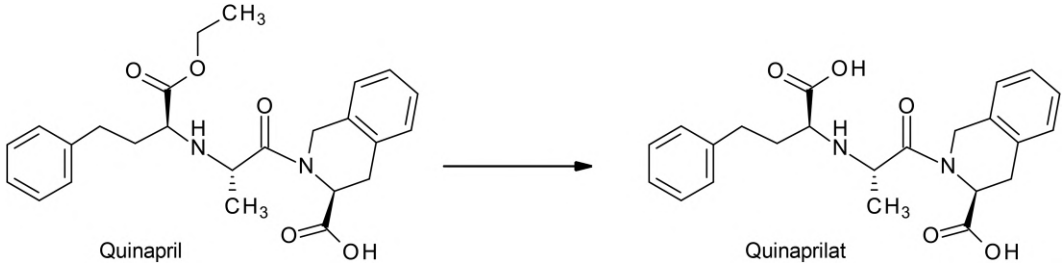
2. Using the unmodified structure of quinapril and the table below, identify all of the *acidic* and *basic* functional groups present in the structure, provide the *normal* pK_a *range* for each functional group, and identify if each functional group would be primarily *ionized* or *unionized* at pH environments of 1.5, 4.8, 6.3, 7.4, and 8.1.



Quinapril

Functional Group	Acidic or Basic	pK_a Range	Primarily Ionized or Unionized				
			1.5	4.8	6.3	7.4	8.1

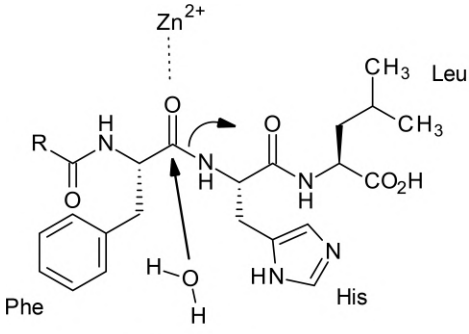
3. Quinapril is a prodrug. It is administered as an oral tablet and converted in vivo to its active metabolite, quinaprilat. Identify the metabolic pathway that converts quinapril to quinaprilat and offer a reason why quinapril is administered orally instead of quinaprilat.



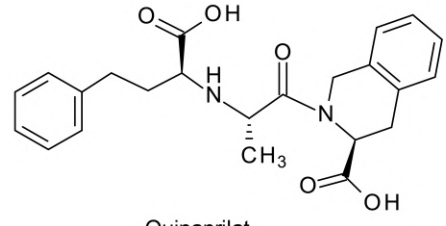
Quinapril Quinaprilat

4. Quinapril inhibits ACE. This enzyme is a relatively nonspecific dipeptidyl carboxypeptidase. It is a zinc protease that converts angiotensin I, a decapeptide, to angiotensin II, an octapeptide. The peptide cleavage is catalyzed by the zinc atom and is shown below. Quinapril,

along with other ACE inhibitors, is a tripeptide mimic that can interact with the enzyme, resulting in enzyme inhibition rather than hydrolysis. Using this information and the structures provided below, identify how quinapril can interact with ACE. Assume that all drug binding interactions are occurring at a pH of 7.4.

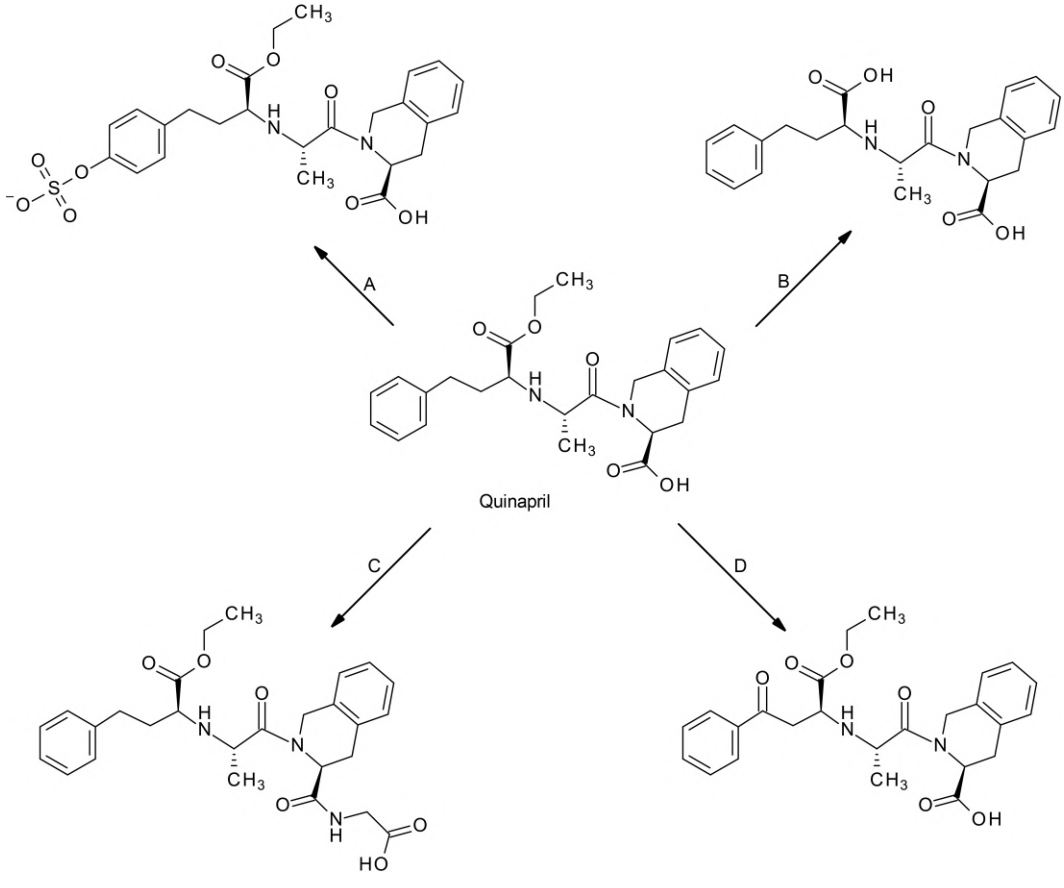


Angiotensin I
R = Asp-Arg-Val-Tyr-Ile-His-Pro

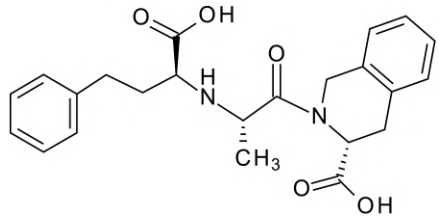


Quinaprilat

5. Shown below are four possible metabolic pathways for quinapril. Identify the metabolic transformations involved in these pathways.

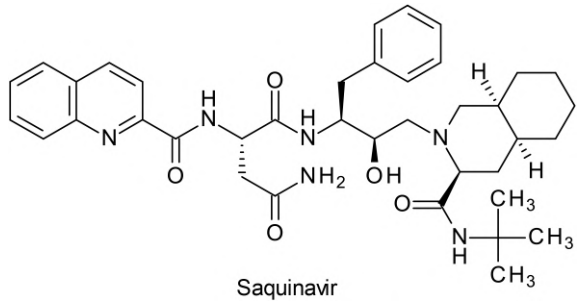


6. Although it is possible for quinapril to undergo all of the above metabolic transformations, pathway B is the major pathway. Other metabolites have been identified but only at very low levels. Provide an explanation for this finding.
7. In evaluating the overall structure of quinapril, identify if it is an *acidic* drug molecule, a *basic* drug molecule, an *amphoteric* drug molecule, or a *nonelectrolyte*.
8. Shown below is a stereoisomer that is significantly less active than quinaliprat. Identify if the stereoisomer is an *enantiomer*, a *diastereomer*, a *geometric isomer*, an *epimer*, and/or a *conformational isomer*. Additionally, provide an explanation as to why this isomer is less active.



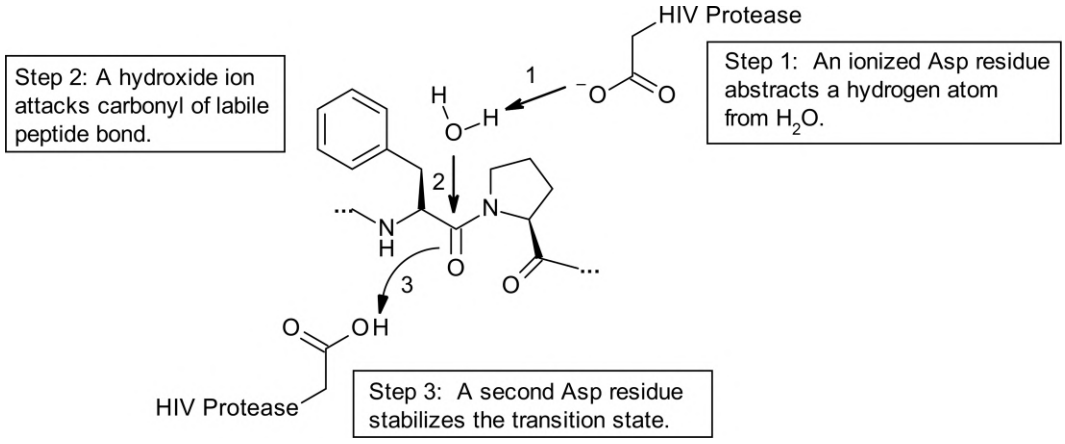
SAQUINAVIR AND OTHER HUMAN IMMUNODEFICIENCY VIRUS PROTEASE INHIBITORS (LEVEL 3)

Shown below is the structure of saquinavir, an antiviral drug used to treat human immunodeficiency virus (HIV) infections.

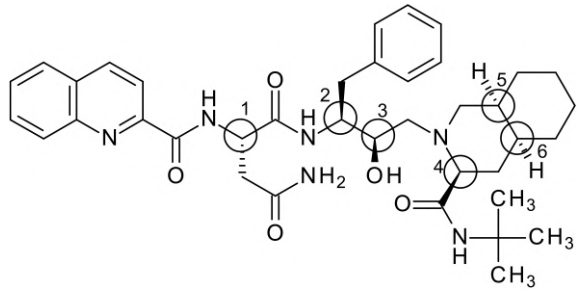


Saquinavir

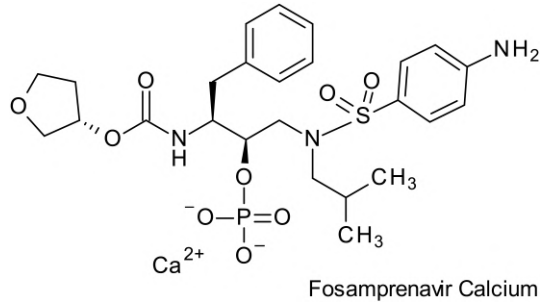
1. Identify the *most* acidic or basic functional group within the structure of saquinavir, provide the *normal* pK_a *range* for this functional group, and identify if it would be primarily *ionized* or *unionized* at a gastric pH of 2.3.
2. Using the functional group you identified in the previous question, draw a water-soluble organic salt. What potential therapeutic benefits would this salt provide?
3. Identify the functional groups present within the structure of saquinavir that allow it to pass through GI membranes and become absorbed into the systemic circulation.
4. Saquinavir inhibits HIV protease, a viral enzyme that cleaves viral precursor proteins into mature, active proteins. As part of its mechanism, HIV protease uses a pair of aspartic acid residues to catalyze the cleavage of a peptide bond between phenylalanine and proline as shown below. HIV protease can also cleave peptide bonds between tyrosine and proline. Using this information and the structure of saquinavir, provide an explanation how saquinavir inhibits HIV protease.



5. The structure of saquinavir contains six chiral centers that have been circled below. Provide an explanation why the alteration of the stereochemistry at any of these chiral centers could lead to a decrease in the ability to inhibit HIV protease.



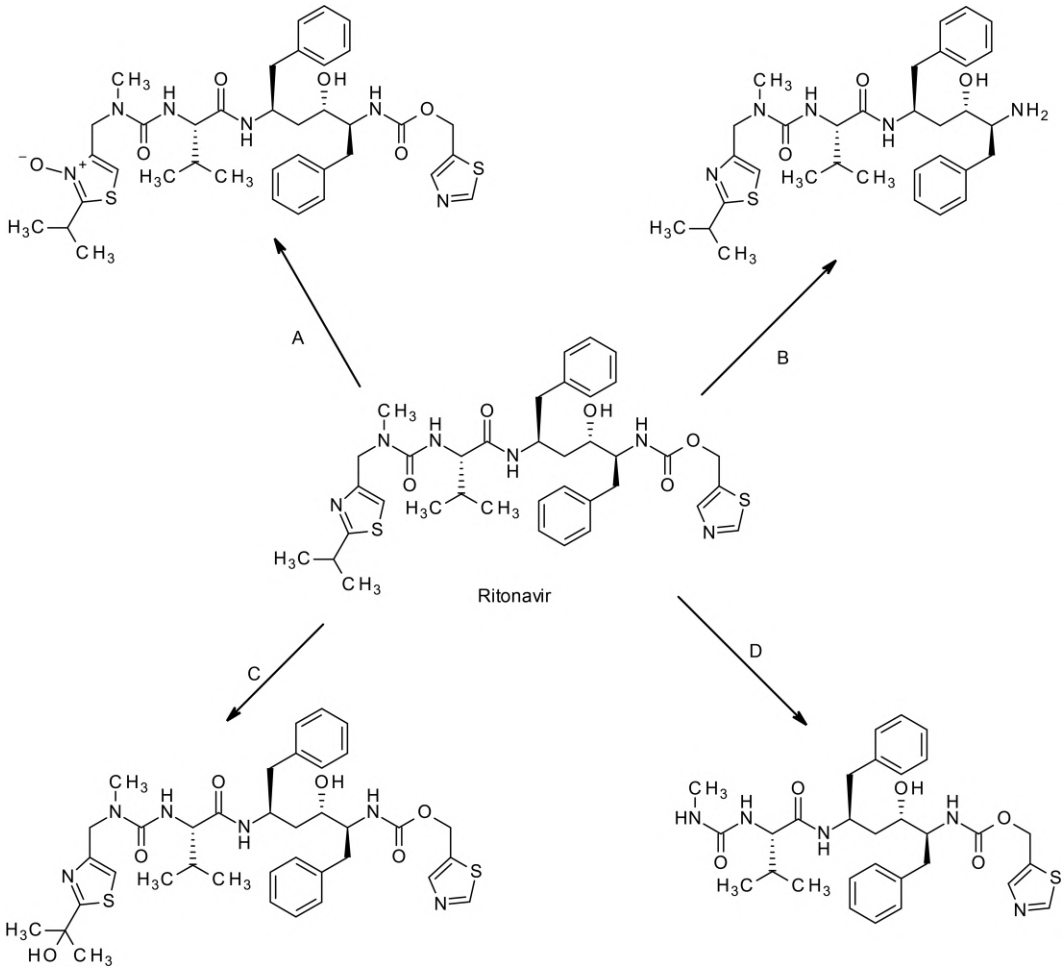
6. Fosamprenavir calcium, shown below, is an orally administered prodrug of amprenavir. Using the structure and name of fosamprenavir, identify the metabolic transformation that is required to convert it to amprenavir, draw the structure of amprenavir, and provide potential advantages for using this prodrug.



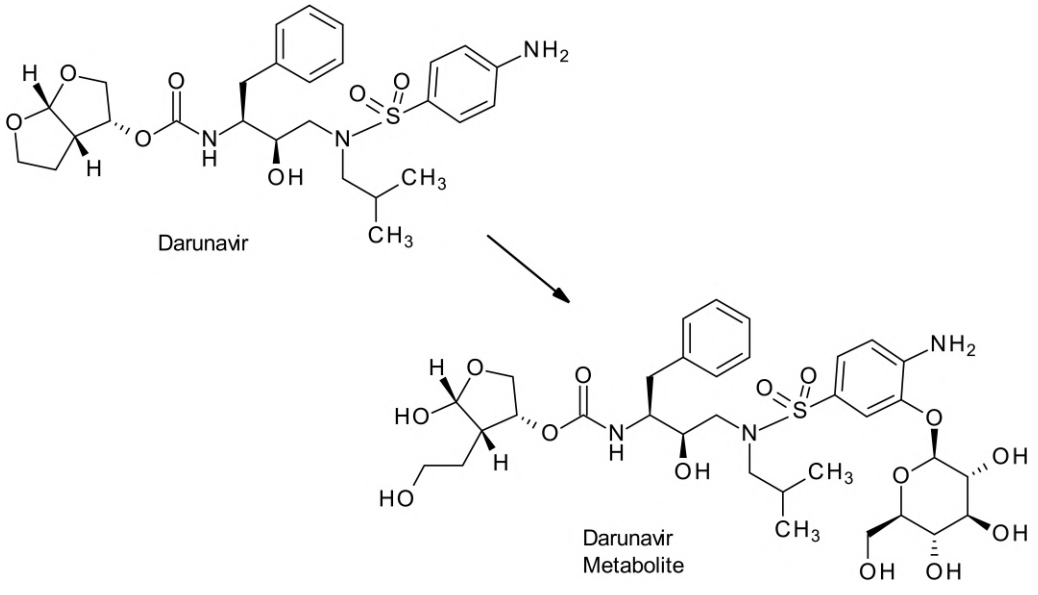
Fosamprenavir Calcium

7. All HIV protease inhibitors contain similar, but not identical, structural features. Compare the structures of saquinavir and amprenavir and identify the structural similarities that allow both of these drugs to bind to the same enzyme. Your answer should include potential binding interactions of these two drugs.

8. Ritonavir is also used to treat HIV infections; however, due to its adverse effects profile, it is rarely used at therapeutic doses. Instead, it is used as a pharmacokinetic enhancer. Ritonavir is both a substrate and an inhibitor of CYP3A4. The ability to inhibit CYP3A4 prevents the metabolism of other HIV protease inhibitors and enhances their actions. Shown below are four possible metabolic pathways for ritonavir. For each pathway, identify the metabolic transformation involved and indicate whether it could be catalyzed by the CYP3A4 isozyme.



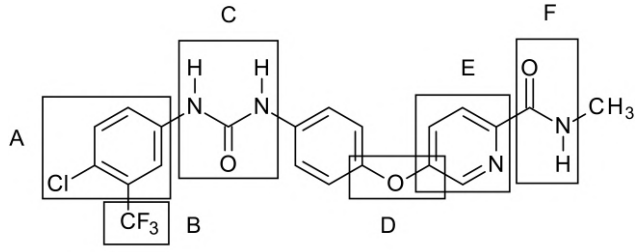
9. Shown below is darunavir, a structural analog of amprenavir, and one of its possible metabolites. Identify the metabolic transformations that would be required to convert darunavir to this metabolite.



SORAFENIB (LEVEL 2)

Because protein tyrosine kinases regulate cellular proliferation, differentiation, and survival, it is no surprise that several neoplastic disorders can be tied to altered protein tyrosine kinase activity. Clinically relevant antineoplastic tyrosine kinase inhibitors interact with the active site of the enzyme via several types of binding interactions. The adenosine triphosphate (ATP) binding domain of the tyrosine kinases contains a hydrophobic domain that includes a significant number of isoleucine, leucine, alanine, and valine residues. At least five binding pockets flank this region in which van der Waals, hydrophobic, hydrogen bonding, and electrostatic interactions occur.

Sorafenib is a tyrosine kinase inhibitor used in the treatment of advanced renal cell carcinoma, a highly vascularized tumor. The drug specifically targets vascular endothelin growth factor 2 (VEGF2), which is instrumental in the generation of new blood vessels.



Sorafenib

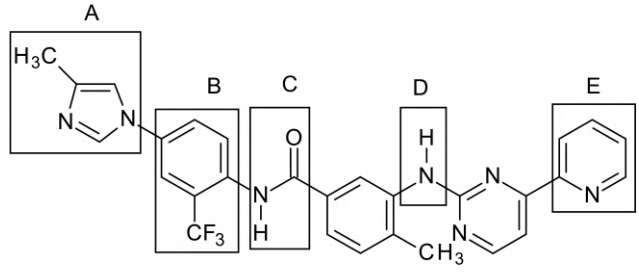
1. Conduct a structural evaluation of sorafenib, focusing on the boxed functional groups, and use the information in the grid to inform your answers to the questions that follow.

	Name of Functional Group	Hydrophilic and/or Hydrophobic	Acidic, Basic, or Neutral (Provide pK_a When Relevant)	Contributes to Aqueous Solubility and/or Absorption	Interaction(s) Possible with Biological Target at Physiologic pH = 7.4
A					
B					
C					
D					
E					
F					

2. Sorafenib interacts with Cys^{919}, Phe^{1047}, and Asp^{1046} via hydrogen bonding and hydrophobic interactions. Identify which functional groups could interact with the side chains of these amino acids. Assume that Asp^{1046} is unionized in the local environment of the enzyme.

Functional Group	Interacts with Cysteine^{919} via a Hydrogen Bonding Interaction	Interacts with Aspartic Acid^{1046} via a Hydrogen Bonding Interaction	Interacts with Phenylalanine^{1047} via a Hydrophobic Interaction
	Yes or No	Yes or No	Yes or No
A			
B			
C			
D			
E			
F			

3. Nilotinib, another tyrosine kinase inhibitor (via Bcr-Abl) indicated for the treatment of Philadelphia Chromosome (Ph^+) positive chronic myelogenous leukemia, also interacts with each of the five binding pockets found within this biological target. This drug interacts with Leu^{285}/Val^{289}, Asp^{391}/Glu^{286}, Thr^{315}, Met^{318}, and Leu^{298}/Val^{299}/Phe^{359} in each of the respective five binding pockets.



Nilotinib

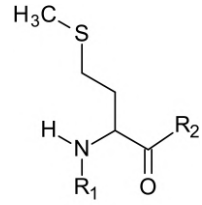
A. Consider the side chains of the amino acids indicated and determine which type(s) of binding interactions is/are possible in each of the five binding pockets. Assume pH = 7.4.

Leu^{285}/Val^{289}	Asp^{391}/Glu^{286}	Thr^{315}	Met^{318}	Leu^{298}/Val^{299}/Phe^{359}

B. Determine which of the boxed functional groups (A–E) can interact with the side chains of the amino acids found in each of the five binding pockets. Indicate the type of interaction(s) possible in the appropriate box. "None" is an acceptable answer. Assume that the drug and the amino acid side chains are both at pH = 7.4.

	Leu^{285}/Val^{289}	Asp^{391}/Glu^{286}	Thr^{315}	Met^{318}	Leu^{298}/Val^{299}/Phe^{359}
A					
B					
C					
D					
E					

C. It has been documented that the pyridyl nitrogen atom (functional group E) of nilotinib participates in a hydrogen bonding interaction with methionine. Draw a diagram that clearly shows which atom(s) within the structure of methionine participate in this interaction.

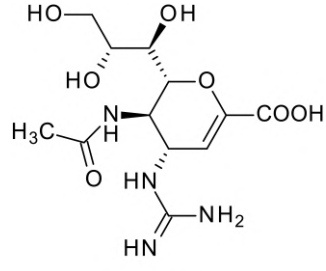


Methionine

4. Sorafenib enters cells via passive diffusion. Using the information in the structure evaluation grid as a starting point, identify which functional groups contribute to the ability of this drug to enter cells via passive diffusion.
5. Nilotinib is considered significantly more hydrophobic than sorafenib (distribution coefficient log *D* is 2.4 and 0.8, respectively). Provide a structural rationale for this property difference.
6. Sorafenib is marketed as a tosylate salt, a lipid-soluble organic salt. Nilotinib is marketed as a hydrochloride monohydrate salt, an inorganic salt. In general, what is the value of each of these types of salts?
7. At least 50% and 69% of a sorafenib and nilotinib dose, respectively, are eliminated fecally. Both drugs undergo CYP3A4 mediated oxidation. List *ALL* possible Phase I metabolic transformations that represent oxidative transformations for each drug.
8. Both drugs are more than 99.5% plasma protein bound, primarily to serum albumin. While bound to albumin, is it possible for either of these drugs to be metabolized or eliminated or interact with their respect biological targets?

ZANAMIVIR AND OSELTAMIVIR (LEVEL 2)

Shown below is the structure of zanamivir. This drug molecule is administered via oral inhalation for the treatment of influenza A and B infections.



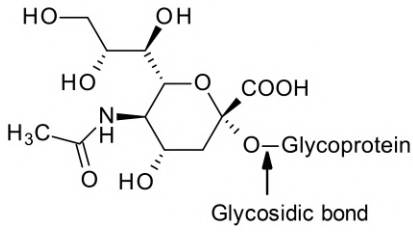
Zanamivir

1. Identify all of the *acidic* and *basic* functional groups, provide the *normal* pK_a *range* for each functional group and identify whether each functional group would be primarily *ionized* or *unionized* at a pulmonary pH of 7.2.

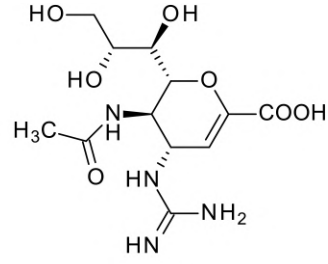
2. Identify all other water-soluble functional groups that are present within the structure of zanamivir.

3. Based on your answers to Questions 1 and 2, explain why zanamivir is administered as an oral inhaler instead of an oral tablet or capsule.

4. Zanamivir exerts its antiviral action by inhibiting neuraminidase, a viral enzyme that is required for the spread of the viral infection. A key component of neuraminidase's action is the hydrolysis of *N*-acetylsialic acid from surface viral glycoproteins. Shown below is the structure of *N*-acetylsialic acid bound to a glycoprotein. Using this structure and the structure of zanamivir, provide an explanation for how zanamivir inhibits neuraminidase.

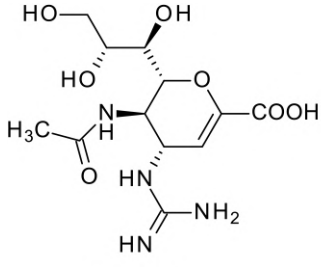


N-Acetylsialic acid bound to glycoprotein

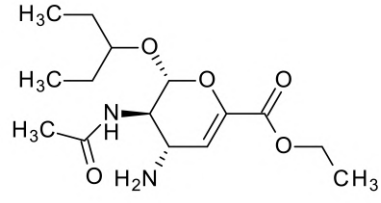


Zanamivir

5. Oseltamivir is a structural analog of zanamivir. It has the same mechanism of action of zanamivir but differs in that it can be administered as either an oral capsule or oral suspension. Conduct a structural comparison of oseltamivir and zanamivir and provide an explanation of why oseltamivir is better suited for oral administration.



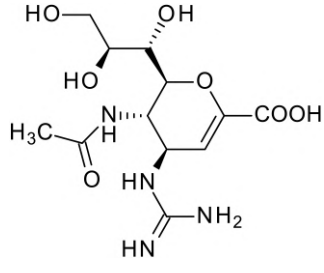
Zanamivir



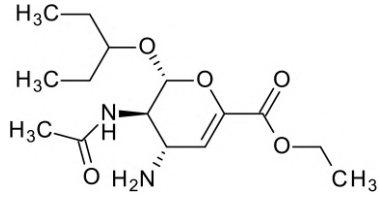
Oseltamivir

6. The pK_a of the primary amine found within the structure of oseltamivir has been reported to be 7.7. What percent of this functional group is ionized at an intestinal pH of 6.5?

7. Shown below is a stereoisomer of zanamivir. Identify if the stereoisomer is an *enantiomer*, a *diastereomer*, a *geometric isomer*, an *epimer*, and/or a *conformational isomer*. Predict whether this stereoisomer's pharmacological activity is likely to be *more active*, *less active*, or *similar to* that of zanamivir.



8. Shown below is the structure of oseltamivir and a list of five metabolic transformations. For each metabolic transformation, indicate whether it is a Phase I or Phase II transformation and whether oseltamivir has a functional group present that can undergo the indicated transformation. When evaluating these metabolic transformations, consider functional groups that are initially present within the structure of oseltamivir as well as those that can be added/revealed through Phase I metabolism. If you answer ***YES***, then draw the appropriate metabolite; if you answer ***NO***, then provide a brief explanation as to why this metabolic transformation is not possible for oseltamivir.



Metabolic Pathways

A. Hydrolysis

B. Allylic oxidation

C. Glucuronide conjugation

D. ω-Oxidation

E. Oxidative *O*-dealkylation

9. Using the information discussed in previous questions, provide an explanation why zanamivir is not metabolized and is excreted unchanged in the urine.

ANSWERS TO CHAPTER QUESTIONS

CHAPTER 2

Structural Analysis Checkpoint

Checkpoint Drug 1: Venetoclax

1. Answers provided in table below.

	Functional Group Name	Contribution to Water and/or Lipid Solubility
A	Halogen (chlorine atom)	Lipid solubility
B	Alicyclic ring, alkyl ring, cycloalkane	Lipid solubility
C	Tertiary amine (piperazine)	Water solubility
D	Heterocyclic ring system (pyrrolopyridine)	Hydrocarbons: lipid solubility
		Nitrogen atoms: water solubility
E	Aromatic ring; phenyl ring; aromatic hydrocarbon	Lipid solubility
F	Sulfonamide	Water solubility
G	Secondary aromatic amine/aniline	Water solubility
H	Ether	Hydrocarbons: lipid solubility
		Oxygen atom: water solubility

2. The sulfonamide and tertiary amine are primarily ionized in most physiologic environments and can participate in ion–dipole interactions (as the ion) with water. In the event that they are unionized, they could participate in hydrogen bonding interactions with water. The nitrogen atoms of the heterocyclic ring system, as well as the secondary aromatic amine, and the oxygen atom of the ether will not be appreciably ionized but can participate in hydrogen bonding interactions with water. Thus, all of these functional groups contribute to the water solubility of venetoclax. The halogen as well as the hydrocarbon chains and rings are not able to ionize or form hydrogen bonds with water and thus contribute to the lipid solubility of venetoclax.

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3. Answers provided in table below.

	Electron Donating or Withdrawing	Resonance or Induction
A	Electron withdrawing	Induction
B	Both	Donates electrons into the aromatic ring through resonance
		Withdraws electrons from adjacent methylene groups through induction
C	Electron donating	Resonance
D	Electron withdrawing	Induction (from aromatic ring)
		Resonance (from ionized sulfonamide)
E	Electron withdrawing	Resonance

Checkpoint Drug 2: Elamipretide

1. Answers provided in the grid below.

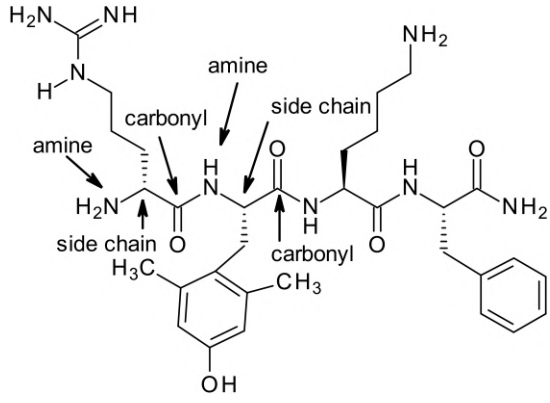
	Name of Functional Group	Character: Hydrophobic, Hydrophilic, or both	Function: Contribute to Solubility or Absorption
A	Guanidine	Hydrophobic (R)	Absorption (R)
		Hydrophilic ($H_2NCNHNH$)	Solubility ($H_2NCNHNH$)
B	Primary amine	Hydrophobic (R)	Absorption (R)
		Hydrophilic (NH_2)	Solubility (NH_2)
C	Amide	Hydrophobic (R)	Absorption (R)
		Hydrophilic ($C{=}ONH_2$)	Solubility ($C{=}ONH_2$)
D	Aromatic hydrocarbon; aromatic ring; phenyl ring	Hydrophobic (R)	Absorption (R)
E	Phenol	Hydrophobic (R)	Absorption (R)
		Hydrophilic (OH)	Solubility (OH)

R = carbon scaffolding.

2. **Part A:** Every amino acid has an amine (basic), a unique side chain, and a carboxylic acid (acidic). As building blocks of proteins, the amine and carboxylic acid of adjacent amino acids are linked to form an amide or peptide linkage (neutral).

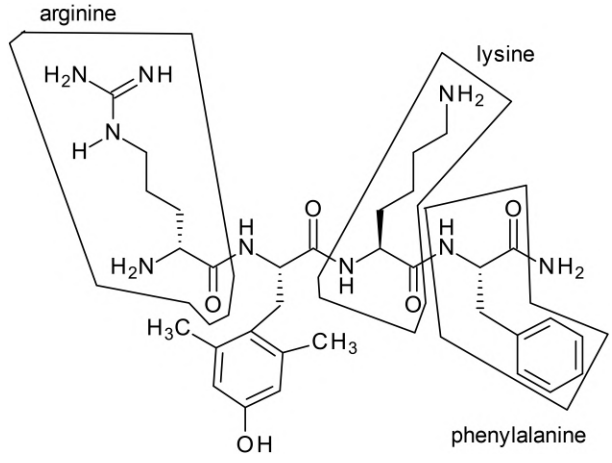
   Part B: As building blocks of endogenous proteins or peptidomimetic drugs, the amine and carboxylic acid of adjacent amino acids are linked to form a peptide bond (amide = neutral). The easiest way to read these kinds of molecules is to look for the pattern "amine-side chain-carbonyl" (representing one amino acid) and to completely ignore the fact that the

amine and adjacent carboxylic acid are really an amide. In the diagram below, the "amine-side chain-carbonyl" pattern for the first two amino acids/amino acid derivatives is shown.



Using this pattern, the first amino acid in the sequence is the amino acid arginine, the second amino acid is a derivative of tyrosine, the third amino acid is lysine, and the fourth amino acid is phenylalanine.

Part C: The portions of the molecule that represent arginine, lysine, and phenylalanine have been boxed.



Part D: The second amino acid is a derivative of tyrosine.

3. Answers provided in the grid below.

	Name of Amino Acid or Amino Acid Derivative	Amino Acid Side Chain Evaluation: Hydrophobic, Hydrophilic, or Both	Amino Acid Side Chain Evaluation: Acidic, Basic, Neutral	Amino Acid Side Chain Evaluation: Nucleophilic, Electrophilic, NA
1	Arginine	Hydrophilic ($NHC{=}NHNH_2$)	Basic	NA
		Hydrophobic (R)		
2	Tyrosine derivative	Hydrophilic (OH)	Acidic	Nucleophilic
		Hydrophobic (R)		
3	Lysine	Hydrophilic (NH_2)	Basic	Nucleophilic
		Hydrophobic (R)		
4	Phenylalanine	Hydrophobic	Neutral	NA

R = carbon scaffolding.

Review Questions

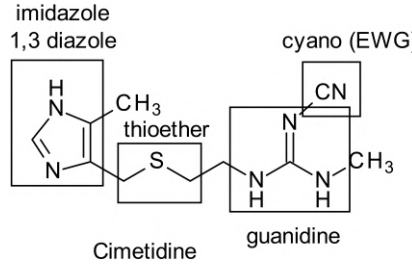
1. Answers provided in the grid below.

Box	Functional Group Name
A	Aliphatic alkane; aliphatic chain
B	Sulfonamide
C	Aniline; aromatic amine
D	Secondary alcohol (or hydroxyl)
E	Aromatic hydrocarbon (or aromatic ring), phenyl ring
F	Carbamate
G	Ether

2. Answers provided in the grid below.

Box	Functional Group Name
A	Ether
B	Tertiary amine
C	Secondary alcohol (or hydroxyl)
D	Cycloalkane; alicyclic ring
E	Ester
F	Quaternary ammonium bromide
G	Alkene

3. **Part A:** Functional groups are identified in the structure shown below.



Part B: The aromatic heterocycle (1,3 diazole) and the cyano group are both electron withdrawing groups. The cyano group will significantly decrease the magnitude of the pK_a value for the guanidine. The experimentally measured pK_a value for the guanidine in cimetidine is in the range of 6.7 to 6.9.

Part C: The guanidine is not protonated at physiologic pH because the cyano group is an electron withdrawing group and pulls (or withdraws) electrons from the guanidine through induction. This decreases the ability of the guanidine to attract protons (H^+); therefore, its basicity will decrease. The experimentally measured pK_a values for cimetidine are all in the range of 6.7 to 6.9. The guanidine and aromatic heterocycle are both basic in character. At pH = 7.4, the pH > pK_a for these functional groups and, therefore, the basic functional groups are predominantly unionized in this pH environment.

4. **Part A:** Answers provided in the grid below.

Name of Two Oxygen Containing Functional Groups	Hydrophilic and/or Hydrophobic	Contribution to Water Solubility and/or Lipid Solubility	Hydrogen Bond Acceptor, Donor, Both, or Neither
Primary alcohol (or hydroxyl)	Hydrophilic (OH); hydrophobic (R)	Water solubility (OH)	Hydrogen bond acceptor and donor
		Lipid solubility (R)	
Secondary alcohol (or hydroxyl)	Hydrophilic (OH); hydrophobic (R)	Water solubility (OH)	Hydrogen bond acceptor and donor
		Lipid solubility (R)	

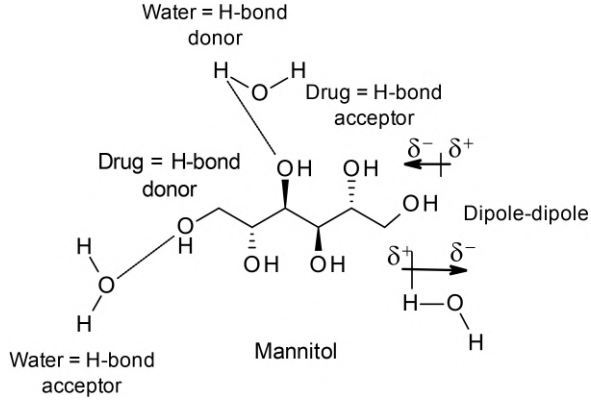
R = carbon scaffolding.

Part B: The primary and secondary alcohols have hydrophilic character. These alcohols (primary and secondary) interact with water as both hydrogen bond acceptors and donors, which means that they make a sizable contribution to the water solubility of mannitol.

Part C: The primary and secondary alcohols have hydrophilic character. The alcohols (primary and secondary) are able to interact with water as both hydrogen bond acceptors and donors, which means that they are able to attract and interact with water.

One mechanism to relieve the swelling in the brain is to remove the excess fluid via the blood brain barrier. Mannitol achieves this by attracting and interacting with water with its six OH groups.

Part D: Answer provided in figure below.



5. **Part A:** Answers provided in the grid below.

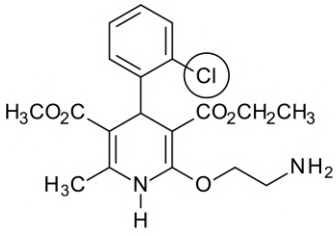
Name of Functional Group	Hydrophilic and/or Hydrophobic	Contribution to Water Solubility and/or Lipid Solubility
Aromatic hydrocarbon	Hydrophobic	Lipid solubility
Ether	Hydrophobic (R)	Lipid solubility (R)
	Hydrophilic (O atom)	Water solubility (O atom)
Tertiary amine	Hydrophobic (R)	Lipid solubility (R)
	Hydrophilic (N atom)	Water solubility (N atom)

R = carbon scaffolding.

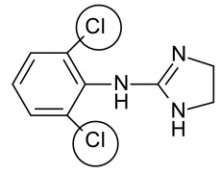
Part B: Diphenhydramine has two aromatic hydrocarbons that are hydrophobic in character and contribute significantly to lipid solubility and drug absorption. These features

contribute to the ability of the drug to easily absorb into the skin, which has considerable lipid character. Please note that the ether and tertiary amine groups have R groups that do contribute, albeit minimally, to the overall hydrophobic character of the drug.

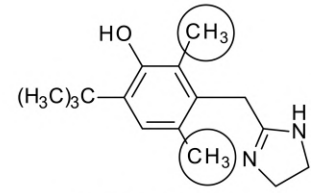
6. Functional groups that influence the shape of each molecule have been circled.



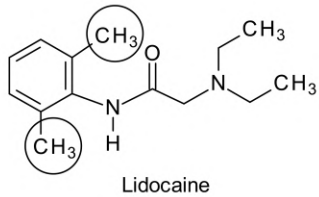
Amlodipine



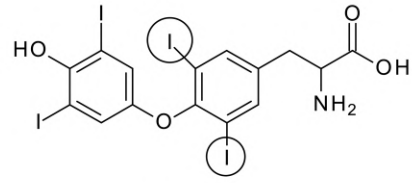
Clonidine



Oxymetazoline



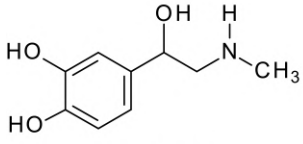
Lidocaine



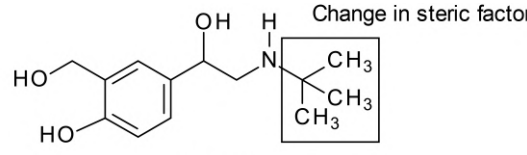
Thyroxine

7. Functional group and explanation provided below.

Part A: The *t*-butyl substituent that is part of the secondary amine is responsible for the β receptor selectivity demonstrated by albuterol.

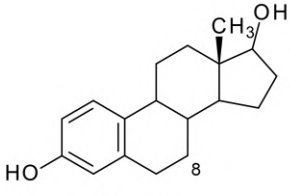


Epinephrine

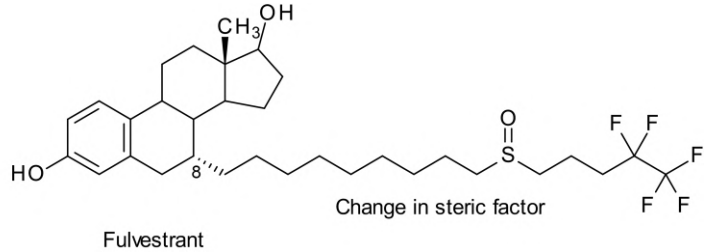


Albuterol

Part B: The 8α substituent is responsible for fulvestrant binding to, but not activating, the estrogen receptor.



Estrogen



Fulvestrant

8. The only structural difference between these two molecules is the presence of a tertiary amine in the parent drug and an *N*-methylated secondary amine in the active metabolite. The parent drug (with the tertiary amine) selectively inhibits the reuptake of serotonin. The active metabolite (desmethyl metabolite) selectively inhibits the reuptake of norepinephrine. This change in the number of methyl substituents represents a change in the steric character of the molecule.

9. **Part A:** The portion of the molecule designated as "C" is the amino acid valine. The side chain of this amino acid is hydrophobic in character and improves the ability of the drug to be absorbed across lipophilic membranes. This characteristic decreases the water solubility of the drug.

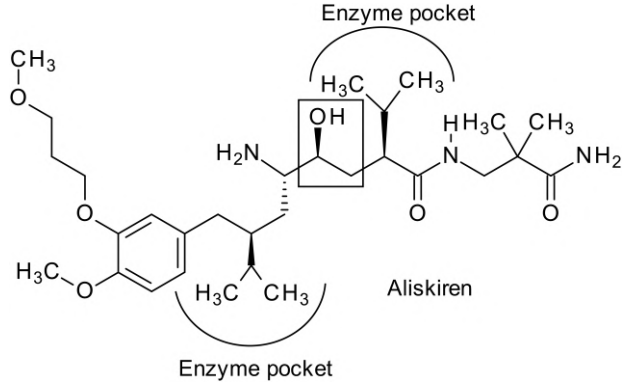
   Part B: The portions of the molecule designated as "A" and "B" resemble the side chain of the amino acid phenylalanine. The side chain of this amino acid is hydrophobic in character and interacts with hydrophobic regions present within the biological target. The most likely interactions would be with the side chains of the aromatic amino acids phenylalanine, tyrosine, and tryptophan; however, it could also interact with the side chains of the aliphatic amino acids alanine, valine, leucine, and isoleucine. The ritonavir side chains "A" and "B" can also participate in π-π stacking interactions with the side chains of phenylalanine, tyrosine, tryptophan, and histidine present within the biological target. It is also possible for these side chains to participate in cation-π interactions with lysine and arginine that are ionized at physiologic pH.

10. **Part A:** The box represents the side chain of the amino acid mimic. The circle represents the carbonyl carbon of the amino acid mimic. In the case of aliskiren, the side chain *mimics* the side chain of valine, and the carbonyl has been replaced with a secondary alcohol.

    Part B: The next sequence [→,□, ○] is a mimic of the amino acid valine.

    Part C: There are likely to be van der Waals and hydrophobic interactions between renin and the amino acid side chains of Leu and Val that are "docked" in the renin enzyme pockets.

    Part D:



11. **Functional group A:** This is a methoxy group (a methyl ether). Because it is directly attached to the aromatic ring, it acts as an electron donating group through resonance.

    Functional group B: This is a tertiary amine that will most likely be ionized at physiologic pH. Because it is more electronegative than its surrounding carbon and hydrogen atoms (and most likely carries a positive charge), it acts as an electron withdrawing group through induction.

    Functional group C: This is a tertiary hydroxyl group (or tertiary alcohol). Because it is not directly attached to an aromatic ring, it cannot donate electrons. Due to its electronegativity, it acts as an electron withdrawing group through induction.

12. **Part A:** Tyrosine (Tyr)—Aspartic Acid (Asp)—Valine (Val)—Glutamine (Gln)

    Part B: Tyrosine: This side chain is one of the largest seen in amino acids and thus is involved in dictating the overall conformation of the peptide. Although the phenyl ring is hydrophobic, the phenolic hydroxyl group can act as both a hydrogen bond donor and acceptor and thus contribute to the overall water solubility.

Aspartic Acid: This side chain is acidic and will most likely be ionized at physiologic pH, which enhances the overall water solubility of the peptide. It will most likely reside in a water-soluble pocket of the overall peptide.

Valine: This is an aliphatic, lipid-soluble, hydrocarbon side chain. It contributes to the overall lipid solubility of the peptide and can contribute to lipid-soluble pockets within the peptide. Additionally, the isopropyl side chain can impart some steric bulk that may influence the overall conformation of the molecule.

