Chapter 3

Receptors

Chapter Outline

3.1. INTRODUCTION

Up to this point in our discussion it appears that a drug is taken, it travels through the body to a target site, and it elicits a pharmacological effect. The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a *receptor*, any biological molecule with which the drug interacts. Allusions were made in Chapter 2 to the binding of a drug to a receptor, which constitutes *pharmacodynamics*. In this chapter, the emphasis will be on pharmacodynamics of general receptors; in Chapter 4, a special class of proteins that have catalytic properties, called enzymes, will be discussed; and in Chapter 6, a nonprotein receptor, deoxyribonucleic acid (*DNA*), will be the topic of discussion. The drug–receptor properties discussed in this chapter will also apply to drug–enzyme and drug–DNA complexes. The receptors discussed in this chapter include some of the major drug targets, such as *guanine nucleotide-binding regulatory protein* (*G protein*)*-coupled receptors* (*GPCRs*), ion channels, nuclear receptors, and receptor tyrosine kinases (RTKs). GPCRs are the largest class of receptors known; about 800 different human genes (~4% of the human genome) are predicted to be members of the GPCR superfamily.[1] Over 80% of hormones use GPCRs for signaling. These seven-transmembrane proteins are activated by a variety of ligands such as peptides, hormones, neurotransmitters, chemokines, lipids, glycoproteins, divalent cations, and light.[2] Binding of these ligands causes a conformational change in the structure of these cell-surface receptors to facilitate interaction of the receptor with a member of the G protein family. G protein activation by the receptor results in the activation of intracellular signal transduction cascades, which leads to a change in the activity of ion channels and enzymes, thereby causing an alteration in the rate of production of intracellular second messengers.[3] Therefore, the GPCRs are involved in the control of every aspect of our behavior and physiology and are linked to numerous diseases, including cardiovascular problems, mental disorders, retinal degeneration, cancer, and acquired immunodeficiency syndrome. Almost half of all drugs target GPCRs by either activating or inactivating them. An *ion channel* is a transmembrane pore that is composed of

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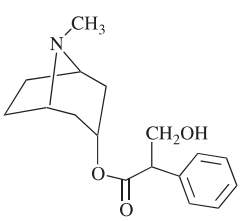
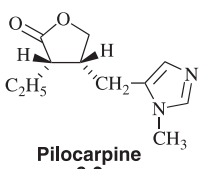
the following elements: a *pore*, which is responsible for the transit of the ion, and one or more *gates* that open and close in response to specific stimuli that are received by the *sensors*. Conformational mobility is an integral component of the function of ion channels; the three states of a channel, closed, open, and activated, are all believed to be regulated by conformational changes. Ligands can gain access to the channel either by membrane permeation or through an open channel state. *Nuclear receptors* are ligand-dependent transcription factors responsible for sensing steroid and thyroid hormones, bile acids, fatty acids, and certain vitamins and prostaglandins.[4] In response to ligand binding, these protein receptors work with other proteins to regulate gene expression, thereby controlling the development, differentiation, metabolism, and reproduction of the organism. Ligand binding to a nuclear receptor results in a conformational change in the receptor, which activates the receptor, resulting in up- or downregulation of gene expression. RTKs are a subclass of cell-surface growth factor receptors having a ligand-dependent enzymatic activity (kinase activity) of catalyzing the transfer of the γ-phosphate group from a nucleoside triphosphate donor, such as adenosine triphosphate, to hydroxyl groups of tyrosine residues of target proteins.[5] The ligand-binding domain, which is usually glycosylated, is connected to the cytoplasmic domain by a single transmembrane helix. RTKs play an important role in the control of most fundamental cellular processes, including the cell cycle, cell migration, cell metabolism and survival, as well as cell proliferation and differentiation, and, therefore, also play a crucial role in carcinogenesis. RTKs function in many signal transduction cascades by which extracellular signals are transmitted through the cell membrane (transmembrane) to the cytoplasm and often to the nucleus, where gene expression may be modified.

In 1878 John N. Langley, a physiology graduate student at Cambridge University, while studying the mutually antagonistic action of the alkaloids atropine (**3.1**; now used as a smooth muscle relaxant in a variety of drugs, such as Prosed) and pilocarpine (**3.2**; Salagen; causes sweating and salivation) on cat salivary flow, suggested that both of these chemicals interacted with some yet unknown substance (no mention of "receptors" was made) in the nerve endings of the gland cells.[6] Langley, however, did not follow-up this notion for over 25 years.



Atropine
3.1



Pilocarpine
3.2

Paul Ehrlich worked for a dye manufacturing company and was fascinated by the observation that dyes could attach so tightly to fabrics that they could not be removed by washing. He also was intrigued by why different bacteria caused different diseases and thought that the toxins generated by bacteria might produce their effects by attaching tightly to specific sites in the cells of the body, just as dyes attach to fabrics. In 1897, Ehrlich suggested his *side chain theory*.[7] According to this hypothesis, cells have side chains attached to them that contain specific groups capable of combining with a particular group of a toxin. Ehrlich termed these side chains *receptors*. Another ground-breaking facet of this hypothesis was that when toxins combined with the side chains, excess side chains are produced and released into the bloodstream. In today's biochemical vernacular, these excess side chains would be called *antibodies*, and they can combine with macromolecular toxins stoichiometrically.

In 1905, Langley[8] (at this time Chair of the Department of Physiology at Cambridge, where he did his graduate studies, and editor of the *Journal of Physiology*, where he published his work on cat salivary flow as a graduate student) studied the antagonistic effects of curare (see Figure 2.16) on nicotine stimulation of skeletal muscle. He concluded that there was a *receptive substance* that received the stimulus and, by transmitting it, caused muscle contraction. This was the first time that attention was drawn to the two fundamental characteristics of a receptor, namely, a *recognition capacity* for specific molecules and an *amplification component*, the ability of the complex between the molecule and the receptor to initiate a biological response.

Receptors are mostly membrane-bound proteins that selectively bind small molecules (*ligands*) and elicit a physiological response. Many receptors are integral proteins that are embedded in the phospholipid bilayer of cell membranes (see Figure 2.26). Since such receptors typically function in the membrane environment, their properties and mechanisms of action depend on the phospholipid milieu. Vigorous treatment of cells with detergents is required to dissociate these proteins from the membrane. Once they become dissociated, however, they generally lose their integrity. Because they usually exist in minute quantities and can be unstable, few membrane-bound receptors have been purified and little structural information is known about most of them. Advances in molecular biology have permitted the isolation, cloning, and sequencing of receptors,[9] and this is leading to further approaches to the molecular characterization of these proteins. However, these receptors, unlike many enzymes, are still typically characterized in terms of their function rather than by their structural properties. The two functional components of receptors, the recognition component and the amplification component, may represent the same or different sites on the same protein. Various hypotheses regarding the mechanisms by which drugs may initiate a biological response are discussed in Section 3.2.4.

3.2. DRUG–RECEPTOR INTERACTIONS

3.2.1. General Considerations

To appreciate the mechanisms of drug action, it is important to understand the forces of interaction that bind drugs to their receptors. Because of the low concentration of drugs and receptors in the bloodstream and other biological fluids, the law of mass action alone cannot account for the ability of small doses of structurally specific drugs to elicit a total response by combination with all, or practically all, of the appropriate receptors. The enlightening calculation shown below supports the notion that something more than mass action is required to get the desired drug–receptor interaction.[10] One mole of a drug contains 6.02×10^{23} molecules (Avogadro's number). If the molecular weight of an average drug is 300 g/mol, then 15 mg (an effective dose for many drugs) will contain $6.02\times10^{23}(15\times10^{-3})/300=3\times10^{19}$ molecules of drug. The human organism is composed of about 3×10^{13} cells. Therefore, each cell will be acted upon by $3\times10^{19}/3\times10^{13}=10^{6}$ drug molecules. One erythrocyte cell contains about 10^{10} molecules. On the assumption that the same number of molecules is found uniformly in all cells, then for each drug molecule, there are $10^{10}/10^{6}=10^{4}$ molecules of the human body! With this ratio of human molecules to drug molecules, Le Chatelier would have a difficult time explaining how the drug could interact and form a stable complex with the desired receptor.