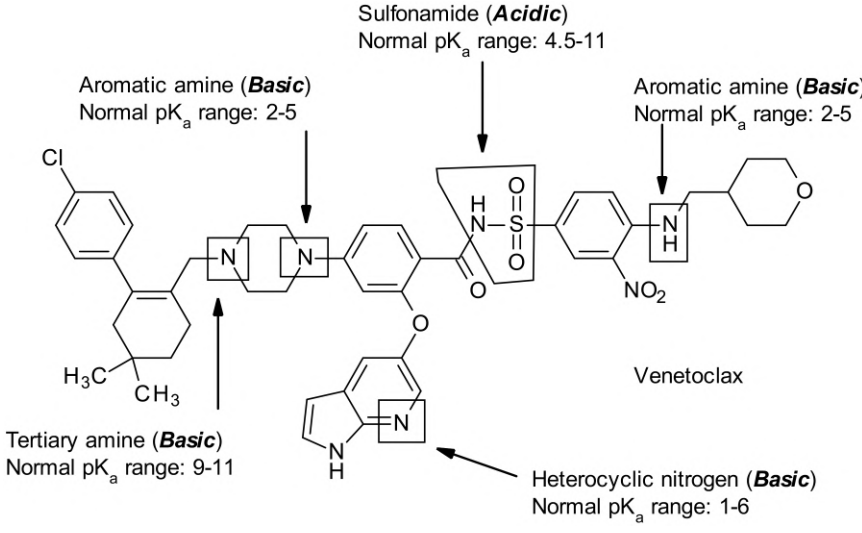
Glutamine: Overall, this side chain is normally classified as a polar, water-soluble side chain due to its ability to act as both a hydrogen bond donor and acceptor. The two methylene carbon atoms allow the amide to be oriented in different locations and may contribute a little to the overall lipid solubility of the peptide.

CHAPTER 3

Structural Analysis Checkpoint

Checkpoint Drug 1: Venetoclax

1. Acidic and basic functional groups along with normal pK_a ranges are shown below.



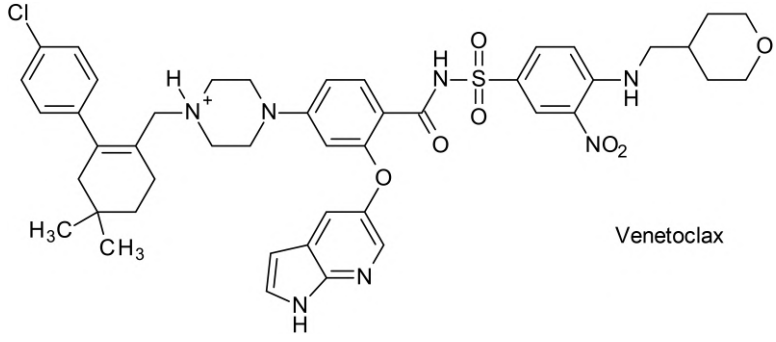
2. Venetoclax is an amphoteric drug because its structure contains both acidic and basic functional groups.

3. There are two main factors that contribute to the enhanced acidity of the sulfonamide functional group. First, this functional group is directly attached to a carbonyl group. Similar to sulfonylureas, this adjacent carbonyl enhances the acidity of the sulfonamide through resonance stabilization of the negative charge. Once the proton leaves, the resulting negative charge can be equally delocalized to all three adjacent oxygen atoms. Second, the nitro group on the adjacent aromatic ring can withdraw electrons from the aromatic ring through resonance. This decreases the electron density of the aromatic ring and causes an inductive effect that withdraws electrons from the adjacent sulfonamide. The overall effect of

this nitro group is somewhat decreased due to the ability of the aromatic amine to donate electrons through resonance into this same aromatic ring. Because the electron withdrawing properties of the nitro group are stronger than the electron donating properties of the aromatic amine, the net result is an electron withdrawing effect that enhances the acidity of the sulfonamide group.

4. Five-membered rings that contain a single nitrogen atom are not basic because the lone pair of electrons on the nitrogen atom is involved in the aromaticity or resonance delocalization of the ring and unavailable for binding to a proton. The presence of a second nitrogen atom within the five-membered ring enhances basicity, as discussed in the chapter; however, in this case, the second nitrogen atom is in the six-membered pyridine ring. This nitrogen atom is basic because the lone pair of electrons on the nitrogen atom is oriented perpendicular to the plane of the aromatic electrons. Thus, the lone pair of electrons are not involved in the aromaticity of the ring and are available to bind to a proton.

5. The ionized form of the tertiary amine is shown below.



Checkpoint Drug 2: Elamipretide

1. Answers provided in the grid below.

	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range (NA is Acceptable)
A	Guanidine	Basic	12.5[a]
B	Primary amine	Basic	9-11
C	Amide	Neutral	NA
D	Phenol	Acidic	9-10

[a] The pK_a range for guanidine can dip as low as 6, especially if the functional group is attached to one or more electron withdrawing groups, as found in the H_2 antagonist class of drugs.

2. Functional group C is an amide. The carbonyl carbon is electron poor due to the electronegativity of the adjacent oxygen atom and the resulting dipole. This carbon atom looks to its neighbor nitrogen atom for electron density. The neighboring nitrogen atom has a nonbonding pair of electrons that is available (via resonance) to donate electron density to the electron deficient carbon atom. Because this nonbonding pair of electrons is busy helping the neighbor electron deficient carbon atom, it is unavailable to participate as a proton acceptor (base). By way of reminder, the electronegative oxygen atom has two pairs of nonbonding electrons, but these electrons are held too tightly to the nucleus of the oxygen atom to participate as a proton acceptor (base). As a result, amides are neutral in character.

3. The ionization states and acid/base character are provided below.

	Stomach (pH = 1) Ionized, Unionized, NA	Acid/Base Character at pH = 1	Intestine (pH = 8) Ionized, Unionized, NA	Acid/Base Character at pH = 8	Urine (pH = 5) Ionized, Unionized, NA	Acid/Base Character at pH = 5
A	Ionized	Acidic	Ionized	Acidic	Ionized	Acidic
B	Ionized	Acidic	Ionized	Acidic	Ionized	Acidic
C	NA	Neutral	NA	Neutral	NA	Neutral
D	Unionized	Acidic	Unionized	Acidic	Unionized	Acidic

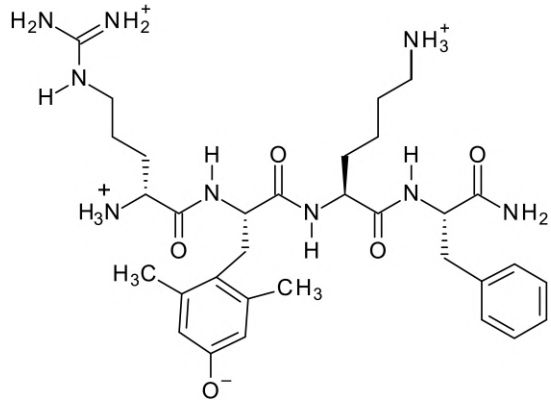
Functional group A (guanidine) is basic in character with a pK_a = ~12.5. It is ionized in all three physiologic locations because the environmental pH < pK_a of the functional group. In its ionized form, a guanidine is a proton donor (acidic).

Functional group B (primary amine) is basic in character with a pK_a range = 9 to 11. It is ionized in all three physiologic locations because the environmental pH < pK_a of the functional group. In its ionized form, a primary amine is a proton donor (acidic).

Functional group C (amide) is neutral in character. It is not an ionizable functional group, so NA should be placed in the grid.

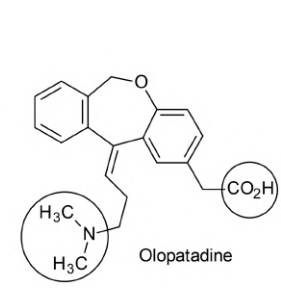
Functional group D (phenol) is acidic in character with a pK_a = 10. It is unionized in all three physiologic locations because the environmental pH < pK_a of the functional group. In its unionized form, a phenol is a proton donor (acidic).

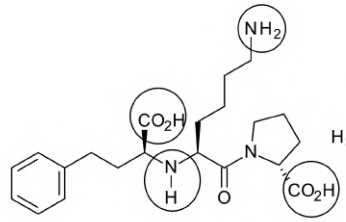
4. The guanidine of arginine, the primary amine of lysine, and the phenol of the tyrosine derivative are all ionizable. Don't forget that this peptide-based drug has an amino and a carboxy terminus! In this case, the carboxy terminus carboxylic acid has been masked as an amide, but the amino terminus primary amine is still present and is ionizable.

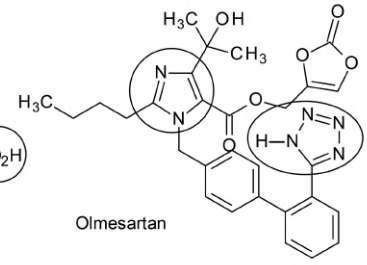


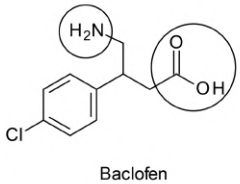
Review Questions

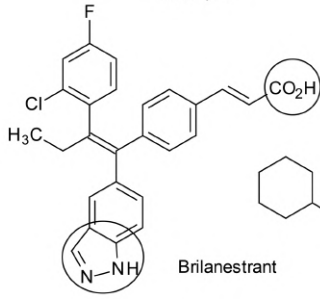
1. Acidic and basic functional groups are circled below and named in the table below.

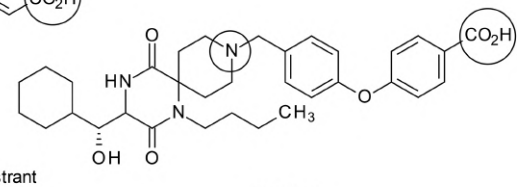






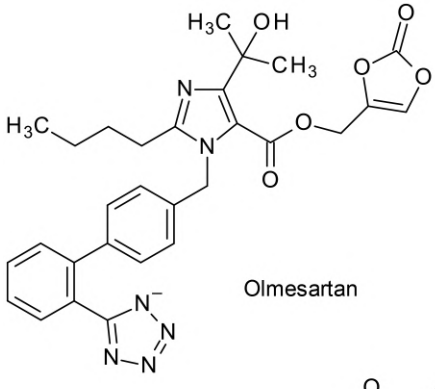


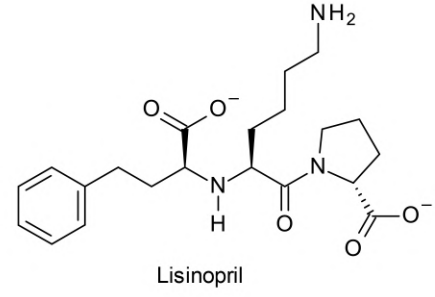


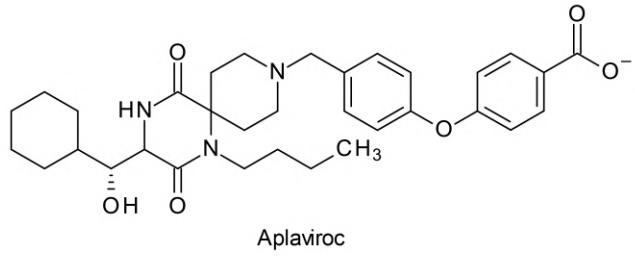


Drug Name	Acidic Functional Groups	Basic Functional Groups
Olapatadine	Carboxylic acid	Tertiary amine
Lisinopril	Carboxylic acid (×2)	Primary amine
		Secondary amine
Olmesartan	Tetrazole	Aromatic heterocycle (imidazole)
Baclofen	Carboxylic acid	Primary amine
Brilanestrant	Carboxylic acid	Aromatic heterocycle
Aplaviroc	Carboxylic acid	Tertiary amine

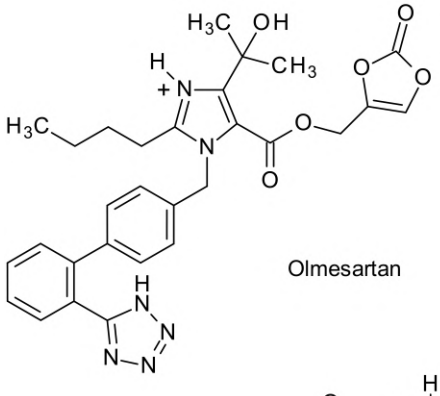
2. Acidic functional groups have been modified to show the ionized form.



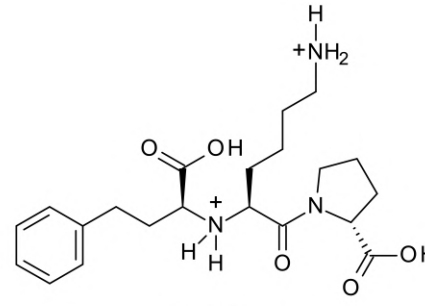




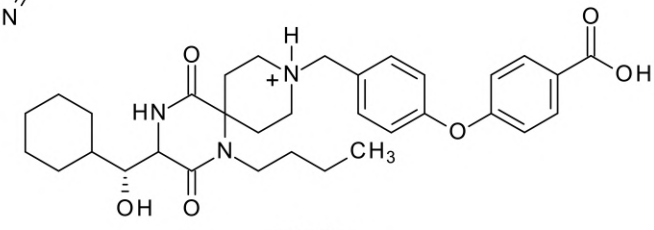
3. Basic functional groups have been modified to show the ionized form.



Olmesartan



Lisinopril



Aplaviroc

4. Functional groups that are acidic when they are in their ionized form are identified below.

Olmesartan	Lisinopril	Aplaviroc
Aromatic heterocycle (imidazole)	Primary amine	Tertiary amine
	Secondary amine	

Functional groups that are basic when they are in their ionized form are identified below.

Olmesartan	Lisinopril	Aplaviroc
Carboxylic acid	Carboxylic acid	Carboxylic acid
Tetrazole		

5. Amphoteric: contains at least one acidic and at least one basic functional group. All of the drugs in Question 1 are amphoteric in nature.

6. Answers provided in the grid below.

Drug Name	Name of Functional Group(s)	Approximate pK_a Value(s)	Acidic, Basic, or Amphoteric
Besafloxacin	Carboxylic acid	pK_a = 2.5-5	Amphoteric
	Aniline	pK_a = 2-5	
	Primary amine	pK_a = 9-11	
Tinlorafenib	Sulfonamide	pK_a = 4.5-11	Amphoteric
	Aniline	pK_a = 2-5	
Nezulcitinib	Tertiary amine (×2)	pK_a = 9-11	Amphoteric
	Aromatic heterocycles (imidazole, 1,2 diazole)	pK_a = 1-6	
	Phenol	pK_a = 9-10	

7. **Part A:** Colestipol (Colestid®) is a polymer than contains numerous basic secondary and tertiary amines as a component of its structure. Evaluating the entire polymeric structure, colestipol is considered basic in character. Because colestipol dissociates into ions in solution, it is therefore classified as an electrolyte.

   Part B: Drugs that contain one or more functional groups that are anionic (negatively charged) when in an environment of pH ~8 exchange for the chloride ion in the polymer. The drug is then associated with the polymer and is eliminated via a fecal route. This results in less drug available to be absorbed into the body (also less bile acids being removed), which likely will reduce the therapeutic effectiveness of the drug because a portion of the dose was removed by the polymer.

   Part C: Atorvastatin (carboxylic acid), sampatrilat (carboxylic acids), and furosemide (carboxylic acid) are all likely to be anionic at pH ~8 and therefore are sequestered by the polymeric drug.

8. **Part A:** Ionizable functional groups are listed in the table below.

Drug Name	Acidic Functional Groups	Basic Functional Groups
Arformoterol	Phenol	Secondary amine
Atorvastatin	Carboxylic acid	
Sampatrilat	Phenol	Primary amine
	Carboxylic acids (×2)	
	Sulfonamide	
Furosemide	Sulfonamide	Aniline
	Carboxylic acid	

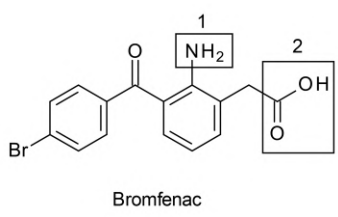
   Part B: The acidic and/or basic character of each drug molecule is provided below.

Drug Name	Acidic Character, Basic Character, or Both Acidic and Basic Character
Arformoterol	Acidic and basic character
Atorvastatin	Acidic character
Sampatrilat	Acidic and basic character
Furosemide	Acidic and basic character

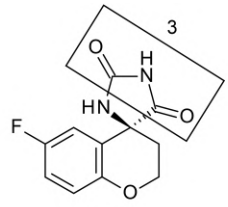
9. The acidic and/or basic character of each drug molecule is provided below.

	Acidic Character, Basic Character, or Both Acidic and Basic Character
Zolmitriptan	Basic character
Xemilofiban	Acidic and basic character
Vilanterol	Acidic and basic character
Terbutaline	Acidic and basic character

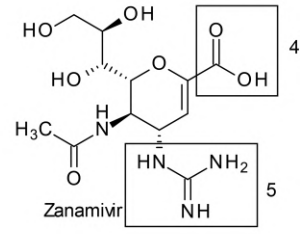
10. Acidic and basic functional groups and their pK_a ranges are provided below.



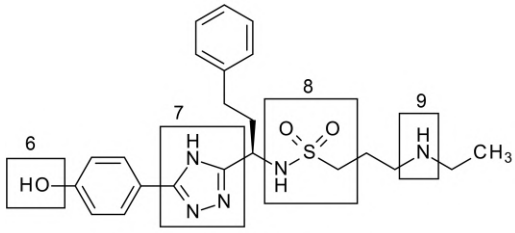
Bromfenac



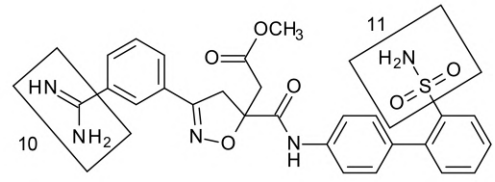
Sorbinil



Zanamivir



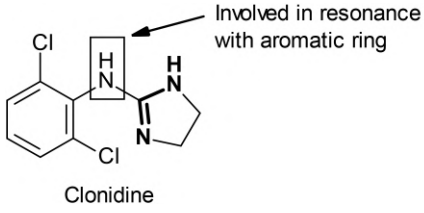
Experimental antidiabetic agent



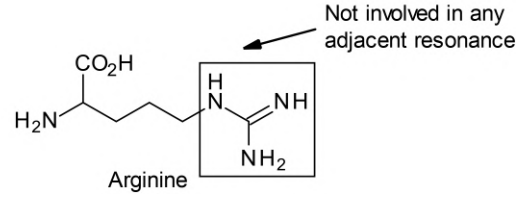
Experimental oral anticoagulant

Drug Name	Acidic Functional Groups	Basic Functional Groups
Bromfenac	Carboxylic acid (2; pK_a = 2.5-5)	Primary aromatic amine; aniline (1; pK_a = 2-5)
Sorbinil	Imide/β-Dicarbonyl (3; pK_a = 4.5-8.5)	
Zanamivir	Carboxylic acid (4; pK_a = 2.5-5)	Guanidine (5; pK_a = 12-13)
Experimental antidiabetic agent	Phenol (6; pK_a = 9-10)	Heterocyclic aromatic nitrogen atoms (7; pK_a = 1-6)
	Sulfonamide (8; pK_a = 4.5-11)	Secondary amine (9; pK_a = 9-11)
Experimental oral coagulant	Sulfonamide (11; pK_a = 4.5-11)	Amidine (10; pK_a = 10-11)

11. Unlike the side chain of the amino acid arginine, the guanidine group of clonidine is directly attached to an aromatic ring. As such, the nitrogen atom directly attached to the aromatic ring (highlighted below) can donate electrons into the aromatic ring, thus making it less available for resonance stabilization of a positive charge. In addition, the aromatic ring of clonidine is attached to two *ortho* chloro groups. Each of these halogens is electron withdrawing and further decreases the basicity of the guanidine group. The combination of these two effects decreases the basicity of the guanidine group by more than four orders of magnitude.



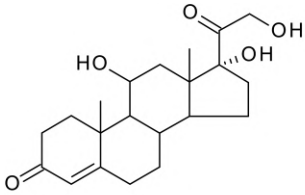
Clonidine



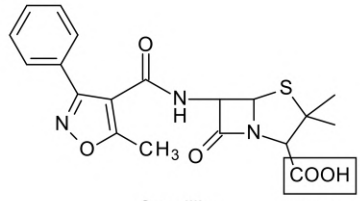
Arginine

12. The acid/base character of each drug molecule is provided below.

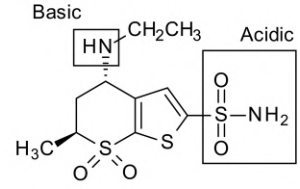
Drug Molecule	Acid/Base Character of Drug Molecule
Hydrocortisone	Nonelectrolyte: does not contain any acidic or basic functional groups
Oxacillin	Acidic: contains only an acidic carboxylic acid
Dorzolamide	Amphoteric: contains a basic secondary amine and an acidic sulfonamide
Diltiazem	Basic: contains only a basic tertiary amine
Amobarbital	Acidic: contains only an acidic imide group



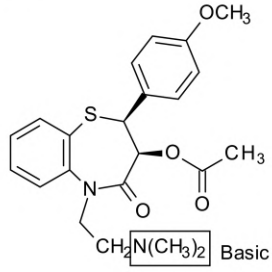
Hydrocortisone



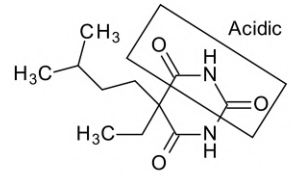
Oxacillin Acidic



Dorzolamide



Diltiazem



Amobarbital

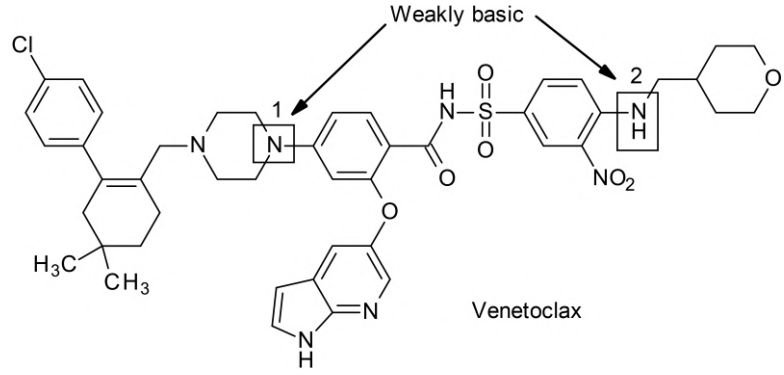
CHAPTER 4

Structural Analysis Checkpoint

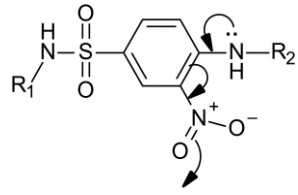
Checkpoint Drug 1: Venetoclax

1. **Part A:** The two aromatic amines are weakly basic due to the presence of other electron withdrawing groups attached to their respective phenyl rings. As discussed in Chapter 3, aromatic amines are much less basic than aliphatic or alicyclic amines due to their ability to donate electrons into the aromatic ring through resonance. This donating ability can be either enhanced or hindered due to the presence of other functional groups. For aromatic amine 1, the *para* carbonyl on the aromatic ring is electron withdrawing, mostly through inductive effects, and enhances the flow of electrons away from the nitrogen and further decreases its basicity. Additionally, the *meta* ether oxygen atom may play a role. Although both the aromatic amine and the ether oxygen can donate electrons into the aromatic ring, the oxygen atom is more electronegative than the nitrogen atom and thus withdraws

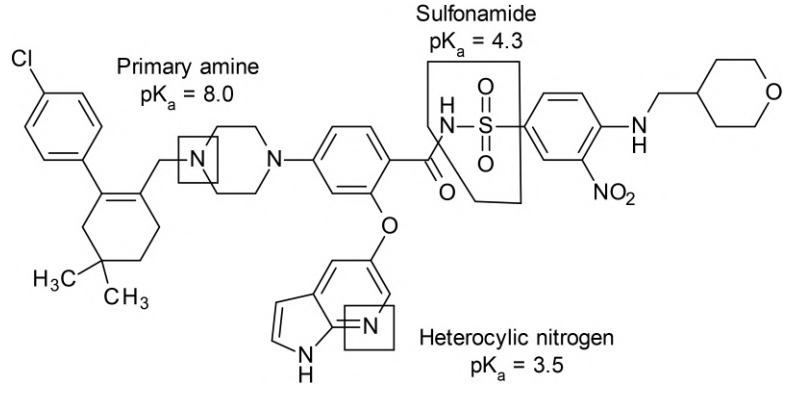
electrons from the nitrogen atom via induction. Both of these effects decrease the availability of the lone pair of electrons and thus the basicity of aromatic amine 1.



For aromatic amine 2, the *ortho* nitro group is electron withdrawing in character and greatly decreases the basicity of the amine through resonance delocalization, as shown below.



Part B: The normal pK_a range for a tertiary amine is 9 to 11. Given this piece of information, the pK_a of 8.0 should be assigned to this basic functional group. The lower basicity may be due to steric hindrance or adjacent functional groups. This leaves the heterocyclic nitrogen atom (***basic***) and the sulfonamide (***acidic***) and the pK_a values of 3.5 and 4.3. The normal pK_a range for a sulfonamide is 4.5 to 11, and the normal pK_a range for heterocyclic nitrogen atoms is 1 to 6. Although both of these pK_a values fall with the normal range for heterocyclic nitrogen atoms, the pK_a value of 3.5 belongs to the heterocyclic nitrogen atom. This is because the pK_a value of 4.3 is only slightly lower than the normal range for sulfonamides, whereas the pK_a value of 3.5 is too far outside this range. A full explanation of the pK_a value for the sulfonamide can be found in the answers for Chapter 3.



2. **Part A:** The primary amine and the heterocyclic nitrogen are primarily ionized, and the sulfonamide is primarily unionized.

   Part B: The primary amine and the sulfonamide are primarily ionized, and the heterocyclic nitrogen is primarily unionized.

Part C: The primary amine and the sulfonamide are primarily ionized, and the heterocyclic nitrogen is primarily unionized.

3. **Part A:** To use the Rule of Nines, the difference between the pH and the pK_a must be an integer (i.e., 1, 2, 3). In evaluating the above nine scenarios, there is only one scenario that meets this criterion: ***the sulfonamide at a urine pH of 5.3***. For this sulfonamide, the absolute value between the pH and the pK_a is equal to 1; thus, there is a 90:10 ratio. Because the sulfonamide ($pK_a = 4.3$) is acidic, it is primarily ionized in a basic environment (pH = 5.3). We can then use this ratio to determine that it is 90% ionized.

   Part B: Two methods allow the Rule of Nines to approximate the percent to which a functional group is ionized, even if the difference between the pH and the pK_a is ***not*** an integer. The first method is to round either the pH of the environment or the pK_a of the functional group so that there is an integral difference. Using the tertiary amine, absolute value between the pH and the pK_a is approximately equal to 6; thus, there is an approximate ratio of 99.9999:0.0001. Because the tertiary amine ($pK_a = 8.0$) is basic, it is primarily ionized in an acidic environment (pH = 1.8). We can then use this ratio to determine that it will be approximately 99.9999% ionized. The second method is to establish a range. Using the sulfonamide, the absolute value between the pH and the pK_a is 2.5. Because the sulfonamide ($pK_a = 4.3$) is acidic, it is primarily unionized in an acidic environment (pH = 1.8). If the absolute value were 2, then 99% would be unionized, and if the absolute value were 3, then 99.9% would be unionized. Because the absolute value lies between 2 and 3, we can conclude that the percent to which the sulfonamide is unionized is somewhere between 99% and 99.9%.

4. **Part A:** As determined in question 1B, the pK_a of 4.3 belongs to the acidic sulfonamide functional group; thus, the ionized form is the Base Form and the unionized form is the Acid Form.

$$\mathrm{pH} = \mathrm{pK_a} + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$3.1 = 4.3 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-1.2 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.063 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \quad \text{or} \quad \frac{0.063}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

   This ratio indicates that for every one molecule that contains the functional group in the acid (or unionized) form, there are 0.063 molecules that contain the functional group in the base (or ionized) form. The following equations can then be used to correctly calculate the percentage of the molecules that are ionized and the percentage that are unionized.

$$0.063 \text{ molecules in Base Form} + 1.0 \text{ molecule in Acid Form} = 1.063 \text{ Total Molecules}$$

$$\text{Base Form} = \text{Ionized Form} \quad \text{and} \quad \text{Acid Form} = \text{Unionized Form}$$

$$\text{Percent in Unionized Form} = \frac{1 \text{ Molecule in Unionized Form}}{1.063 \text{ Total Molecules}} \times 100\% = 94.1\%$$

$$\text{Percent in Ionized Form} = \frac{0.063 \text{ Molecules in Ionized Form}}{1.063 \text{ Total Molecules}} \times 100\% = 5.9\%$$

The question asks for the percent that is ionized, so the correct answer is 5.9%.

Part B: As determined in question 1B, the pK_a of 8.0 belongs to the basic tertiary amine functional group; thus, the ionized form is the Acid Form, and the unionized form is the Base Form.

$$pH = pK_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$7.3 = 8.0 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-0.7 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.20 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \quad \text{or} \quad \frac{0.20}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that for every one molecule that contains the functional group in the acid (or ionized) form, there are 0.20 molecules that contain the functional group in the base (or unionized) form. The following equations can then be used to correctly calculate the percentage of the molecules that are ionized and the percentage that are unionized.

$$0.20 \text{ molecules in Base Form} + 1.0 \text{ molecule in Acid Form} = 1.20 \text{ Total Molecules}$$

$$\text{Base Form} = \text{Unionized Form and Acid Form} = \text{Ionized Form}$$

$$\text{Percent in Unionized Form} = \frac{0.20 \text{ Molecule in Unionized Form}}{1.20 \text{ Total Molecules}} \times 100\% = 16.7\%$$

$$\text{Percent in Ionized Form} = \frac{1.0 \text{ Molecules in Ionized Form}}{1.2 \text{ Total Molecules}} \times 100\% = 83.3\%$$

The question asks for the percent that is ionized, so the correct answer is 83.3%.

Part C: As determined in Question 1B, the pK_a of 3.5 belongs to the basic heterocyclic nitrogen atom; thus, the ionized form is the Acid Form, and the unionized form is the Base Form. The functional group is 30% ionized. This means that for every 100 molecules of venetoclax, 30 of them have an ionized heterocyclic nitrogen atom and 70 have an unionized heterocyclic nitrogen atom. Thus, the [Base Form]/[Acid Form] ratio is 70:30. Using this ratio and the given pK_a, the pH can be determined.

$$pH = pK_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$pH = 3.5 + \log \frac{70}{30}$$

$$pH = 3.5 + \log 2.3$$

$$pH = 3.5 + 0.36$$

$$pH = 3.86$$

Checkpoint Drug 2: Elamipretide

1. Answer provided in table below.

	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range
A	Guanidine (arginine side chain)	Basic	~12.5
B	Primary amine (lysine side chain)	Basic	~10.5
C	Phenol (modified tyrosine side chain)	Acidic	~10.5
D	Primary amine (peptide backbone)	Basic	~10.5

2. To determine the extent to which a functional group is ionized in a given physiological environment (qualitatively or quantitatively), you must know several facts and select how to set the problem up. You need to know the pK_a of the functional group and the pH of the environment (facts) and then, to set the problem up, you must know whether the functional group is acidic or basic.

   For example, using the Henderson-Hasselbalch equation to solve the problem qualitatively, you must be able to identify the base form (ionized form for acids; unionized form for bases) and the acid form (unionized form for acids; ionized form for bases) of the drug molecule. When using qualitative methods, remember that when $pH < pK_a$ (for acids), the functional group is predominantly unionized; but, when $pH < pK_a$ (for bases), the functional group is predominantly ionized.

3. Answers provided in the tables below.

	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range	Primarily Ionized (> 50%) Primarily Unionized (< 50%) pH = 2
A	Guanidine (arginine side chain)	Basic	~12.5	Primarily ionized
B	Primary amine (lysine side chain)	Basic	~10.5	Primarily ionized
C	Phenol (modified tyrosine side chain)	Acidic	~10.5	Primarily unionized
D	Primary amine (amino terminus)	Basic	~10.5	Primarily ionized

	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range	Primarily Ionized (> 50%) Primarily Unionized (< 50%) pH = 5
A	Guanidine (arginine side chain)	Basic	~12.5	Primarily ionized
B	Primary amine (lysine side chain)	Basic	~10.5	Primarily ionized
C	Phenol (modified tyrosine side chain)	Acidic	~10.5	Primarily unionized
D	Primary amine (amino terminus)	Basic	~10.5	Primarily ionized

	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range	Primarily Ionized (> 50%) Primarily Unionized (< 50%) pH = 7.4
A	Guanidine (arginine side chain)	Basic	~12.5	Primarily ionized
B	Primary amine (lysine side chain)	Basic	~10.5	Primarily ionized
C	Phenol (modified tyrosine side chain)	Acidic	~10.5	Primarily unionized
D	Primary amine (amino terminus)	Basic	~10.5	Primarily ionized

Functional group A (guanidine) is basic in character with a pK_a = ~12.5. It is primarily ionized in all three physiologic locations because the environmental pH < pK_a of the functional group. In its ionized form, a guanidine is a proton donor (acidic).

Functional group B (primary amine) is basic in character with a pK_a = ~10.5. It is primarily ionized in all three physiologic locations because the environmental pH < pK_a of the functional group. In its ionized form, a primary amine is a proton donor (acidic).

Functional group C (phenol) is acidic in character with a pK_a = ~10.5. It is primarily unionized in all three physiologic locations because the environmental pH < pK_a of the functional group.

Functional Group D (amino terminus primary amine) is basic in character with a pK_a = ~10.5. It is primarily ionized in all three physiologic locations because the environmental pH < pK_a of the functional group. In its ionized form, a primary amine is a proton donor (acidic).

4. Answers provided in the tables below.

	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range	Percent Ionized pH = 5.4
A	Guanidine (arginine side chain)	Basic	~12.5	99.9999921%
B	Primary amine (lysine side chain)	Basic	~10.5	99.99921%
C	Phenol (modified tyrosine side chain)	Acidic	~10.5	0.00079%
D	Primary amine (amino terminus)	Basic	~10.5	99.99921%

	Name of Functional Group	Character: Acidic, Basic, Neutral	pK_a Value or Range	Percent Ionized pH = 8.5
A	Guanidine (arginine side chain)	Basic	~12.5	99.99%
B	Primary amine (lysine side chain)	Basic	~10.5	99.01%
C	Phenol (modified tyrosine side chain)	Acidic	~10.5	0.99%
D	Primary amine (amino terminus)	Basic	~10.5	99.01%

Part A: pH = 5.4

For the guanidine functional group at pH=5.4:

$$\text{pH} = \text{pK}_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$5.4 = 12.5 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-7.1 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.000000079 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } \frac{0.000000079}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that for every one molecule that contains the functional group in the acid (or ionized) form, there are 0.000000079 molecules that contain the functional group in the base (or unionized) form. The following equations can then be used to correctly calculate the percentage of the molecules that are ionized and the percentage that are unionized.

$$0.000000079 \text{ molecules in Base Form} + 1.0 \text{ molecule in Acid Form} = 1.000000079 \text{ Total Molecules}$$

$$\text{Base Form} = \text{Unionized Form and Acid form} = \text{Ionized Form}$$

$$\text{Percent in Unionized Form} = \frac{0.000000079 \text{ Molecule in Unionized Form}}{1.000000079 \text{ Total Molecules}} \times 100\% = 0.0000079\%$$

$$\text{Percent in Ionized Form} = \frac{1.0 \text{ Molecule in Ionized Form}}{1.000000079 \text{ Total Molecules}} \times 100\% = 99.9999921\%$$

The question asks for the percent that is ionized, so the correct answer is 99.9999921%.

For both primary amine functional groups at pH = 5.4:

$$\text{pH} = \text{pK}_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$5.4 = 10.5 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-5.1 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.0000079 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } \frac{0.0000079}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that for every one molecule that contains the functional group in the acid (or ionized) form, there are 0.0000079 molecules that contain the functional group in the base (or unionized) form. The following equations can then be used to correctly calculate the percentage of the molecules that are ionized and the percentage that are unionized.

$$0.0000079 \text{ molecules in Base Form} + 1.0 \text{ molecule in Acid Form} = 1.0000079 \text{ total molecules}$$

$$\text{Base Form} = \text{Unionized Form and Acid Form} = \text{Ionized Form}$$

$$\text{Percent in Unionized Form} = \frac{0.0000079 \text{ Molecule in Unionized Form}}{1.0000079 \text{ Total Molecules}} \times 100\% = 0.00079\%$$

$$\text{Percent in Ionized Form} = \frac{1.0 \text{ Molecule in Ionized Form}}{1.0000079 \text{ Total Molecules}} \times 100\% = 99.99921\%$$

The question asks for the percent that is ionized, so the correct answer is 99.99921%.

For the phenol functional group at pH = 5.4:

$$\log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$5.4 = 10.5 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-5.1 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.0000079 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } \frac{0.0000079}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that for every one molecule that contains the functional group in the acid (or unionized) form, there are 0.0000079 molecules that contain the functional group in the base (or ionized) form. The following equations can then be used to correctly calculate the percentage of the molecules that are ionized and the percentage that are unionized.

0.0000079 molecules in Base Form + 1.0 molecule in Acid Form = 1.0000079 Total Molecules

$$\text{Base Form} = \text{Ionized Form and Acid Form} = \text{Unionized Form}$$

$$\text{Percent in Ionized Form} = \frac{0.0000079 \text{ Molecule in Unionized Form}}{1.0000079 \text{ Total Molecules}} \times 100\% = 0.00079\%$$

$$\text{Percent in Unionized Form} = \frac{1.0 \text{ Molecule in Ionized Form}}{1.0000079 \text{ Total Molecules}} \times 100\% = 99.99921\%$$

The question asks for the percent that are ionized, so the correct answer is 0.00079%.

Part B: pH = 8.5

For the guanidine functional group at pH = 8.5

$$\text{pH} = \text{pK}_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$8.5 = 12.5 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-4 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.0001 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } \frac{0.0001}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that for every one molecule that contains the functional group in the acid (or ionized) form, there are 0.0001 molecules that contain the functional group in the

base (or unionized) form. The following equations can then be used to correctly calculate the percentage of the molecules that are ionized and the percentage that are unionized.

$$0.0001 \text{ molecules in Base Form} + 1.0 \text{ molecule in Acid Form} = 1.0001 \text{ Total Molecules}$$

$$\text{Base Form} = \text{Unionized Form and Acid Form} = \text{Ionized Form}$$

$$\text{Percent in Unionized Form} = \frac{0.0001 \text{ Molecule in Unionized Form}}{1.0001 \text{ Total Molecules}} \times 100\% = 0.009999\%$$

$$\text{Percent in Ionized Form} = \frac{1.0 \text{ Molecule in Ionized Form}}{1.0001 \text{ Total Molecules}} \times 100\% = 99.99\%$$

The question asks for the percent that is ionized, so the correct answer is 99.99%.

For both primary amine functional groups at pH = 8.5:

$$\text{pH} = \text{pK}_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$8.5 = 10.5 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-2 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.01 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } \frac{0.01}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that for every one molecule that contains the functional group in the acid (or ionized) form, there are 0.0000079 molecules that contain the functional group in the base (or unionized) form. The following equations can then be used to correctly calculate the percentage of the molecules that are ionized and the percentage that are unionized.

$$0.01 \text{ molecules in Base Form} + 1.0 \text{ molecule in Acid Form} = 1.01 \text{ Total Molecules}$$

$$\text{Base Form} = \text{Unionized Form and Acid Form} = \text{Ionized Form}$$

$$\text{Percent in Unionized Form} = \frac{0.01 \text{ Molecule in Unionized Form}}{1.01 \text{ Total Molecules}} \times 100\% = 0.99\%$$

$$\text{Percent in Ionized Form} = \frac{1.0 \text{ Molecule in Ionized Form}}{1.01 \text{ Total Molecules}} \times 100\% = 99.01\%$$

The question asks for the percent that is ionized, so the correct answer is 99.01%.

For the phenol functional group at pH = 8.5:

$$\log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$8.5 = 10.5 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-2 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.01 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } \frac{0.01}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that for every one molecule that contains the functional group in the acid (or unionized) form, there are 0.0000079 molecules that contain the functional group in the base (or ionized) form. The following equations can then be used to correctly calculate the percentage of the molecules that are ionized and the percentage that are unionized.

$$0.01 \text{ molecules in Base Form} + 1.0 \text{ molecule in Acid Form} = 1.01 \text{ Total Molecules}$$

$$\text{Base Form} = \text{Ionized Form and Acid Form} = \text{Unionized Form}$$

$$\text{Percent in Ionized Form} = \frac{0.01 \text{ Molecules in Unionized Form}}{1.01 \text{ Total Molecules}} \times 100\% = 0.99\%$$

$$\text{Percent in Unionized Form} = \frac{1.0 \text{ Molecule in Ionized Form}}{1.01 \text{ Total Molecules}} \times 100\% = 99.001\%$$

The question asks for the percent that are ionized, so the correct answer is 0.99%.

Review Questions

1. Answers provided in the grid below.

Drug (pK_a Value)	Name of Functional Group	Acidic/Basic
Carvedilol (7.8)	Secondary amine	Basic
Ketoprofen (5.94)	Carboxylic acid	Acidic
Dasolampanel (3.93)	Tetrazole	Acidic
Dasolampanel (6.73)	Secondary amine	Basic
Haloperidol (8.6)	Tertiary amine	Basic

2. The acidic, basic, or neutral environments are provided below.

Saliva (pH = 6.4)	Stomach (pH = 2)	Duodenum (pH = 5.4)	Plasma (pH = 7.4)	Urine (pH = 5.7)
Acidic	Acidic	Acidic	Basic (nearly neutral)	Acidic

3. Predominant ionization states are provided below.

Drug (pK_a value)	Saliva (pH = 6.4)	Stomach (pH = 2)	Duodenum (pH = 5.4)	Plasma (pH = 7.4)
Carvedilol (7.8)	Ionized	Ionized	Ionized	Ionized
Ketoprofen (5.94)	Ionized	Unionized	Unionized	Ionized
Dasolampanel (3.93)	Ionized	Unionized	Ionized	Ionized
Dasolampanel (6.73)	Ionized	Ionized	Ionized	Unionized
Haloperidol (8.6)	Ionized	Ionized	Ionized	Ionized

4. Tolbutamide contains an acidic functional group (sulfonylurea; pK_a 5.4), and it is predominantly ionized when the environmental pH > pK_a. As stated in the chapter text, the Rule of Nines can be used in lieu of the Henderson-Hasselbalch equation if the difference between the pH and pK_a is an integer, as it is in this scenario. Using this rule, we can calculate the approximate percent ionization in each of these environments (duodenum pH = 5.4; saliva pH = 6.4; plasma pH = 7.4) by simply determining the magnitude of the difference between the pH and pK_a. In the duodenum, the pH = pK_a; therefore, 50% of the carboxylic acid is ionized at any given time and 50% is unionized. In the saliva, the difference between the pH

and the pK_a values is 1; therefore, 90% of the carboxylic acid functional groups is ionized and 10% is unionized. In the plasma, the difference between the pH and the pK_a values is 2; therefore, 99% of the carboxylic acid functional groups is ionized and 1% is unionized.

5. **Part A:** Qualitative approach (predominantly ionized or unionized?)

Dasolampanel contains a tetrazole (acidic; $pK_a = 3.93$) in an environment pH = 7.4. For acids, if $pH > pK_a$, then the functional group is predominantly ionized.

Dasolampanel contains a secondary amine (basic; $pK_a = 6.73$) in an environment pH = 7.4. For basic functional groups, if the $pH > pK_a$, then the functional group is predominantly unionized.

Part B: Quantitative approach (% ionized and % unionized?)

Answer for the Acidic Functional Group (Dasolampanel):

The form of the Henderson-Hasselbalch equation used for acidic functional groups is shown below.

$$pH = pK_a + \log \frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]} \quad \text{or} \quad pH = pK_a + \log \frac{[\text{Basic Form}]}{[\text{Acidic Form}]}$$

When using this equation to determine whether the functional group is predominantly ionized or unionized, we need to know the pK_a of the functional group (tetrazole in dasolampanel $pK_a = 3.93$) and the pH of the environment (plasma pH = 7.4).

$$7.4 = 3.93 + \log \frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]}$$

$$3.47 = \log \frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]}$$

The fact that the number on the left is positive indicates that the tetrazole is predominantly in its unprotonated (basic, ionized) form at pH = 7.4.

To determine the percentage of ionized and unionized drug in the plasma, we need to calculate the antilog of both sides of this equation. In this case, the antilog of 3.47 is 2,951. This means that for every one molecule that is protonated (unionized tetrazole), 2,951 molecules are unprotonated (ionized tetrazole).

Calculation of the percentage of ionized drug and unionized drug at pH = 7.4 requires one additional step.

2,931 molecules in Ionized Form + 1.0 molecule in Unionized Form = 2,932 Total Molecules

$$\text{Percent of Molecules in Ionized Form} = \frac{2{,}931 \text{ Molecules in Ionized Form}}{2{,}932 \text{ Total Molecules}} \times 100\% = 99.96\%$$

$$\text{Percent of Molecules in Unionized Form} = \frac{1 \text{ Molecule in Unionized Form}}{2{,}932 \text{ Total Molecules}} \times 100\% = 0.04\%$$

Answer for the Basic Functional Group (Dasolampanel):

The form of the Henderson-Hasselbalch equation used for basic functional groups is show below.

$$pH = pK_a + \log \frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]}$$

When using this equation to determine whether the functional group is predominantly ionized or unionized, we need to know the pK_a of the functional group (secondary amine in dasolampanel $pK_a = 6.73$) and the pH of the environment (plasma pH = 7.4).

$$7.4 = 6.73 + \log \frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]}$$

$$0.67 = \log \frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]}$$

The fact that the number on the left is positive indicates that the secondary amine is predominantly in its unprotonated (basic, unionized) form at pH = 7.4.

To determine the percentage of ionized and unionized drug in the plasma, we need to calculate the antilog of both sides of the equation. In this case, the antilog of 0.67 is 4.68. This means that for every one molecule that is protonated (ionized secondary amine), there are 4.86 molecules that are unprotonated (unionized secondary amine).

Calculation of the percentage of ionized drug and unionized drug at pH = 7.4 requires one additional step.

$$1 \text{ molecule in Ionized Form} + 4.86 \text{ molecules in Unionized Form} = 5.86 \text{ Total Molecules}$$

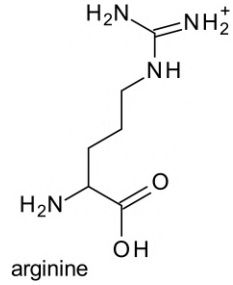
$$\text{Percent of Molecules in Unionized Form} = \frac{4.86 \text{ Molecules in Unionized Form}}{5.86 \text{ Total Molecules}} \times 100\% = 82.9\%$$

$$\text{Percent of Molecules in Ionized Form} = \frac{1 \text{ Molecule in Ionized Form}}{5.86 \text{ Total Molecules}} \times 100\% = 17.1\%$$

6. Based on ionization only (which allows for ion–dipole interactions with water) ketoprofen is the only one of the four drugs that is unionized in the duodenum. Although this molecule can participate in other types of interactions with water (e.g., H-bonding) it cannot participate in ion–dipole interactions because it is not ionized in that environment.

   Based on ionization only (which allows for ion–dipole interactions with water) dasolampanel is the only one of the four drugs that has two ionized functional groups in the duodenum. This molecule is able to interact with water via both the ionized secondary amine and the ionized tetrazole.

7. To determine if a drug can participate in an ionic interaction with the ionized arginine residue found within the active site of COX-1, we first need to determine what the ionized form of arginine looks like at pH = 7.4. Using the Henderson-Hasselbalch equation to determine if this functional group is predominantly ionized in the plasma (pH = 7.4), we learn that 99.9991% of the molecules of arginine are ionized in that environment (see structure of ionized arginine side chain below).



arginine

For a drug to participate in an ionic interaction with the side chain of this arginine residue, it must contain a functional group that is negatively charged in the same environment.

Because we have already determined that ketoprofen is predominantly ionized at pH = 7.4 (via the carboxylic acid), it stands to reason that ketoprofen can participate in this critical ionic interaction with the ionized arginine residue.

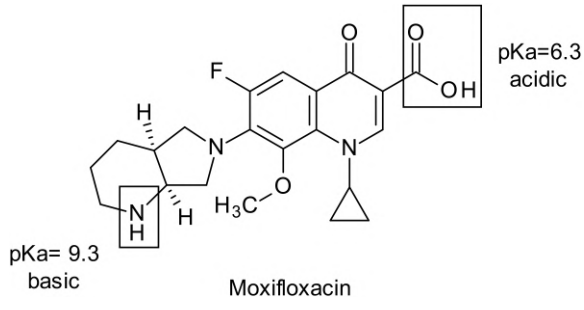
Haloperidol contains a basic tertiary amine (piperidine) and, when ionized, is positively charged. As we determined in Question 3, haloperidol is predominantly ionized in this environment (pH = 7.4). Because an ionic interaction requires that an ion pair form between one negatively and one positively charged functional group, it stands to reason that the positively charged tertiary amine (piperidine) in haloperidol cannot participate in an ionic interaction with the positively charged side chain of the arginine residue.

8. Acidic and basic functional groups are identified below.

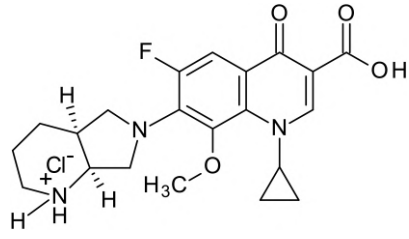
Functional Group Name	Acidic or Basic	Saliva (pH = 6.4)	Stomach (pH = 2)	Duodenum (pH = 5.4)	Plasma (pH = 7.4)	Urine (pH = 5.7)
Secondary amine	Basic (pK_a = 9-11)	Ionized	Ionized	Ionized	Ionized	Ionized
Phenol	Acidic (pK_a = 9-10)	Unionized	Unionized	Unionized	Unionized	Unionized

There are no physiologic environments in which batefenterol is predominantly in its unionized form. For the drug to be absorbed via passive diffusion, we can see that in the stomach the phenol is primarily in its unionized form; however, only a very small fraction of the secondary amine is in its unionized form in that environment at any given time. We know that an equilibrium exists between the unionized and ionized form of the drug and that the equilibrium is re-established as the unionized drug is absorbed, so we might anticipate that little by little batefenterol will be absorbed from the stomach.

9. **Part A:** Acidic and basic functional groups and their respective pK_a values are shown below.



Part B: The hydrochloride salt form of the drug is ionized (secondary amine is in its protonated form) and, therefore, capable of participating in an ion–dipole interaction with water. This enhances the solubility of the drug in an aqueous solution.



Part C:

Answer for Acidic Functional Group (Carboxylic Acid)

The form of the Henderson-Hasselbalch equation used for acidic functional groups is shown below.

$$pH = pK_a + \log\frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]} \quad \text{or} \quad pH = pK_a + \log\frac{[\text{Basic form}]}{[\text{Acidic}]}$$

When using this equation to determine whether the functional group is predominantly ionized or unionized, we need to know the pK_a of the functional group (carboxylic acid in moxifloxacin $pK_a = 6.3$) and the pH of the environment (formulation pH = 7.4).

$$7.4 = 6.3 + \log\frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]}$$

$$1.1 = \log\frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]}$$

The fact that the number on the left is positive indicates that the carboxylic acid is predominantly in its unprotonated (basic, ionized) form at pH = 7.4.

To determine the percentage of ionized and unionized drug in the formulation, we need to calculate the antilog of both sides of this equation. In this case, the antilog of 1.1 is 12.59. This means that for every one molecule that is protonated (unionized carboxylic acid; acidic), there are 12.59 molecules that are unprotonated (ionized carboxylic acid; basic).

Calculation of the percentage of ionized drug and unionized drug at pH = 7.4 requires one additional step.

$$1.1 \text{ Molecules in Ionized Form} + 1.0 \text{ Molecule in Unionized Form} = 2.1 \text{ Total Molecules}$$

$$\text{Percent of Molecules in Ionized Form} = \frac{1.1 \text{ Molecules in Ionized Form}}{2.1 \text{ Total Molecules}} \times 100\% = 52.38\%$$

$$\text{Percent of Molecules in Unionized Form} = \frac{1 \text{ Molecule in Unionized Form}}{2.1 \text{ Total Molecules}} \times 100\% = 47.62\%$$

Answer for Basic Functional Group (Secondary Amine/Piperazine)

The form of the Henderson-Hasselbalch equation used for basic functional groups is show below.

$$pH = pH_a + \log\frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]}$$

When using this equation to determine whether the functional group is predominantly ionized or unionized, we need to know the pK_a of the functional group (secondary amine in moxifloxacin $pK_a = 9.3$) and the pH of the environment (formulation pH = 7.4).

$$7.4 = 9.3 + \log\frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]}$$

$$-1.9 = \log\frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]}$$

The fact that the number on the left is negative indicates that the secondary amine is predominantly in its protonated (acidic, ionized) form at pH = 7.4.

To determine the percentage of ionized and unionized drug in the formulation, we need to calculate the antilog of both sides of the equation. In this case, the antilog of –1.9 is 0.012589. This means that for every one molecule that is unprotonated (unionized secondary amine), there are 0.012589 molecules that are protonated (ionized secondary amine).

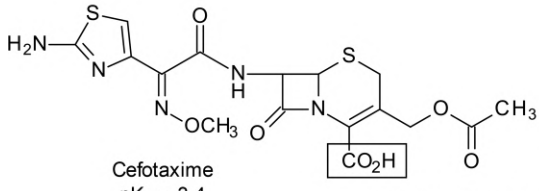
Calculation of the percentage of ionized drug and unionized drug at pH = 7.4 requires one additional step.

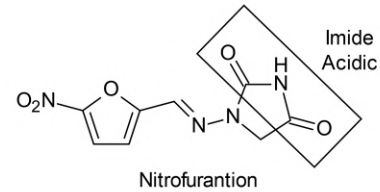
$$1\ \text{Molecule in Ionized Form} + 0.012589\ \text{Molecules in Unionized Form} = 1.012589\ \text{Total Molecules}$$

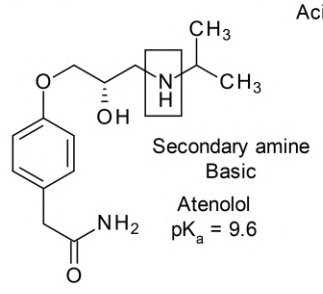
$$\text{Percent of Molecules in Ionized Form} = \frac{1\ \text{Molecule in Ionized Form}}{1.012589\ \text{Total Molecules}} \times 100\% = 99.87\%$$

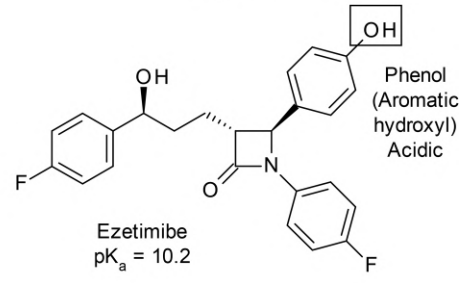
$$\text{Percent of Molecules in Unionized Form} = \frac{0.012589\ \text{Molecules in Unionized Form}}{1.012589\ \text{Total Molecules}} \times 100\% = 0.13\%$$

10. **Part A:** Functional groups are matched with the appropriate pK_a values below.









Part B: Ionization states and explanations are provided below.

Drug (pK_a Value)	Stomach (pH = 1.8)	Urine (pH = 6.1)	Cell (pH = 7.4)
Cefotaxime (3.4)	Primarily unionized	Primarily ionized	Primarily ionized
Nitrofurantoin (7.1)	Primarily unionized	Primarily unionized	Primarily ionized
Atenolol (9.6)	Primarily ionized	Primarily ionized	Primarily ionized
Ezetimibe (10.2)	Primarily unionized	Primarily unionized	Primarily unionized

Cefotaxime contains an acidic carboxylic acid. At a urine pH of 6.1 and a cellular pH of 7.4, the environment (i.e., the pH) is more basic than the functional group (i.e., the pH > pK_a). An acidic functional group is primarily ionized in a basic environment. In contrast, at a stomach pH of 1.8, the environment is more acidic than the functional group (i.e., the pH < pK_a). An acidic functional group is primarily unionized in an acidic environment.

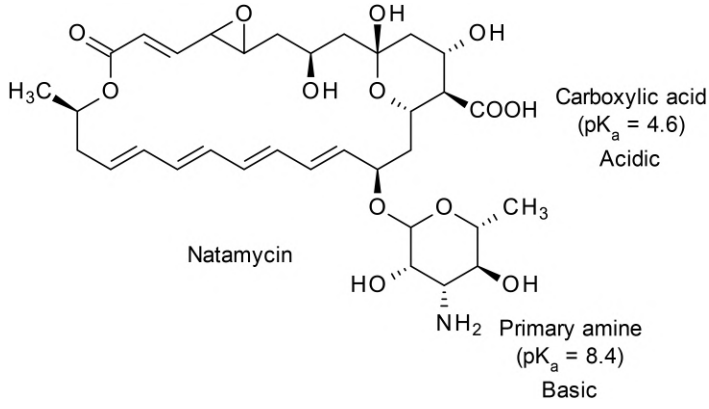
Nitrofurantion contains an acidic imide group. At a stomach pH of 1.8 and a urinary pH of 6.1, the environment (i.e., the pH) is more acidic than the functional group (i.e., the pH < pK_a). An acidic functional group is primarily unionized in an acidic environment. At a cellular pH

of 7.4, the environment is slightly more basic than the functional group (i.e., the pH > pK_a). An acidic functional group is primarily ionized in a basic environment.

Atenolol contains a basic secondary amine with a pK_a value that is greater than any of the above three environments. In all of these situations, the environment is more acidic than the functional group (i.e., the pH < pK_a). This basic functional group is primarily ionized in all three of these acidic environments.

Ezetimibe contains an acidic phenol with a pK_a value that is greater than any of the above three environments. In all of these situations, the environment is more acidic than the functional group (i.e., the pH < pK_a). This acidic functional group is primarily unionized in all three of these acidic environments.

11. **Part A:** Functional groups are matched with the appropriate pK_a below.



Part B: Calculations for percent ionization at a pH = 6.2 are provided below.

For the carboxylic acid, the pK_a = 4.6 and the pH = 6.2. Using the Henderson-Hasselbalch equation gives the following:

$$6.2 = 4.6 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

Solving the equation provides a ratio of [Base Form]/[Acid Form].

$$1.6 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$39.8 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \quad \text{or} \quad \frac{39.8}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

Thus, for every one molecule that contains this functional group in the acid form, there are 39.8 molecules that contain this functional group in the base form. Because the functional group is acidic, the base form is equal to the ionized form and the acid form is equal to the unionized form.

$$39.8 \text{ Molecules in Base Form} + 1.0 \text{ Molecule in Acid Form} = 40.8 \text{ Total Molecules}$$

$$\text{Base Form} = \text{Ionized Form, and Acid Form} = \text{Unionized Form for this functional group}$$

$$\text{Percent in Ionized Form} = \frac{39.8 \text{ Molecules in Ionized Form}}{40.8 \text{ Total Molecules}} \times 100\%$$

$$\text{Percent in Ionized Form} = 97.5\%$$

For the primary amine, the $pK_a = 8.4$ and the pH = 6.2. Using the Henderson-Hasselbalch equation gives the following.

$$6.2 = 8.4 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

Solving the equation provides a ratio of [Base Form]/[Acid Form].

$$-2.2 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.0063 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } \frac{0.0063}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

Thus, for every one molecule that contains this functional group in the acid form, there are 0.0063 molecules that contain this functional group in the base form. Because the functional group is basic, the base form is equal to the unionized form, and the acid form is equal to the ionized form.

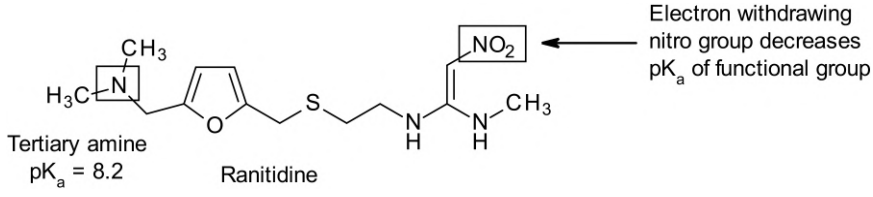
0.0063 Molecules in Base Form + 1.0 Molecule in Acid Form = 1.0063 Total Molecules

Base Form = Unionized Form and Acid Form = Ionized Form for this functional group

$$\text{Percent in Ionized Form} = \frac{1 \text{ Molecule in Ionized Form}}{1.0063 \text{ Total Molecules}} \times 100\%$$

$$\text{Percent in Ionized Form} = 99.4\%$$

12. The functional group with the pK_a value of 8.2 is a tertiary amine as shown below. The nitro group adjacent to the other nitrogen atoms is electron withdrawing and greatly decreases the basicity and pK_a value for this functional group.



We can use the Henderson-Hasselbalch equation to solve this problem. Because the ionized form of a basic functional group can also be designated as its protonated form or its conjugate acid form, either of the following equations can be used.

$$\text{pH} = \text{pK}_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$\text{or pH} = \text{pK}_a + \log \frac{[\text{Unprotonated Form}]}{[\text{Protonated Form}]}$$

Therefore, if 70% of this functional group is ionized:

$$\text{pH} = 8.2 + \log \frac{[30]}{[70]}$$

$$\text{pH} = 8.2 + \log \frac{[30]}{[70]} = 8.2 + (-0.37) = 7.83$$

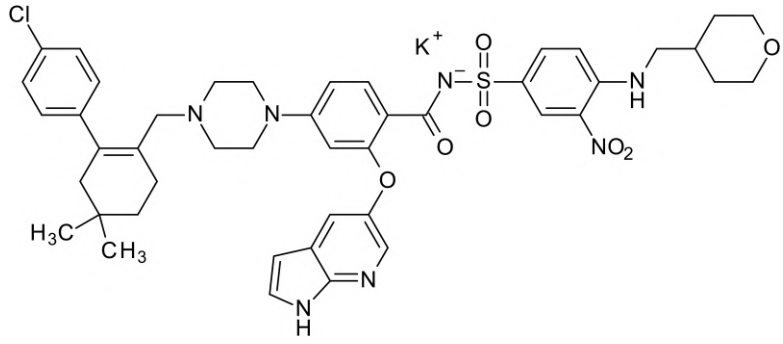
Because a pH of 7.83 does not exist physiologically, this percent ionization could only occur in an exogenously prepared solution.

CHAPTER 5

Structural Analysis Checkpoint

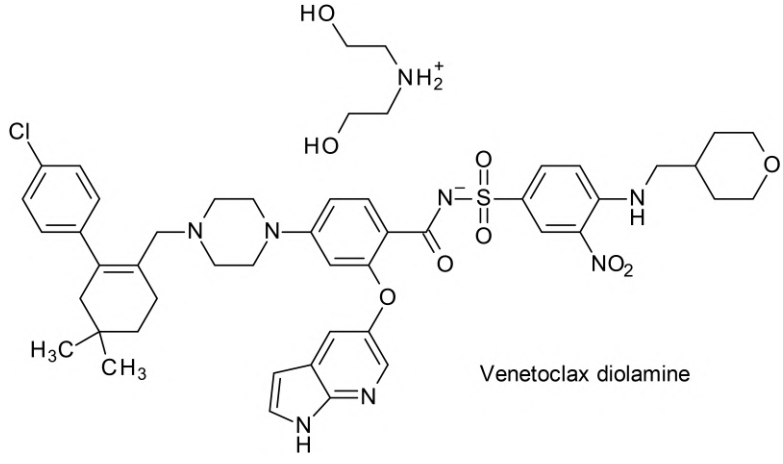
Checkpoint Drug 1: Venetoclax

1. The potassium salt of venetoclax is shown below.



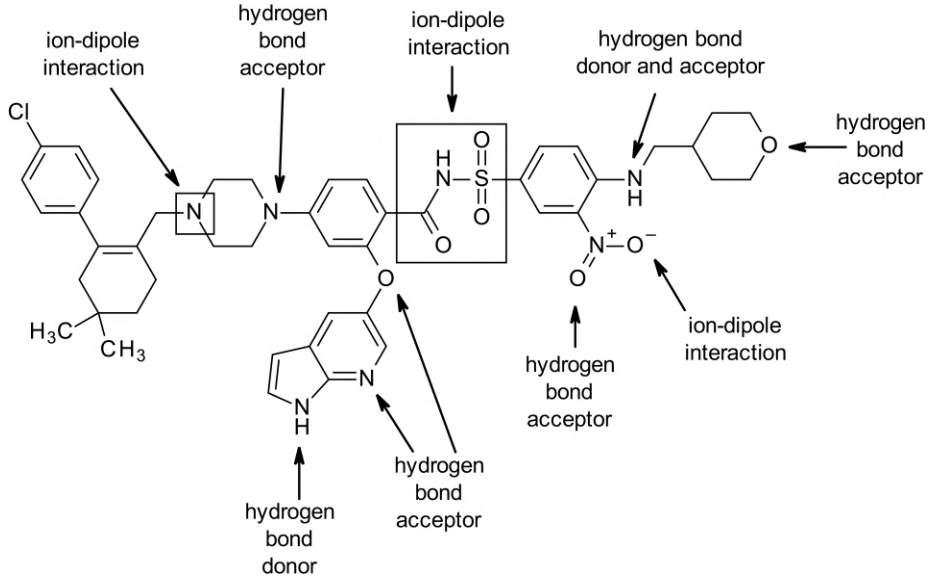
Part A: In Chapter 3, we identified five ionizable functional groups. Four of these five functional groups are basic while one is acidic. To form a salt, it is necessary to combine an acid and a base in a solution. In this case, an inorganic potassium salt was formed, so it can be concluded that the potassium ion came from KOH, a strong inorganic molecule. The addition of KOH to a solution containing venetoclax causes a significant increase in the pH of the solution. All four basic functional groups are primarily unionized, while the acidic sulfonamide is primarily ionized. Under these conditions, the negatively charged sulfonamide reacts with the positively charged potassium to form the salt shown in the answer to Question 1.

Part B: Shown below is a diolamine salt of venetoclax. Another base that could be used to make a salt with the sulfonamide is tromethamine.



Part C: Water-soluble organic salts are able to provide enhanced solvation and dissolution as compared with inorganic salts. The inorganic potassium ion is able to form ion-dipole bonds with water (as the ion). This is also true with the nitrogen atom of diolamine salt; however, the two hydroxyl groups allow additional hydrogen bonds with water, thus further enhancing water solubility beyond what is possible with the potassium salt.

2. The structure of venetoclax contains a large number of functional groups that can form hydrogen bonds or ion–dipole interactions with water. These have been highlighted below. Please note that the tertiary amine and the sulfonamide (boxed) is primarily ionized in the pH of the small intestine and would participate in ion–dipole interactions with water. These functional groups provide sufficient water solubility for venetoclax to dissolve in the gastrointestinal (GI) tract. The remaining functional groups (aromatic and alicyclic rings, alkyl chains, and halogens) provide sufficient lipid solubility to allow venetoclax to traverse the GI membrane once it is dissolved.



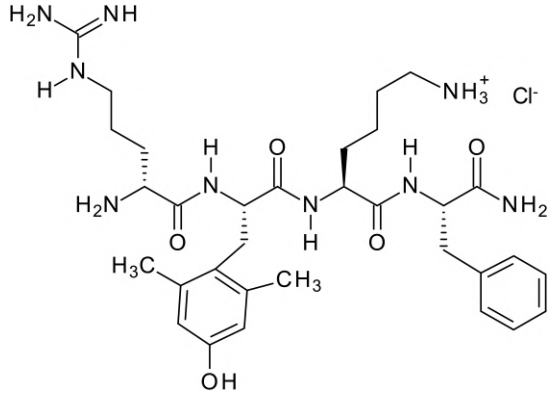
3. As compared with the original structure of venetoclax, the addition of a hydroxyl group enhances water solubility because this functional group can form hydrogen bonds with water. Additionally, the addition of a hydroxyl group allows for the formation of water- and lipid-soluble ester prodrugs. As discussed in the chapter, esterases are ubiquitous throughout the human body, allowing the release of the active drug in the liver, the blood stream, the GI tract, or the target tissue.

4. The chlorine group is lipid soluble; therefore, removing it and replacing it with a hydrogen atom would decrease the overall lipid solubility, or increase the overall water solubility, of venetoclax. Additionally, because the chlorine group is electron withdrawing through induction, the aromatic ring would no longer be electron deficient. This could affect charge transfer interactions and other interactions between the aromatic ring and its biological target. A full explanation of these types of interactions is provided in the next chapter. Additionally, electron withdrawing groups on aromatic rings decrease the metabolic oxidation of the ring. This is discussed in Chapter 8. Finally, because a hydrogen atom is smaller than a chlorine atom, the *para* position of this aromatic ring would be less sterically hindered.

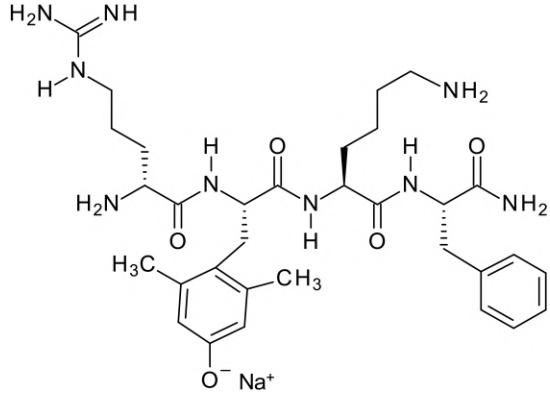
Checkpoint Drug 2: Elamipretide

1. **Part A:** Elamipretide can be formulated as a sodium or potassium salt (phenol) or as a hydrochloride or hydrobromide salt (guanidine and primary amine).

Part B: The hydrochloride salt of the primary amine of lysine is shown below.



The sodium salt of the phenol is shown below.



Part C: When comparing the sodium salt of elamipretide with the parent drug, it is important to note that the sodium salt allows for an ion–dipole interaction with water that will enhance the overall hydrophilic character of the drug molecule. This enhances water solubility and therefore improve distribution in the plasma. This also translates into better solubility and dissolution of the sodium salt of the drug when compared with the parent drug.

2. Elamipretide is a molecule that is significantly ionized across a range of pH values. The likelihood of it being water soluble at pH = 7.4 for IV administration is fairly good. If you are concerned that the substituted phenol and the aromatic hydrocarbon represent significant enough hydrophobic character to warrant formation of a water-soluble organic salt, then consider formulation as a tartrate salt (in **Figure 5-3** there are other examples). Because elamipretide contains several basic function groups (guanidine and two primary amines), it is possible to formulate the drug as a lactate salt.

3. **Part A:** We know that the smaller the value of log *P*, the more water soluble the drug molecule is. In this case cLog *P* = 0.3677 for elamipretide and the cLog *P* values for the HMG CoA reductase inhibitors are all larger than this value (although rosuvastatin isn't significantly larger!). That means that elamipretide is likely to be significantly more water soluble than the HMG CoA reductase inhibitors.

   Part B: The structure evaluation conducted in Chapter 2 revealed that there is significant hydrophilic character present in elamipretide (phenol [OH], primary amines, guanidine, amides). This supports the small cLog *P* value that indicates that elamipretide is water soluble.

   Part C: Several structural modifications could be made to increase the lipophilic character of elamipretide. A lipid-soluble salt (e.g., stearate) could be formed with the basic functional groups. A lipid-soluble ester prodrug (e.g., benzoate) of the phenol could be developed. Converting the amino terminus (primary amine) to an acetamide or other lipophilic amide is another option, but only if this primary amine isn't involved in a critical ionic interaction or ion–dipole interaction (as the ionic component) with the biological target.

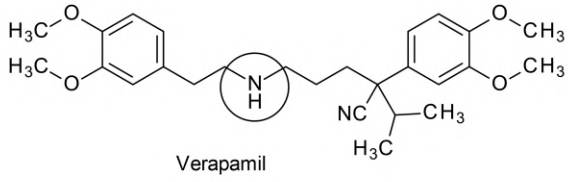
4. **Part A:** As mentioned in the answer to Question 3C, the amino terminus primary amine group can be converted to a lipophilic amide to improve lipid solubility of the drug molecule (provided that key ionic and/or ion–dipole interactions with the amine are not required). This type of transformation also protects the molecule from degradation from aminopeptidases because the enzyme would no longer recognize the drug molecule as a potential substrate. (*Note*: If the other primary amine [side chain of lysine] were converted into a lipophilic amide, the drug molecule would become more lipid soluble but would still remain subject to degradation via aminopeptidases.)

   The one problem with this strategy is that amidases are not as ubiquitous as esterases, and there is the potential that the amide would not undergo bioactivation (hydrolysis) to form an appreciable amount of the component carboxylic acid and amine (parent drug). My earlier warning about the types of interactions that are required with this primary amine determine whether the amide would represent a prodrug. *Remember*: A prodrug must be able to be metabolically cleaved to produce the active drug!

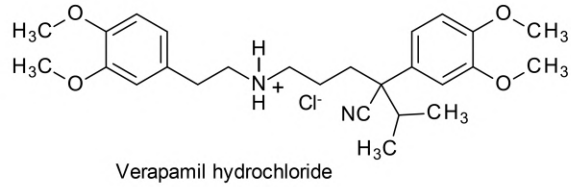
   Part B: Lipid-soluble ester prodrugs enhance the overall lipid solubility of the drug molecule and, therefore, improve absorption across lipophilic members (e.g., GI wall, skin, cell membranes). This causes an increase in cLog *P* to reflect less water solubility and more lipid solubility. This improved solubility enhances oral bioavailability. These types of prodrugs can also be used in depot-based formulations, which increase the duration of action of the product administered.

Review Questions

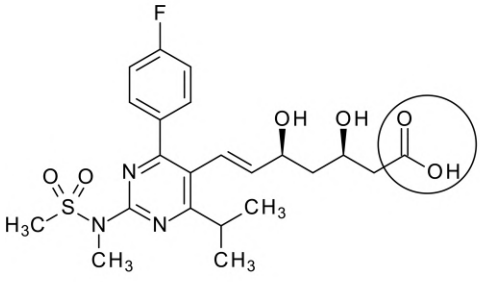
1. **Parts A and B:** Functional groups required to form salts have been circled and modified below.



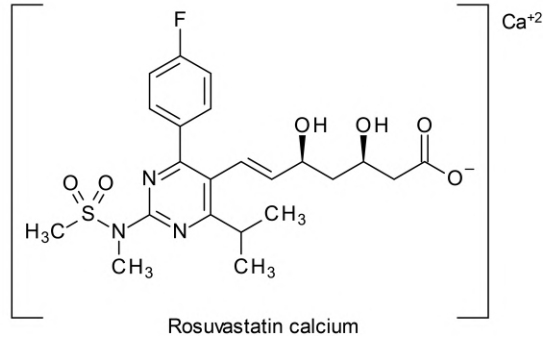
Verapamil



Verapamil hydrochloride



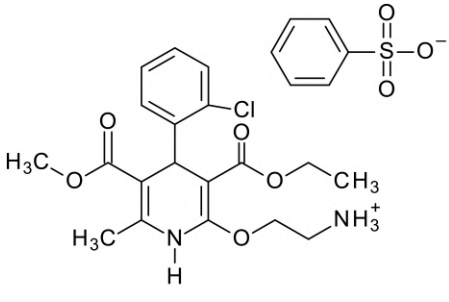
Rosuvastatin



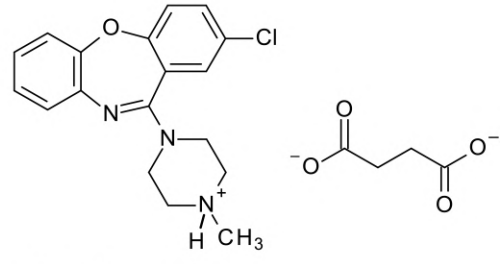
Rosuvastatin calcium

Part C: The salts formed are both inorganic salts.

2. Amlodipine besylate and loxapine succinate are examples of organic salts. Each drug has been modified below to represent the form present at pH = 7.4.



Amlodipine besylate



Loxapine succinate

3. **Part A:** Answers are provided in the grid below.

Name of Functional Group	Hydrophilic and/or Hydrophobic	Acidic, Basic, or Neutral	Contribution to Aqueous Solubility and/or Absorption
Ether	Hydrophilic (O)	Neutral	Aqueous solubility (O)
	Hydrophobic (R)		Absorption (R)
Aromatic hydrocarbon	Hydrophobic	Neutral	Absorption
Tertiary amine	Hydrophilic (N)	Basic (ionized at pH = 7.4)	Aqueous solubility (N)
	Hydrophobic (R)		Absorption (R)

R = carbon scaffolding.

Part B: Citalopram has a balance between functional groups that are hydrophobic (aromatic hydrocarbon) and can contribute to the ability of the drug to be absorbed/cross lipophilic membranes and groups that are hydrophilic (ether, ionized tertiary amine) that will allow for dissolution/solubility in the aqueous contents of the stomach.

Part C: Citalopram hydrobromide is more hydrophilic due to the presence of an ionized functional group that can participate in strong ion–dipole interactions with water.

4. Log P = –1 can be translated to mean for every one molecule that partitions into the organic phase, there are 10 molecules that partition into the aqueous phase. The more negative the value of log P, the more water soluble the drug is. When you compare the log *P* values for oxytetracycline and doxycycline, you can see that the number is becoming more positive, meaning the drug is becoming less water soluble. If you analyze the functional group composition of each of these tetracyclines, you see that doxycycline contains one less hydroxyl group and therefore is likely to be slightly less hydrophilic than oxytetracycline.

5. **Part A:** The structure evaluation is provided in the grid below.

Name of Functional Group	Hydrophilic and/or Hydrophobic	Acidic, Basic, or Neutral	Contribution to Aqueous Solubility and/or Absorption
Retinol			
Cycloalkene	Hydrophobic	Neutral	Absorption
Aliphatic chain and alkene groups	Hydrophobic	Neutral	Absorption
Primary alcohol (or hydroxyl)	Hydrophilic (OH)	Neutral	Aqueous solubility (OH)
	Hydrophobic (R)		Absorption (R)
Tretinoin			
Cycloalkene	Hydrophobic	Neutral	Absorption
Aliphatic chain and alkene groups	Hydrophobic	Neutral	Absorption
Carboxylic acid	Hydrophilic (COOH)	Acidic	Aqueous solubility (COOH)
	Hydrophobic (R)		Absorption (R)
Adapalene			
Aromatic hydrocarbon	Hydrophobic	Neutral	Absorption
Cycloalkane	Hydrophobic	Neutral	Absorption
Ether	Hydrophilic (O)	Neutral	Aqueous solubility (O)
	Hydrophobic (R)		Absorption (R)
Carboxylic acid	Hydrophilic (COOH)	Acidic	Aqueous solubility (COOH)
	Hydrophobic (R)		Absorption (R)

R = carbon scaffolding.

Part B: Acne is characterized by areas of skin inflammation and irritation. Topical application of a medication to treat acne allows for local treatment of only the affected areas and hopefully prevents unnecessary systemic distribution.

Retinol contains functional groups that are hydrophobic in character (cycloalkene, alkenes) that contribute to absorption of the drug into the affected skin and associated blemishes. Adapalene contains functional groups that are hydrophobic in character (cycloalkane; aromatic hydrocarbons) that contribute to absorption of the drug into the affected skin and associated blemishes. Additional evaluation of adapalene reveals the presence of a hydrophilic carboxylic acid. Despite the presence of this ionizable functional group, adapalene is considered primarily hydrophobic in character.

Part C: Tretinoin contains a mixture of hydrophilic (carboxylic acid, ionized at most physiologic pHs) and hydrophobic functional groups (cycloalkene, alkenes). This provides sufficient hydrophobic/hydrophilic balance to allow the drug to be solubilized in the aqueous contents of the GI tract and then absorbed across the lipophilic membrane of the GI tract.

Part D: Isotretinoin contains functional groups that are hydrophobic in character (cycloalkane, alkenes) that contribute to absorption of the drug into the affected skin and associated blemishes. Given that this agent has systemic side effects, one can assume that this agent is able to cross the skin and enter systemic circulation. The hydrophilic, ionizable carboxylic acid contributes to the ability of the drug to be solubilized in the plasma.

6. **Part A:** The structure evaluation is provided in the grid below.

Functional Group Name	Hydrophilic and/or Hydrophobic	Contribution to Aqueous Solubility and/or Absorption
Naftifine (marketed as HCl salt)		
Aromatic hydrocarbon	Hydrophobic	Absorption
Tertiary amine (basic, ionized at most physiological pHs)	Hydrophilic (N)	Aqueous solubility (N)
	Hydrophobic (R)	Absorption (R)
Alkene	Hydrophobic	Absorption
Undecylenic acid (marketed as calcium salt [powder] or zinc salt [cream])		
Alkene	Hydrophobic	Absorption
Alkane	Hydrophobic	Absorption
Carboxylic acid (acidic, ionized at most physiological pHs)	Hydrophilic (COOH)	Aqueous solubility (COOH)
	Hydrophobic (R)	Absorption (R)
Fluconazole		
Halogenated aromatic hydrocarbon	Hydrophobic (R)	Absorption (R)
1,2,4 Triazole rings	Hydrophilic (Ns)	Aqueous solubility (Ns)
	Hydrophobic (R)	Absorption (R)
Tertiary alcohol	Hydrophilic (OH)	Aqueous solubility (OH)
	Hydrophobic (R)	Absorption (R)

R = carbon scaffolding.

Part B: Naftifine and undecylenic acid contain several functional groups (e.g., aromatic hydrocarbon, alkene, aliphatic alkane) that contribute to the hydrophobic character of these molecules. Because they are predominantly hydrophobic in character, this allows for enhanced absorption across lipophilic membranes.

The therapeutic goal for these patients is to treat a fungal infection that is localized on their feet; therefore, medication does not need to be administered systemically. To be effective, the medication used must be able to penetrate both the skin and the fungal cell wall, both of which have a hydrophobic component associated with them.

Part C: For a drug to be given orally, it must have hydrophilic character to allow for solubility in the aqueous contents of the stomach and hydrophobic character to allow for absorption across the lipophilic membranes of the GI tract. A balance in these two types of structural characteristics is necessary for a drug to be administered orally.

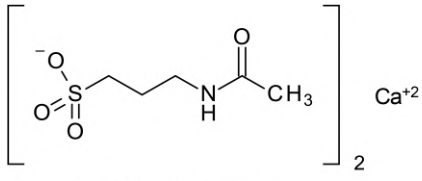
In fluconazole there is a halogenated aromatic hydrocarbon present. This functional group is hydrophobic and contributes to drug absorption. The heteroatoms in the 1,2,4 triazole rings are hydrophilic in character, as is the tertiary alcohol. These three functional groups contribute significantly to the water solubility of this agent.

Part D: This agent contains ethers (several) and other oxygen containing functional groups (ketones), all of which can interact with water via H-bonding (as H-bond acceptors). This suggests that this agent has more hydrophilic character than the antifungal agents (used topically) discussed above. Although there is a halogenated aromatic hydrocarbon (chloro substituent) and a cycloalkene present that contribute to the hydrophobic character of the drug, this agent is poorly absorbed across lipophilic membranes and is not utilized topically to treat fungal infections.

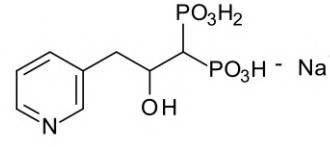
7. **Part A:** Hydrocortisone sodium succinate is a water-soluble organic salt. This increases the hydrophilic character of hydrocortisone sufficiently to become water soluble enough to be administered IM or IV. The strategy used to allow hydrocortisone to be administered IM or IV was via formation of a water-soluble organic salt.

   Part B: Hydrocortisone valerate is hydrophobic ester of hydrocortisone. This structural change further increases the hydrophobic character of hydrocortisone and decreases its aqueous solubility. Without sufficient balance between hydrophilic and hydrophobic character, this agent is unlikely to be sufficiently bioavailable if administered via an oral route and is not soluble enough to allow for IM or IV administration.

8. Both of these agents are very hydrophilic in character (acamprosate calcium: sulfonate salt, amide; risedronate sodium: bisphosphonate, secondary alcohol, pyridine) and are likely to be very water soluble. As a result, their elimination pathway is unlikely to include the need for Phase I or Phase II metabolic transformations.



Acamprosate calcium



Risedronate sodium

9. The acidic or basic character of each drug is shown below.

Orphenadrine citrate	Orphenadrine is basic
Losartan potassium	Losartan is acidic
Atorvastatin sodium	Atorvastatin is acidic
Bazedoxifene acetate	Bazedoxifene is basic
Abacavir sulfate	Abacavir is basic
Enalapril maleate	Enalapril is basic
Tetracycline phosphate	Tetracycline is basic

Omeprazole magnesium	Omeprazole is acidic
Tramadol hydrochloride	Tramadol is basic
Doxycycline calcium	Doxycycline is acidic
Butaconazole nitrate	Butaconazole is basic

10. **Part A:** We know that the partition coefficient of a drug molecule is defined as the ratio of the solubility of the unionized drug in an organic solvent to the solubility of the same unionized drug in an aqueous environment. Log $P = 1$ can be translated to mean for every 10 molecules that partition into the organic phase, there is one molecule that partitions into the aqueous phase. The more positive the value of log P, the more lipid soluble the drug is. In this case, both vitamin A and vitamin E have cLog P values that are much larger than one, which means that they are very hydrophobic in character. Administration of a drug molecule (or in this case a vitamin) via the skin requires that the drug/vitamin possess considerable hydrophobic character for it to be absorbed into and potentially across the lipophilic skin.

    Part B:

    1. Vitamin E contains an ionizable phenol functional group. This group can be converted into an inorganic salt (e.g., potassium salt) that can participate in an ion–dipole interaction with water and therefore enhance the aqueous solubility of the vitamin. Vitamin E could also be modified to form a water-soluble organic salt (e.g., a tromethamine salt). Again, the ionized drug can participate in an ion–dipole interaction with water and, therefore, enhances the aqueous solubility of the vitamin.

    2. Vitamin A does not contain an ionizable functional group, so it cannot form inorganic salts or a water-soluble organic salt. It can, however, be formulated as a water-soluble prodrug (e.g., sodium succinate ester) that can be metabolically cleaved to the parent vitamin. In this case, the inactive vitamin prodrug is able to participate in the ion–dipole interactions with water to improve the water solubility of the prodrug, and then metabolic hydrolysis will release the less aqueous soluble vitamin. Another option is to oxidize the primary alcohol to the corresponding carboxylic acid and then form an inorganic salt that will confer additional aqueous solubility. In this scenario, the carboxylic acid form of the vitamin represents one of the active forms of the vitamin and represents most of the activity of vitamin A.

11. **Part A:** Functional group names and properties are provided below.

Functional Group Name	Hydrophilic and/or Hydrophobic	Contribution to Aqueous Solubility and/or Absorption
TERCONAZOLE		
Halogenated aromatic hydrocarbon	Hydrophobic	Absorption
Ether(s)	Hydrophobic (R)	Absorption (R)
	Hydrophilic (O)	Solubility (O)
Aromatic hydrocarbon	Hydrophobic	Absorption
Aromatic heterocycle (1,2,4 triazole)	Hydrophobic (R)	Absorption (R)
	Hydrophilic (N)	Solubility (N)
Aniline (piperazine)	Hydrophobic (R)	Absorption (R)
	Hydrophilic (N)	Solubility (N)
Tertiary amine (piperazine)	Hydrophobic (R)	Absorption (R)
	Hydrophilic (N)	Solubility (N)

Functional Group Name	Hydrophilic and/or Hydrophobic	Contribution to Aqueous Solubility and/or Absorption
FLUCONAZOLE		
Halogenated aromatic hydrocarbon	Hydrophobic (R)	Absorption (R)
	Hydrophilic (F)	Solubility (F)
Aromatic heterocycle (1,2,4 triazole)	Hydrophobic (R)	Absorption (R)
	Hydrophilic (N)	Solubility (N)
Tertiary alcohol	Hydrophobic (R)	Absorption (R)
	Hydrophilic (OH)	Solubility (OH)
MICONAZOLE		
Halogenated aromatic hydrocarbon	Hydrophobic	Absorption
Aromatic heterocycle (1,3 diazole; imidazole)	Hydrophobic (R)	Absorption (R)
	Hydrophilic (N)	Solubility (N)
Ether	Hydrophobic (R)	Absorption (R)
	Hydrophilic (O)	Solubility (O)

R = carbon scaffolding.

Part B: Both miconazole and terconazole contain several hydrophobic functional groups (e.g., halogenated aromatic hydrocarbon, aromatic hydrocarbon). This hydrophobic character promotes absorption across membranes, including the vaginal wall and yeast cell walls. Vaginal secretions in premenopausal women are typically pH = 7.4. All of the basic aromatic and nonaromatic heterocycles in both drugs likely exist predominantly in their unionized form, which is also favorable for absorption across membranes.

Vaginal yeast infections are considered topical skin/skinfold infections and therefore it is effective to treat them with a topical agent.

Part C: For drugs to be administered orally, they must have a balance of hydrophilic character (for solubility in the aqueous contents of the stomach) and hydrophobic character (for absorption across the GI wall). Fluconazole has many hydrophilic functional groups that can participate in H-bonding with water (halogenated aromatic hydrocarbon; tertiary alcohol; 1,2,4 triazoles). These functional groups contribute to drug solubility in the aqueous contents of the stomach. These functional groups also have a hydrophobic component (all of the carbon-based R groups) that contribute to the ability of the drug to be absorbed across the GI wall). Because fluconazole has a balance of hydrophilic and hydrophobic character, it can be administered via an oral route.

12. **Loperamide**:
    - Swallow oral tablet
    - Tablet dissolves in aqueous contents of stomach (requires drug to be water soluble)
        - The amide, piperidine (tertiary amine), and tertiary alcohol all have hydrophilic character and contribute to the water solubility of the drug.
    - Do not want absorption into systemic circulation via stomach lining
        - The piperidine (tertiary amine pK_a = 9-11) is predominantly ionized in the stomach pH = 1 (pH < pK_a).
    - Distribution into intestine; smaller fraction in ionized form; retain water solubility
        - The piperidine (tertiary amine pK_a = 9-11) is somewhat less (but still predominantly) ionized in the intestine pH = 8 (pH < pK_a).
    - Interaction with receptor located in intestine; no need for absorption out of the GI system and into systemic distribution

Scopolamine:

- Place patch on skin
- Absorption of drug from patch into skin lipophilic bilayer (requires hydrophobic character)
  - The aromatic ring and bicyclic heterocycle contribute to hydrophobic character of drug and allow for absorption and transport through skin.
- Transport through skin and dissolved into circulation (requires drug to be water soluble)
  - The bicyclic heterocycle (tertiary amine $pK_a = 9\text{-}11$) is primarily ionized in the plasma $pH = 7.4$ ($pH < pK_a$); the ester, ether, and primary alcohol are hydrophilic in character and contribute to the water solubility of the drug.
- Distribution to peripheral receptors (requires drug to be water soluble)
  - The bicyclic heterocycle (tertiary amine $pK_a = 9\text{-}11$) is primarily ionized in the plasma $pH = 7.4$ ($pH < pK_a$); the ester, ether and primary alcohol are hydrophilic in character and contribute to the water solubility of the drug.
- Interaction with receptor located in periphery

CHAPTER 6

Structural Analysis Checkpoint

Checkpoint Drug 1: Venetoclax

1. Answers provided in the table below.

	Functional Group	Types of Binding Interactions	Amino Acids Capable of Forming Specific Binding Interaction
A	Halogenated phenyl ring (or aromatic ring)	(1) van der Waals; hydrophobic	(1) Tyr, Phe, Trp (better interaction)[a]; Val, Leu, Ile, Met
		(2) π–π Stacking	(2) Tyr, Phe, Trp
		(3) Charge transfer (as electron poor aromatic ring)	(3) Tyr, Trp
		(4) Cation-π interactions	(4) Lys, Arg, His[b]
B	Tertiary amine	(1) Ionic	(1) Asp, Glu
		(2) Ion-dipole (as the ion)	(2) Ser, Thr, Tyr, Cys, Asn, Gln, Met
C	Sulfonamide[c]	(1) Ionic	(1) Lys, Arg, His[b]
		(2) Ion-dipole (as the ion)	(2) Ser, Thr, Tyr, Cys, Asn, Gln, Trp, His[b]
D	Heterocyclic non-aromatic ring (ether containing alicyclic ring)	*Ether oxygen atom*	
		(1) Ion-dipole (as the dipole)	(1) Lys, Arg, His[b]
		(2) Dipole-dipole	(2) Asn, Gln
		(3) Hydrogen bond acceptor	(3) Ser, Thr, Tyr, Trp, Cys, Asn, Gln, His[b]
		Hydrocarbon ring	
		van der Waals; hydrophobic	Val, Leu, Ile, Met, Ala (better interaction)[a]; Tyr, Phe, Trp

[a] Stronger van der Waals interactions occur when aromatic rings interact with aromatic rings and when aliphatic rings and chains interact with aliphatic rings and chains; however, all of the listed amino acids could possibly interact with the indicated functional groups.