The driving force for the drug–receptor interaction can be considered as a low energy state of the drug–receptor complex (Scheme 3.1), where k_{on} is the rate constant for formation of the drug–receptor complex, which depends on the concentrations of the drug and the receptor, and k_{off} is the rate constant for breakdown of the complex, which depends on the concentration of the drug–receptor complex as well as other forces. The biological activity of a drug is related to its affinity for the receptor, i.e., the stability of the drug–receptor complex. This stability is commonly measured by how difficult it is for the complex to dissociate, which is represented by its K_d, the dissociation constant for the drug–receptor complex at equilibrium (Eqn (3.1)). Note that

$$K_d = \frac{[\text{drug}]\,[\text{receptor}]}{[\text{drug - receptor complex}]} \tag{3.1}$$

because K_d is a *dissociation* constant, the smaller the K_d, the larger the concentration of the drug–receptor complex, the more stable is that complex, and the greater is the affinity of the drug for the receptor. K_d roughly represents the concentration of the drug required to reach an equilibrium of 50% in the drug–receptor complex. To give you an idea of the affinity of a typical drug for its target, it has been estimated that the median K_d for enzyme inhibitor drugs on the market is about 20 nM[11] (at 20 nM concentration of drug, the enzyme is 50% in the drug–enzyme complex). Formation of the drug–receptor complex involves an elaborate equilibrium. Solvated ligands (such as drugs) and solvated proteins (such as receptors) generally exist as an equilibrium mixture of several conformers each. To form a complex, solvent molecules that occupy the binding site of the receptor must be displaced by the drug to produce a solvated complex; interactions between the drug and the receptor are stronger than the interactions between the drug and receptor with the solvent molecules.[12] Drug–receptor complex formation is also entropically unfavorable; it causes a loss in conformational degrees of freedom for both the protein and the ligand, as well as the loss of three rotational and three translational degrees of freedom.[13] Therefore, highly favorable enthalpic contacts (interactions) between the receptor and the drug must compensate for the entropic loss.

3.2.2. Important Interactions (Forces) Involved in the Drug–Receptor Complex

Interactions involved in the drug–receptor complex[14] are the same forces experienced by all interacting organic molecules and include covalent bonding, ionic (electrostatic) interactions, ion–dipole and dipole–dipole interactions, hydrogen bonding, charge-transfer interactions, hydrophobic interactions, cation–π interactions, halogen bonding, and van der Waals interactions. Weak interactions usually are possible only when molecular surfaces are close and complementary, that is, bond strength is distance dependent. The spontaneous formation of a bond between atoms occurs with a decrease in free energy, that is, a noncovalent bond will occur only when there is a negative ΔG, which is the sum of an enthalpic term (ΔH) and an entropic term ($-T\Delta S$). The change in free energy (*binding energy*) is related to the binding equilibrium constant (K_{eq}) according to Eqn (3.2). Therefore, at physiological temperature (37 °C), changes in free energy of a

$$\Delta G^0 = -\,\mathrm{RT}\ \ln K_{eq} \tag{3.2}$$

few kilocalories per mole can have a major effect on the establishment of good secondary interactions. In fact, if the K_{eq} were only 0.01 (i.e., 1% of the equilibrium mixture in the form of the drug–receptor complex), then a ΔG^0 of interaction of −5.45 kcal/mol would shift the binding equilibrium

$$\text{drug} + \text{receptor} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{drug-receptor complex}$$

SCHEME 3.1 Equilibrium between a drug, a receptor, and a drug–receptor complex

constant to 100 (i.e., 99% in the form of the drug–receptor complex). It would be desirable for observed interactions to be additive; however, molecular interactions tend to behave in a highly nonadditive fashion.[15] A particular interaction may be worth different amounts of free energy depending on the specific molecular structure involved. The multiplicity of interactions in one protein–ligand complex is a compromise of attractive and repulsive forces; solvation processes, long-range interactions, and conformational changes are often neglected. Also, it is very easy to be misled by drug–receptor interactions in crystal structures, which present static views of interactions and do not take into account the energy cost of displacement of water molecules from the binding site.[16]

Generally, the bonds formed between a drug and a receptor are weak noncovalent interactions; consequently, the effects produced are reversible. Because of this, a drug becomes inactive as soon as its concentration in the extracellular fluids decreases, generally by metabolism (see Chapter 8). Often it is desirable for the drug effect to last only for a limited time so that the pharmacological action can be terminated. In the case of central nervous system (CNS) stimulants and depressants, for example, a prolonged action could be harmful. Sometimes, however, the effect produced by a drug should persist, and even be irreversible. For example, it is most desirable for a *chemotherapeutic agent*, a drug that acts selectively on a foreign organism or tumor cell, to form an irreversible complex with its receptor so that the drug can exert its toxic action for a prolonged period.[17] In this case, a covalent bond would be desirable.