[b] The side chain of histidine is primarily unionized at a pH = 7.4. The small fraction that is ionized could participate in a cation-π interaction or an ionic bond, while the unionized fraction can serve as a dipole in an ion–dipole interaction or as a hydrogen bond donor or acceptor.

[c] The carbonyl group has been included in the box because it provides additional resonance delocalization of the negative charge of the sulfonamide (see Chapter 3 Questions/Answers).

2. Answers provided in the table below.

	Functional Group	Types of Binding Interactions	Possible with DNA (Yes or No) and Why or Why Not
A	Halogenated phenyl ring (or aromatic ring)	(1) van der Waals; hydrophobic	(1) *Yes,* the purine and pyrimidine rings have a dipole, so dipole-induced dipole interactions are possible. Hydrophobic interactions are less likely due to the normal stacking of DNA bases (i.e., less displacement of water).
		(2) π–π stacking	(2) *Yes*, this could occur with the purine and pyrimidine bases.
		(3) Charge transfer (as electron poor aromatic ring)	(3) *Yes*, this could occur with the purine and pyrimidine bases.
		(4) Cation-π interactions	(4) *No*, positive charges are not normally present in DNA.
B	Tertiary amine	(1) Ionic	(1) *Yes*, ionic bonds could form with the phosphate groups of DNA.
		(2) Ion-dipole (as the ion)	(2) *Yes*, this could occur with dipoles present in the purine and pyrimidine bases as well as the deoxyribose sugar.
C	Sulfonamide	(1) Ionic	(1) *No*, positive charges are not normally present in DNA.
		(2) Ion-dipole (as the ion)	(2) *Yes*, this could occur with dipoles present in the purine and pyrimidine bases as well as the deoxyribose sugar.
D	Heterocyclic non-aromatic ring (ether-containing alicyclic ring)	*Ether oxygen atom*	
		(1) Ion-dipole (as the dipole)	(1) *Yes*, this could occur with dipoles present in the purine and pyrimidine bases as well as the deoxyribose sugar.
		(2) Dipole-dipole	(2) *Unlikely,* but could occur with partially positive carbon atoms in the purine and pyrimidine bases.
		(3) Hydrogen bond (acceptor)	(3) *Yes*, hydrogen bond donors exist in purine and pyrimidine rings as well as the deoxyribose sugar.
		Hydrocarbon ring	
		van der Waals; hydrophobic	*No*, these interactions generally occur with alicylic rings and chains, not the heterocylic bases in DNA.

3. Yes, it is possible for venetoclax to form covalent bonds with proteins, enzymes, and other biomolecules; however, the probability of this occurring is somewhat low. None of the functional groups present within the structure of venetoclax are inherently highly reactive; thus, alkylation, acylation, or phosphorylation reactions would not be expected to occur. It is, however, possible for one or more of these functional groups to be metabolically transformed to a more highly reactive intermediate similar to the rearrangement reactions discussed in the chapter. If this were to occur, then a covalent bond could be formed.

Checkpoint Drug 2: Elamipretide

1. Answers provided in the table below.

	Functional Group Name	Interactions Possible (as Drawn)	Interactions Possible (at pH = 7.4)	Amino Acid Whose Side Chain Can Interact with the Functional Group at pH = 7.4
A	Guanidine	1. Hydrogen bonding (acceptor and donor)	1. Ionic	1. Glu, Asp
		2. Ion-dipole (as the dipole)	2. Ion-dipole (as the ion)	2. Ser, Thr, Met, Tyr, Cys, Gln, Asn, Trp
B	Primary amine	1. Hydrogen bonding (acceptor and donor)	1. Ionic	1. Glu, Asp
		2. Ion-dipole (as the dipole)	2. Ion-dipole (as the ion)	2. Ser, Thr, Met, Tyr, Cys, Gln, Asn, Trp
C	Amide	1. Hydrogen bonding (acceptor and donor)	1. Hydrogen bonding (acceptor and donor)	1. Ser, Thr, Met, Tyr, Cys, Gln, Asn, Trp. His
		2. Ion-dipole (as the dipole)	2. Ion-dipole (as the dipole)	2. Glu, Asp, Lys, Arg
D	Phenol	1. Hydrogen bonding (acceptor and donor)	1. Hydrogen bonding (acceptor and donor)	1. Ser, Thr, Met, Tyr, Cys, Gln, Asn, Trp, His
		2. Ion-dipole (as the dipole)	2. Ion-dipole (as the dipole)	2. Glu, Asp, Lys, Arg

2. Answers provided in the table below.

Interaction Type	Functional Groups
Cation-π	A, B, D
Ionic	A, B
Chelation	A, B
van der Waals/Hydrophobic	D
Ion-dipole (as the dipole)	C, D
Ion-dipole (as the ion)	A, B
H-bonding (donor and acceptor)	C, D

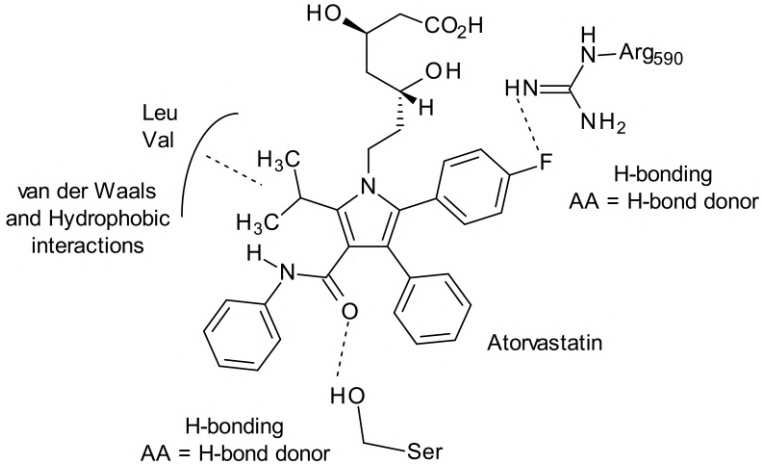
Review Questions

1. Answers provided in the table below.

Name of Functional Group	Acidic, Basic, or Neutral (As Drawn)	Ionized, Unionized or not Ionizable (at pH = 7.4)	Hydrogen Bond Acceptor, Donor, Both, or Neither (at pH = 7.4)	Amino Acids Whose Side Chain Can Interact with the Functional Group via Hydrogen Bonding (at pH = 7.4)[a]
Carbamate (×2)	Neutral	Not ionizable	Both	Ser, Thr, Tyr, Cys, Asn, Gln, Trp, His, Met
Secondary alcohol (or hydroxyl)	Neutral	Not ionizable	Both	Ser, Thr, Tyr, Cys, Asn, Gln, Trp, His, Met
Aromatic hydrocarbon	Neutral	Not ionizable	Neither	None
Secondary Amine	Basic	Ionized	Neither	None
Aliphatic alkane	Neutral	Not ionizable	Neither	None

[a] "None" is a possible answer.

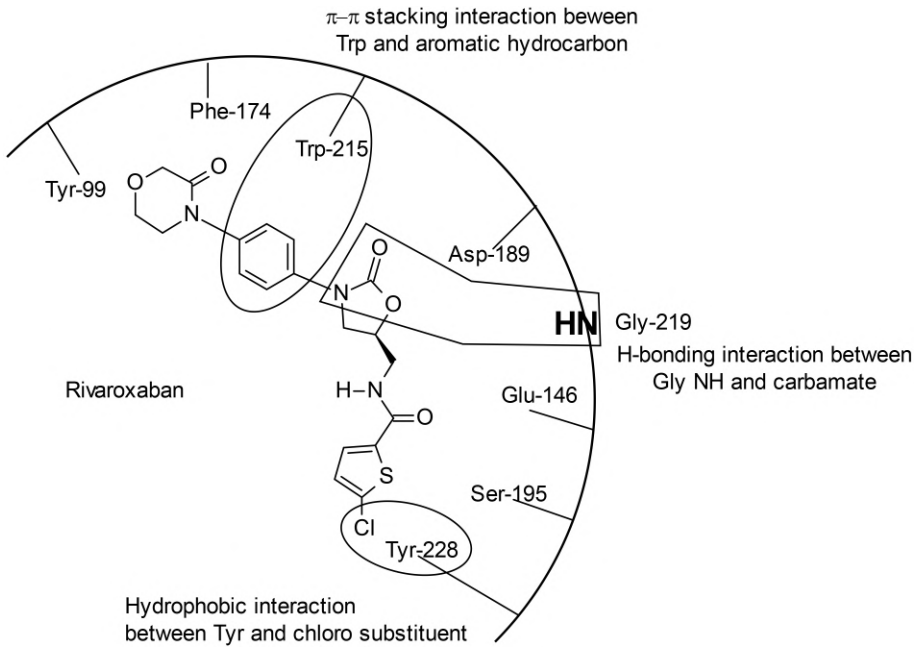
2. The diagram below is labeled to show the interactions between the isopropyl, *para*-fluoro phenyl, and amide functional groups and three active site amino acid side chains within HMG CoA reductase.



3. Possible interactions are provided in the grid below.

Name of Functional Group	Acidic, Basic, Neutral (as Drawn)	H-bond Acceptor, Donor, Both, or Neither (at pH = 7.4)	Interaction Possible with Serine (at pH = 7.4)	Interaction Possible with Glutamic Acid (at pH = 7.4)	Interaction Possible with Lysine (at pH = 7.4)	Interaction Possible with Tryptophan (at pH = 7.4)
Ertapenem						
Amide (mono substituted)	Neutral	Both	Hydrogen bonding; dipole-dipole	Ion dipole Drug = dipole	Ion dipole Drug = dipole	Hydrogen bonding; dipole-dipole
Amide (Lactam)	Neutral	Acceptor only	Hydrogen bonding; dipole-dipole	Ion-dipole Drug = dipole	Ion-dipole Drug = dipole	Hydrogen bonding; dipole-dipole
Carboxylic acid	Acidic pK_a = 2.5-5	Neither (ionized)	Ion-dipole Drug = ion	None	Ionic	Ion-dipole Drug = ion
Phenol (R-OH)	Acidic pK_a = 9-10	Both	Hydrogen bonding; dipole-dipole	Ion-dipole Drug = dipole	Ion-dipole Drug = dipole	OH: Hydrogen bonding; dipole-dipole R: van der Waals; Hydrophobic: π–π stacking
Thioether	Neutral	Acceptor only	Hydrogen bonding	None	Ion-dipole FG = dipole	Hydrogen bonding; van der Waals; hydrophobic interaction
Primary amine	Basic pK_a = 9-11	Neither (ionized)	Ion-dipole Drug = Ion	Ionic	None	Ion dipole Drug = ion; Cation-π interaction FG = cation
Alkene	Neutral	Neither	None	None	None	van der Waals; hydrophobic interaction

4. The functional groups that interact with the amino acid side chains in the S1 and S4 pockets as well as with the backbone glycine NH are circled and the interactions labeled.



5. Functional group evaluation to predict interactions within PAR-1 receptor binding site.

Name of Functional Group	Acidic, Basic, or Neutral	Hydrogen Bond Acceptor, Donor, Both, or Neither	Interaction Possible with the PAR-1 (Thrombin) Receptor	Name of One Amino Acid That Can Participate in the Interaction Identified in the Previous Column with the Functional Group (at pH = 7.4)
Ester	Neutral	H-bond acceptor	H-bonding; ion-dipole (as the dipole); dipole-dipole	Ser, Thr, Tyr, Cys, Gln, Asn, Trp, His
Cycloalkane	Neutral	Neither	van der Waals; hydrophobic	Val, Leu, Ile, Met, Phe, Tyr, Trp
Carbamate	Neutral	H-bond donor and acceptor	Ion-dipole; dipole-dipole; H-bonding	Lys, Arg (ion); Ser, Thr, Tyr, Cys, Gln, Asn, Trp; His, Met (dipole and H-bonding)
Alkene	Neutral	Neither	van der Waals; hydrophobic	Val, Leu, Ile, Met
Aromatic heterocycle (pyridine)	Basic	H-bond acceptor	Ion-dipole (as the dipole); dipole-dipole; H-bonding	Lys, Arg (ion); Ser, Thr, Tyr, Cys, Gln, Asn, Trp, His (dipole and H-bonding)
Halogenated aromatic hydrocarbons	Neutral	H-bond acceptor	H-bonding	Ser, Thr, Tyr, Cys, Gln, Asn, Trp, His

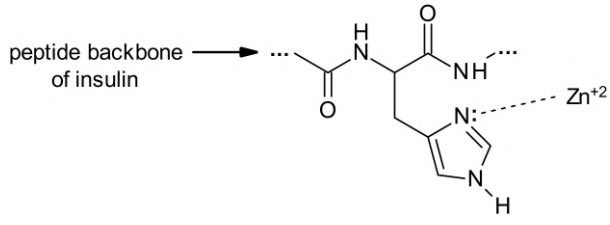
6. Functional groups and their hydrogen bonding properties are provided below.

Bictegravir	Emtricitabine	Tenofovir
Amide (H-bond acceptor and donor)	Ether (H-bond acceptor)	Ester (H-bond acceptor)
Lactam (H-bond acceptor)	Urea (H-bond acceptor)	Phosphamide (H-bond acceptor and donor)
Ketone (H-bond acceptor)	Primary alcohol (H-bond acceptor and donor)	Aromatic heterocycle (H-bond acceptor)
Halogenated aromatic hydrocarbon (H-bond acceptor)	Fluoro (H-bond acceptor)	Ether (H-bond acceptor)
Ether (H-bond acceptor)	Thiourea (H-bond acceptor)	Primary amine (H-bond acceptor and donor)
Tertiary amine (H-bond acceptor)	Primary amine (H-bond acceptor and donor)	

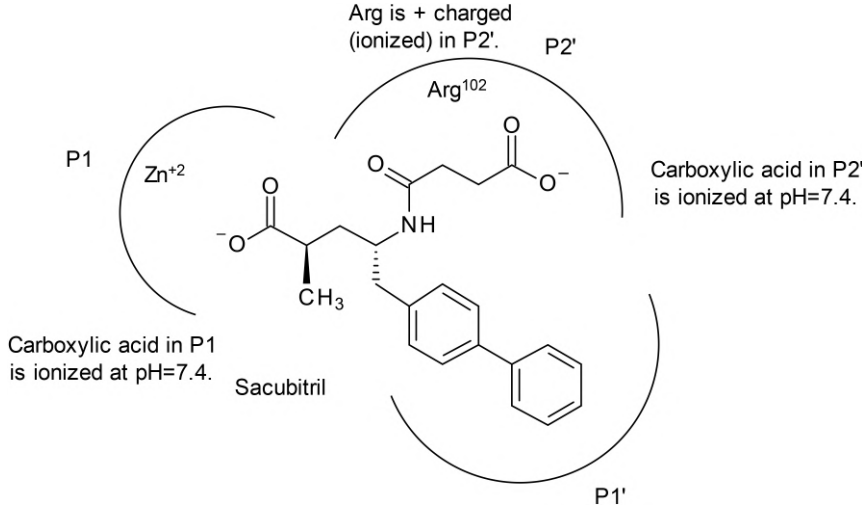
7. **Part A:** An ionic interaction occurs between the ionized carboxylic acid found in the side chain of aspartic acid and the ionized primary amine found in the structure of dopamine.

   Part B: The serine hydroxyl group is a neutral functional group and is not ionizable in any physiologic environment. The catechol is acidic in character and at physiologic pH the pH < pK_a and the catechol is unionized. Both of these functional groups are able to participate in hydrogen bonding or dipole–dipole interactions.

8. The positively charged zinc atom complexes with the lone pair of electrons on the imidazole ring. Although the zinc atom can also complex with an amide carbonyl found on the peptide backbone of insulin, it is much more likely to complex with the histidine side chain because this side chain is less sterically hindered and thus more available.

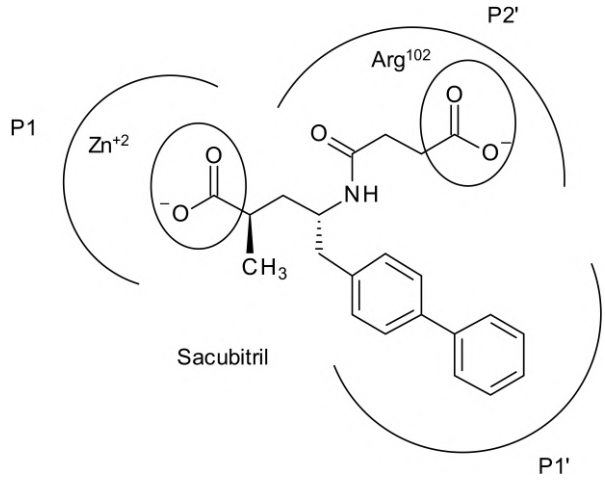


9. **Part A:** Ionization states are shown below.



Part B: In the P1 pocket, the zinc atom and the ionized carboxylic acid can participate in an ionic interaction. In the $P2^1$ pocket, the ionized arginine residue can participate in an ionic interaction with the ionized carboxylic acid.

Part C: Functional groups that can participate in ionic interactions are circled below.



Part D: In the $P1^1$, pocket there can be van der Waals, hydrophobic, or π-π stacking interactions with the biphenyl functional group. The three amino acids most likely to participate in these interactions are Phe, Tyr, and Trp.

10. Both nonoxonyl-9 and cannabidiol contain unbranched long aliphatic and/or alicyclic alkanes as well as aromatic rings. These functional groups can participate in hydrophobic interactions and van der Waals interactions.

11. Drug molecules that covalently bind to their biological targets can demonstrate enhanced selectivity for their targets and a prolonged duration of action. Let's look at each of these properties individually. The enhancement in selectivity is primarily achieved via the use of prodrugs that are converted to reactive intermediates (active drug) in close proximity to their biological targets. Proximity of the reactive intermediate and the biological target is important to maximize so as to avoid unnecessary misadventures between the reactive intermediate and other proteins present. In fact, it should be noted that those drugs that bind covalently *that are not prodrugs*, and therefore do not require in vivo bioactivation, are generally no more selective than analogous drugs that interact via noncovalent means. Although drugs that form a covalent bond with their biological target do have a longer duration of action than drug molecules that participate in noncovalent interactions, it is important to be cautious when championing this property. It should be no surprise that the effects of covalently bound drugs are not as readily reversed as those associated with drugs that participate in noncovalent interactions. As a result, there is a possibility that the overall duration of drug action may be too long for the desired therapeutic effect (representing a potential disadvantage).

    As you might expect, the possibility for a drug misadventure, as mentioned previously, represents the major disadvantage associated with drugs that form covalent bonds with their biological targets. Because the active form of the drug is highly reactive, the potential for the drug to react with a nearby protein can be significant. As a result, special preparation and administration guidelines *may* need to be in place to ensure that drug activation occurs in close proximity to the desired biological target.

12. Answers provided in the table below.

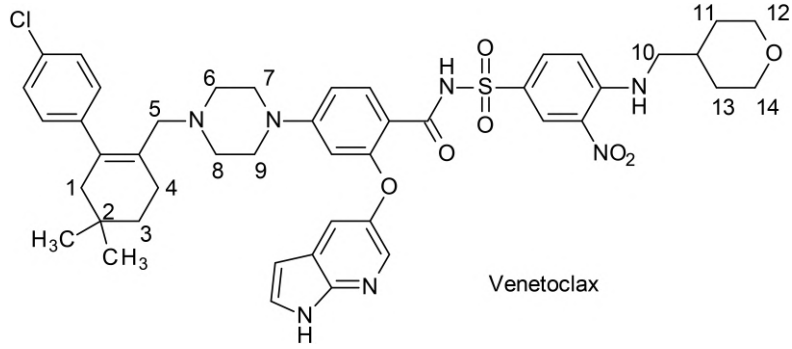
Sclareol		Vanillin		Nerolidol	
Interaction Type	**Functional Group**	**Interaction Type**	**Functional Group**	**Interaction Type**	**Functional Group**
Hydrophobic	Cycloalkane, aliphatic alkane, alkene	van der Waals, hydrophobic	Phenol (specifically the aromatic ring)	van der Waals, hydrophobic	Aliphatic alkanes and alkenes
Hydrogen bond (acceptor and donor)	Tertiary alcohol (or hydroxyl)	Hydrogen bond (acceptor)	Phenol, ether, aldehyde	Hydrogen bond (acceptor and donor)	Tertiary alcohol (or hydroxyl)
Ion-dipole (as the dipole)	Tertiary alcohol (or hydroxyl)	Hydrogen bond (donor)	Phenol		
		Ion-dipole (as the dipole)	Phenol, ether, aldehyde		

CHAPTER 7

Structural Analysis Checkpoint

Checkpoint Drug 1: Venetoclax

1. The structure of venetoclax does not contain any chiral centers; however, there are 14 potential prochiral centers. These have been identified below. With the exception of prochiral center 2, the metabolic addition of a functional group to any of these carbon atoms would result in a chiral center. For prochiral center 2, the addition of a functional group to either of the adjacent methyl groups would create a chiral center. At this point, we are only looking at ***potential*** prochiral centers. In Chapter 8, after we have discussed all of the metabolic pathways, we revisit this question to see which of these potential prochiral centers can actually be converted to a chiral center.



Venetoclax

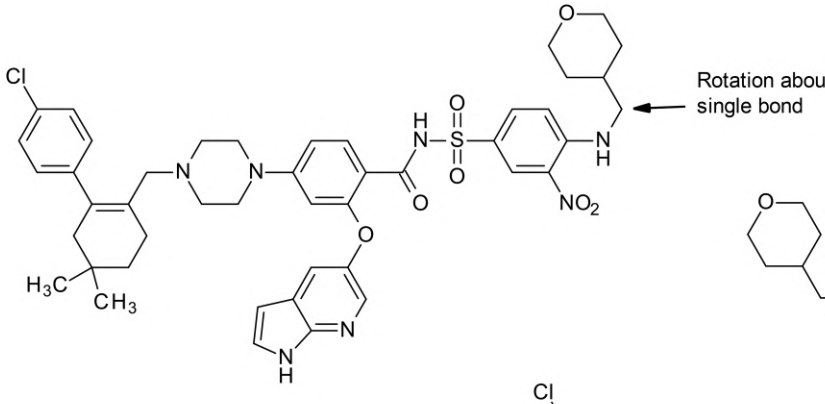
2. **Part A:** No. Drug molecules need to have at least one chiral center to have enantiomers. The structure of venetoclax does not contain any chiral centers, so it is not possible for it to have enantiomers.

   Part B: No. Drug molecules need to have at least two chiral centers to have diastereomers. The structure of venetoclax does not contain any chiral centers, so it is not possible for it to have diastereomers.

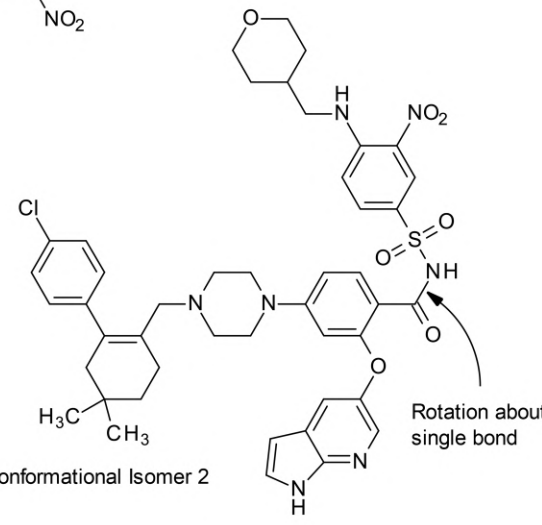
Part C: No. Geometric isomers are a specialized type of diastereomers that occur due to the presence of an alicyclic ring or a double bond. The structure of venetoclax does contain two alicyclic rings; however, as mentioned above, neither of these contains a chiral center. The cyclohexene ring that is attached to the *para* chlorophenyl ring does contain a double bond; however, due to the geometry and rigidity of a six-membered ring, it is not able to have *cis* and *trans* isomers. To have geometric isomers, the double bond needs to be in an aliphatic chain and not a ring.

Part D: Yes. Conformational isomers result from the rotation of single bonds with a drug molecule. Because the structure of venetoclax contains numerous single bonds that can undergo free rotation, it is possible for venetoclax to have multiple conformational isomers.

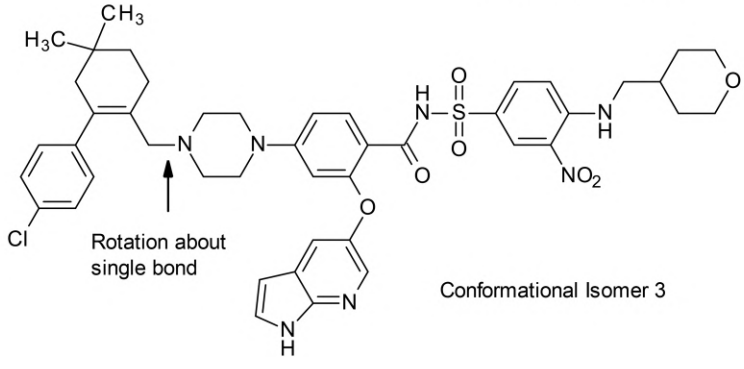
3. Shown below are three conformational isomers of venetoclax. Although all of these conformational isomers are possible, conformational isomer 2 requires rotation of a bond involved in the resonance stabilization of the sulfonamide and may be less likely than the other two.



Conformational Isomer 1



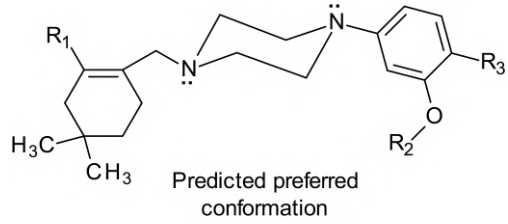
Conformational Isomer 2



Conformational Isomer 3

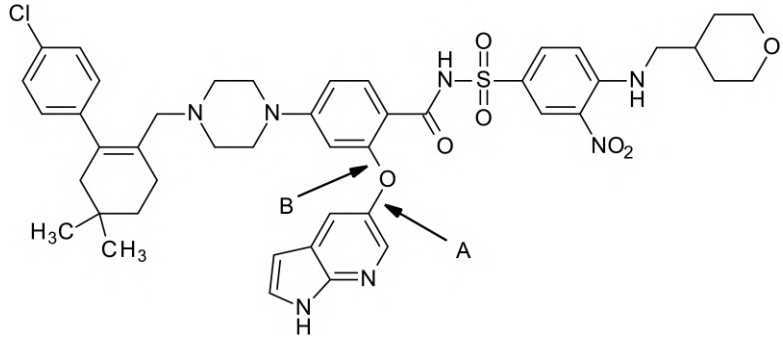
4. Cyclohexane and other six-membered, nonaromatic, heterocyclic ring systems (i.e., a piperazine ring) can adopt either a chair or boat conformation. Of these two options, chair conformations are preferred due to lower steric hindrance (or repulsion) and the presence of staggered bonds (as compared with eclipsed bonds seen in boat formations). Similar

to chair-chair inversion seen with cyclohexane, the ability of nitrogen atoms to undergo inversion of their lone-pair of electrons allows for the minimization of steric hindrance (or repulsion) and the maximization of attractive forces. In the case of venetoclax, the nitrogen atoms in the piperazine ring are attached to aromatic and alicyclic rings. For steric reasons, these groups should be oriented such that they both occupy equatorial positions as shown below.



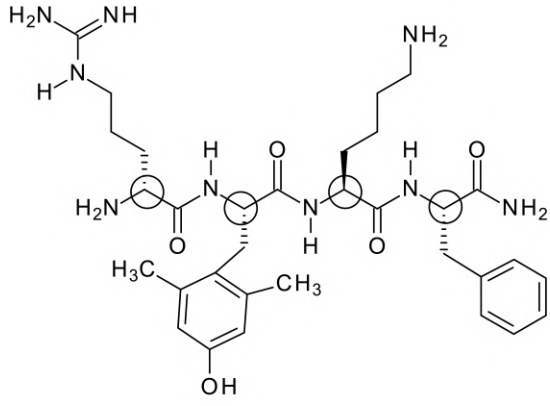
Predicted preferred conformation

5. The pyrrolopyridine ring can assume a variety of conformational orientations relative to its adjacent aromatic ring and the *meta* piperazine ring and the *ortho* side chain attached to this ring. The pyrrolopyridine ring can rotate about two bonds indicated in the structure below. Due to the rigidity and distance of the piperazine ring, it would not be expected to influence the conformation of the pyrrolopyridine ring. Rotation about bond A would allow the pyrrolopyridine ring to lie parallel to the adjacent aromatic ring, perpendicular to the adjacent aromatic ring, and any intermediate orientation. Minimal steric factors affect this rotation. Rotation about bond B would allow similar orientations; however, rotations approaching 135° to 180° would cause increasingly significant steric interactions with the *ortho* side chain and would not be favored. In terms of bond B, the orientation shown below of the pyrrolopyridine with respect to the *ortho* side chain provides the least steric hindrance.



Checkpoint Drug 2: Elamipretide

1. There are four chiral carbons present in elamipretide.



Elamipretide

2. For the arginine component of elamipretide, there are three additional prochiral carbons.

   For the tyrosine derivative component of elamipretide, there is one additional prochiral carbon.

   For the lysine component of elamipretide, there are four additional prochiral carbons.

   For the phenylalanine component of elamipretide, there is one additional prochiral carbon.

3. **Part A:** Enantiomers must contain at least one chiral carbon. Elamipretide contains four chiral carbons. To produce an *enantiomer* of elamipretide (*S,S,R,S* = configuration as drawn), all of the chiral carbons would need to be in their opposite configuration (*R,R,S,R*).

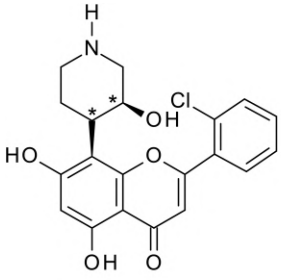
   Part B: For a molecule to have diastereomers, it must contain at least two chiral carbons. Elamipretide contains four chiral carbons. To produce a *diastereomer* of elamipretide (*S,S,R,S* = configuration as drawn), at least one of the chiral carbons must be in its opposite configuration (*R,S,R,S*). There are many, many diastereomers possible for elamipretide!

4. Geometric isomers are *not* possible for elamipretide because it does not contain a double bond or alicyclic ring system.

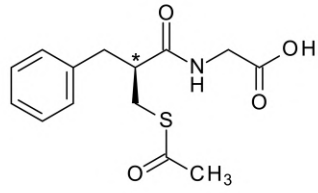
5. Because elamipretide contains numerous rotatable single bonds, not only at the ends of the molecule but in the middle of the molecule, it is considered conformationally flexible. In addition, it does not contain any double bonds or rigid ring systems.

Review Questions

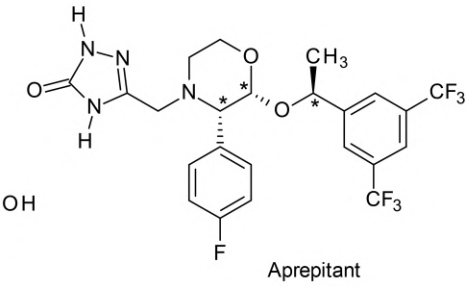
1. Chiral carbon atoms are identified below with asterisks. Circled carbon atoms are identified below as being prochiral or not prochiral.



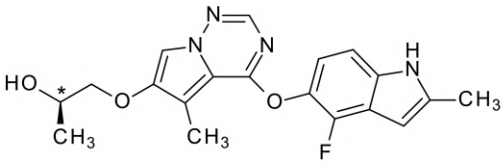
Alvocidib



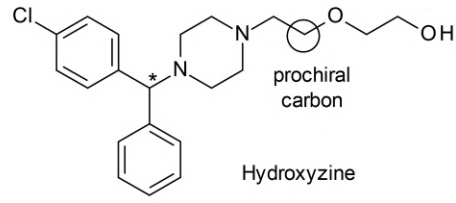
Ecadotril



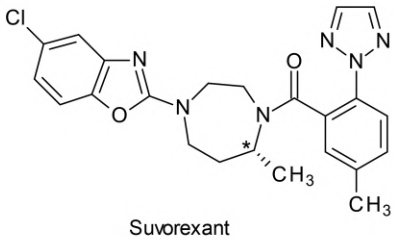
Aprepitant



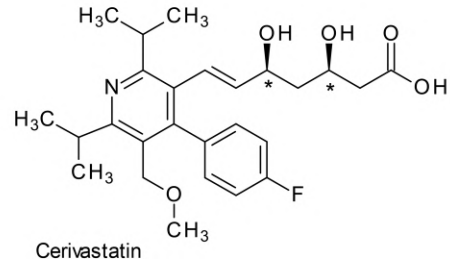
Brivanib



Hydroxyzine



Suvorexant



Cerivastatin

2. The *R* enantiomer is drawn. This is based on the following priorities:

   Priority #1: Aromatic heterocycle (pyridine)

   Priority #2: CH_2-to geminally substituted cyclopentane

   Priority #3: CH_2CH_2-O

   Priority #4: CH_2CH_2-NH

3. *Similar properties:* molecular weight, infrared (IR) and nuclear magnetic resonance (NMR) spectral properties, log *P* values, water/lipid solubility balance, dissolution rates, pK_a values of any acidic or basic functional group, and the percent ionization of these functional groups at any given pH value.

   Different property: direction that the molecule rotates plane polarized light. D/L

4. Correct matches are shown below.

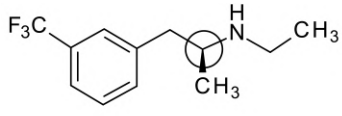
(+)/(–) matches with	Direction that enantiomer rotates plane polarized light
	Dextrorotatory/levorotatory
d/l matches with	Direction that enantiomer rotates plane polarized light
	Dextrorotatory/levorotatory
D/L matches with	Absolute configuration
	Steric arrangement of atoms about a chiral carbon
R/S matches with	Absolute configuration Steric arrangement of atoms about a chiral carbon

5. **Part A:** Only one (+)/(–) designation that indicates the net rotation of plane polarized light.

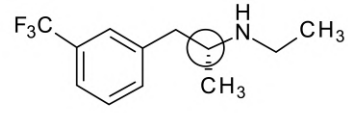
   Part B: Yes, it can have an enantiomer (nonsuperimposable mirror image), a diastereomer (nonsuperimposable nonmirror image), and conformational isomers (nonsuperimposable isomers that differ due to free rotation of atoms about single bonds). There is not enough information provided to determine if a geometric isomer is possible (e.g., no information related to presence of rings or double bonds).

6. Sotalol is sold as a racemate. It is possible that the two biological targets (potassium channel and β receptor) have stereochemical differences for their interaction requirements. As an example, let's assume that when the *R* enantiomer interacts with the β receptor, the hydroxyl group is oriented in a direction that allows it to participate in a required ion–dipole interaction with a charged functional group located within the biological target. In the *S* enantiomer, this hydroxyl group is oriented in the opposite direction of the *R* enantiomer and is not able to participate in the required ion–dipole interaction with the biological target. At the potassium channel, the binding requirements for this same hydroxyl group are different, thus allowing the *S* enantiomer to participate in a key hydrogen bond but not the *R* enantiomer.

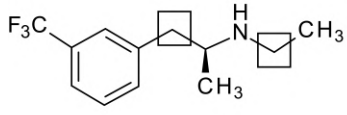
7. Chiral carbons are circled. Prochiral carbons are boxed.



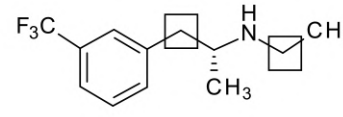
D-Fenfluramine



L-Fenfluramine



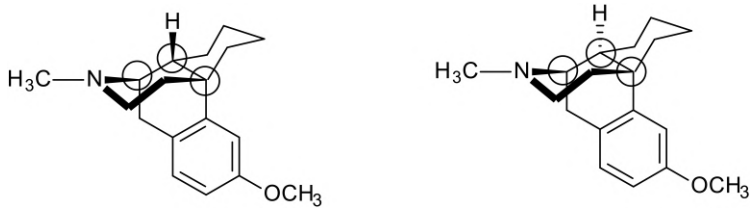
D-Fenfluramine



L-Fenfluramine

Fenfluramine is available as a mixture of D/L isomers; however, the L-isomer causes significant drowsiness. The L-isomer has a methyl group pointed behind the plane of the paper, whereas the D-isomer has a methyl group pointed in front of the plane of the paper. Since drowsiness is associated with the L-isomer, one might guess that this methyl group is important in activating the biological target that causes drowsiness (or prevents alertness).

8. Chiral centers have been circled below.



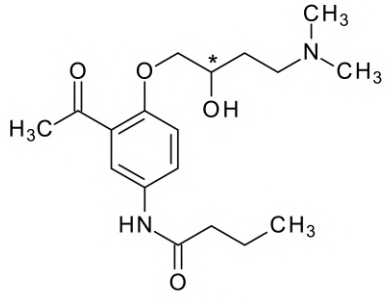
Similarities to enantiomers:	Same molecular formula, not superimposable
Differences from enantiomers:	Not mirror images, different chemical and physical properties, may have different pharmacological activities

At least one of the stereocenters has not changed configuration between the two structures; therefore, these two isomers are diastereomers.

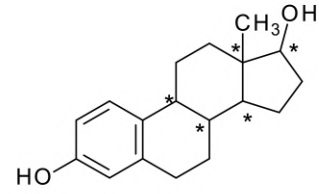
9. Priority of the double bond substituents using the CIP system are listed below.

Priority #1:	Aromatic ring directly attached to the ether oxygen atom
Priority #2:	Aromatic ring attached to methylene carbon then to ether oxygen atom
Priority #1′:	Ethyl chain attached to tertiary amine
Priority #2′:	Hydrogen atom

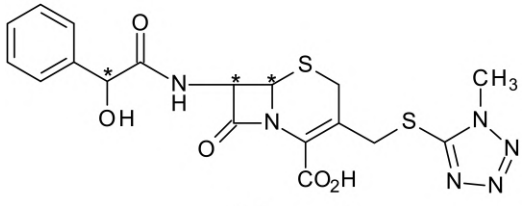
10. **Part A:** Chiral centers are highlighted with an asterisk. The carbon atom circled on the structure of nifedipine is not chiral since the ring is symmetrical at that carbon atom.



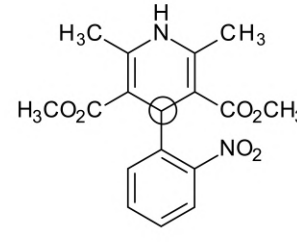
Acebutolol
1 chiral center



Estradiol
5 chiral centers



Cefamandole
3 chiral centers



Nifedipine
0 chiral centers

Part B: Acebutolol, estradiol, and cefamandole can have enantiomers, while nifedipine cannot. For a drug molecule to have an enantiomer, its structure must contain at least one chiral center. Acebutolol, estradiol, and cefamandole all meet this criterion. Because the structure of nifedipine does not contain a chiral center, it cannot have enantiomers.

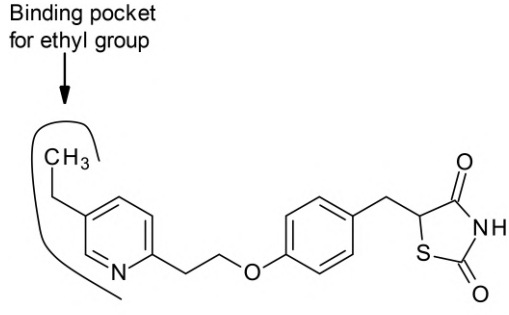
Part C: Estradiol and cefamandole can have diastereomers, while acebutolol and nifedipine cannot. For a drug molecule to have a diastereomer, its structure must contain at least two chiral centers. Because the structures of estradiol and cefamandole have five and three chiral centers, respectively, these two drug molecules meet this criterion and can have diastereomers. Acebutolol (one chiral center) and nifedipine (0 chiral centers) do not meet this criterion and thus cannot have diastereomers.

Part D: Estradiol and cefamandole can have geometric isomers, while acebutolol and nifedipine cannot. Geometric isomers are a specialized type of diastereomer and result from the restricted rotation about a carbon-carbon bond. Geometric isomers can occur due to the presence of either a double bond or an alicyclic ring. Both estradiol and cefamandole contain alicyclic rings and thus can have geometric isomers. Acebutolol and nifedipine do not meet the above criteria and thus cannot have geometric isomers. Please note that the double bonds seen in the 1,4-dihydropyridine ring of nifedipine reside in a six-membered ring and thus cannot participate in the formation of geometric isomers.

11. Enantiomers have identical physical and chemical properties, with the exception of the direction in which they rotate plane polarized light. Thus, water solubility and the percent ionization of the carboxylic acid at a pH of 7.4 would be expected to be identical. The major difference, and most important aspect of enantiomers, is their relative abilities to interact with three dimensional biological targets. Hepatic metabolism and active renal reabsorption depend on binding to metabolizing enzymes and transport proteins, respectively, and would be expected to be different. Adverse effects can be due to the interaction of these drug molecules with other biological targets and/or the formation of a specific metabolite. Differences in potency can result in differential metabolism (i.e., one enantiomer may be inactivated quicker than the other) or differential binding to the biological target.

12. For the purposes of this question, each bond has been rotated by 180°. Other rotations are also possible.

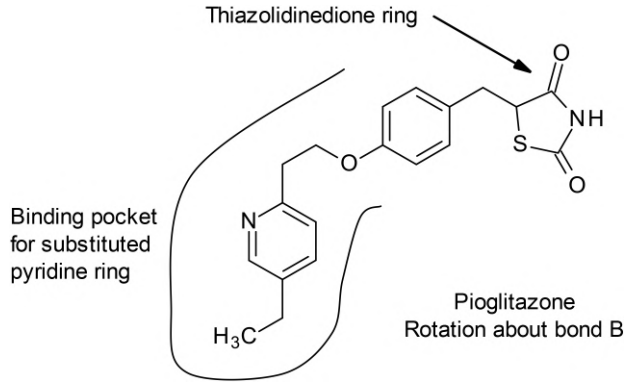
    Rotation about bond A alters the orientation of the ethyl chain, such that the terminal carbon atom is now further away from the nitrogen atom of the pyridine ring. If the binding site for this ethyl chain matched this orientation (shown in the figure below), then this conformational isomer should demonstrate enhanced binding. On the other hand, if the binding site did not match this orientation, then this conformational change would decrease the interaction of pioglitazone with its receptor.



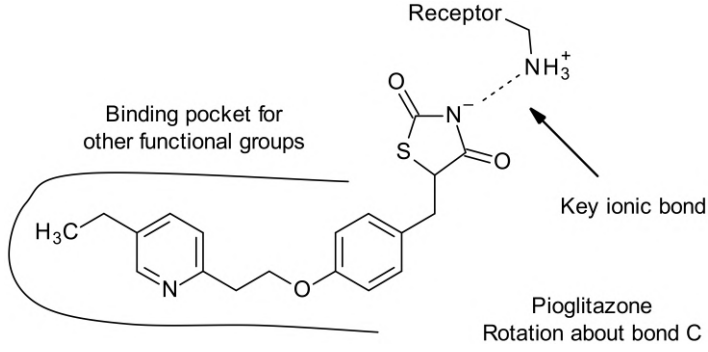
Pioglitazone
Rotation about bond A

Rotation about bond B alters the orientation of the *para*-ethyl pyridine ring relative to the thiazolidinedione ring. If the binding sites of the *para*-ethyl pyridine ring and the thiazolidinedione ring matched this orientation (shown in figure below), then this conformational isomer should demonstrate enhanced binding. As with the first example, if the binding sites

did not match this orientation, then this conformational change would decrease the binding of pioglitazone to its receptor.



Rotation about bond C alters the orientation of the acidic thiazolidinedione ring relative to the rest of the molecule. Since ionic interactions often play a key role in the binding of a drug to its biological target, it is important that ionizable functional groups are correctly orientated to form these bonds. If the binding site for pioglitazone matches the orientation shown below, then this conformational isomer should demonstrate enhanced binding. If this orientation did not match the binding site of pioglitazone, then this conformational change would participate in fewer interactions.



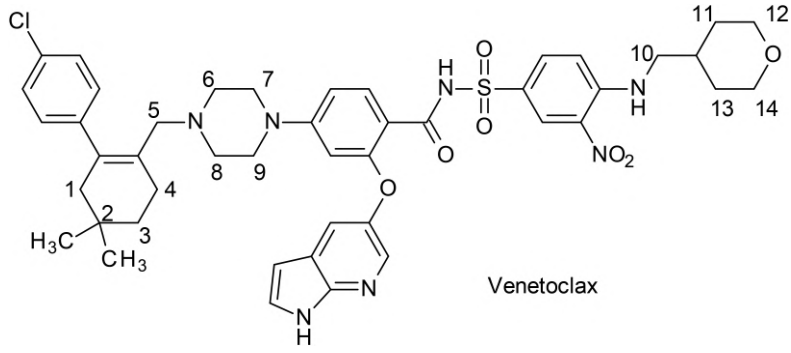
CHAPTER 8

Structural Analysis Checkpoint

Checkpoint Drug 1: Venetoclax

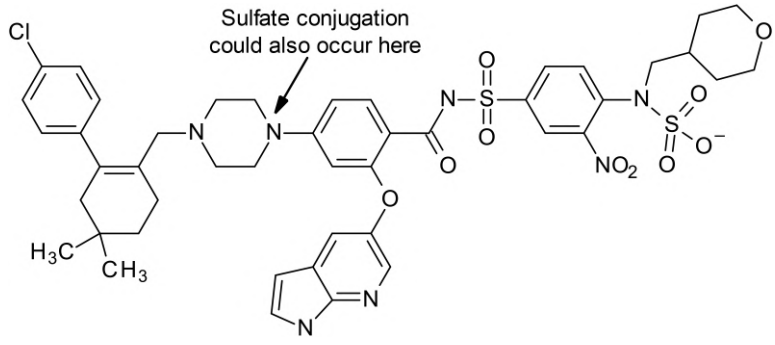
1. Shown below is the answer provided in Chapter 7. Of the 14 potential prochiral centers, only four of these (1-4) are valid. Carbon atoms 1 and 4 are allylic carbon atoms and can undergo allylic oxidation that would make these carbon atoms chiral. Either one of the methyl groups attached to carbon atom 2 can undergo ω oxidation, thus making carbon atom 2 chiral. Alicyclic rings can be oxidized at C_3 and C_4 positions. Carbon atom 3 resides at either the C_3 position relative to carbon atom 5 or the C_4 position relative to the *para*-chlorophenyl ring. Oxidation of this carbon atom would create a chiral center; however, the probability of this metabolic transformation is decreased due to steric hindrance by the adjacent dimethyl substitution. Oxidation at carbon atoms 5 to 10 can occur during the process of oxidative *N*-dealkylation. While the initial oxidation of these carbon atoms produces a chiral center, the resulting carbinolamine is unstable and readily forms an

aldehyde. The same is true with carbon atoms 12 and 14 as they can be involved with oxidative *O*-dealkylation. Oxidation at carbon atoms 11 and 13 is highly unlikely. As mentioned above, alicyclic rings can be oxidized, but this normally occurs at the C_3 and C_4 positions of a cyclohexane ring and not at the carbon atoms adjacent to the aliphatic chain.

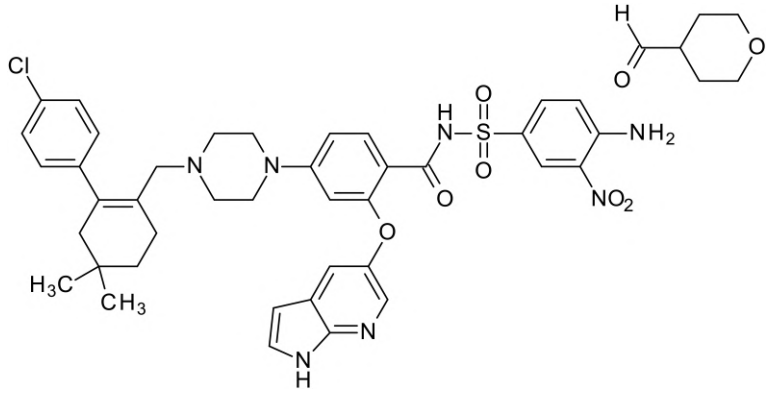


2. **Metabolic Pathway A: No.** Benzylic oxidation is a *Phase I* metabolic transformation that requires an aliphatic carbon atom that is directly attached to an aromatic ring. While the structure of venetoclax contains four aromatic rings, only two of these are directly attached to an aliphatic carbon. Neither of these aliphatic carbon atoms have a hydrogen atom attached and thus cannot be oxidized

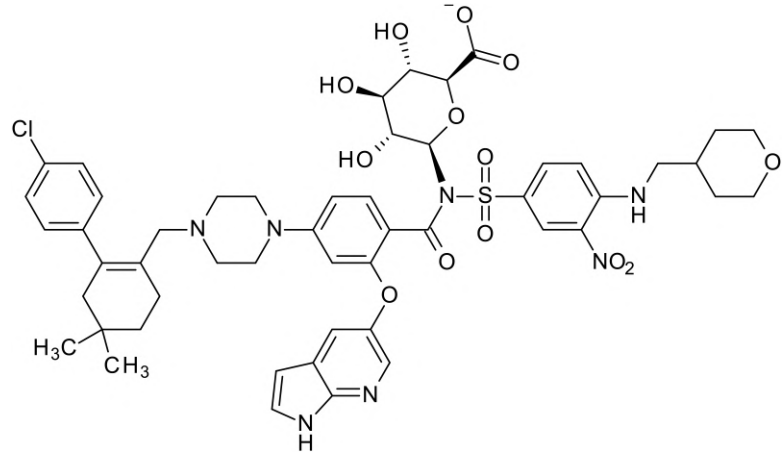
   Metabolic Pathway B: Yes. Sulfate conjugation is a *Phase II* metabolic transformation. Sulfate conjugation can occur with aromatic amines. The structure of venetoclax contains two aromatic amines.



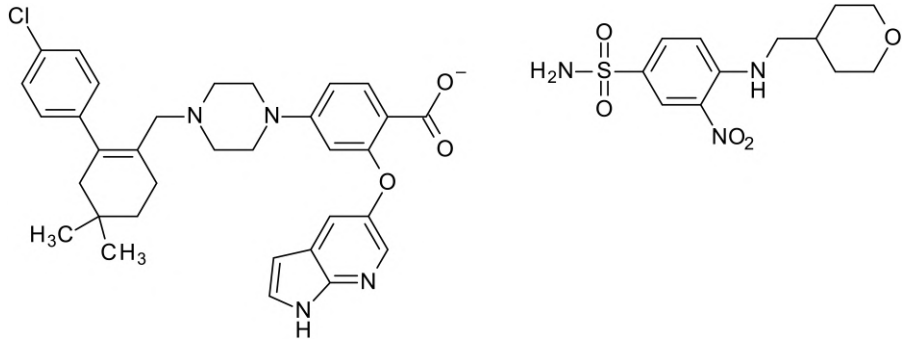
   Metabolic Pathway C: Yes. Oxidative *N*-dealkylation is a *Phase I* metabolic transformation. As discussed in Question 1, venetoclax has six possible oxidative *N*-dealkylation sites. The metabolite shown below results from oxidation at the least sterically hindered (or most accessible) site.



Metabolic Pathway D: Yes. Glucuronic conjugation is a *Phase II* metabolic transformation. Glucuronide conjugation can occur with the sulfonamide (shown), the tertiary amine, and the two aromatic amines. It should be noted that due to steric hindrance and the electron withdrawing properties of the nitro group, glucuronide conjugation of venetoclax is a minor metabolic pathway.



Metabolic Pathway E: Yes. Hydrolysis is a *Phase I* metabolic transformation. The bond between the benzyl carbonyl and the sulfonamide can be hydrolyzed.



Metabolic Pathway F: No. Alkene oxidation is a *Phase I* metabolic transformation that can occur with nonaromatic carbon-carbon double bonds. Although the structure of venetoclax contains a double bond in the cyclohexene ring, neither carbon atom involved in this double bond bears a hydrogen atom. Thus, this metabolism cannot occur.

3. All three of these phenyl rings are either electronically deactivated and/or sterically hindered, thus minimizing the probability that they would undergo aromatic oxidation. The *para*-chloro phenyl ring is very accessible to metabolism; however, the electron withdrawing chloro group deactivates the ring. The aromatic ring in the middle of the molecule is attached to both electron withdrawing groups (the carbonyl) and electron donating groups (the ether oxygen and the aromatic amine); however, due to its position in the molecule and the size of the rings and chains to which it is attached, it is not very accessible to metabolizing enzymes. It is thus highly unlikely that aromatic oxidation would occur here at this aromatic ring. Similarly, the aromatic ring with the nitro group is highly sterically hindered and not very accessible. Additionally, the nitro group is a strong electron withdrawing group and electronically deactivates the ring from aromatic oxidation.

4. There are two sites of metabolic transformation: the nitro group and the tetrahydropyran ring (i.e., the oxygen containing alicylic ring). The nitro group is first reduced to a primary

aromatic amine. This is followed by acetylation. The tetrahydropyran ring first undergoes oxidative *O*-dealkylation to give a primary hydroxyl group and an aldehyde. The aldehyde is further oxidized by ALDH enzymes to a carboxylic acid. The carboxylic acid then undergoes amino acid conjugation to yield the final metabolic product.

Checkpoint Drug 2: Elamipretide

1. The cLog *P* for elamipretide is 0.3677, which indicates significant water solubility (the amount of hydrophilic character of guanidine, primary amines, phenol, and multiple amides is greater than the hydrophobic character of phenol, aromatic hydrocarbon). The ability of the guanidine and two primary amines to be predominantly ionized in most physiologic environments contributes to the overall water solubility of elamipretide. Because renal elimination requires a drug to be highly water soluble, it is likely that elamipretide can be eliminated without the need for Phase I or Phase II metabolic transformation.
2. **No,** a Phase I transformation does not need to occur prior to a Phase II transformation. The two primary amines can directly undergo a Phase II acetylation transformation as well as a Phase II *N*-methylation. The phenol can directly undergo a phase II POMT catalyzed *O*-methylation as well as Phase II sulfation and glucuronidation transformations.
3. *Primary amine (amino terminus):* oxidative deamination; N-oxidation

   Primary amine (lysine): oxidative deamination; N-oxidation

   Phenol: benzylic oxidation of CH_3; benzylic oxidation of CH_2 (prochiral carbon); aromatic hydroxylation (*ortho, para*)

   Aromatic hydrocarbon: benzylic oxidation of CH_2 (prochiral carbon); aromatic hydroxylation (*ortho, para*)
4. This prodrug must undergo the following metabolic transformations to become the active drug:

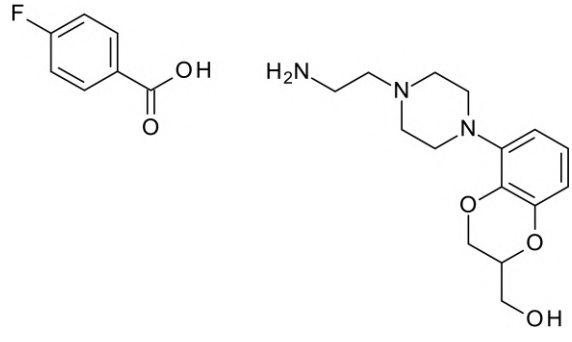
   Primary amine (amino terminus): amide hydrolysis

   Phenol: oxidative-*O*-dealkylation

Review Questions

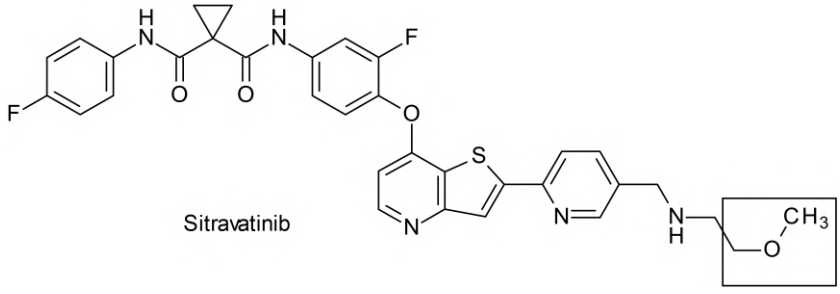
1. **Part A:** *para* and *ortho* aromatic hydroxylation; oxidative *O*-dealkylation; oxidative *N*-dealkylation; N-oxidation; amide hydrolysis; alcohol oxidation

   Part B: Metabolic products are shown below.

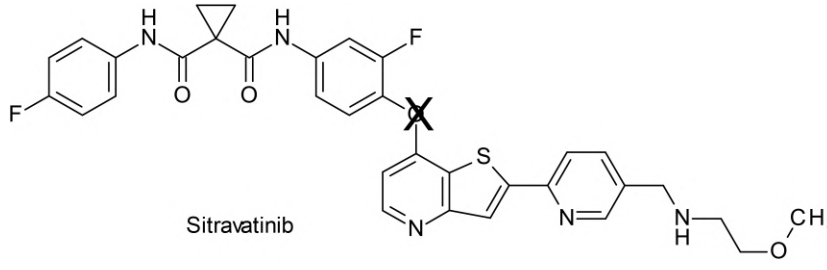


   Part C: Phase II metabolic transformations can occur on the primary alcohol. If this functional group were replaced by an SH group, then potential H-bonding interactions could still occur, but Phase II metabolic transformations could not occur.

2. **Part A**: The ether that can participate in oxidative *O*-dealkylation has been boxed.

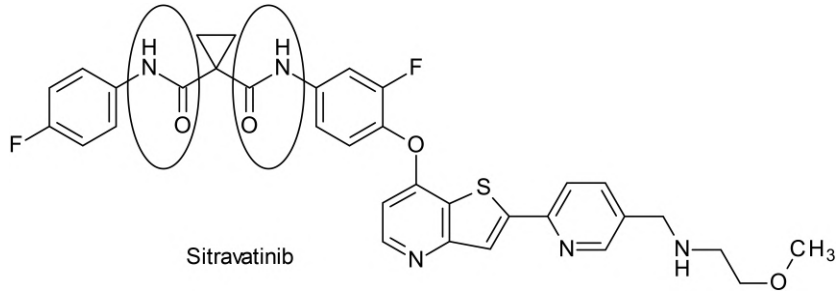


Part B: The crossed-out ether cannot undergo oxidative *O*-dealkylation because the carbon atoms attached to the ether oxygen atom do not bear any hydrogen atoms and therefore cannot undergo oxidation.

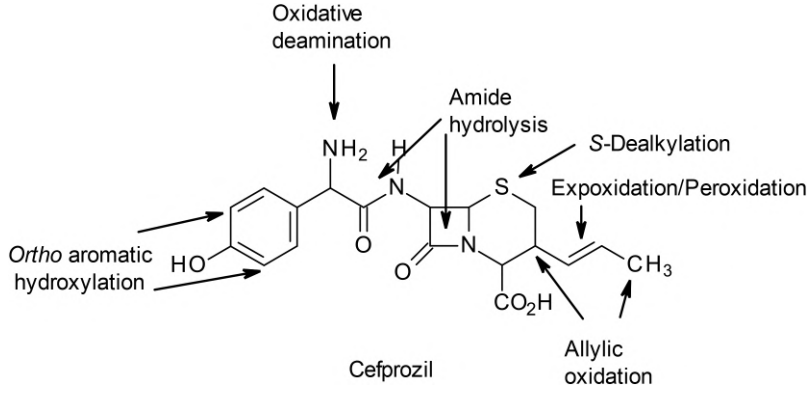


Part C: The boxed halogenated aromatic hydrocarbon cannot undergo *para* aromatic hydroxylation because there is a fluoro substituent in the *para* position. As a result, the carbon atom at the *para* position does not bear a hydrogen atom and is not eligible for oxidation.

Part D: The functional groups that can undergo hydrolysis are circled below.



3. All possible Phase I metabolic transformations are provided below.



Names of Phase I transformations possible:

Aromatic hydroxylation (*ortho*)

Oxidative deamination

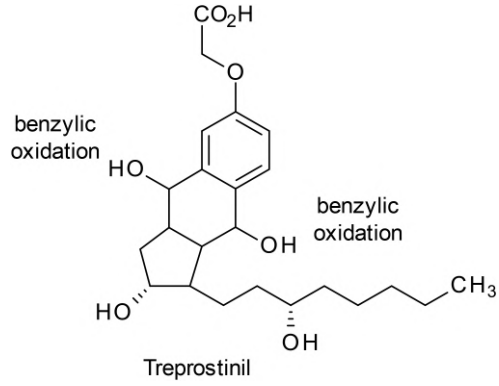
Amide hydrolysis

S-dealkylation

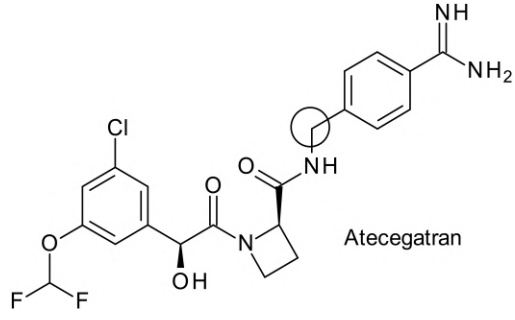
Epoxidation/peroxidation

Allylic oxidation

4. **Part A:** Yes, treprostinil can undergo benzylic oxidation.



Part B: No, Atecegatran cannot undergo benzylic oxidation. There is one benzylic carbon (circled atom) present in the molecule, however it is attached to a nitrogen heteroatom. As a result, benzylic oxidation does not occur.



5. Potential Phase I and Phase II metabolic transformations are provided below.

Dobutamine	Hydroxyzine	Fludrocortisone	Tolvaptan
para/*ortho* Aromatic hydroxylation	*para*/*ortho* Aromatic hydroxylation	Allylic oxidation	Omega oxidation
Oxidative *N*-dealkylation	Oxidative *N*-dealkylation	Alcohol oxidation	Omega-1 oxidation
Oxidative deamination	*N*-oxidation	Reduction	*para*/*ortho* Aromatic hydroxylation
Benzylic oxidation	Oxidative *O*-dealkylation		Benzylic oxidation (aromatic heterocycle)
Phase II: Sulfation	Alcohol oxidation		*N*-oxidation
Phase II: Glucuronidation	Phase II: Glucuronidation	Phase II: Glucuronidation	Phase II: None directly
Phase II: Methylation	Phase II: Sulfation	Phase II: Sulfation	

Isalmadol	Pravastatin	Lisinopril
para/ortho Aromatic hydroxylation	Allylic oxidation	Oxidative deamination
Ester hydrolysis	Alcohol oxidation	Oxidative *N*-dealkylation
Oxidative *N*-dealkylation	Epoxidation/peroxidation	Amide hydrolysis
N-oxidation	Ester hydrolysis	Benzylic oxidation
	Reduction	*para/ortho* Aromatic hydroxylation
Phase II: Sulfation	Phase II: Amino acid conjugation	Phase II: Acetylation
	Phase II: Sulfation	Phase II: Amino acid conjugation

6. Answers provided in table below.

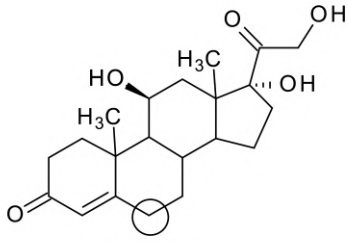
	The molecule can undergo a Phase II metabolic transformation without having to undergo a Phase I transformation first.	The molecule can only undergo a phase II metabolic transformation after undergoing a Phase I transformation.
Besafloxacin	TRUE (carboxylic acid: glucuronide conjugation, amino acid conjugation) (primary amine: acetylation, methylation)	
Acetaminophen	TRUE (phenol: glucuronide conjugation, sulfate conjugation, methylation)	
Neocromil	TRUE (carboxylic acid: glucuronide conjugation, amino acid conjugation)	
Aprepitant		TRUE
Lovastatin	TRUE (secondary alcohol; glucuronide conjugation, amino acid conjugation)	
Gemopatrilat	TRUE (carboxylic acid: glucuronide conjugation, amino acid conjugation)	
Procaine	TRUE (aniline: acetylation, glucuronide conjugation)	

7. **Part A:** Phase I metabolic transformation are provided in table below.

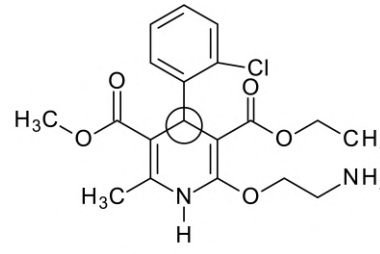
	Oxidative *O*-dealkylation	Benzylic Oxidation	Ester Hydrolysis	*para* Aromatic Hydroxylation	*ortho* Aromatic Hydroxylation
Clofibrate	No	No	Yes	No	Yes
Fenofibrate	No	No	Yes	Yes	Yes
Gemfibrozil	Yes	Yes	No	Yes	Yes

Part B: Gemfibrozil is the only drug molecule that is able to be ionized at physiologic pH and participate in an ionic interaction with the biological target. It is drawn in its active form.

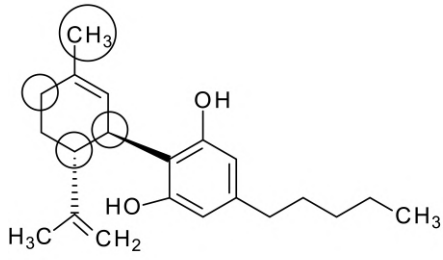
8. Possible site for allylic oxidation are shown below. Lazertinib has only one alkene and the only allylic carbon is already fully oxidized. Be careful not to consider the 1,2 diazole because it is an aromatic heterocycle. It can undergo benzylic oxidation but cannot undergo allylic oxidation!



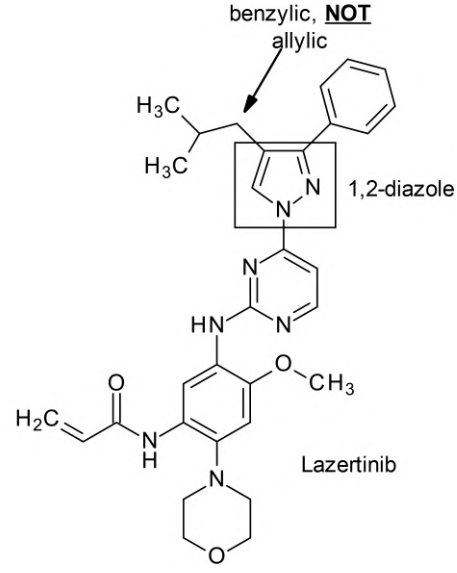
Hydrocortisone



Amlodipine



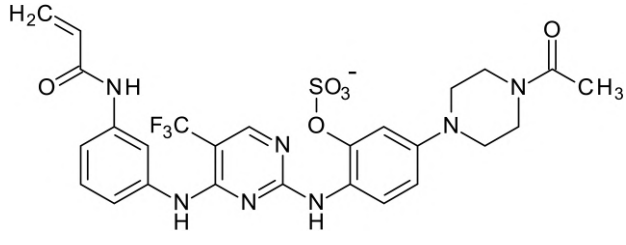
Cannabidiol



Lazertinib

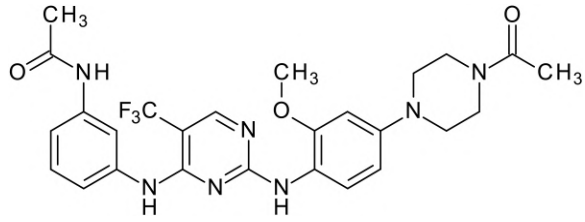
9. Thyroxine (sulfation, glucuronidation, amino acid conjugation, acetylation, and methylation), linzagolix (glucuronidation and amino acid conjugation), oxymetazoline (glucuronidation and sulfation), and tucidinostat (glucuronidation and acetylation) can all undergo a Phase II metabolic transformation without having to first undergo a Phase I metabolic transformation.

10. Metabolite transformations are identified below.



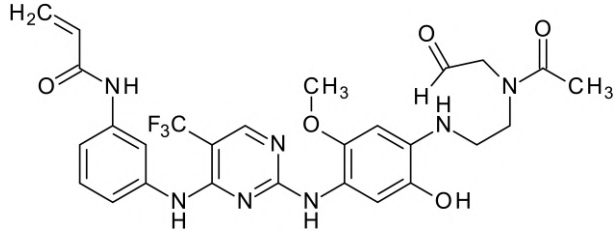
Oxidative *O*-dealkylation (Phase I)

Sulfation (Phase II)



Amide hydrolysis (Phase I)

Acetylation (Phase II)



Oxidative *N*-dealkylation (Phase I)

ortho or *para* Aromatic hydroxylation (Phase I)

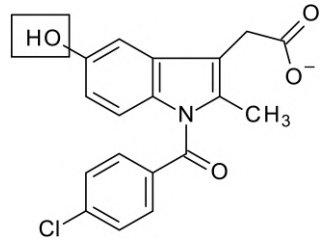
11. Procainamide: Acetylation catalyzed by *N*-acetyltransferase

    Tetracycline: Sulfation catalyzed by sulfotransferase

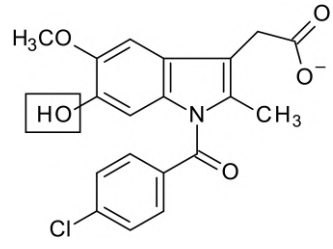
    Naproxen: Amino acid conjugation (with glycine) catalyzed by *N*-acetyltransferase

12. **Part A:** Oxidative deamination (NO): Indomethacin does not contain a functional group that is eligible for oxidative deamination. This Phase I transformation typically occurs with primary amines but can also occur with some secondary amines. Additionally, the carbon atom adjacent to the amine must be attached to at least one hydrogen atom.

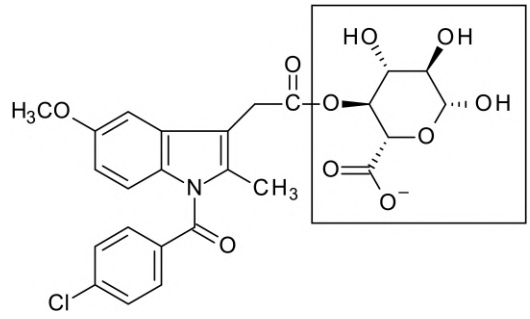
    Part B: Oxidative *O*-dealkylation (YES)



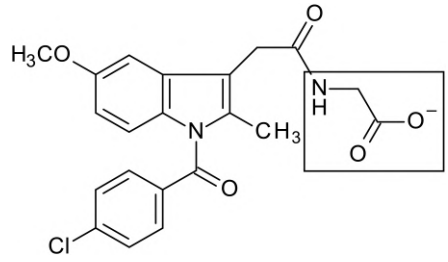
    Part C: Aromatic hydroxylation (YES)



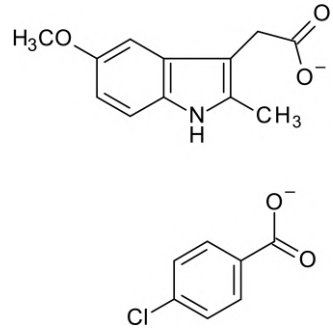
Part D: Glucuronide conjugation (YES)



Part E: Amino acid conjugation (YES: glycine shown, could also be glutamine)



Part F: Hydrolysis (YES: amide)



Part G: Oxidative dehalogenation (NO): While the structure of indomethacin does contain a chlorine atom at the *para* position of the lower aromatic ring, the adjacent carbon atom is not attached to a hydrogen atom; therefore, this metabolic transformation cannot occur.

CHAPTER 9

Structural Analysis Checkpoint

Checkpoint Drug 1: Venetoclax

1. The alteration of functional groups on the aromatic ring can change the solubility of the drug molecule, the electronics of the aromatic ring, and/or the overall steric size of the aromatic ring and drug molecule. In examining the six functional groups and the activity profile, the difference in steric size does not explain the activity profile. The NO_2 and CN groups enhance activity whereas the OCH_3, a functional group of similar size, causes an

elimination of activity. The same is true with solubility. Although the OH and OCH_3 groups are more water soluble than the original chloro group, the CH_3 has similar solubility to the chloro group. All three of these functional groups cause either a decrease or elimination of activity. Thus, the changes in activity must be due to the electronic differences in the functional group. The original Cl group is electron withdrawing due to induction. Replacement with functional groups that are electron withdrawing due to induction—the F group—retain activity whereas replacement with functional groups that are electron withdrawing through resonance—the NO_2 or CN groups—enhance activity. As discussed in Chapter 2, the ability to donate or withdraw electrons through resonance is stronger than the ability to donate or withdraw electrons through induction. The CH_3, OH, and OCH_3 all decrease or eliminate activity. All of these functional groups are electron donating: the CH_3 through induction, and the OH and OCH_3 through resonance. *Given this information, the following SAR statements can be made:*

- Electron withdrawing functional groups on this aromatic ring enhance activity while electron donating groups detract from activity.
- Those functional groups that withdraw electrons through resonance enhance the activity to a greater extent than those that can only withdraw electrons through induction.
- Functional groups can donate electrons through resonance eliminate activity.

2. The original pyrrolopyrimidine bicyclic ring can interact with its biological target through hydrogen bonds, van der Waals interactions, π–π stacking, and cation-π interactions. In evaluating these four analogs, the major difference among them is their ability to form specific types of hydrogen bonds. The pyrrole nitrogen (i.e., the one in the five-membered ring) acts as a hydrogen bond donor whereas the pyrimidine nitrogen (i.e., the one in the six-membered ring) acts as a hydrogen bond acceptor. The only analog to retain activity is Analog B. This bicyclic indole ring retains the nitrogen atom that can act as a hydrogen bond donor. Please note that the location/position of the hydrogen bond donor is the same. Analog D also has a nitrogen atom that can act as a hydrogen bond donor; however, because the pyrrolopyrimidine is now attached to the rest of the molecule via a different carbon atom, this hydrogen bond donor is located or positioned differently. As such, the strength of the hydrogen bond to the biological target is not as strong, leading to the 10-fold loss in activity. Analogs A and C lack a hydrogen bond acceptor in the aromatic ring and are thus inactive. Given this information, the following SAR statement can be made:

   - The pyrrolopyridine ring provides a key hydrogen bond (as the donor) to its biological target. This bicyclic ring can be altered as long as the position of the hydrogen bond donor is unaltered.

3. Replacing the ether oxygen atom with a secondary amine enhances the water solubility of venetoclax. The ether oxygen atom can form hydrogen bonds with water (as the acceptor); however, the secondary amine will be primarily ionized at physiologic pH and thus be able to form ion–dipole interactions with water (as the ion). An ion-dipole bond enhances water solubility to greater extent than a hydrogen bond. Additionally, the presence of an ionized amine would allow this isostere of venetoclax to participate in ionic or ion-dipole bonds with its biological target. This is assuming that complementary functional groups are present on the biological target. The presence of an ionized amine could also cause a decrease in the overall binding activity. The tetrahydropyran ring may reside in a hydrophobic pocket, with the most important interactions occurring via van der Waals interactions with the carbon atoms. If this is true, then the presence of an ionized secondary amine may decrease drug binding and activity. Given that "O" and "NH" are pseudoatoms and classic isosteres, it would be expected that these groups would be similar in size and thus not alter activity due to steric reasons. The same is true for the methylene carbon.

Replacing the ether oxygen with a methylene group decreases water solubility and increases lipid solubility of the drug. As mentioned above, the ether oxygen atom can form hydrogen bonds with water while the methylene group cannot. In terms of receptor binding, if the ether oxygen was essential in forming a hydrogen bond (as the acceptor) with its biological target, then this isosteric substitution could decrease the activity of this analog. Conversely, if the tetrahydropyran ring resides in a hydrophobic pocket, this isosteric replacement could enhance van der Waals interactions with its biological target.

4. The answer is "NO," with the key word being "easily." The easiest way to convert a drug molecule to a more water- or lipid-soluble prodrug is to form an ester as these functional groups can be easily hydrolyzed back to the active drug at various locations within the body. To form an ester, the structure of the drug must contain either a carboxylic acid or a hydroxyl group that is easily accessible to esterase enzymes. Because neither of these functional groups is present within the structure of venetoclax, it cannot be easily converted to a prodrug. For the sake of completeness, it should be noted that it is theoretically possible to make a prodrug from the sulfonamide functional group; however, this would create a sterically hindered site that may be difficult to access by metabolizing/activating enzymes.

Checkpoint Drug 2: Elamipretide

1. Possible binding interactions are provided in the table below.

	Amino Terminus	Carboxy Terminus
SS-31 (elamipretide)	Ionic Ion dipole Cation-π Chelation	Cation-π π-π stacking Hydrophobic H-bonding Ion-dipole (dipole)
SS-02	Ionic Ion-dipole (ion) Ion-dipole (dipole) Cation-π H-bonding π-π stacking Hydrophobic	Ionic Ion-dipole (ion) Ion-dipole (dipole) H-bonding Cation-π Chelation
SS-20	Ionic Ion-dipole (ion) Cation-π π-π stacking Hydrophobic	Ionic Ion-dipole (ion) Ion-dipole (dipole) H-bonding Cation-π Chelation

SS-02 is the only analog that can participate in H-bonding and ion-dipole (as the dipole) at the amino terminus (via the phenol OH) at physiologic pH. These types of interactions might be important for μ receptor activation.

2. At physiologic pH, the basic functional groups (guanidine and primary amine) found within elamipretide and the two analogs are ionized and able to interaction via an ionic interaction with a phosphate head. In all three molecules, the two amino acids with basic side chains are always separated by one amino acid and are therefore always in the same proximity to one another. Additionally, the flexibility of the lysine and arginine side chains allows all three of these molecules to place their respective basic functional groups in the same location.

3. In elamipretide and in SS-20, the tyrosine derivative is located between the amino acids with the basic side chains. In SS-02, the phenylalanine residue is located between the

amino acids with the basic side chains. The basic side chains are required for an important electrostatic interaction with the phosphate head found within cardiolipin. It is possible that cardiolipin also requires a particular type of interaction (ion-dipole, H-bonding, or dipole-dipole) between these two basic amino acids for optimal binding. If this is the case, then SS-02 would not have the right type of functionality to participate in this important interaction (ion-dipole, H-bonding, or dipole-dipole) with cardiolipin.

4. **Part A:** There are several reasons why a lipophilic ester prodrug might be valuable including improving bioavailability and the related absorption across lipophilic membranes. Prodrugs also protect drug molecules from metabolic and/or enzymatic degradation and may prevent metabolic transformations that lead to rapid drug elimination.

   Part B: This prodrug form of elamipretide contains amides at both the amino and carboxy terminus. Amino- and carboxypeptidases require their substrates to contain either a primary amine or carboxylic acid, respectively. To fool these enzymes, these functional groups can be "protected" or "masked" as amides. Once the primary amine and/or carboxylic acid is modified to be an amide (prodrug), then the amino- and carboxypeptidases no longer recognize the drug as a substrate, thus extending the half-life of the drug. Because amides are relatively easily cleaved to the corresponding carboxylic acid and amine (Phase I metabolic transformation catalyzed by amidases), they serve as valuable prodrugs.

Review Questions

1. **Part A:** Insulin #1: Inactive. Three disulfide bonds must be present to allow insulin to adopt the correct conformation. In this scenario, the amino and carboxy terminal amino acid residues are identical to that found in endogenous insulin; however, if the amino acids at the amino or carboxy termini of the A chain are different than that found in the endogenous hormone, then the key receptor interactions may not occur and the corresponding analog would be biologically inactive.

   Part B: Insulin #2: Inactive. Only six amino acids can be removed from the amino terminus of the B chain and still retain biological activity. In this case, 10 amino acids are missing from the amino terminus of the B chain.

   Part C: Insulin #3: Inactive. There is a good chance that this insulin analog will be inactive due to the number of *D*-amino acids present. The change in stereochemistry in so many locations will more than likely not only change the shape of the insulin molecule (amino acid side chains may not be located in the right place in space to allow for the appropriate secondary and ternary structural features to form) but also may influence how many amino acids can interact with the insulin receptor.

2. Glipizide contains an acidic sulfonylurea functional group whereas repaglinide contains an acidic carboxylic acid functional group. This molecular modification is an example of the use of a nonclassical bioisostere. There are several other molecular modifications that you might have identified, including shortening the hydrocarbon chain between the arylsulfonylurea and the amide functional groups, reversal of the placement of the amide carbonyl group, and replacement of an aromatic heterocycle with an aromatic hydrocarbon.

3. Insulin lispro contains a proline residue as part of its primary structure; however, it is in closer proximity to the carboxy terminus as a result of a structural exchange of the Lys_{29} with the Pro_{28}. You might imagine that the size of the "hook" is now smaller (only one amino acid in length) and that the likelihood of insulin dimerization will decrease. This

is in fact exactly what happens! As a result of this structural modification, there is more monomer present in the formulation and the time that it takes for the insulin dimer present to dissociate into two monomers is likely to be shorter than that required for Humulin R.

Insulin aspart does not contain a proline residue as part of its primary structure. This means that there is no "hook" present within the carboxy terminus of the B chain of this insulin analog. This means that this analog does not dimerize and only needs to dissociate from the insulin stabilizer present in the formation (which is true for all insulin formulations) to be an active drug. This takes less time; therefore, the onset and duration of action are shorter than Humulin R.

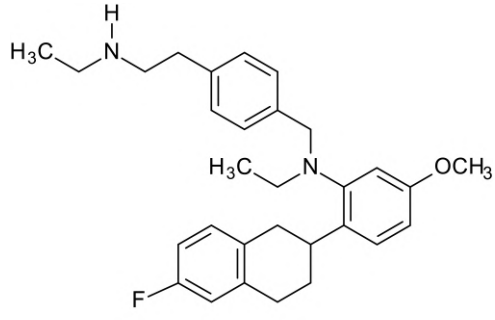
Note: Although insulin glulisine is also considered an ultra-short acting insulin, the rationale for this has nothing to do with the primary structure (Pro_{28} is present), but rather has to do with the type of insulin stabilizer used in the formulation.

4. Tazarotene is formulated as a topical agent in the treatment of several skin disorders. For this drug to penetrate the affected skin, it must be highly hydrophobic in character. Although the carboxylic acid is important for biological activity, it is very hydrophilic and limits the ability of the drug to be appropriately formulated for topical use. Formation of the ethyl ester significantly decreases the hydrophilic character of this functional group and not only improves the overall characteristics from a formulation perspective but also improves the ability of the drug to penetrate the skin.

5. For many years, ciprofloxacin represented the gold standard to which other fluoroquinolones were compared. Thousands of analogs were synthesized, with only a handful exhibiting equal or better action against gram-negative pathogens. When looking at the analog series A–D, there is a progressive addition of hydrocarbon character associated with the N atom substituent. Once this substituent contains more than three carbons, there is a complete loss of biological activity. One could surmise that the interaction between the drug and its biological target allows for a finite amount of hydrophobicity/bulk in this location, and once this hydrophobic space is exceeded, the interaction of the drug with the biological target suffers significantly.

6. **Analog A:** Replace phenol OH with F.

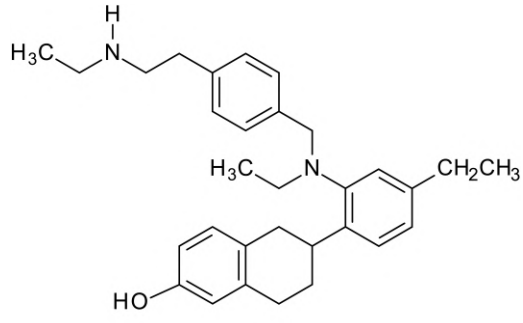
   This decreases the potential for Phase I catechol formation and Phase II sulfation, glucuronidation, and *O*-methylation catalyzed by POMT.



Analog A

Analog B: Replace oxygen atom in methyl ether substituent with a methylene unit (CH_2).

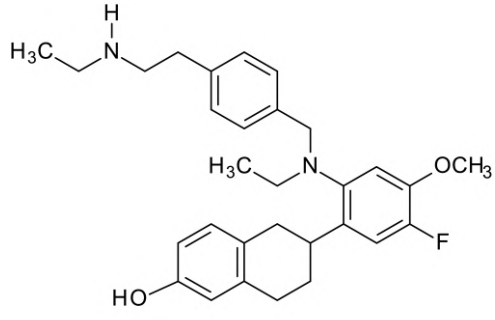
This decreases the potential for Phase I oxidative *O*-dealkylation (potentially followed by Phase II sulfation, glucuronidation, and *O*-methylation catalyzed by POMT).



Analog B

Analog C: Insert fluoro substituent *ortho* to the methyl ether substituent.

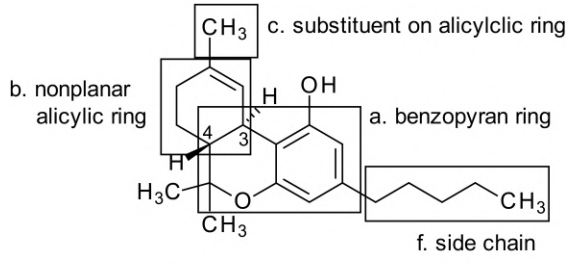
This decreases the potential for Phase I aromatic hydroxylation (potentially followed by Phase II sulfation, glucuronidation, and *O*-methylation catalyzed by POMT).



Analog C

Several other functional groups can undergo metabolic transformation (e.g., oxidative *N*-dealkylation of the secondary or tertiary amines; benzylic oxidation of the carbon atoms attached to aromatic hydrocarbon); however, isosteric modification is not available. In these cases, other strategies can be employed (e.g., steric hindrance) to limit the potential for metabolic transformation at these sites.

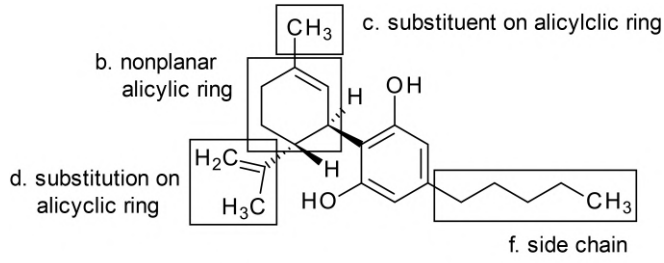
7. **Part A:** Each of the structural requirements has been boxed and labeled.



Tetrahydrocannabinol

d: not a requirement; not present
e: not a requirement; not present

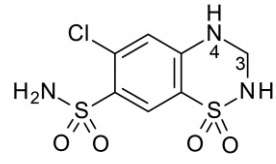
Part B: This analog meets nearly all of the structural requirements to have cannabinoid activity. Unfortunately, it does not contain the benzopyran ring system and, therefore, is devoid of cannabinoid activity. Based on what we learned in Chapter 7, the benzopyran ring system likely provides much needed conformational restriction to allow for the correct spatial orientation of the rest of the functional groups. This conformational restriction permits the functional groups to optimally interact with the cannabinoid receptor.



Tetrahydrocannabinol analog

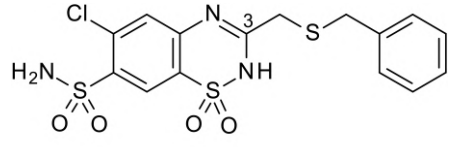
a: required; not present
e: not a required; not present

8. **Pair #1**: Hydrochlorothiazide has greater diuretic effect. Saturation of the 3,4-double bond found in chlorothiazide enhances diuretic effect.



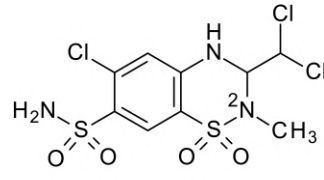
Hydrochlorothiazide

Pair #2: Benzthiazide has a longer duration of action. Lipophilic groups at C-3 increase duration of action.



Benzthiazide

Pair #3: Methylclothiazide has a longer duration of action. *N*-2 substitution increases duration of action.



Methylclothiazide

9. **Part A:** Gemfibrozil is able to interact with PPARα due to the presence of an ionizable carboxylic acid. In its ionized form, gemfibrozil is able to participate in an ion–dipole interaction with the side chain of the tyrosine residue found within the biological target. Fenofibrate,

a prodrug, must undergo a Phase I metabolic transformation (ester hydrolysis) to "reveal" the underlying ionizable carboxylic acid.

Part B: The active form of fenofibrate (carboxylic acid) contains a *para* chloro substituent on the aromatic hydrocarbon. Fibrates with a *para* chloro substituent have a significantly longer half-life than those that do not have a *para* chloro substituent or chloro containing substituent.

10. A check mark or comment has been included in the table to indicate if the drug molecule follows the SAR.

	Epoprostenol (PGI_2)	Iloprost	Treprostinil
C-1 carboxylic acid to participate in required ionic or ion/dipole interaction	✓	✓	✓
C-11 and C-15 hydroxyl groups to participate in H-bonding interactions	✓	✓	✓
Cyclopentane ring system + C-5 and C-13 double bonds (to maintain geometry)	✓	✓	Cyclopentane ring present; C-5 double bond replaced by aromatic hydrocarbon
C-6 oxygen (increases potency and undergoes rapid metabolism to inactive drug)	✓	Not present	Not present
Additional functional groups capable of H-bonding interactions (decreases receptor affinity)	Not present	Not present	Not present

11. **Part A:** The replacement of the secondary aromatic amine with a methylene group is an example of a classic divalent isosteric modification. Although the size of a methylene group is similar to that of a secondary amine, its solubility and electronic properties are very different. The secondary aromatic amine is more water soluble than the methylene group and can participate in hydrogen bonds (as both a donor and an acceptor), ion-dipole bonds (as the dipole), and dipole-dipole bonds. Additionally, the lone pair of electrons on this amine can contribute to resonance through its ability to donate electrons into the two adjacent aromatic rings. In contrast, the methylene group is more lipid soluble and cannot form hydrogen bonds, ion-dipole bonds, or dipole-dipole bonds. It can participate in van der Waals interactions; however, this binding interaction is very different from that of the original secondary aromatic amine. Therefore, this isosteric change would be expected to alter the ability of dasatinib to interact with/bind to its biological target.

Part B: The replacement of a chloro group with a methyl group is an example of a classic monovalent isosteric modification. As discussed in Chapter 8, aromatic rings with electron withdrawing groups (such as the chloro group) are less likely to undergo aromatic oxidation than are unsubstituted rings or those with electron donating groups (such as a methyl group). Therefore, this isosteric change would most likely lead to an increase in aromatic hydroxylation, which could then be followed by Phase II conjugation. Additionally, this isosteric change adds a methyl group that can easily be oxidized to either a benzylic hydroxyl group or a carboxylic acid. The removal of the electron withdrawing chloro group also makes the other *ortho* methyl group more susceptible to oxidation. In summary, this isosteric change would be expected to increase the rate of metabolism of dasatinib and decrease its duration of action.

12. **Part A:** The initials SAR represent a **S**tructural **A**ctivity **R**elationship, a term that literally defines the relationship between the chemical structure of a drug molecule and the physiologic, pharmacological, pharmacokinetic, and therapeutic effects it provides. The structure of a drug molecule refers to its functional groups and their stereochemical orientation to one another. The activity of a drug molecule can refer to its pharmacological actions, its route of administration, its ability to bind to specific receptors, its ability to be metabolized by a specific pathway (or not), its duration of action, and/or its ability to cause specific drug interactions or adverse effects.

    Part B: The most important component of any SAR is the **why** or **how** component. Without this component, an SAR simply becomes something to memorize. Stating that "Drug A has a higher affinity for its target receptor than any other drug in its pharmacological class" ***does not*** allow for any application, nor does it provide any information to design any additional drugs. An example of a more complete, and proper, SAR would state, "The structure of Drug A contains a hydrogen bond acceptor *meta* to the acidic functional group on the aromatic ring. This greatly enhances the affinity of Drug A for its target receptor."

CHAPTER 10

Aliskiren (Level 1)

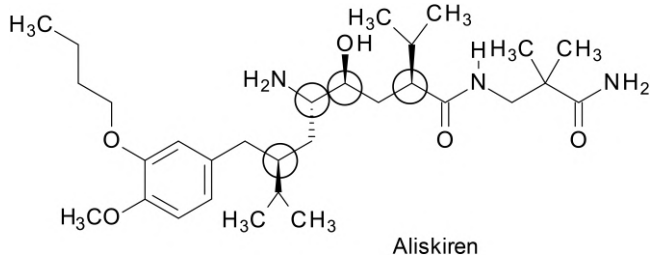
1. Functional group names and other information provided below.

	Name of Functional Group	Character: Hydrophilic and/or Hydrophobic	Character: Acidic, Basic, or Neutral (Provide pK_a When Relevant)	Function: Contribute to Aqueous Solubility and/or Contribute to Absorption	Function: Interaction(s) Possible with Biological Target at Physiologic pH = 7.4	Function: Amino Acids That Can Interact with the Functional Group via Ion–Dipole Interactions at pH = 7.4[a]
A	Ether	Hydrophilic (O) Hydrophobic (R)	Neutral	Solubility (O) Absorption (R)	H-bonding (A) Dipole–dipole Ion–dipole (as the dipole)	Asp, Glu, Lys, Arg
B	Aromatic hydrocarbon	Hydrophobic	Neutral	Absorption	Hydrophobic van der Waals π-π stacking	None
C	Aliphatic alkane	Hydrophobic	Neutral	Absorption	Hydrophobic van der Waals	None
D	Primary amine	Hydrophilic (NH_2) Hydrophobic (R)	Basic pK_a ~9-11	Solubility (NH_2) Absorption (R)	Ion–dipole (as the ion) Ionic	Ser, Thr, Cys, Tyr, Asn, Gln, His, Trp, Met
E	Secondary alcohol	Hydrophilic (OH) Hydrophobic (R)	Neutral	Solubility (OH) Absorption (R)	H-bonding (A + D) Dipole–dipole Ion–dipole (as the dipole)	Asp, Glu, Lys, Arg

	Name of Functional Group	Character: Hydrophilic and/or Hydrophobic	Character: Acidic, Basic, or Neutral (Provide pK_a When Relevant)	Function: Contribute to Aqueous Solubility and/or Contribute to Absorption	Function: Interaction(s) Possible with Biological Target at Physiologic pH = 7.4	Function: Amino Acids That Can Interact with the Functional Group via Ion–Dipole Interactions at pH = 7.4[a]
F	Amide	Hydrophilic ($CONH_2$)	Neutral	Solubility ($CONH_2$)	H-bonding (A)	Asp, Glu, Lys, Arg
		Hydrophobic (R)		Absorption (R)	Dipole–dipole	
					Ion–dipole (as the dipole)	

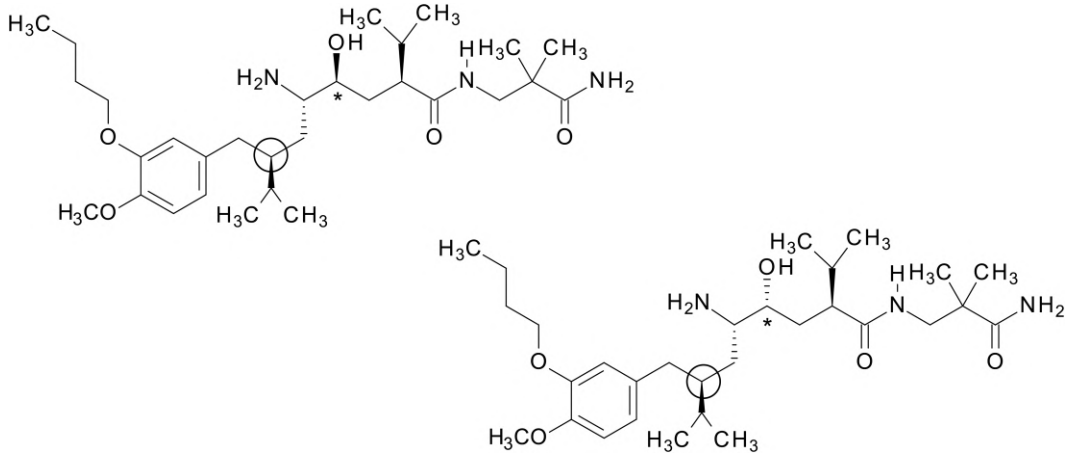
R = carbon scaffolding.
[a] "None" is a possible answer.

2. Chiral carbon atoms have been circled.



Aliskiren

If there are two or more chiral carbon atoms, then it is possible for the drug to have diastereomeric forms. In the case of aliskiren, there are four chiral carbon atoms; therefore, there are a lot of potential diastereomeric forms. ***A quick reminder on how to determine if a pair of isomers is diastereomeric:*** If one chiral carbon is held constant (e.g., *R*-isomer) and you vary at least one additional chiral carbon, then the pair of isomers will be diastereomeric to one another. In the example below, the chiral center that is circled is held constant and the (*) chiral center is varied. The pair of isomers drawn are diastereomers.



For geometric isomers to be possible, there must be restricted rotation around a carbon-carbon bond (e.g., presence of a double bond or alicyclic ring). Evaluation of aliskiren reveals the absence of double bonds and alicyclic rings; therefore, geometric isomers cannot exist.

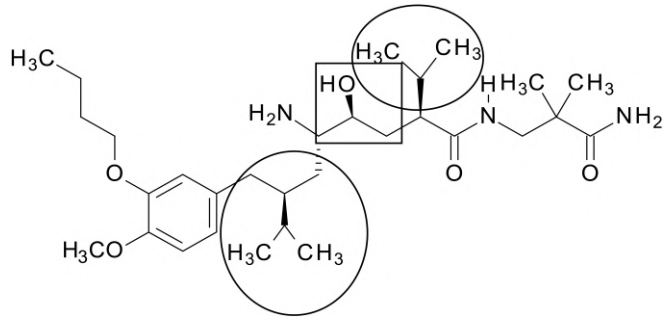
3. Oral bioavailability depends on the drug's ability to dissolve in the aqueous contents of the gastrointestinal (GI) tract and its ability to cross the lipid bilayer membrane. The hydrophilic character of the drug promotes aqueous solubility. Evaluation of the entire molecule (not just the boxed functional groups) for hydrophilic character reveals two ethers, two amides, a primary amine (in its ionized form), and a secondary alcohol that contribute meaningfully to the hydrophilic character of the drug. (***NOTE:*** the drug is sold as a hemifumarate salt that further increases the overall water solubility of the drug). The hydrophobic character of the drug promotes absorption across the lipid bilayer. Evaluation of the entire molecule (not just the boxed functional groups) for hydrophobic character reveals an aromatic hydrocarbon, a lipophilic ether, and several aliphatic alkanes. The drug is formulated as a water-soluble salt and has ample hydrophilic character. Unfortunately, it does not have sufficient hydrophobic character to allow for meaningful absorption.

4. Metabolic transformations and identification of oxidative or nonoxidative provided below.

	Name of Metabolic Transformation	Oxidative or Nonoxidative
A	Oxidative *O*-dealkylation	Oxidative
B	Alcohol oxidation	Oxidative
C	Oxidative deamination	Oxidative
D	Oxidative *O*-dealkylation[a]	Oxidative
E	Amide hydrolysis[a]	Non-oxidative

[a]This metabolite has been identified as one of the two primary metabolites produced.

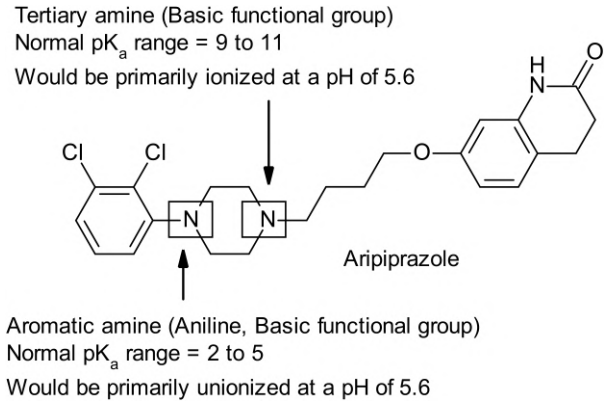
5. Functional groups mimicking Leu and Val are circled. The nonhydrolyzable hydroxyethylene group is boxed.



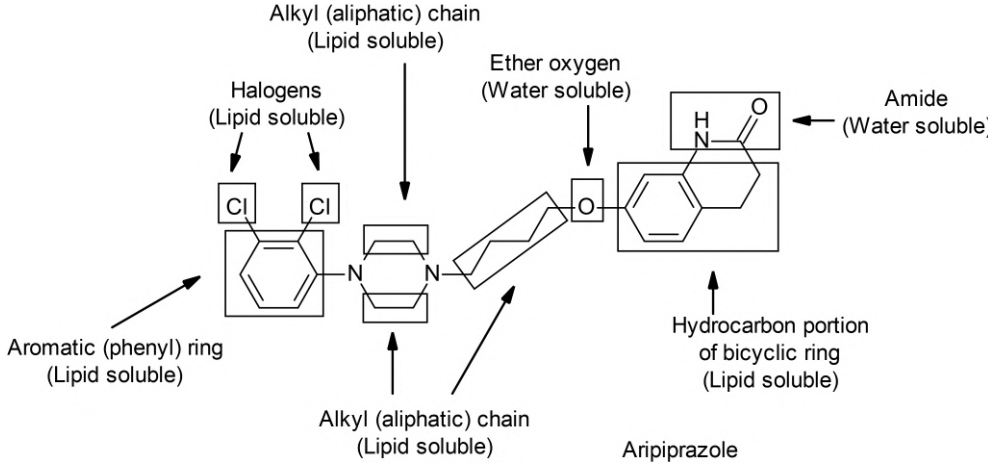
6. It is important to first determine whether these drugs are basic, acidic, or amphoteric in nature. Based on a structural evaluation of the entire aliskiren molecule (not just the boxed functional groups), there is one basic functional group (primary amine) and no acidic functional groups present. The same evaluation for amlodipine yields identification of two basic functional groups (primary amine and dihydropyridine ring) and no acidic functional groups. Based on this evaluation, both drugs are basic, and both would be bound to α_1-acid glycoprotein (plasma protein). It is important to remember that plasma protein binding interactions are likely to happen when a drug is > 90% plasma protein bound. Given the extent to which amlodipine is plasma protein bound, it is highly likely that a plasma protein binding interaction will occur when aliskiren displaces amlodipine from the glycoprotein binding site. When this type of interaction occurs, there is a greater fraction of amlodipine present in its unbound form. This leads to an increase in pharmacological activity, resulting in hypotension (excessively low blood pressure).

Aripiprazole (Level 1)

1. Acidic and basic function groups are identified along with ranges and ionization state at pH = 5.6.



2. All other functional groups are identified along with their water or lipid nature.



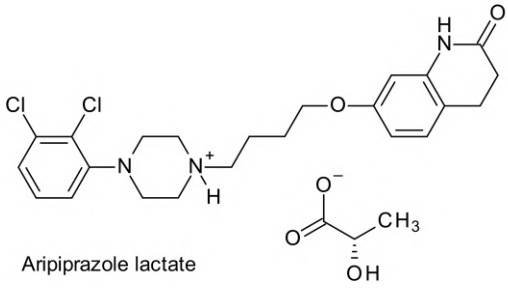
3. The tertiary amine, aromatic amine, amide, and ether oxygen atom provide adequate water solubility that allows aripiprazole to dissolve in the aqueous contents of the gastrointestinal (GI) tract. Once dissolved, aripiprazole must pass through the GI mucosal membrane to enter the blood stream. The dichloro phenyl ring, the alkyl chain, and the hydrocarbon portion of the bicyclic ring provide adequate lipid solubility to allow for absorption. The tertiary amine is primarily ionized within the GI tract, whereas the aromatic amine is primarily unionized once it leaves the stomach. Remember that ionization is an equilibrium process with some fraction of unionized molecules present at all times. According to Le Chatelier's principle, as soon as one unionized molecule passes through the GI membrane, the equilibrium resets to provide additional unionized molecules. This same principle also allows ionizable functional groups to penetrate the blood brain barrier and enter the central nervous system (CNS). The same functional groups that allowed aripiprazole to pass through the GI mucosal membrane also provide sufficient lipid solubility to allow it to cross the blood brain barrier and reach its target receptors within the CNS.

4. Aromatic amines are much less basic than primary, secondary, and tertiary aliphatic or alicyclic amines. The reason for this decreased basicity lies in the fact that an aromatic amine can donate its lone pair of electrons, through resonance, into the aromatic ring, thus

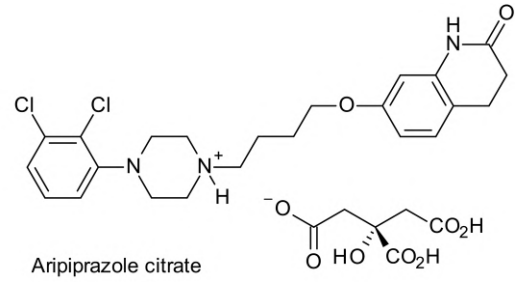
making these electrons less available to bind with a proton. Within the structure of aripiprazole, the nitrogen atom of the aromatic amine is directly adjacent to *ortho* and *meta* chlorine atoms. Chlorine has a higher electronegativity than nitrogen and, through induction, each acts as an electron withdrawing group. This further decreases the availability of the lone pair of electrons on the nitrogen, resulting in a decrease in basicity. Thus, the pK_a for this particular aromatic amine would be expected to be at the lower end of this range (i.e., closer to 2).

5. The answer here is ***No***. Tolbutamide and losartan are acidic drug molecules due to the presence of an acidic sulfonylurea and a tetrazole functional group, respectively. Aripiprazole is a basic drug molecule due to the presence of a tertiary amine and an aromatic amine. Acidic drug molecules primarily bind to albumin whereas basic drug molecules primarily bind to α_1-glycoprotein. Plasma protein binding interactions (also known as plasma protein displacement interactions) occur due to the nonspecific nature of the plasma proteins that bind and transport drug molecules. These types of interactions are only therapeutically important when the drug molecules are highly plasma protein bound (i.e., > 90%). Although this is true of all three drugs, the difference in their acid/base nature significantly decreases the probability of a drug interaction due to plasma protein displacement.

6. Shown below are two examples of water-soluble organic salts using lactic acid and citric acid. There are several other water-soluble organic acids that could be used (e.g., fumaric acid, malic acid, maleic acid, tartaric acid).

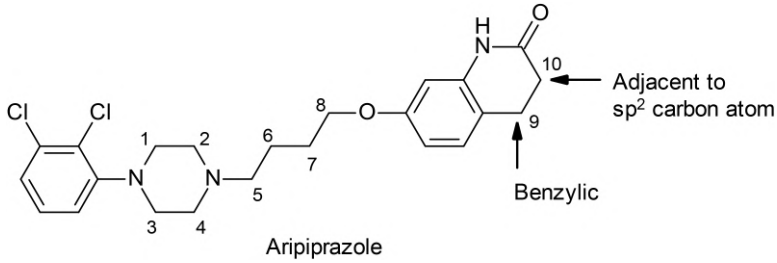


Aripiprazole lactate



Aripiprazole citrate

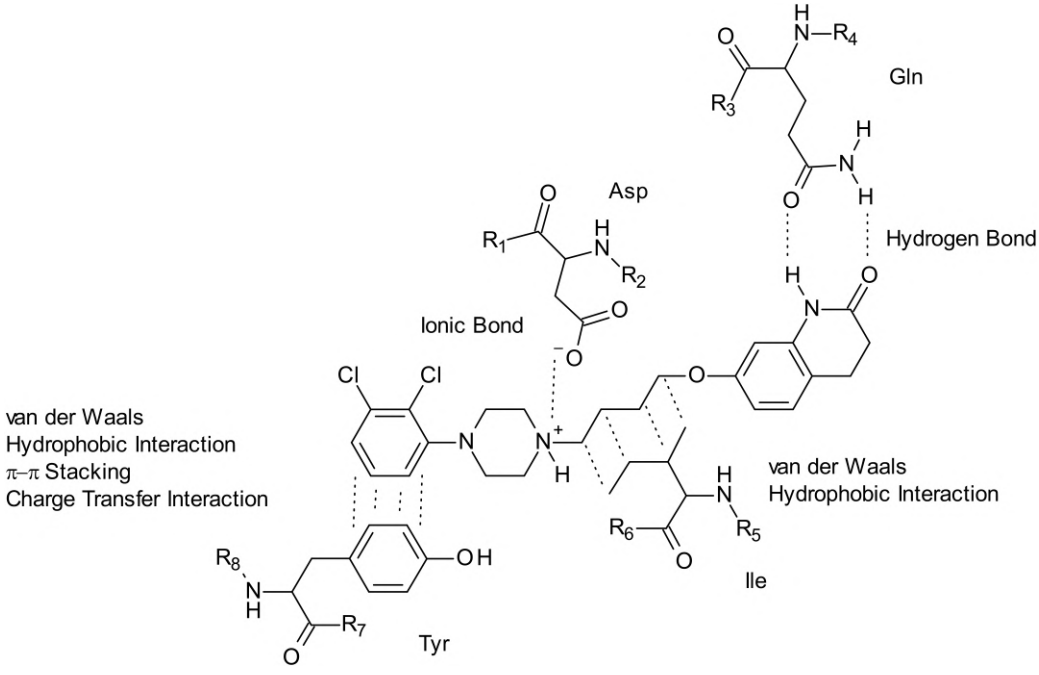
7. There are only two methylene groups that would be expected to generate a chiral center via metabolism: the benzylic carbon atom and the carbon atom adjacent to the sp^2 carbonyl. Oxidation at either of these two positions adds a hydroxyl group and generate a chiral center. Oxidation of carbon atoms 1 to 5 leads to oxidative *N*-dealkylation and the initial formation of an aldehyde. Similarly, oxidation of carbon atom 8 leads to oxidative *O*-dealkylation and the initial formation of an aldehyde. Oxidation of methylene groups in the middle of an alkyl chain and adjacent to an sp^3 hybridized center generally does not occur.



Aripiprazole

8. One example is shown below. Other binding interactions are possible among these four functional groups and four amino acids. Isoleucine could form van der Waals and hydrophobic interactions with the halogenated aromatic ring, whereas tyrosine could form the same

types of interactions with the alkyl chain. Aspartic acid could form an ion–dipole interaction (as the ion) with the amide, whereas glutamine could form an ion–dipole interaction (as the dipole) with the ionized tertiary amine. Please note that it is possible for tyrosine to form hydrogen bonds with the amide and an ion–dipole interaction (as the dipole) with the ionized tertiary amine. However, in the scenario given in this question, if tyrosine were used to form an interaction with either the amide or the ionized tertiary amine, neither aspartic acid nor glutamine could be used to form van der Waals and hydrophobic interactions with the halogenated aromatic ring.

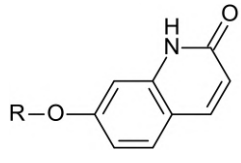


9. **Metabolite A:** Aromatic oxidation, Phase I

Note: This is somewhat of an unexpected metabolite because the presence of halogen substituents on the aromatic ring generally deactivates these rings from oxidation. The difference in this particular drug molecule is the presence of an adjacent aromatic amine. This aromatic amine can donate electrons into the ring via resonance and either override or neutralize the electronic effects of the chloro groups, thus allowing for aromatic oxidation.

Metabolite B: Benzylic oxidation, Phase I

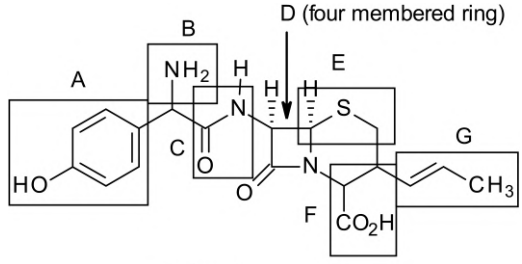
Note: Due to the ability to form a conjugated system, this secondary hydroxyl group readily undergoes dehydration to form the following metabolite.



Metabolite C: Oxidative *N*-dealkylation followed by oxidation of the resulting aldehyde to a carboxylic acid by aldehyde dehydrogenase, Phase I

Cefprozil (Level 1)

1. Functional group names and other information is provided below.



Cefprozil

	Name of Functional Group	Character: Hydrophilic and/or Hydrophobic	Character: Acidic, Basic, or Neutral (Provide pK_a When Relevant)	Function: ↑ Solubility and/or ↑ Absorption	Function: Interaction(s) Possible with Biological Target at Physiologic pH (7.4)	Function: Amino Acids That Can Interact with Functional Group via H-bonding (at pH = 7.4)[a]
A	Phenol	Hydrophilic (OH) Hydrophobic (Ar)	Acidic (pK_a 9-10)	Solubility (OH) Absorption (Ar)	OH: H-bonding (A+D) Dipole-dipole Ion-dipole (as the dipole) Ar: van der Waals Hydrophobic π-π Stacking Cation-π (as the π cloud)	Ser, Tyr, Trp, His, Thr, Cys, Asn, Gln, Met
B	Primary amine	Hydrophilic (NH_2) Hydrophobic (R)	Basic (pK_a 9-11)	Solubility (NH_2) Absorption (R)	Ion-dipole (as the ion) Ionic Cation-π (as the cation)	None
C	Amide	Hydrophilic (CONH) Hydrophobic (R)	Neutral	Solubility (CONH) Absorption (R)	H-bonding (A + D) Dipole-dipole Ion-dipole (as the dipole)	Ser, Tyr, Thr, Cys, Asn, Gln, Trp, His, Met
D	Lactam (cyclic amide)	Hydrophilic (CON) Hydrophobic (R)	Neutral	Solubility (CON) Absorption (R)	H-bonding (A) Dipole-dipole Ion-dipole (as the dipole)	Ser, Tyr, Thr, Cys, Asn, Gln, Trp, His
E	Thioether	Hydrophobic	Neutral	Absorption	Hydrophobic van der Waals H-binding (A)	None
F	Carboxylic acid	Hydrophilic (COOH) Hydrophobic (R)	Acidic (pK_a 2.5-5)	Solubility (COOH) Absorption (R)	Ion-dipole (as the ion) Ionic	None
G	Alkene	Hydrophobic	Neutral	Absorption	Hydrophobic van der Waals	None

A = H-bond acceptor; Ar = aromatic hydrocarbon; D = H-bond donor; R = carbon skeleton.
[a] "None" is a possible answer.

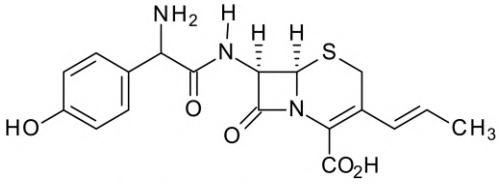
2. Cefprozil contains two acidic functional groups (phenol and carboxylic acid) and one basic functional group (primary amine). Because this drug contains both acidic and basic functional groups, it is considered ***amphoteric*** in nature.

3. Functional groups, their acidic or basic nature, the pK_a that matches, and the ionization status at the various pH levels are shown below.

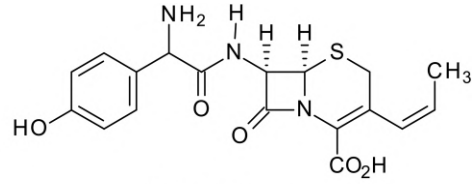
Name of Functional Group	Acidic or Basic (pK_a)	Ionized or Unionized at pH = 5 (Saliva)	Ionized or Unionized at pH = 1 (Stomach)	Ionized or Unionized at pH = 7.4 (Plasma)	Ionized or Unionized at pH = 8 (Intestine)	Ionized or Unionized at pH = 6 (Urine)
Phenol	Acidic pK_a=10	Unionized	Unionized	Unionized	Unionized	Unionized
Carboxylic acid	Acidic pK_a=1.7	Ionized	Unionized	Ionized	Ionized	Ionized
Primary amine	Basic pK_a=7.2	Ionized	Ionized	Likely 50%/50% ionized/ unionized	Unionized	Ionized

4. Drug absorption is enhanced as hydrophobic character is increased. Cefprozil contains several hydrophobic functional groups, including the aromatic ring portion of the phenol, the thioether and the alkenes. In the stomach (pH = 1) the carboxylic acid and the phenol are predominantly unionized; however, the primary amine is primarily ionized. In the intestine (pH = 8) the phenol and the primary amine are unionized and the carboxylic acid is primarily ionized. This means that cefprozil will always be predominantly in an ionized form regardless of the GI location. *A quick reminder:* An equilibrium exists between the ionized form and the unionized form of a drug molecule. In the case of cefprozil only a small fraction of the drug is completely unionized at any point regardless of its location within the GI tract. When cefprozil is in its unionized form, there is sufficient hydrophobic character to permit the drug to cross the lipid bilayer membranes of the GI tract and enter systemic circulation. Based on this evaluation, it is possible that drug absorption can occur in both GI compartments.

5. As described in Chapter 7, geometric isomers are not mirror images of one another and are not superimposable. These isomers are a result of the presence of a carbon-carbon double bond. This double bond causes conformational restriction and forces the double bond substituents to be positioned in one of two orientations. Either the methyl group and the bulk of the cefprozil molecule are on opposite sides of the double bond (*E* geometric isomer) or they are on the same side of the double bond (*Z* geometric isomer). Geometric isomers have different physical and chemical properties. In this example, the geometric isomers have the same pharmacological action. In general, geometric isomers may have similar or different pharmacological activities. ***NOTE:*** In the case of cefprozil, the *E*-isomer is predominant and has much more activity against gram (-) organisms than the *Z*-isomer.

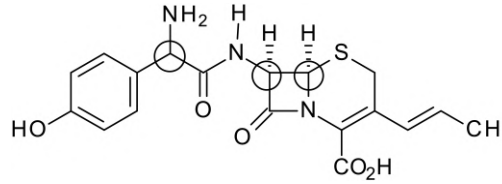


E (entgeggen)
Geometric Isomer



Z (zusammen)
Geometric Isomer

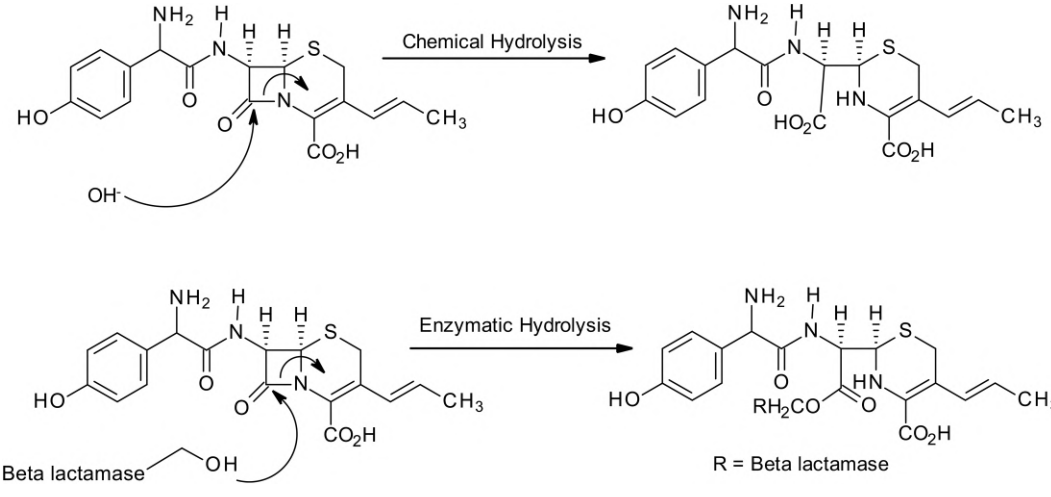
6. There are three chiral centers within the structure of cefprozil. Because there is more than one chiral center, diastereomers are possible. Diasteromeric pairs occur when one chiral center is held constant (e.g., *S*-enantiomer) and another chiral center changes stereochemical orientation (e.g., from *R*-enantiomer to *S*-enantiomer). In the case of cefprozil, diastereomeric pairs occur when two centers are held constant and the third chiral center changes stereochemical orientation or when one center is held constant and the other two chiral centers change stereochemical orientations. Diastereomers are not superimposable (like enantiomers) and are not mirror images (unlike enantiomers). Diastereomers are expected to have different physical and chemical properties.



7. The metabolic transformations are listed below.

	Name of Transformation	Phase I or Phase II
A	Oxidative deamination	Phase I
B	Aromatic hydroxylation	Phase I
C	Amide hydrolysis	Phase I
D	Sulfate conjugation	Phase II
E	Allylic oxidation	Phase I
F	Amino acid conjugation (with glycine)	Phase II

8. The hydrolysis of the β-lactam bond by chemical and enzymatic mechanisms is shown below.



Levonorgestrel (Level 3)

1. Answers provided below.

	Name of Functional Group	Character: Hydrophobic, Hydrophilic, or both	Function: Contribute to Aqueous Solubility or Absorption
A	Ketone	Hydrophobic (R)	Absorption (R)
		Hydrophilic (C=O)	Solubility (C=O)
B	Alkene	Hydrophobic	Absorption
C	Cycloalkane	Hydrophobic	Absorption
D	Alkyne	Hydrophobic	Absorption
E	Tertiary alcohol	Hydrophobic (R)	Absorption (R)
		Hydrophilic (OH)	Solubility (OH)

R = carbon scaffolding.

2. Both the ketone and tertiary alcohol contain electronegative oxygen atoms, which inductively attract/withdraw electron density from the carbon atoms attached to them. This means that both the tertiary alcohol and ketone are electron withdrawing groups based on an inductive effect.

   The tertiary alcohol is attached to aliphatic carbon atoms and therefore cannot participate in any resonance effects. The ketone is part of an α, β unsaturated ketone and there is a limited amount of resonance contribution as an electron withdrawing group.

3. Levonorgestrel has several functional groups that are exclusively hydrophobic in character. This should promote absorption (lipid solubility) across the skin if the drug is formulated as a patch. Distribution into the plasma is facilitated by both the ketone and tertiary alcohol, which are both hydrophilic in character and able to participate in H-bonding with water due to the electronegativity differences between the C and O atoms or O and H atoms, respectively. This hydrophilic character contributes to aqueous solubility in the blood.

   Because levonorgestrel has functional groups that contribute to both the hydrophobic and hydrophilic character (as just described) of the drug, it is possible for it to be administered orally. Dissolution and solubility in the aqueous contents of the stomach, as well as distribution into the plasma, are facilitated by functional groups that contribute to the hydrophilic character of the drug. Absorption across the lipophilic GI membranes and then across target cell membranes is facilitated by functional groups that contribute to the hydrophobic character of the drug.

4. In Chapter 3, we described a nonelectrolyte as a drug that does not dissociate into ions in solution (contains only neutral functional groups) and an electrolyte as a drug that does dissociate into ions in solution (contains one or more acidic or basic functional groups or a quaternary amine). Levonorgestrel is a nonelectrolyte. It does not contain any ionizable (acidic or basic) functional groups and is comprised only of neutral functional groups.

5. In Chapter 3, we discovered that human serum albumin (albumin) binds to a variety of endogenous substances and drugs. It is fairly nonspecific in its binding requirements although it prefers to interact with acidic molecules more so than basic molecules and

prefers to interact with drugs that are hydrophobic in character more so than those that are hydrophilic in character. Levonorgestrel has no acidic or basic functional groups but is comprised of functional groups that contribute a significant amount of hydrophobic character. Because of this hydrophobic character, levonorgestrel is suitable for binding to albumin.

6. In Chapter 5, we learned that log *P* represents a ratio of the solubility of the unionized drug in an organic solvent to the solubility of the same unionized drug in an aqueous environment. The structural evaluation of levonorgestrel reveals that this drug is unable to be ionized, so we are really looking at the ratio of the solubility of the drug in an organic solvent to the solubility of the same drug in an aqueous environment.

   Part A: If we want the log *P* value to decrease, we need to increase the amount of drug that is soluble in an aqueous environment (larger denominator in the ratio). This means that our structural modification should add hydrophilic character to the drug molecule. In Chapter 5, we learned that a water-soluble prodrug could be created (e.g., create a sodium phosphate ester or a sodium succinate ester). The prodrug would be metabolically cleaved to reveal the parent drug. Another solution is to modify the drug structure by adding one or more hydrophilic functional groups (e.g., alcohol, amine). *A word of caution with this last recommendation:* Functional group addition must not interfere with the critical binding interactions between a drug and its biological target.

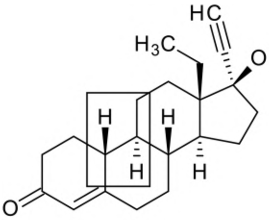
   Part B: If we modify the molecule to increase the hydrophilic character of the molecule, then we have also increased the water solubility of the molecule.

   Part C: Norgestimate has a log $P = 4.11$, which is larger than levonorgestrel. The numerator represents the solubility of the drug in an organic solvent. So, from a structural evaluation perspective, why is norgestimate more lipid soluble than levonorgestrel? As you can see, the tertiary alcohol has been converted into a lipid-soluble ester prodrug. This modification enhances the lipid solubility of the drug and contributes to the increase in log *P* value. In addition, the ketone has been converted into an oxime, which can be metabolically converted into the parent ketone. The oxime is actually more water soluble than the parent ketone, so this modification is not what is enhancing the lipid solubility and causing an increase in the log *P* value.

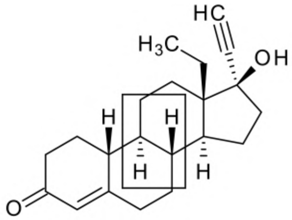
7. Both ethinyl estradiol and levonorgestrel contain a hydrophobic steroid scaffold (infrastructure) on which several substituents are positioned. The hydrophobic steroid skeleton resides in the hydrophobic cavity found as part of both the estrogen and progesterone receptors. Additional structural evaluation reveals that levonorgestrel contains a ketone (H-bond acceptor) and a tertiary alcohol (H-bond acceptor and donor) on opposite ends of the steroid skeleton. Ethinyl estradiol has a phenol (H-bond acceptor and donor) and a tertiary alcohol (H-bond acceptor and donor) on opposite ends of that steroid skeleton. Given that the estrogen receptor requires that receptor agonists interact with both the hydrophobic cavity as well as via two critical H-bonding interactions, it is no surprise that levonorgestrel has some agonist activity at the estrogen receptor.

8. **Part A:** As you learned in Chapter 7, the *trans* designation refers to substituents being located on opposite sides of a double bond or ring system in geometric isomers.

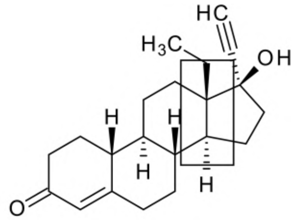
Part B: In levonorgestrel there are several places where the *trans* designation is relevant. Each of the following shows a unique *trans* relationship within levonorgestrel.



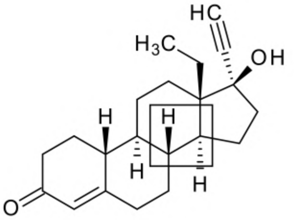
Levonorgestrel



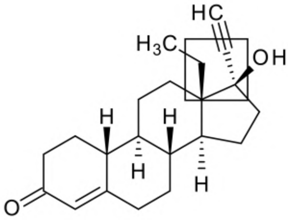
Levonorgestrel



Levonorgestrel

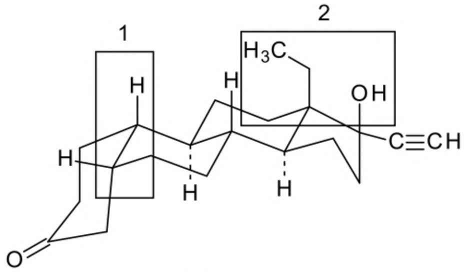


Levonorgestrel



Levonorgestrel
(*trans*: ethyl and ethynyl groups)

Parts C and D: When functional groups are *cis* to one another, they are found on the same side of a double bond or ring system. In the case of the levonorgestrel analog that has a mixture of *cis/trans* relationships, there are several *cis* relationships, two of which are boxed. Only one of these, box #1, has an effect on the overall shape of the steroid skeleton. As you can see from the picture below, the steroid is no longer flat as a result of the *cis* ring junction found in box #1. This would make interaction with the progesterone receptor's hydrophobic cavity less than optimal. Not only is the steroid skeleton a different shape but also the ketone is now located in a different place in space and is unlikely able to interact via H-bonding with the estrogen receptor. For both types of receptors, this *cis* configuration places a hydrophilic functional group (ketone) in the space that should be occupied only with hydrophobic functional groups. (*Note*: The structural activity relationships [SARs] for the progesterone receptor require the presence of the double bond in the A ring, so this analog does not bind to and activate the progesterone receptor.)



cis/trans mixture

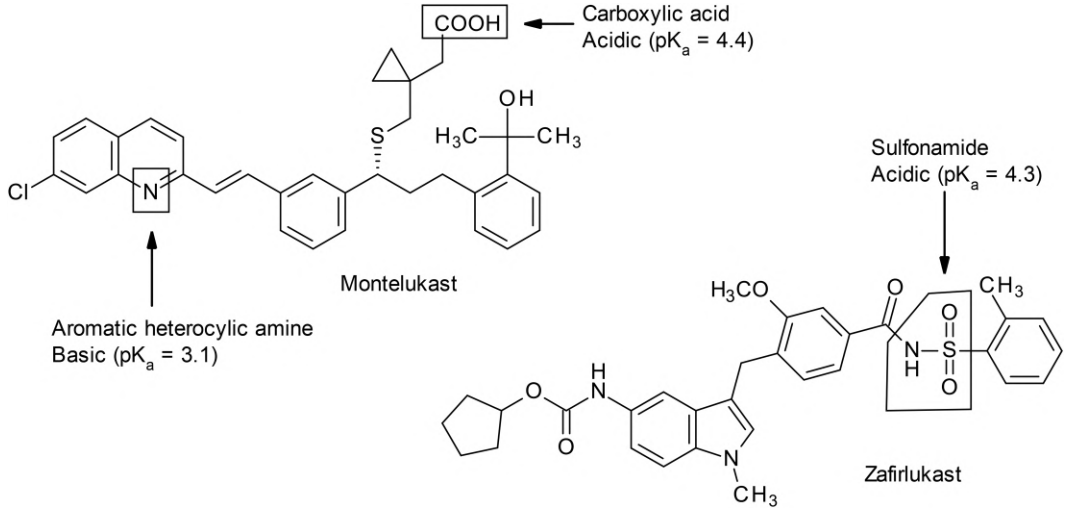
9. **Part A:** As we learned in Chapter 8, a drug that is subject to first-pass metabolism is metabolized by the liver prior to reaching systemic circulation. This decreases the amount of drug that is bioavailable. Drugs that are lipid soluble are typically subject to first-pass metabolism.

Part B: As we identified earlier in this evaluation, levonorgestrel contains a significant amount of hydrophobic character. You might have expected that it was sufficiently hydrophobic to undergo first-pass metabolism.

Part C: *Possible Phase I transformations:* allylic oxidation; epoxidation/peroxidation. *Possible Phase II transformations:* glucuronidation; sulfation (minor).

Montelukast and Zafirlukast (Level 2)

1. Shown below are the reported pK_a values for the functional groups. As discussed in Chapter 3, pK_a ranges can occasionally overlap; therefore, it is possible for a carboxylic acid to have a pK_a value of 3.1 and an aromatic heterocyclic amine to have a pK_a value of 4.4. Please note that even if these pK_a values were switched, the answers to the ionization questions would remain the same.

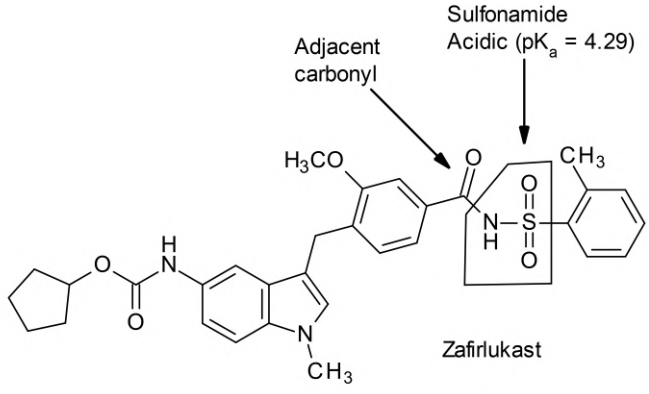


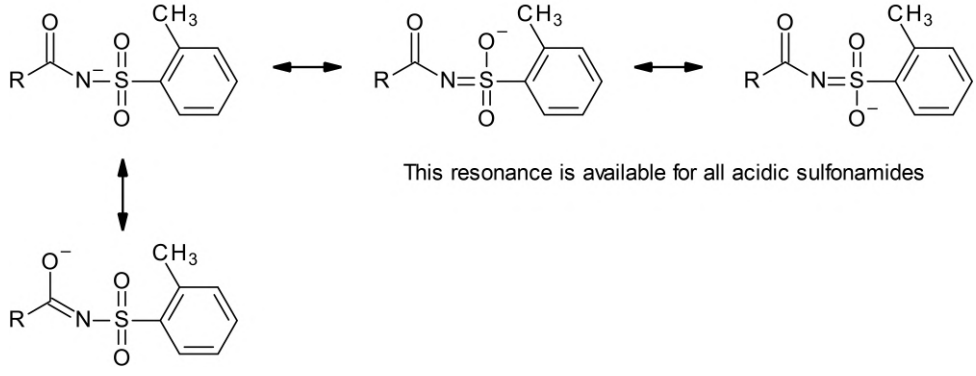
Functional Group	Acidic or Basic	Primarily Ionized or Unionized				
		1.9	5.4	6.1	7.2	8.3
Carboxylic Acid	Acidic	Unionized	Ionized	Ionized	Ionized	Ionized
Aromatic heterocyclic amine	Basic	Ionized	Unionized	Unionized	Unionized	Unionized
Sulfonamide	Acidic	Unionized	Ionized	Ionized	Ionized	Ionized

2. To use the Rule of Nines, the difference between the pH and the pKa must be an integer (i.e., 1, 2, 3). In evaluating the above 15 scenarios, there are three scenarios that meet this criterion: the ***carboxylic acid of montelukast*** (pK_a = 4.4) at a urine pH of 5.4, ***the aromatic heterocyclic amine of montelukast*** (pK_a = 3.1) at a cellular pH of 6.1, and the ***sulfonamide of zafirlukast*** (pK_a = 4.3) at a solution pH of 8.3. For the carboxylic acid of montelukast, |pH – pKa| is equal to 1; thus, there is a 90:10 ratio. Because the carboxylic acid (pK_a = 4.4) would be primarily ionized in a basic environment (pH = 5.4), we can use this ratio to determine that it would be 90% ionized. For the aromatic heterocyclic amine of montelukast, |pH – pKa| is equal to 3; thus, there is a 99.9:0.1 ratio. Because the aromatic heterocyclic amine (pK_a = 3.1) is a basic functional group, it would be primarily unionized

in a basic environment (pH = 6.1). We can use this information to predict that it would be 0.1% ionized. For the sulfonamide of zafirlukast, |pH – pKa| is equal to 4; thus, there is a 99.99:0.01 ratio. Because the sulfonamide ($pK_a = 4.3$) is an acidic functional group, it would be primarily ionized in a basic environment (pH = 8.3). Thus, we can use this information to predict that it would be 99.99% ionized.

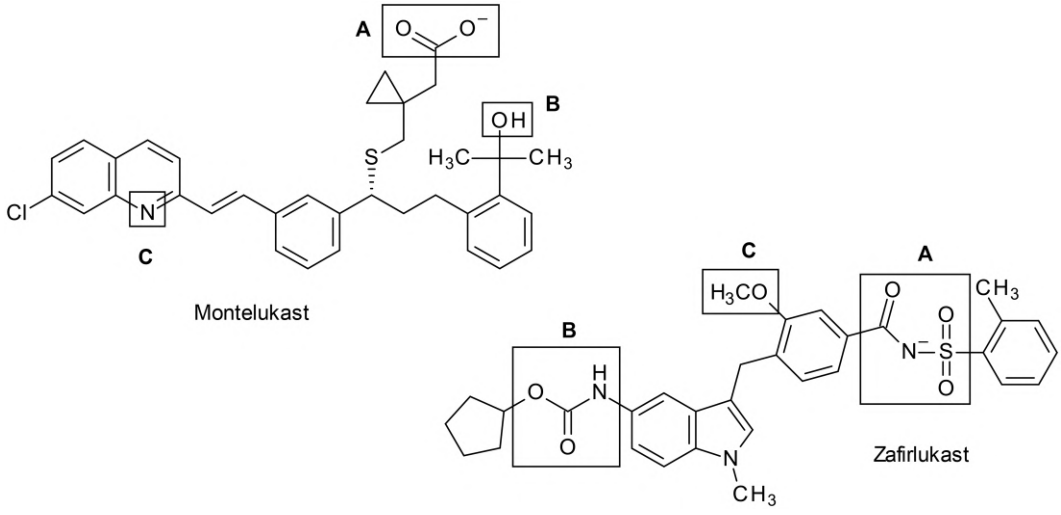
3. The presence or absence of specific functional groups can affect the acidity or basicity of ionizable functional groups. In the case of zafirlukast, the adjacent carbonyl group allows for increased resonance stabilization of a negative charge. As shown below, once the acidic proton dissociates, the resulting negative charge can be equally distributed among the nitrogen atom and the three oxygen atoms. This enhanced resonance stabilization increases the acidity of the sulfonamide, resulting in a lower pK_a value.





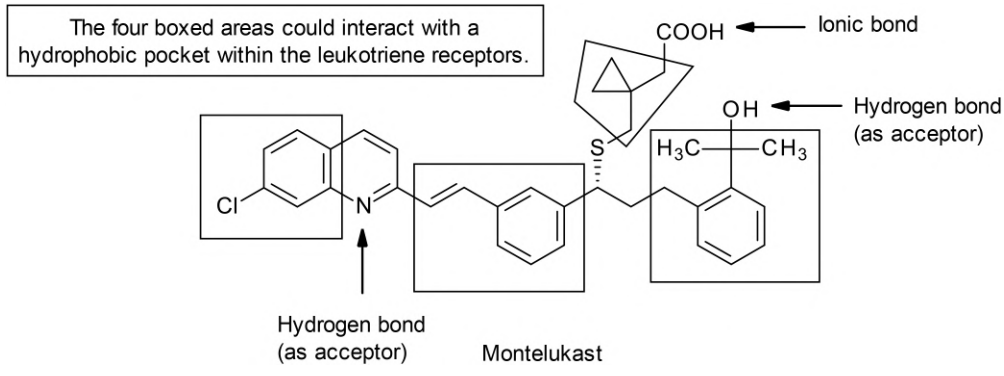
4. A sodium salt is an inorganic salt of the parent drug. The primary purpose of using an inorganic salt is to enhance the water solubility of a drug molecule. This in turn enhances its solvation and dissolution with the aqueous environment of the gastrointestinal (GI) tract. Because montelukast must be administered as an inorganic salt and zafirlukast does not have this requirement, this indicates that zafirlukast has higher water solubility than montelukast. In evaluating their structures, it is found that montelukast and zafirlukast each contain three water soluble functional groups; the remainder of their structures is comprised of alkyl chains, aromatic rings, a thioether, and a halogen. These latter functional groups bestow lipid solubility to their respective drug molecules. A key difference between these two structures is the overall nature of their water-soluble functional groups. The sulfonamide of zafirlukast has a wider charge distribution (four atoms) than does the

carboxylic acid of montelukast (two atoms; **Comparison A**). Additionally, the carbamate group of zafirlukast has the ability to form more hydrogen bonds with water than does the tertiary hydroxyl group of montelukast (**Comparison B**). The ability to act as hydrogen bond acceptors would be expected to be similar for the methoxy group of zafirlukast and the aromatic heterocyclic amine of montelukast (**Comparison C**). Please note that the nitrogen atom of the indole ring within the structure of zafirlukast is an extremely weak base ($pK_a < 0.1$) and cannot participate in hydrogen bonds. This is because the lone pair of electrons on the nitrogen atom are required for the aromaticity of the indole ring.

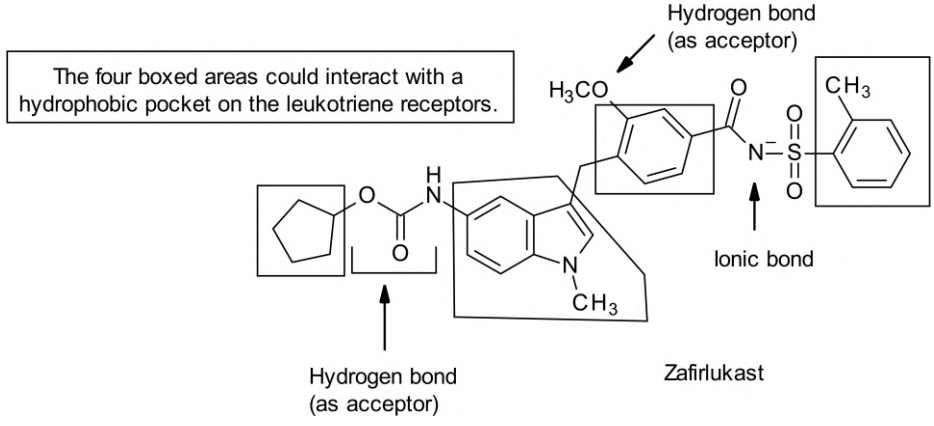


In addition, the overall lipid-soluble character of montelukast is greater than that of zafirlukast. The structure of montelukast contains 34 aromatic or aliphatic carbon atoms (as well as a halogen atom) whereas the structure of zafirlukast contains only 28. The combination of a lower water-soluble nature and a higher lipid-soluble nature is responsible for the need to utilize a sodium salt for the oral administration of montelukast.

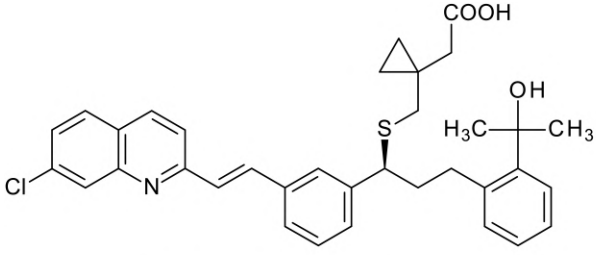
5. Let us evaluate these two drug molecules separately. The structure of montelukast contains a carboxylic acid that could participate in an ionic bond with the leukotriene receptors. It also contains two functional groups, the tertiary hydroxyl group and the aromatic heterocyclic amine, that could participate in a hydrogen bonding interaction as an acceptor. As shown below, the structure of montelukast contains four separate regions that could interact with the three hydrophobic pockets of the receptor via van der Waals and hydrophobic interactions.

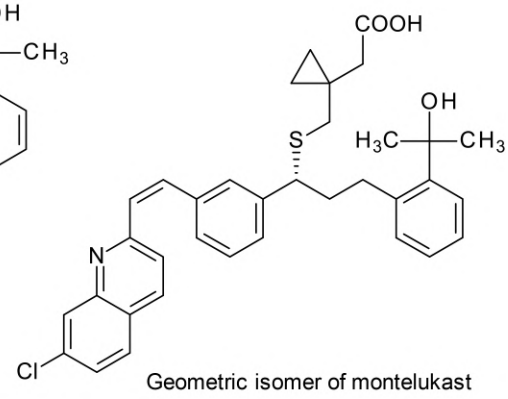


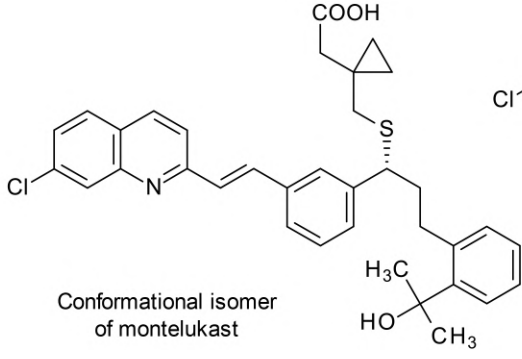
Similarly, the structure of zafirlukast contains a sulfonamide group that could participate in an ionic bond with the leukotriene receptor as well as two functional groups, the methoxy group and the carbamate, that could participate in a hydrogen bonding interaction as an acceptor. As shown below, the structure of zafirlukast also contains four separate regions that could interact with the three hydrophobic pockets within the receptor.



6. The structure of montelukast contains one chiral center, one nonaromatic double bond, and numerous rotatable single bonds. Thus, it can have an ***enantiomer,*** a ***geometric isomer***, and numerous ***conformational isomers***. It cannot have diastereomers. Examples are shown below.

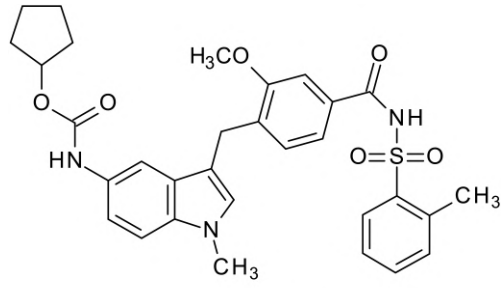






The structure of zafirlukast does not contain a chiral center or a nonaromatic double bond; therefore, it cannot have an enantiomer, diastereomers, or geometric isomers. Due to the

presence of a number of freely rotatable single bonds, zafirlukast can have ***conformational isomers***. One example is shown below.



Conformational isomer of zafirlukast

7. Plasma proteins are used by the human body to transport endogenous molecules in the plasma from one cell to another. Given that the plasma is water soluble, these proteins are primarily required to carry those endogenous molecules that have a high level of lipid solubility (e.g., estradiol, cholesterol, hydrocortisone). This is also true for exogenously administered drug molecules. Drug molecules that have a more lipid-soluble character have a greater affinity for plasma proteins than do those that have a more water-soluble character. Thus, using the calculated log *P* values provided for montelukast and zafirlukast, it would be expected that these two drug molecules would be ***highly plasma protein bound***.

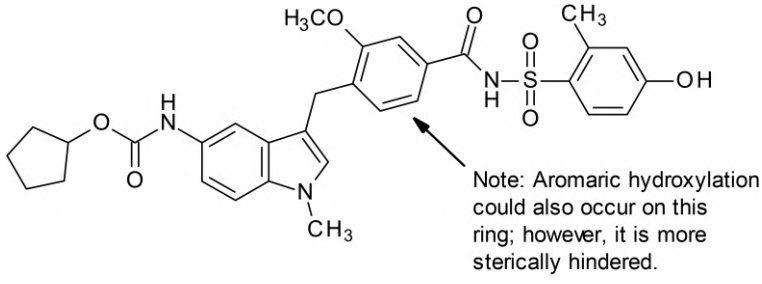
   The primary purpose of drug metabolism is to enhance the removal of the drug molecule from the human body. Drug molecules that already possess adequate water solubility, and thus can be readily eliminated, generally undergo minimal or no metabolism whereas drug molecules that are highly lipid soluble often undergo extensive metabolic transformation. Thus, using the calculated log *P* values provided for montelukast and zafirlukast, it would be expected that these two drug molecules would undergo ***extensive hepatic metabolism***.

8. The metabolic transformations are listed below.

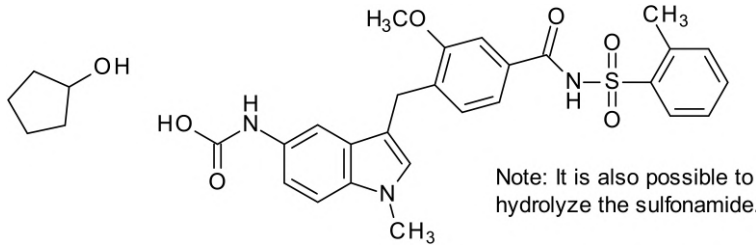
   - ***Metabolite A:*** Phase II; Glucuronic acid conjugation
   - ***Metabolite B:*** Phase I; *S*-oxidation
   - ***Metabolite C:*** Phase I; ω-oxidation
   - ***Metabolite D:*** Phase I; Benzylic oxidation

9. **Pathway A (Methylation): No.** Methylation is a ***Phase II*** metabolic transformation that requires a catechol, a phenol, an amine, or a sulfhydryl functional group. Because none of these functional groups are present within the structure of zafirlukast, this metabolic transformation cannot occur.

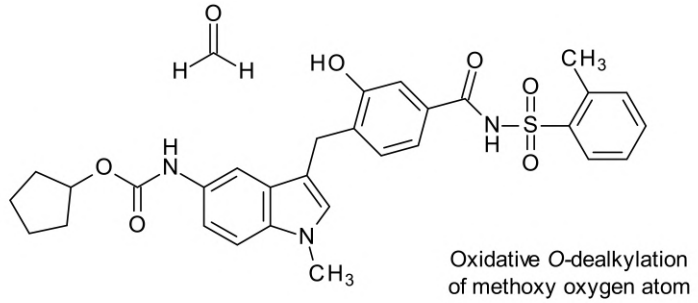
   Pathway B (Aromatic Oxidation): Phase I; Yes

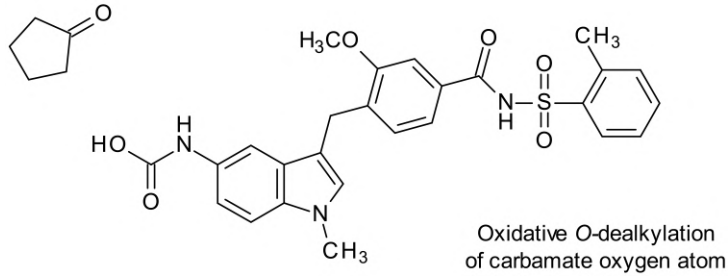


Pathway C (Hydrolysis): Phase I; Yes

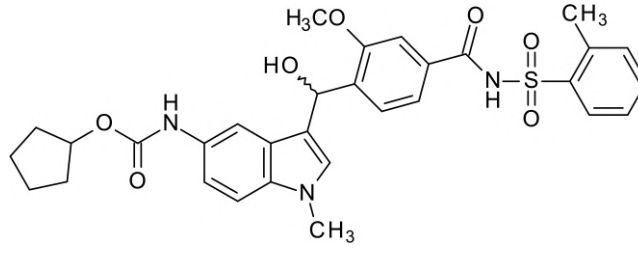


Pathway D (Oxidative *O*-Dealkylation): Phase I; Yes





Pathway E (Benzylic Oxidation): Phase I; Yes

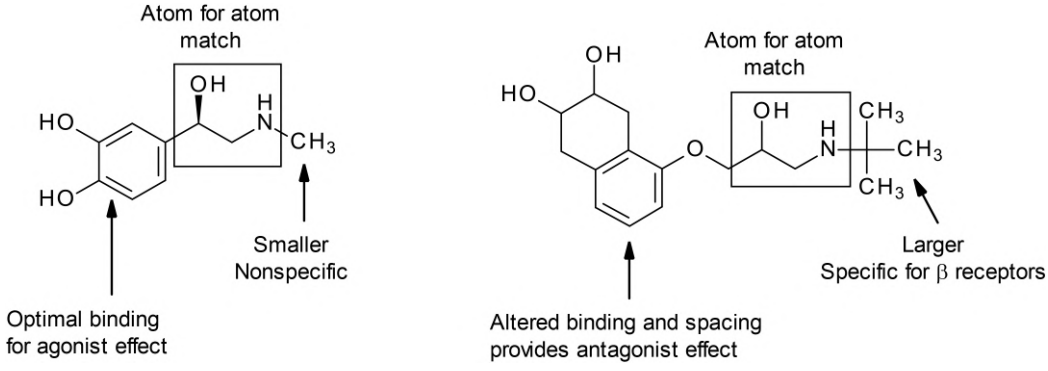


Nadolol and Other β-Adrenergic Antagonists (Level 3)

1. Functional group names and hydrophilic or hydrophobic character provided below.

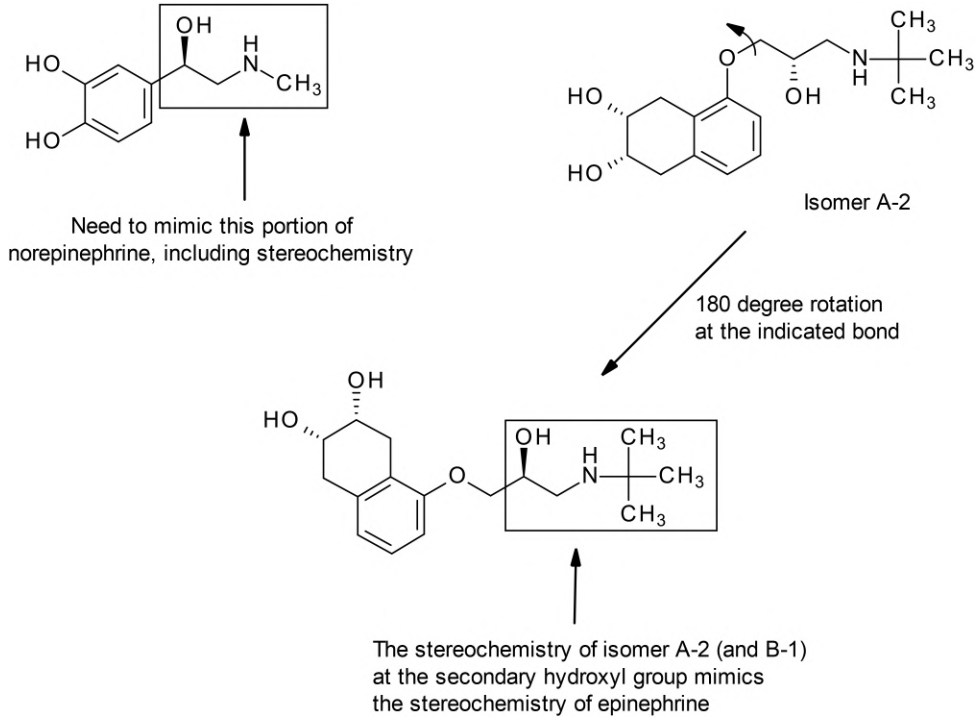
	Functional Group Name	Hydrophilic or Hydrophobic
A	Secondary hydroxyl (secondary alcohol)	**Hydrophilic** due to its ability to form hydrogen bonds with water as either a donor or an acceptor
B	Ether oxygen	**Hydrophilic** due to its ability to form hydrogen bonds with water as an acceptor
C	Alkyl group; alkyl chain; aliphatic chain	**Hydrophobic** due to its inability to ionize or form hydrogen bonds with water; hydrocarbon functional groups enhance lipid solubility
D	Secondary amine	**Hydrophilic** due to its ability to ionize and form ion–dipole interactions with water
E	Alkyl group; *t*-butyl group	**Hydrophobic** due to its inability to ionize or form hydrogen bonds with water; hydrocarbon functional groups enhance lipid solubility
F	Aromatic ring; phenyl ring	**Hydrophobic** due to its inability to ionize or form hydrogen bonds with water; hydrocarbon functional groups enhance lipid solubility

2. Epinephrine is a naturally occurring hormone that acts as an agonist on both α- and β-adrenergic receptors. The secondary amine, the secondary hydroxyl group, and the phenolic hydroxyl groups present within the structure of epinephrine provide key binding interactions with both the α- and β-adrenergic receptors. As discussed in Chapter 9, replacement of the *N*-methyl group of epinephrine with a larger alkyl group, such as the *t*-butyl group on nadolol, provides selectivity for β-adrenergic receptors due to a steric effect. The secondary amine, methylene, and secondary hydroxyl group of nadolol provides an atom for atom mimic of epinephrine. The major difference in these two structures lies in the spacing between the basic nitrogen atom and the aromatic ring systems as well as the positioning/orientation of the phenolic or alicyclic hydroxyl groups. The orientation seen in epinephrine produces an agonist effect when it binds to the β-adrenergic receptor while the larger bicyclic ring system produces an antagonist effect.

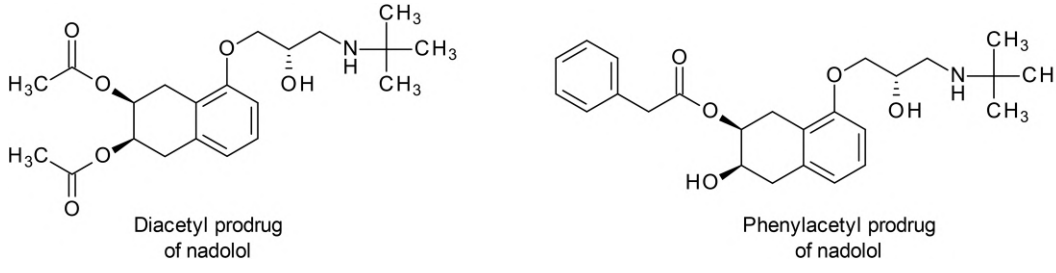


3. Epinephrine is a naturally occurring endogenous agonist at the β-adrenergic receptors. The structure of epinephrine contains one chiral center with an *R* configuration that maximizes the binding interactions of the secondary hydroxyl group with the adrenergic receptors.

In examining the four stereoisomers, the secondary hydroxyl groups in isomers A-2 and B-1 have the same stereochemistry as that seen in epinephrine and would be expected to exhibit better binding and pharmacological activity than those that had the opposite stereochemistry. This is illustrated below.

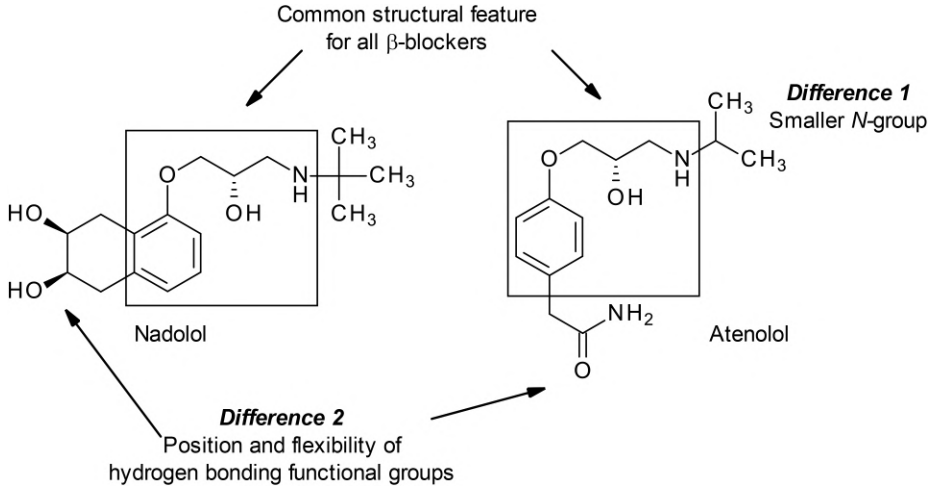


4. The use of salts and esters are two common chemical strategies used to enhance the oral absorption of a drug. Inorganic salts and water-soluble organic salts are commonly used to enhance the dissolution of a drug whereas lipid-soluble esters are commonly used as prodrugs to enhance the lipid solubility of drugs. Based on its low log *P* value, it appears that nadolol has sufficient water solubility but lacks sufficient lipid solubility to traverse the GI membranes. Thus, the use of lipid-soluble esters could be used to obtain a better water/lipid solubility balance. The alicyclic hydroxyl groups are a little less sterically hindered than the secondary hydroxyl group in the alkyl chain and could be esterified. Two examples of lipid-soluble esters of isomer B-1 are shown below.



5. Both nadolol and atenolol share a key structural backbone that is found in all β-blockers. This backbone has been boxed in the structures below. The two structural differences are the *N*-alkyl groups and the substitutions on the respective aromatic rings. It can be concluded that one or both of these differences is responsible for the β_1 adrenergic receptor selectivity. Although this question assumes no prior knowledge of β-blocker SAR, you

should be able to ***postulate potential binding differences***. The isopropyl group of atenolol may optimize interactions with the β_1 receptor while the larger and slightly more bulky *t*-butyl found within the structure of nadolol does not. Similarly, the location and availability of functional groups capable of hydrogen bonding interactions may optimize binding interactions with the β_1 receptor. Both nadolol and atenolol have functional groups capable of acting as both hydrogen bond donors and/or acceptors; however, the amide present within the structure of atenolol is *para* to the ether oxygen atom, while the alicyclic hydroxyl groups within the structure of nadolol are oriented in a different direction. Additionally, the amide functional group is located on a flexible alkyl chain while the alicyclic hydroxyl groups have a more ridged conformation. The combination of these structural differences and binding interactions may contribute to atenolol selectively blocking the β_1-adrenergic receptor.



6. Binding interactions and corresponding amino acids provided below.

	Functional Group	Types of Binding Interactions	Amino Acids Capable of Forming Specific Binding Interactions[a]
A	Amide	Ion-dipole (as the dipole)	Asp, Glu (with carbon atom or hydrogen atoms); Arg, Lys, His[b] (with oxygen or nitrogen atoms)
		Dipole-dipole	Ser, Thr, Tyr, Cys, Asn, Gln, Met, His[b]
		Hydrogen bond (donor and/or acceptor)	Ser, Thr, Tyr, Cys, Met, Asn, Gln, Trp, His[b]
B	Phenyl ring; aromatic ring; aromatic hydrocarbon	van der Waals; hydrophobic	Tyr, Phe, Trp (better interaction[c]); Val, Leu, Ile, Met, Ala
		π–π Stacking	Tyr, Phe, Trp
		Cation-π interaction	Arg, Lys, His (less likely[b])
C	Secondary hydroxyl	Ion-dipole (as the dipole)	Asp, Glu (with hydrogen atom of hydroxyl); Arg, Lys, His[b] (with oxygen atom of hydroxyl)
		Dipole-dipole	Ser, Thr, Tyr, Cys, Asn, Gln, Met, His[b]
		Hydrogen bond (donor and/or acceptor)	Ser, Thr, Tyr, Cys, Met, Asn, Gln, Trp, His[b]

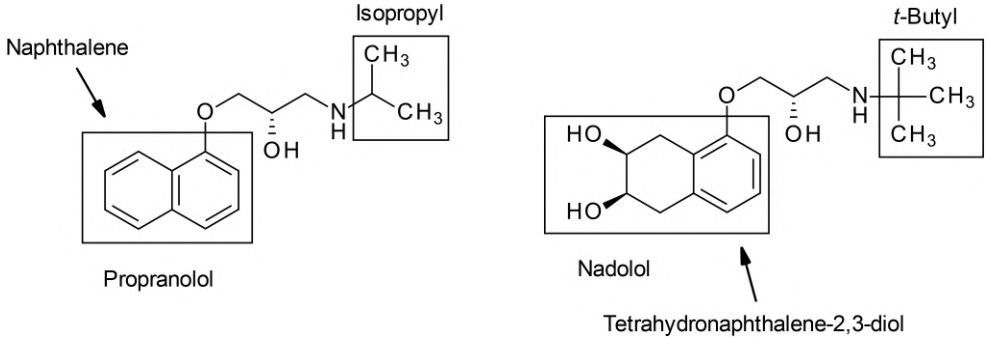
	Functional Group	Types of Binding Interactions	Amino Acids Capable of Forming Specific Binding Interactions[a]
D	Secondary amine	Ionic	Asp, Glu
		Ion-dipole (as the ion)	Ser, Thr, Tyr, Cys, Asn, Gln, Met
E	Alkyl group; aliphatic hydrocarbon	van der Waals; hydrophobic	Val, Leu, Ile, Ala (better interaction[c]); Tyr, Phe, Trp

[a] At a pH of 7.4, the side chains of Glu, Asp, Lys, and Arg are primarily ionized; therefore, they can only participate in ionic bonds and ion–dipole interactions (as the ion).

[b] The side chain of histidine is primarily unionized at a pH = 7.4. The small fraction that is ionized could form an ion–dipole interaction with a partially negatively charged atom or participate in a cation-π interaction, while the unionized fraction can serve as a hydrogen bond donor or acceptor. Additionally, in its unionized form it can serve as the dipole in an ion–dipole interaction.

[c] Stronger van der Waals interactions occur when aromatic rings interact with aromatic rings and alkyl chains interact with alkyl chains; however, all of the listed amino acids could possibly interact with the indicated functional group.

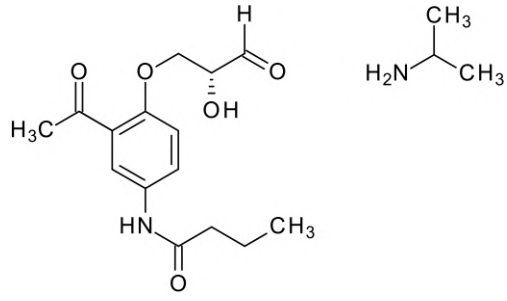
7. In comparing the structures of propranolol and nadolol, there are two sites of structural variation. The *t*-butyl group within the structure of nadolol is slightly larger and more lipid soluble than the isopropyl group within the structure of propranolol; however, the major difference lies in the ring systems. The structure of propranolol contains a lipid-soluble, bicyclic naphthalene ring while the structure of nadolol contains a more water-soluble bicyclic ring. The two alicyclic hydroxyl groups within the structure of nadolol can form hydrogen bonds with water, an interaction not possible with propranolol. Thus, propranolol is expected to be more lipid soluble than nadolol. A comparison of their respective log *P* values verifies this expectation. The log *P* value for nadolol is 1.3 (given in Question 4), and the log *P* value for propranolol is 3.1. Because lipid-soluble drugs are more likely to undergo extensive first-pass metabolism than water-soluble analogs, it can be concluded that propranolol is extensively metabolized while nadolol is excreted unchanged. Additionally, because propranolol is more lipid soluble than nadolol, it can better penetrate the blood brain barrier and provide beneficial effects in the treatment of migraine headaches and anxiety.



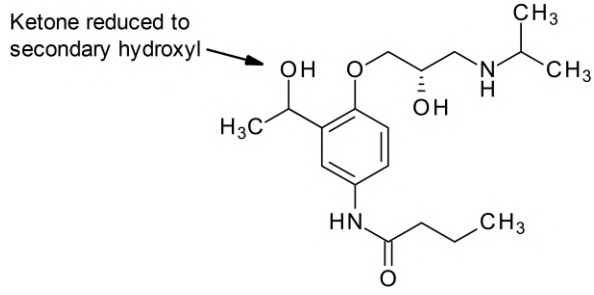
8. **Part A:** Hydrolysis of the amide followed by acetylation of the resulting aromatic amine.

Part B:

Pathway A: Oxidative deamination is a Phase I transformation. ***YES***. Although direct oxidative deamination is possible for secondary amines, oxidative *N*-dealkylation to a primary amine normally occurs prior to oxidative deamination.

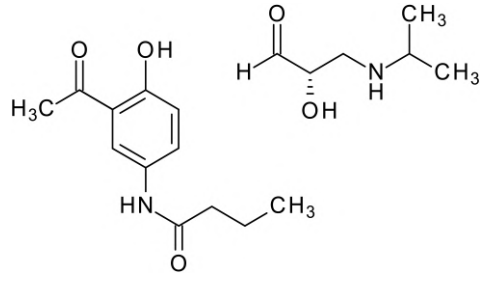


Pathway B: Reduction: Phase I, ***YES.***

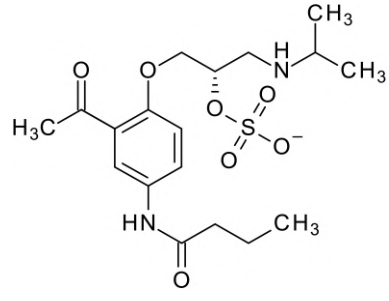


Pathway C: Benzylic oxidation is a Phase I transformation. ***NO***, it is not possible for acebutolol because the only benzylic carbon atom is a ketone and lacks a hydrogen atom.

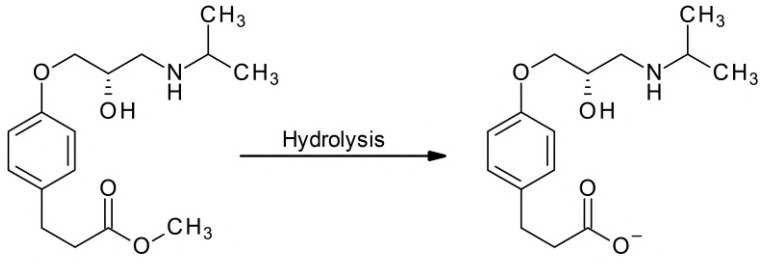
Pathway D: Oxidative *O*-dealkylation is a Phase I transformation. ***YES,*** it is possible; however, due to the location of the ether oxygen atom, it is sterically hindered and the probability of this metabolic transformation is low.



Pathway E: Sulfate conjugation: Phase II, ***YES***.



9. In comparing the structures of esmolol, acebutolol, and atenolol, it is found that the structure of esmolol contains an ester functional group while the structures of acebutolol and atenolol contain amide functional groups. Esterase enzymes are readily available within the human body and can quickly hydrolyze ester functional groups. The hydrolysis of esmolol is shown below. The metabolite is inactive because its binding interactions with the β_1 receptor have been significantly altered. An ionized carboxylic acid is unable to form the same binding interactions with the β_1 receptor as the original ester and is thus inactive.



Esmolol

Ofloxacin (Level 1)

1. Functional group names and other information provided below.

	Name of Functional Group	Hydrophobic and/or Hydrophilic	Contributes to Aqueous Solubility and/or Absorption
A	Tertiary amine + aniline/ aromatic amine (piperazine)	Hydrophobic (R)	Absorption (R)
		Hydrophilic (both N atoms)	Solubility (both N atoms)
B	Halogen	Hydrophobic (R)	Absorption (R)
		Hydrophilic (F)	Solubility (F)
C	Aniline	Hydrophobic (R)	Absorption (R)
		Hydrophilic (N)	Solubility (N)
D	Ketone	Hydrophobic (R)	Absorption (R)
		Hydrophilic (C=O)	Solubility (C=O)
E	Carboxylic acid	Hydrophobic (R)	Absorption (R)
		Hydrophilic (COOH)	Solubility (COOH)
F	Alkene	Hydrophobic (R)	Absorption (R)
G	Ether	Hydrophobic (R)	Absorption (R)
		Hydrophilic (O)	Solubility (O)

R = carbon scaffolding.

2. **Part A:** halogen; ether (inductive); ketone; carboxylic acid

   Part B: ketone (carbon atom)

   Part C: piperazine (tertiary amine + aniline/aromatic amine)

   Part D: ether (resonance), aniline (resonance)

3. Functional group names and other information provided below.

	Name of Functional Group	Acidic, Basic, or Neutral	pK_a Value/Range
A	Piperazine	Basic	pK_a 9-11 (methylated N atom)
			pK_a 2-5 (aniline/aromatic amine)
B	Halogen	Neutral	
C	Aniline	Basic	pK_a 2-5
D	Ketone	Neutral	
E	Carboxylic acid	Acidic	pK_a 2.5-5
F	Alkene	Neutral	
G	Ether	Neutral	

Because ofloxaxin contains at least one acidic and one basic functional group, it is considered amphoteric.

4. Functional group ionization at different pH environments shown below.

Name of Functional Group	pH = 2 (Stomach)	pH = 7.4 (Plasma)	pH = 5 (Urine)	pH = 8.5 (Large Intestine)
Piperazine (CH_3)	Ionized	Ionized	Ionized	Ionized
Piperazine[a] (aniline/ aromatic amine)	Ionized	Unionized	50%/50%	Unionized
Aniline	Ionized	Unionized	50%/50%	Unionized
Carboxylic acid	Unionized	Ionized	50%/50%	Ionized

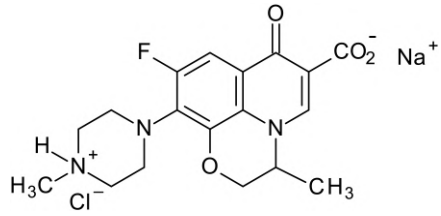
[a] Note: Only one of the two nitrogen atoms in this piperazine ring is ionized at a time. The more basic nitrogen atom, and the one that is more likely to be ionized, is the nitrogen atom with a methyl substituent.

Part A: Four possible atoms/functional groups can be ionized in any given pH environment. In the urine (pH = 5), all four functional groups are either primarily ionized or are at least 50% ionized. This confers considerable hydrophilic character to the molecule, which already has a number of functional groups that are hydrophilic in character (halogen, ketone, ether) but are not ionizable, making it remarkably water soluble. As a result, elimination via the kidneys is expected, without the need for any Phase I or Phase II metabolism.

Part B: Conducting a similar qualitative evaluation at pH = 6.5, we find that two of the four atoms/functional groups are predominantly ionized at pH = 6.5. This confers considerable water solubility to a molecule that already has a number of functional groups that are hydrophilic in character (halogen, ketone, ether) but are not ionizable. The considerable hydrophilic character makes the molecule suitable for delivery as an aqueous solution.

Piperazine (CH_3)	Primarily ionized
Piperazine (aniline/aromatic amine)	Primarily unionized
Aniline	Primarily unionized
Carboxylic acid	Primarily ionized

5. **Part A:** Ofloxacin can be formulated as both a hydrochloride and as a sodium salt. Both types of inorganic salts enhance the water solubility of the drug.



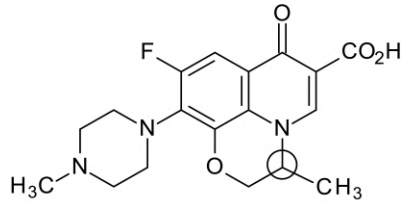
Part B: Ofloxacin could be modified to form a lipid-soluble salt (e.g., benzathine or stearate salts). It could also be modified to form a lipid-soluble ester prodrug.

6. **Part A:** The top half of ofloxacin has a fluorine atom, a ketone, and a carboxylic acid. At physiologic pH, these functional groups participate in H-bonding interactions (H-bond acceptor) via the fluorine and ketone functional groups and ion–dipole interactions (as the ion) with the carboxylic acid functional group (in its ionized form).

Part B: The bottom half of ofloxacin includes a piperazine, an ether, an aniline, and some hydrocarbon scaffolding. At physiologic pH, these functional groups participate in H-bonding interactions (H-bond acceptor) via the ether group, ion–dipole interactions (as the ion) via the piperazine group (in its ionized form), and through hydrophobic interactions with the hydrocarbon scaffolding present.

Part C: The fluoroquinolones self-associate into two pairs at the biological target. Each pair of drugs is stacked together via π-π stacking interactions. One drug pair then interacts with the other drug pair via the interactions mentioned in part B.

7. **Part A:** Chiral center has been circled.



Ofloxacin

Part B: Enantiomers are possible at the chiral center. Diastereomers are not possible because there is only one chiral carbon. Geometric isomers are not possible because there are no double bonds or ring junctions that can adopt an alternate conformation. Conformational isomers are possible primarily via the piperazine ring because it can adopt either a boat or chair conformation.

Part C: Given that the chiral center is located on the bottom side of the molecule, it is likely that the interactions associated with drug pair associations will be affected by a change in stereochemistry at this center. Interactions with the DNA target are not affected by a change in the stereochemistry at this center. Drug-drug pairing is also unaffected by a change in the stereochemistry at this center.

8. **Part A:** Ofloxacin is highly hydrophilic in character and has several functional groups that are predominantly ionized in every physiologic compartment. As a result, it is considered very water soluble. First-pass metabolism typically occurs on molecules that are highly hydrophobic in character; therefore, it is unlikely that ofloxacin would undergo first-pass metabolism. Given that ofloxacin has 98% oral bioavailability, this confirms that first-pass metabolism does not occur.

Part B: *Phase I transformations:* Oxidative *N*-dealkylation; N-oxidation; oxidative *O*-dealkylation; aromatic hydroxylation (*ortho* and *para*). *Phase II transformations:* Glucuronidation; amino acid conjugation.

Part C: Because ofloxacin is primarily excreted unchanged via a renal route and a small portion undergoes liver-based metabolic transformations, patients diagnosed with moderate-to-severe kidney and/or liver disease should not be given ofloxacin.

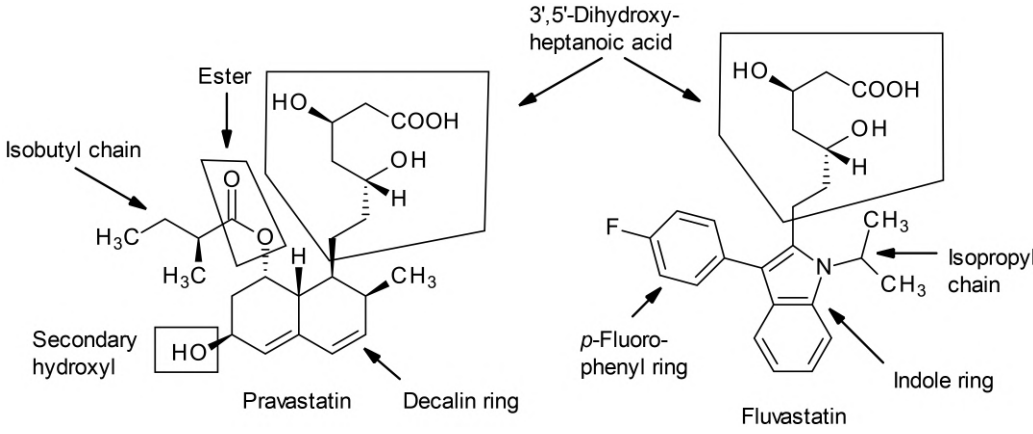
Pravastatin and Fluvastatin (Level 2)

1. Functional groups and their solubility effects are provided below.

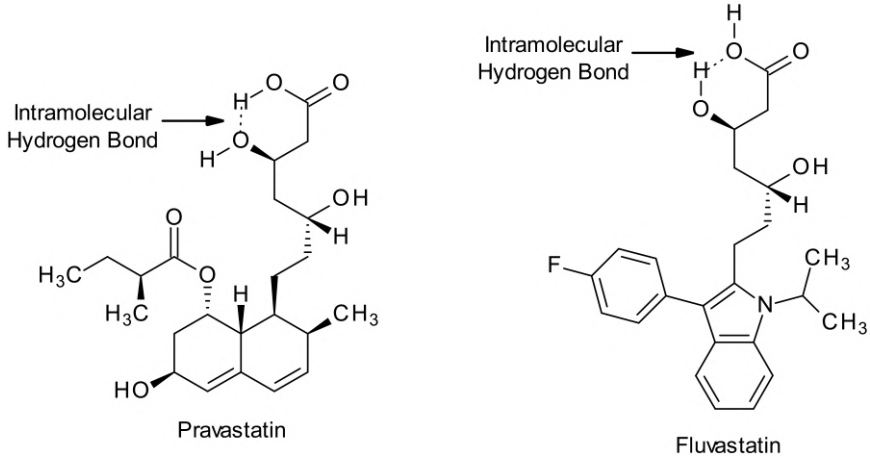
	Functional Group Name	Solubility Effect of Functional Group
A	Alkyl group; alkyl chain; aliphatic chain	**Hydrophobic** due to its inability to ionize or form hydrogen bonds with water; hydrocarbon functional groups enhance lipid solubility
B	Ester	**Hydrophilic** due to its ability to form hydrogen bonds with water as an acceptor
C	Secondary hydroxyl (secondary alcohol)	**Hydrophilic** due to its ability to form hydrogen bonds with water as either a donor or an acceptor
D	Halogen; fluorine	Effects can vary; fluorine can form hydrogen bonds with water as an acceptor; however, studies have shown that the substitution of a hydrogen atom for a fluorine atom tends to slightly enhance lipid solubility
E	Aromatic ring system; heterocyclic aromatic ring; indole	**Hydrophobic** due to its inability to ionize or form hydrogen bonds with water; the lone pair of electrons present on the nitrogen atom are involved in the aromaticity of the ring system and therefore are not available to form hydrogen bonds; the remaining hydrocarbons comprising the ring system enhance lipid solubility
F	Carboxylic acid	**Hydrophilic** due to its ability to ionize and form ion dipole interactions with water

2. The log *P* value is a logarithmic expression of the ratio of a drug molecule's solubility in a lipid environment when compared with an aqueous environment and assumes that all ionizable functional groups are present in their unionized form. The log *P* value of any given drug molecule is the result of the additive contributions and interrelationships of all of its functional groups. In evaluating the structures of pravastatin and fluvastatin, both drug molecules contain a 3,5-dihydroxyheptanoic acid group, and they differ in the ring systems attached to this group. The carboxylic acid and the two secondary hydroxyl groups contribute to the water solubility of these drug molecules; however, it would be expected that these contributions would be similar for both drug molecules. In evaluating the different ring systems and their substituents, it is apparent that the structure of pravastatin contains an additional secondary hydroxyl group as well as two ester oxygen atoms. These functional groups are capable of forming additional hydrogen bonds and thus contribute to the overall water solubility of pravastatin. The remaining functional groups of pravastatin-the decalin ring and the isopropyl side chain-contribute to its overall lipid solubility. In contrast, the remaining functional groups of fluvastatin—aromatic rings, an aliphatic chain, and a halogen—contribute to its overall lipid solubility. It should be noted that the lone pair of electrons present within the indole ring are required for the aromaticity of the ring

and therefore are not available to form hydrogen bonds or accept protons. Because the structure of pravastatin contains more hydrophilic functional groups than the structure of fluvastatin, it has a lower log *P* value.



3. The presence or absence of specific functional groups can affect the acidity or basicity of ionizable functional groups. In the case of pravastatin and fluvastatin, as well as other drug molecules within this chemical/pharmacological class, the 3-hydroxyl group affects the acidity of the carboxylic acid. In general, nonaromatic hydroxyl groups are electron withdrawing due to the high electronegativity of the oxygen atom; however, in the case of pravastatin and fluvastatin, the position of the 3-hydroxyl group orients it to form intramolecular hydrogen bonds with the carboxylic acid. These hydrogen bonds decrease the ability of the proton of the carboxylic acid to dissociate, therefore decreasing its acidity and increasing its pK_a value. Please note the hydroxyl group can serve as either a donor or an acceptor and that both hydrogen bonds are possible for pravastatin and fluvastatin. Additionally, the alkyl chain directly adjacent to the carboxylic acid in both drugs can donate electrons through induction and thus lower the acidity and increase the pK_a values. This is similar to what was discussed in Chapter 6 with glutamic acid and aspartic acid.



4. To solve this problem, we need to use the Henderson-Hasselbalch equation. Because the functional group (carboxylic acid) is acidic, the ionized form ($R\text{-}CO_2^-$) is the Base Form and the unionized form ($R\text{-}CO_2H$) is the Acid Form.

$$pH = pK_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$5.20 = 4.56 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.64 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$4.36 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \text{ or } \frac{4.36}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that for every one molecule that contains the functional group in the acid (or unionized) form, there are 4.36 molecules that contain the functional group in the base (or ionized) form. The following equations can then be used to correctly calculate the percent of the molecules that are ionized and the percent that are unionized.

$$4.36 \text{ Molecules in Base Form} + 1.0 \text{ Molecule in Acid Form} = 5.36 \text{ Total Molecules}$$

$$\text{Base Form} = \text{Ionized Form and Acid Form} = \text{Unionized Form}$$

$$\text{Percent in Ionized Form} = \frac{4.36 \text{ Molecules in Ionized Form}}{5.36 \text{ Total Molecules}} \times 100\% = 81.3\%$$

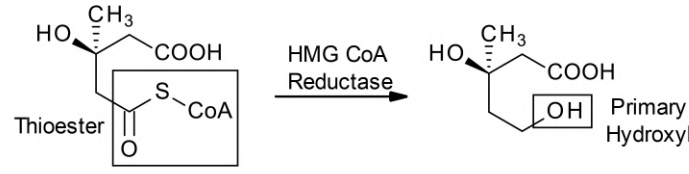
$$\text{Percent in Unionized Form} = \frac{1 \text{ Molecule in Unioinized Form}}{5.36 \text{ Total Molecules}} \times 100\% = 18.7\%$$

Because the question asked for the percent of fluvastatin that would be unionized, the correct answer is 18.7%.

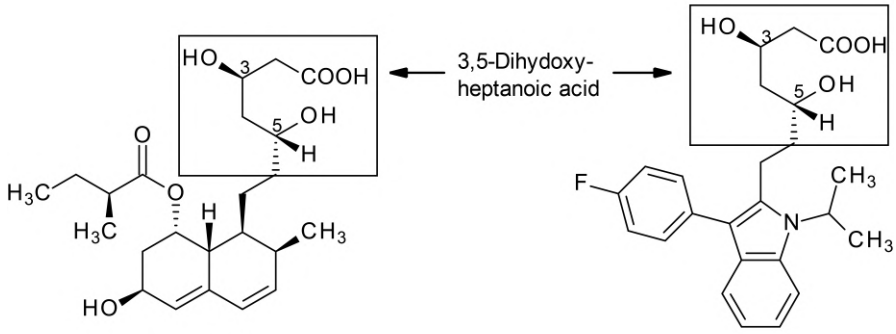
5. The Rule of Nines provides a quick and easy way to determine the ratio between the percent ionized and the percent unionized for a given functional group as long as the difference between the pH and pK_a is an integer (i.e., 1, 2, 3). Additionally, the Rule of Nines only provides a ratio, and additional steps are required to correctly assign numeric values to ionized and unionized forms. In Question 4, the absolute value of the difference between the pH (5.20) and the pK_a (4.56) is 0.64. This value is greater than 0 and less than 1. If this value were 0, then there would be a 50%:50% ratio between the ionized and unionized forms of the functional group. If this value were 1, then there would be a 90%:10% ratio between the ionized and unionized forms of the functional group. Because the value lies somewhere between 0 and 1, then the ratio lies somewhere between these two values. This fact helps to verify that the answer for Question 4 (81.3% ionized to 18.7% unionized) is correct. The sole purpose of this question is to demonstrate that the Rule of Nines can quickly provide a range that should match the result from your Henderson-Hasselbalch calculation.

6. In the reaction catalyzed by HMG CoA reductase, the thioester of HMG CoA is reduced to a primary hydroxyl group. The 3,5-dihydoxyheptanoic acid found within the structures of pravastatin and fluvastatin very nicely mimics the structure of mevalonic acid, the product of this reaction. Unlike the natural substrate, pravastatin and fluvastatin cannot be reduced. Because they bear structural similarity to both the substrate and the product, they can interact with the enzyme in a similar manner as the substrate; however, because they lack the functional group that is normally reduced, they are not transformed by the

enzyme and act as enzyme inhibitors. The respective ring systems of pravastatin and fluvastatin most likely occupy binding sites that are adjacent to the active site of the enzyme.



HMG CoA Mevalonic Acid



Pravastatin Fluvastatin

7. The addition of a functional group affects the overall electronic distribution, water/lipid solubility balance, and steric dimensions of the parent drug molecule. The electronic effects would be expected to be minimal due to the low electronegativity values of carbon and hydrogen. The additional carbon atom increases lipid solubility, which could affect the ability of the analog to dissolve; however, the use of a sodium salt (similar to what is done with fluvastatin) should still allow for adequate dissolution. This then leaves the steric effect as the most probable cause for the loss of activity with this analog. There are two aspects to consider. First, the addition of an extra carbon atom may not be permitted due to the steric dimensions of the target enzyme, HMG CoA reductase. The simple addition of a methyl group (or carbon atom) can significantly alter the interaction of a drug molecule with its biological target. Although this may be the cause for the loss of activity, a much bigger alteration in the steric dimension of fluvastatin has been introduced with this conformational restriction. In evaluating the structure of fluvastatin, it is found that there is free rotation about the bond that connects the indole ring to the *para*-fluoro aromatic ring. This allows flexibility and the opportunity for these two rings to be oriented in a manner that allows optimal interactions with HMG CoA reductase. In contrast, the conformational restriction introduced in the fluvastatin analog creates a large, planar, tetracycline ring system with no flexibility. The ability of this analog to interact with HMG CoA reductase would therefore depend on the availability of a complementary large, flat, hydrophobic area within the active site of the enzyme. Receptor binding studies have shown that the *para*-fluoro aromatic ring of fluvastatin cannot be coplanar with the indole ring and that conformational restriction, such as that shown in this question, abolishes therapeutic activity.

8. The metabolic transformations are

   - Oxidative *N*-dealkylation
   - Aromatic oxidation followed by glucuronide conjugation

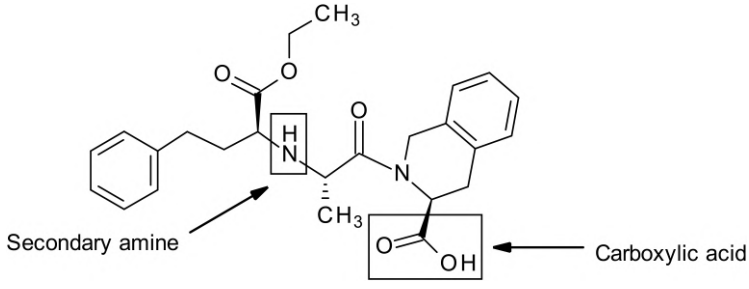
9. The epimer results from the oxidation of the 3β hydroxyl group to a ketone by alcohol dehydrogenase followed by reduction to the 3α epimer. The loss of activity results from the fact that the compound no longer mimics HMG CoA or mevalonic acid. The 3α epimer contains a hydroxyl group that is oriented in the opposite direction and either fails to form a crucial hydrogen bond or sterically inhibits interaction with the active site of HMG CoA reductase.

Quinapril (Level 1)

1. Functional groups are their solubility effects are shown below.

	Functional Group Name	Solubility Effect of Functional Group
A	Phenyl ring; aromatic ring; aromatic hydrocarbon	***Hydrophobic*** due to its inability to ionize or form hydrogen bonds; hydrocarbon functional groups enhance lipid solubility
B	Ethyl ester	Contains both ***hydrophilic*** and ***hydrophobic*** properties; the two oxygen atoms can act as hydrogen bond acceptors and can contribute to water solubility; the ethyl group can neither ionize nor form hydrogen bonds and thus contributes to lipid solubility
C	Secondary amine	***Hydrophilic*** due to its ability to ionize and participate in hydrogen bonding (acceptor and donor) in its unionized form
D	Amide	***Hydrophilic*** due to its ability to form hydrogen bonds as a hydrogen bond acceptor (*Note*: Does not participate as a hydrogen bond donor due to the lack of hydrogen atom)
E	Carboxylic acid	***Hydrophilic*** due to its ability to ionize and participate in hydrogen bonding (acceptor and donor) in its unionized form

2. Acidic and basic functional groups and other information is provided below.

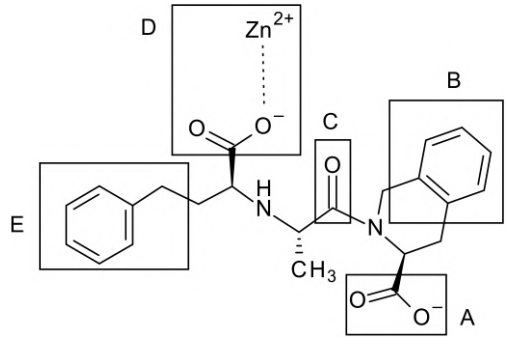


Functional Group	Acidic or Basic	pK_a Range	Primarily Ionized or Unionized				
			1.5	4.8	6.3	7.4	8.1
Secondary amine	Basic	9-11	Ionized	Ionized	Ionized	Ionized	Ionized
Carboxylic acid	Acidic	2.5-5	Unionized	Ionized[a]	Ionized	Ionized	Ionized

[a] There is a possibility that this functional group could be > 50% unionized at a pH of 4.8; however, because the pK_a values of carboxylic acids present in the structures of other drug molecules within this chemical/pharmacological class tend to range from 2.3 to 3.5, this functional group will most likely be primarily ionized at this pH value.

3. Ester hydrolysis converts quinapril to quinaprilat. Esterases are ubiquitous within the human body and can readily convert ester prodrugs to their active forms. In evaluating the structures of quinapril and quinaprilat, the only difference in these two molecules is the presence of a second carboxylic acid in quinaprilat instead of the ethyl ester present in quinapril. Both of these molecules possess a sufficient number of water-soluble functional groups to allow for their dissolution within the aqueous content of the GI tract. Quinaprilat has three ionizable functional groups (two carboxylic acids and a secondary amine) along with an amide that is capable of forming hydrogen bonds. This greatly enhances the water solubility of quinaprilat to the extent that it has difficulty traversing the GI membrane despite the presence of hydrophobic rings and alkyl chains. To optimize the water/lipid balance, an ester prodrug is used. The ethyl ester is more hydrophobic than the carboxylic acid and in combination with the other hydrophobic groups allows for better passage across the GI membrane.

4. Drug binding interactions are explained below.



Because ACE is a nonspecific dipeptidyl carboxypeptidase, it is able to hydrolyze a dipeptide sequence from the carboxylic acid end of a peptide. For this enzyme to be able to cleave a dipeptide sequence (i.e., two amino acids), substrates must have a minimum length of three amino acids. Quinaprilat and other ACE inhibitors act as tripeptide mimics. These drug molecules retain key peptide features (i.e., peptide bonds, side chains similar to those found on amino acids) but are not able to be hydrolyzed. Thus, they are able to interact in a similar manner as the substrate does. The table below lists five key interactions of quinaprilat with ACE.

	Drug Binding Interaction	Explanation
A	Ionic bond	This carboxylic acid mimics the C-terminal carboxylic acid of an ACE substrate and forms an ionic bond with the side chain of either Lys or Arg.
B	van der Waals and/or hydrophobic binding with hydrophobic amino acids (e.g., Phe, Tyr, Leu, Ile)	Because ACE is a nonspecific carboxypeptidase, quinapril does not need to exactly mimic Leu, the C-terminal amino acid of angiotensin I. Similar to the side chain of Leu, this ring system is hydrophobic and can participate in similar types of interactions as Leu.
C	Hydrogen bond acceptor	Because ACE is a dipeptidyl carboxypeptidase, it does not cleave the terminal peptide bond. Instead, the terminal peptide bond can participate in hydrogen bonds. The carbonyl of the amide bond can act as a hydrogen bond acceptor and interact with Ser, Thr, Tyr, Trp, Gln, Asn, or Cys.

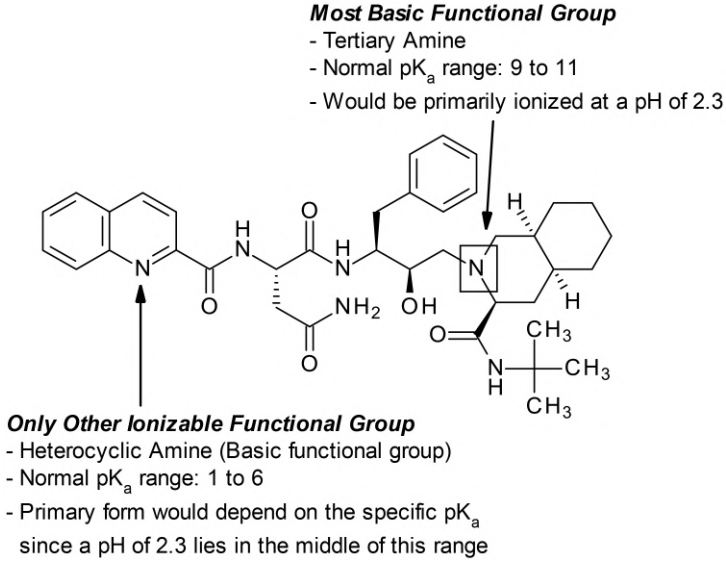
	Drug Binding Interaction	Explanation
D	Ionic bond (metal complexation)	This carboxylic acid would be located very close to the zinc atom shown participating in the mechanism of angiotensin I metabolism. As such, at pH = 7.4, it can form an ionic bond (or a metal complexation) with the zinc atom.
E	van der Waals and/or hydrophobic binding with hydrophobic amino acids (e.g., Phe, Tyr, Trp)	This aromatic ring mimics the Phe side chain of angiotensin I and can participate in similar interactions.

5. Metabolic transformations involved in the pathways are provided below.
   - **Pathway A:** Aromatic oxidation followed by sulfate conjugation of the resulting phenol
   - **Pathway B:** Ester hydrolysis
   - **Pathway C:** Amino acid conjugation (with glycine)
   - **Pathway D:** Benzylic oxidation followed by oxidation of the resulting secondary alcohol
6. Remember that the major purpose of drug metabolism is to enhance the removal of the drug from the body, and the number of metabolic transformations required to achieve this goal varies from drug molecule to drug molecule. In the case of quinapril, ester hydrolysis can occur quickly and at many locations within the body. The resulting metabolite, quinaprilat, contains three ionizable functional groups and is easily eliminated from the body without the need for further metabolism.
7. Because the structure contains one basic functional group and one acidic functional group, it is classified as an ***amphoteric*** drug molecule.
8. The structure of quinaprilat contains three chiral centers, one within the bicyclic ring and two others within the heterocyclic chain. Additionally, it lacks a double bond (outside of those within the aromatic ring). The stereoisomer that is shown has the same stereochemical configuration at two chiral centers and the opposite stereochemical configuration at the third chiral center. Additionally, the stereoisomer contains the exact same conformation as quinaprilat. Thus, this stereoisomer is both a ***diastereomer*** and an ***epimer*** of quinaprilat.

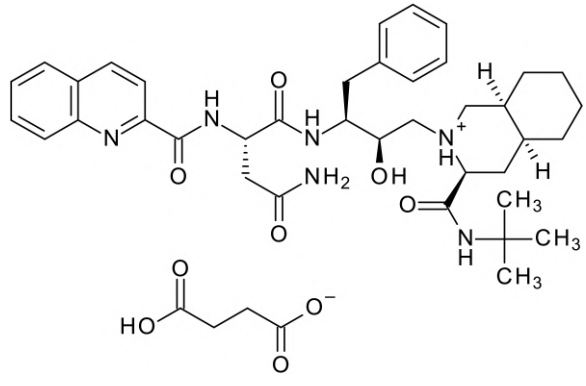
   The primary reason that this stereoisomer is significantly less active is due to its inability to correctly interact with ACE. Human enzymes such as ACE have binding sites that are comprised of L-amino acids. Alteration of the stereochemistry of the carboxylic acid converts it to a D-amino acid mimic. This causes the carboxylic acid to be pointed in the wrong place in space within the enzyme binding site. This change in location of the carboxylic acid can decrease the binding affinity of the molecule due to either steric hindrance or the inability to form a strong ionic bond with the enzyme. Additionally, because this is a diastereomer of quinaprilat, it has different physical and chemical properties that may play a role in its decreased activity.

Saquinavir and Other Human Immunodeficiency Virus Protease Inhibitors (Level 3)

1. The most basic functional group has been identified along with pK_a range and other information.

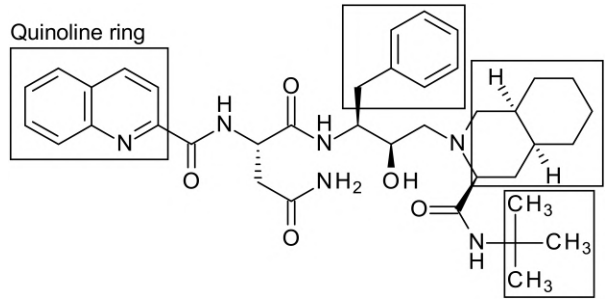


2. Water-soluble organic salts enhance the solvation, dissolution, and water solubility of drug molecules. Therapeutically, this can contribute to enhanced oral absorption as well as the preparation of concentration intravenous, ophthalmic, and otic solutions. Shown below is a succinate salt of saquinavir.

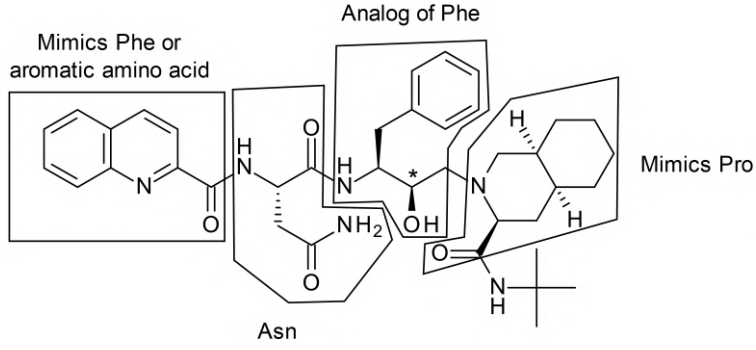


3. The ability of a drug molecule to be orally absorbed from the GI tract into the systemic circulation requires a balance between water- and lipid-soluble functional groups. The water-soluble functional groups allow the drug to dissolve in the GI tract, and the lipid-soluble functional groups allow the drug to pass through the GI membrane and enter the systemic circulation. The lipid-soluble functional groups within the structure of saquinavir have been

boxed below. Please note that while the nitrogen atom of the quinoline ring can participate in hydrogen bonds, this aromatic ring system (as a whole) enhances lipid solubility.

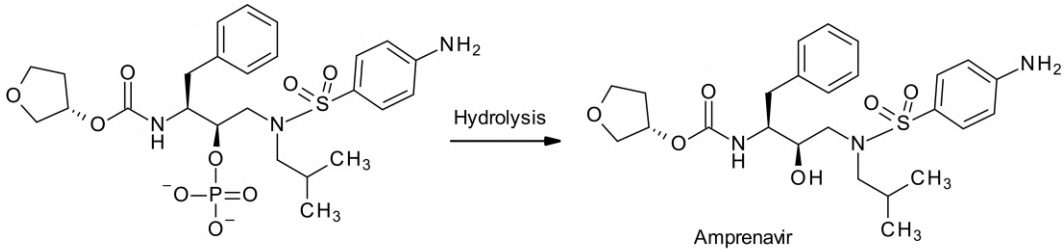


4. Saquinavir is able to inhibit HIV protease by acting as a stable mimic of the substrate for this enzyme. The structure of saquinavir is largely peptidic in nature, as shown below. It contains three amide (i.e., peptide) bonds and functional groups that either match or mimic the side chains of amino acids normally found in the substrates for HIV protease. The labile peptide bond between the phenylalanine and the proline residues has been replaced with a stable hydroxyethyl group. The tetrahedral carbon atom (indicated with the asterisk) that is attached to the secondary hydroxyl group provides a stable mimic of peptide bond hydrolysis. Due to these structural characteristics, saquinavir is considered a peptidomimetic and is able to bind to the active site of HIV. Because the labile bond has been replaced with a stable mimic, HIV protease is not able to cleave the bond and is thus inhibited by the binding of saquinavir.



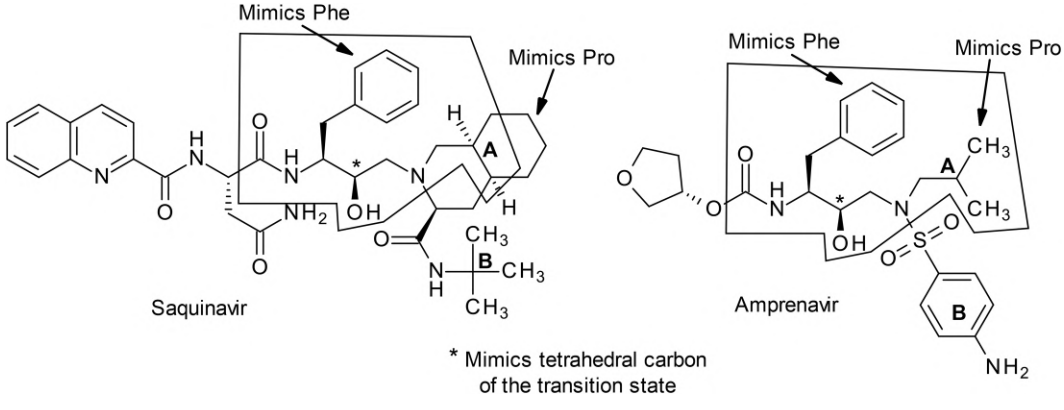
5. As discussed in the previous question, saquinavir acts as a stable mimic of the substrates for HIV protease. To serve as a stable mimic of a protein, the stereochemistry of the amino acids (or amino acid analogs) must match that of the naturally occurring amino acids. Chiral centers 1, 2, and 4 match the normal stereochemistry found in naturally occurring L-amino acids; therefore, changing the stereochemistry of any of these chiral centers would likely result in a decreased ability to effectively mimic a protein substrate. The stereochemistry of chiral center 3 is also essential as it provides a stable mimic of peptide bond hydrolysis. Chiral centers 5 and 6 are part of the bicyclic ring that mimics proline. This bicyclic ring is larger than proline, and the *cis* orientation of the hydrogen atoms more than likely optimizes the binding of this larger ring to a hydrophobic pocket in the enzyme binding site. While the stereochemistry of chiral centers 5 and 6 may not be as ***essential*** as those at chiral centers 1 to 4, the optimal binding of this bicyclic ring would have been tested and established in the development of saquinavir.

6. The information provided in the question provides multiple clues to the correct answers. Fosamprenavir is the prodrug, and amprenavir is the active metabolite. The only difference in these names is the "Fos" prefix. Examining the structure of fosamprenavir reveals the presence of a phosphate functional group. It can thus be concluded that the phosphate group of fosamprenavir is removed to produce the active drug amprenavir. The metabolic transformation that would remove a phosphate group is a hydrolysis reaction.



The phosphate functional group enhances the water solubility of amprenavir. Increasing the water solubility of a drug enhances its solvation and dissolution. This is required for oral absorption as well as the preparation of concentrated solutions. Given that fosamprenavir is administered orally, this prodrug is used to enhance the water solubility of amprenavir and to provide a better water/lipid solubility balance for oral absorption.

7. The first step in comparing the structural similarities of drug molecules is to ensure that all overlapping (or similar) functional groups are properly aligned. Because we have already examined the structure of saquinavir, we use this as our prototype and leave it unaltered. To align the functional groups of amprenavir with those of saquinavir, all that needs to be done is to rotate about the sulfonamide nitrogen atom. This reveals an atom-for-atom match for a large portion of the structures of saquinavir and amprenavir (indicated by the polygons in the structures below). Both drugs have a mimic of Phe, a tetrahedral carbon atom that mimics the transition state of HIV protease, and a mimic of Pro. Additionally, the distance between carbon A in the bicyclic ring of saquinavir and the central *t*-butyl carbon B is exactly the same as that seen between carbon A of amprenavir's isobutyl chain and the center of its aromatic ring (B). This indicates that these two hydrophobic functional groups are located in similar positions and can provide similar binding interactions (i.e., van der Waals interactions) with hydrophobic binding pockets present within the structure of HIV protease. As a final note here, amprenavir was initially drawn in a manner in which all of the functional groups did ***not*** overlap with those of saquinavir. This was done intentionally. Drug structures that you will encounter may look or be drawn differently, depending on the source. A key skill to master is the ability to look at drug structures, identify the similarities, and, if necessary, redraw the structures so that you can easily compare them.



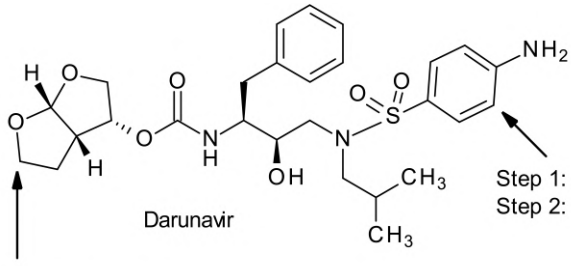
8. **Metabolic Path A:** *N*-Oxidation; this metabolic transformation can be catalyzed by CYP450 enzymes (including the CYP3A4 isozyme) as well as FMO enzymes.

   Metabolic Path B: Hydrolysis; this metabolic transformation is catalyzed by hydrolase enzymes, not CYP3A4 isozymes.

   Metabolic Path C: ω-1 Oxidation (or oxidation of aliphatic carbon atoms); this metabolic transformation could be catalyzed by the CYP3A4 isozyme. Given that the oxidized carbon atom is directly adjacent to a heterocyclic aromatic ring, this could also be labeled as benzylic oxidation.

   Metabolic Path D: Oxidative *N*-dealkylation; this metabolic transformation could be catalyzed by the CYP3A4 isozyme.

9. There are two sites of metabolism. It does not matter which site is metabolized first; however, in both cases, step 1 must occur prior to step 2.



Darunavir

Step 1: Aromatic oxidation, ***phase I***
Step 2: Glucuronide conjugation, ***phase II***

Step 1: Oxidative *O*-dealkylation, ***phase I***
Step 2: Reduction of resulting aldehyde, ***phase I***

Sorafenib (Level 2)

1. Functional group names and other information provided below.

	Name of Functional Group	Hydrophilic and/or Hydrophobic	Acidic, Basic, or Neutral (Provide pK_a When Relevant)	Contributes to Aqueous Solubility and/or Absorption	Interaction(s) Possible with Biological Target at Physiologic pH = 7.4
A	Halogenated aromatic hydrocarbon	Hydrophobic	Neutral	Absorption	Cl: Dipole-dipole Ion-dipole (as the dipole)
					Ar: van der Waals Hydrophobic π-π Stacking Cation-π interaction Charge transfer interaction
B	Halogenated aliphatic alkane	Hydrophilic (F)	Neutral	Solubility	H-bonding (A) Dipole–dipole Ion–dipole (as the dipole)
C	Urea	Hydrophilic (HNCONH)	Neutral	Solubility (HNCONH)	H-bonding (A + D) Dipole–dipole Ion–dipole (as the dipole)
		Hydrophobic (R)		Absorption (R)	

	Name of Functional Group	Hydrophilic and/or Hydrophobic	Acidic, Basic, or Neutral (Provide pK_a When Relevant)	Contributes to Aqueous Solubility and/or Absorption	Interaction(s) Possible with Biological Target at Physiologic pH = 7.4
D	Ether	Hydrophilic (O)	Neutral	Solubility (O)	H-bonding (A) Dipole–dipole Ion–dipole (as the dipole)
		Hydrophobic (R)		Absorption (R)	
E	Pyridine (Azine)	Hydrophilic (N)	Basic (pK_a 1-5)	Solubility (N)	Pyridine N atom: H-bonding (A) Dipole–dipole Ion–dipole (as the dipole)
		Hydrophobic (R)		Absorption (R)	R: van der Waals Hydrophobic π-π Stacking Cation-π interactions Charge transfer interactions
F	Amide	Hydrophilic (CONH)	Neutral	Solubility (CONH)	H-bonding (A+ D) Dipole–dipole Ion–dipole (as the dipole)
		Hydrophobic (R)		Absorption (R)	

R = carbon scaffolding.

2. Functional group interactions provided below.

Functional Group	Interacts with Cysteine[919] via a Hydrogen Bonding Interaction Yes or No	Interacts with Aspartic Acid[1046] via a Hydrogen Bonding Interaction Yes or No	Interacts with Phenylalanine[1047] via a Hydrophobic Interaction Yes or No
A	No	No	Yes
B	Yes	Yes	No
C	Yes	Yes	No
D	Yes	Yes	No
E	Yes	Yes	Yes
F	Yes	Yes	No

3. **Part A:** Possible types of binding interactions are listed below.

Leu[285]/Val[289]	Asp[391]/Glu[286]	Thr[315]	Met[318]	Leu[298]/Val[299]/Phe[359]
Hydrophobic	Ionic Ion–dipole (as the ion)	H-bonding Dipole–dipole Ion–dipole (as the dipole)	Hydrophobic Dipole–dipole H-bonding (A)	Hydrophobic van der Waals π-π Stacking Cation-π interactions

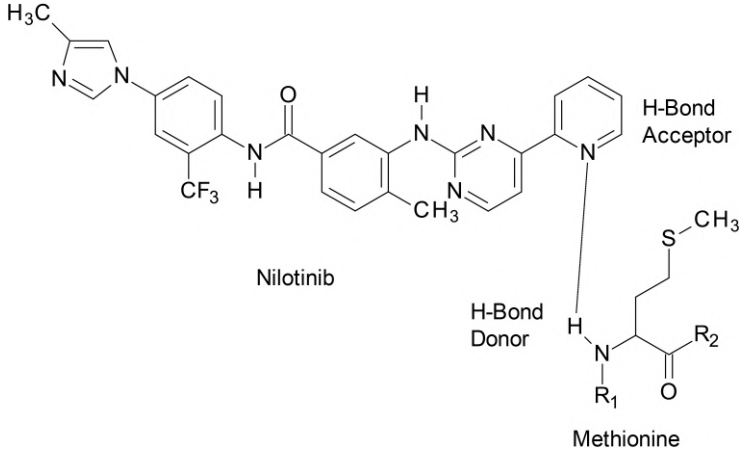
Part B: Possible types of binding interactions between amino acids and functional groups are provided below.

	Leu^{285}/Val^{289}	Asp^{391}/Glu^{286}	Thr^{315}	Met^{318}	Leu^{298}/Val^{299}/ Phe^{359}
A	Hydrophobic	Ion–dipole (functional group as the dipole)	H-bonding (A) Dipole–dipole	Hydrophobic	Hydrophobic π-π Stacking
B	Hydrophobic van der Waals	None	H-bonding (A) Dipole–dipole	Hydrophobic	Hydrophobic van der Waals π-π Stacking Cation-π interactions
C	None	Ion–dipole (functional group as the dipole)	H-bonding (A+D) Dipole–dipole	H-bonding (Met is acceptor)	None
D[a]	None	Ion–dipole (functional group as the dipole)	H-bonding (A+D) Dipole–dipole	H-bonding (Met is acceptor)	None
E[b]	Hydrophobic	Ion–dipole (functional group as the dipole)	H-bonding (A) Dipole–dipole	None	Hydrophobic π-π Stacking Cation-π interactions

[a] Aniline-like nitrogen atom is not ionized at physiologic pH.

[b] Pyridine (azine) nitrogen atom is not ionized at physiologic pH.

Part C: Interaction between the pyridyl nitrogen atom and methionine is shown below.



4. The halogenated aromatic hydrocarbon, aromatic hydrocarbon, and the pyridine ring carbon atoms all contribute significantly to the hydrophobic character of sorafenib. It is important to note that the basic pyridine ring is unlikely to be ionized at physiologic pH ($pK_a = 6 < pH = 7.4$). Although there appears to be significant hydrophilic character (halogenated aliphatic alkane, urea, ether, the nitrogen atom of the pyridine, amide) present in this molecule, sorafenib is practically insoluble in water. The lack of water solubility and clearly identifiable hydrophobic character allow for passive diffusion of the drug across the cellular lipid bilayer membrane.

5. Based on the information found in the structure evaluation grid for sorafenib, there are several functional groups that contribute to the overall hydrophobic character of the

molecule (e.g., halogenated aromatic hydrocarbon, aromatic hydrocarbon, carbon atoms of pyridine/azine ring). Similar evaluation of nilotinib yields two aromatic rings, a pyrimidine ring (between functional groups D and E), a pyridine ring (functional group E), and even some hydrophobic character in the histidine ring (functional group A) that contribute to the overall hydrophobic character of the molecule. When you compare the sheer number of functional groups that contribute to the overall hydrophobic character for each drug, nilotinib wins!

6. The value of lipid-soluble organic salts is to decrease the water solubility and increase the lipid solubility of the parent drug (in this case sorafenib) (see Chapter 5). Typically, lipid-soluble salts are used in the formation of lipid-soluble suspensions. In addition, they can improve the oral bioavailability of acid labile drug molecules and improve the palatability of liquid solutions. *p*-Toluenesulfonic acid (tosylic acid) is considered a strong organic acid and forms a strong counter-ion when dissociated from the drug molecule. Sorafenib is administered as a film-coated tablet for adults and as a liquid suspension for children.

   The value associated with the formation of inorganic salts is due to the improved aqueous solubility, solvation, and dissolution that results. In general, inorganic salts enhance the absorption of drugs that are administered orally because they improve both solvation and dissolution properties.

7. **Sorafenib:**

   - Aromatic hydroxylation (*ortho, para*)
   - *N*-oxidation
   - Oxidative *N*-dealkylation (amide)

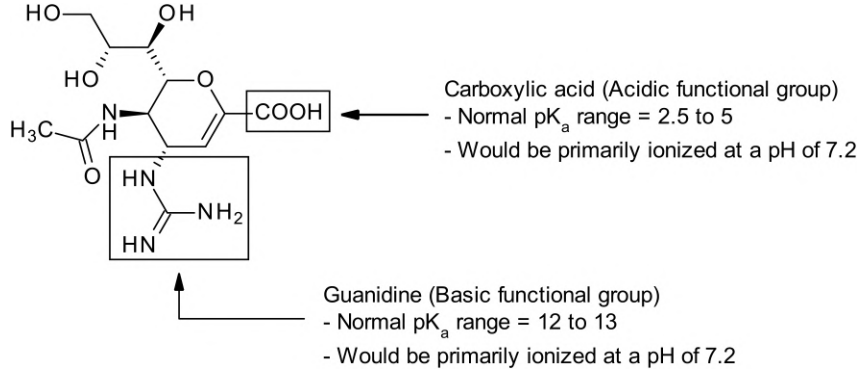
   Nilotinib:

   - Aromatic hydroxylation (*ortho, para*)
   - Benzylic oxidation
   - N-oxidation

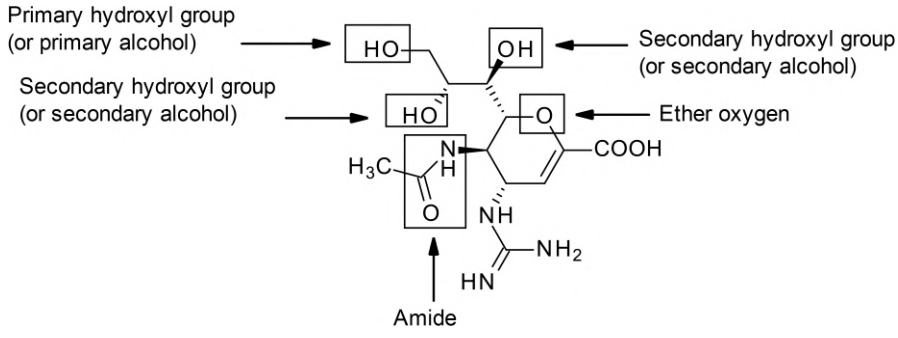
8. ***No,*** when a drug molecule is bound to a serum protein, including serum albumin, it cannot undergo metabolic transformations, be eliminated from the body, or effectively interact with its biological target. Only the fraction of the drug "unbound" is eligible to exert its therapeutic effect and is subject to metabolic transformations. It should be noted that extensive protein binding can effectively increase the half-life of the drug.

Zanamivir and Oseltamivir (Level 2)

1. Acidic and basic function groups are identified along with ranges and ionization state at pH = 7.2.

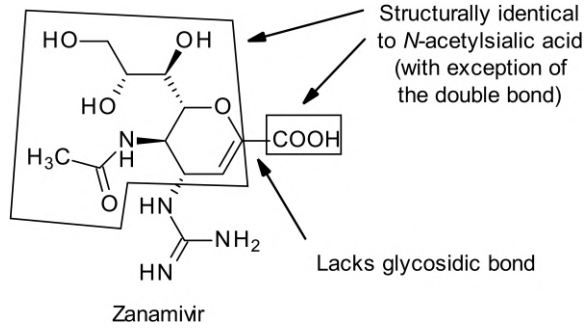


2. The amide and hydroxyl groups can act as hydrogen bond donors and acceptors and thus can form hydrogen bonds with water. The ether oxygen most likely contributes the least to water solubility; however, it can function as a hydrogen bond acceptor.



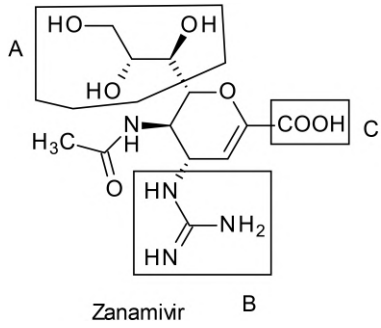
3. The structure of zanamivir contains multiple hydrophilic functional groups that allow it to easily dissolve within the aqueous contents of the GI tract. The carboxylic acid and the guanidine functional groups are extensively ionized at an intestinal pH = 5, which further increases the overall water solubility of the molecule. Although the structure of zanamivir contains a hydrocarbon chain and ring, the overall balance between water and lipid solubility hinders its ability to effectively cross the GI membrane. The oral absorption of zanamivir has been reported to be between 1% and 5%. Due to this, zanamivir must be administered via oral inhalation.

4. Zanamivir is a stable mimic of *N*-acetylsialic acid. As shown below, the structure of zanamivir retains many of the structural features of *N*-acetylsialic acid but lacks a cleavable glycosidic bond.

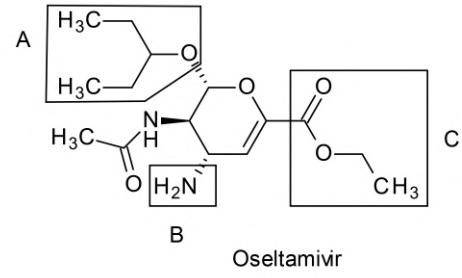


The structural similarity allows zanamivir to interact with neuraminidase in the same manner as *N*-acetylsialic acid. Ionic or ion–dipole interactions with the carboxylic acid remain the same, as does the ability to form hydrogen bonds with the hydroxyl groups and the amide. Because zanamivir lacks a cleavable glycosidic bond, it can occupy the binding site without being metabolically transformed. The most significant difference between zanamivir and *N*-acetylsialic acid is the substitution of a secondary hydroxyl group with a guanidine group. Studies have shown that this basic functional group can form ionic interactions with neuraminidase. In summary, zanamivir structurally mimics the natural substrate for neuraminidase and is able to bind to the active site of the enzyme. This binding inhibits the ability of neuraminidase to cleave *N*-acetylsialic acid from viral glycoproteins, thus inhibiting the spread of a viral infection.

5. When comparing these two structures, there are three key structural differences. In all three instances, the functional group on oseltamivir is less water soluble (or more lipid soluble) than the analogous functional group on zanamivir.



Zanamivir



Oseltamivir

The glycerol side chain of zanamivir (**A**) is much more water soluble than the alkyl ether of oseltamivir (**A**). Although both drug molecules contain a basic functional group within their structure, the guanidine group in zanamivir (**B**) is more basic and more ionized than the primary amine (**B**) in oseltamivir. Finally, the ionizable carboxylic acid of zanamivir (**C**) has been esterified in oseltamivir (**C**). This ethyl ester is no longer acidic, cannot ionize, and therefore generates a more hydrophobic prodrug. Once it is orally absorbed, the ester is hydrolyzed to produce the active metabolite of oseltamivir. Returning to Question 3, the key reason that zanamivir is not orally active is that it does not possess adequate lipid solubility to traverse the GI mucosal membrane. By incorporating these three structural changes, oseltamivir has a better water/lipid solubility balance that allows it to be administered orally in the treatment of influenza infections.

6. To solve this problem, we need to use the Henderson-Hasselbalch equation. Because the functional group, a primary amine, is basic, the ionized form ($R\text{-}NH_3^+$) is the Acid Form and the unionized form ($R\text{-}NH_2$) is the Base Form.

$$pH = pK_a + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$6.5 = 7.7 + \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$-1.2 = \log \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

$$0.063 = \frac{[\text{Base Form}]}{[\text{Acid Form}]} \quad \text{or} \quad \frac{0.063}{1} = \frac{[\text{Base Form}]}{[\text{Acid Form}]}$$

This ratio indicates that for every one molecule that contains the functional group in the acid (or ionized) form, there are 0.063 molecules that contain the functional group in the base (or unionized) form. The following equations can then be used to correctly calculate the percentage of the molecules that are ionized and the percentage that are unionized.

$$0.063 \text{ Molecules in Base Form} + 1.0 \text{ Molecule in Acid Form} = 1.063 \text{ Total Molecules}$$

$$\text{Base Form} = \text{Unionized Form and Acid Form} = \text{Ionized Form}$$

$$\text{Percent in Ionized Form} = \frac{1 \text{ Molecule in Ionized Form}}{1.063 \text{ Total Molecules}} \times 100\% = 94.1\%$$

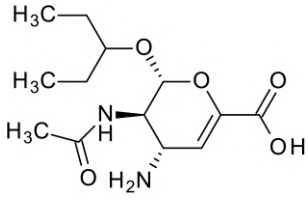
$$\text{Percent in Unionized Form} = \frac{0.063 \text{ Molecules in Unioinized Form}}{1.063 \text{ Total Molecules}} \times 100\% = 5.9\%$$

Because the question asks for the percent that are ionized, the correct answer is 94.1%.

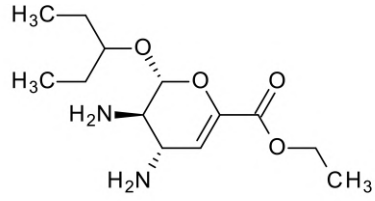
7. This stereoisomer has the opposite stereochemical configuration at all five chiral centers and has the exact same conformation as zanamivir; therefore, this is the ***enantiomer*** of zanamivir. None of the other stereochemical designations are correct.

   Given that zanamivir and oseltamivir exert their antiviral activity by mimicking *N*-acetylsialic acid, alteration of the stereochemistry decreases the resemblance to *N*-acetylsialic acid and would be predicted to cause a decrease in binding affinity to neuraminidase and a decreased antiviral effect.

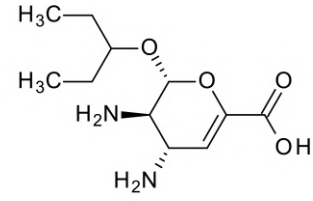
8. **Pathway A: Hydrolysis:** Phase I transformation. ***YES***, both the ester and amide functional groups can undergo hydrolysis. Ester hydrolysis produces the active metabolite of oseltamivir.



Ester hydrolysis

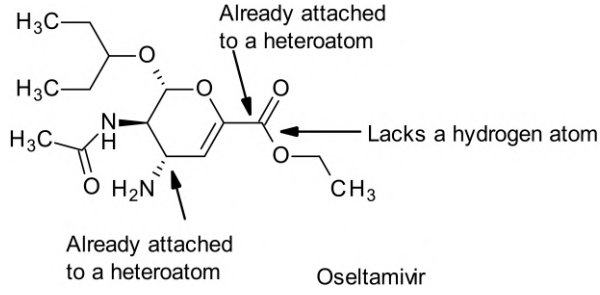


Amide hydrolysis



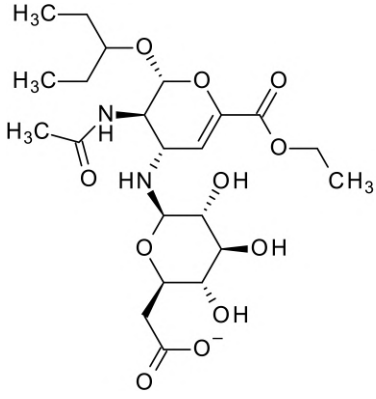
Ester and amide hydrolysis

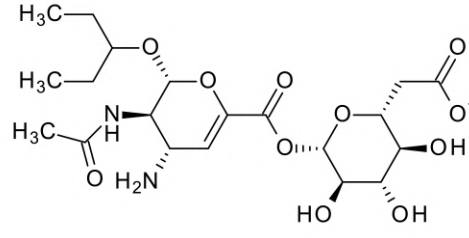
Pathway B: Allylic oxidation: Phase I transformation. ***NO***, it is not possible because both allylic carbon atoms are already attached to heteroatoms. Additionally, one allylic carbon atom lacks a hydrogen atom that is required for oxidation.



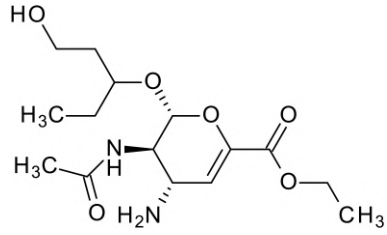
Oseltamivir

Pathway C: Glucuronide conjugation: Phase II transformation. ***YES***, it can occur directly with the secondary amine or with the carboxylic acid that results from Phase I ester hydrolysis.

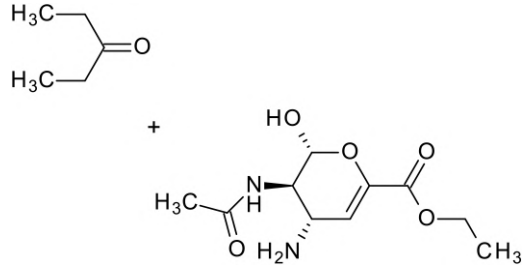




Pathway D: ω-Oxidation: Phase I, ***YES.***



Pathway E: Oxidative *O*-dealkylation: Phase I, ***YES.***



9. Zanamivir is very highly water soluble. Its chemical structure contains two ionizable functional groups as well as several others that can form hydrogen bonds with water. As such, zanamivir can be readily excreted without further metabolic transformation. Remember that the major purpose of drug metabolism is to enhance the removal of the drug from the body. If a drug molecule can be readily removed from the body, there is no need for metabolic transformations.

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