In the following subsections, the various types of drug–receptor interactions are discussed briefly. These interactions are applicable to all types of receptors, including enzymes and DNA, that are described in this book.

3.2.2.1. Covalent Bonds

The *covalent bond* is the strongest bond, generally worth anywhere from −40 to −110 kcal/mol in stability. It is seldom formed by a drug–receptor interaction, except with enzymes and DNA. These bonds will be discussed further in Chapters 5 and 6.

3.2.2.2. Ionic (or Electrostatic) Interactions

For protein receptors at *physiological pH* (generally taken to mean pH 7.4, the pH of blood), basic groups such as the amino side chains of arginine, lysine, and, to a much lesser extent, histidine, are protonated and, therefore, provide a cationic environment. Acidic groups, such as the carboxylic acid side chains of aspartic acid and glutamic acid, are deprotonated to give anionic groups.

Drug and receptor groups will be mutually attracted provided they have opposite charges. This *ionic interaction* can be effective at distances farther than those required for other types of interactions, and they can persist longer. A simple ionic interaction can provide a $\Delta G^0 = -5$ kcal/mol, which declines by the square of the distance between the charges. If this interaction is reinforced by other simultaneous interactions, the ionic interaction becomes stronger ($\Delta G^0 = -10$ kcal/mol) and persists longer. The antidepressant drug pivagabine (Tonerg) is used as an example of a molecule that can hypothetically participate in an ionic interaction with an arginine residue (Figure 3.1).

3.2.2.3. Ion–Dipole and Dipole–Dipole Interactions

As a result of the greater electronegativity of atoms such as oxygen, nitrogen, sulfur, and halogens relative to that of carbon, C–X bonds in drugs and receptors, where X is an electronegative atom, will have an asymmetric distribution of electrons; this produces electronic dipoles. These dipoles in a drug molecule can be attracted by ions (*ion–dipole interaction*) or by other dipoles (*dipole–dipole interaction*) in the receptor, provided charges of opposite sign are properly aligned. Because the charge of a dipole is less than that of an ion, a dipole–dipole interaction is weaker than an ion–dipole interaction. In Figure 3.2, the insomnia drug zaleplon (Sonata) is used to demonstrate these interactions, which can provide a $\Delta G^0 = -1$ to -7 kcal/mol.

3.2.2.4. Hydrogen Bonds

Hydrogen bonds are a type of dipole–dipole interaction formed between the proton of a group X–H, where X is an electronegative atom, and one or more other electronegative atoms (Y) containing a pair of nonbonded electrons. The most significant hydrogen bonds occur in molecules where

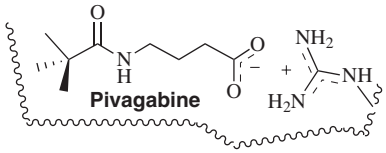


FIGURE 3.1 Example of an electrostatic (ionic) interaction. Wavy line represents the receptor cavity.

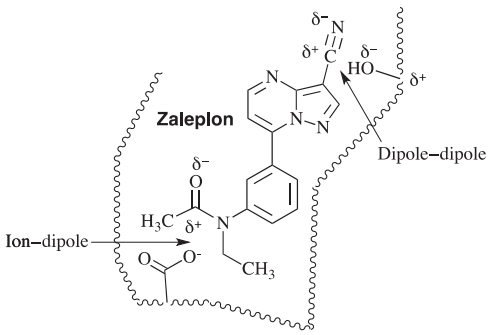
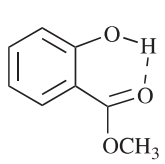


FIGURE 3.2 Examples of ion–dipole and dipole–dipole interactions. Wavy line represents the receptor cavity.

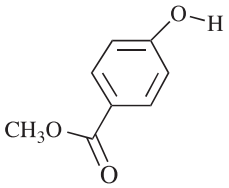
X and Y are N and O and, to a lesser extent, F[18]; interesting special cases of weak hydrogen bonding for X=C also have been described.[19] X removes electron density from the hydrogen so it has a partial positive charge, which is strongly attracted to the nonbonded electrons of Y. The interaction is denoted as a dotted line, –X–H···Y–, to indicate that a covalent bond between X and H still exists, but that an interaction between H and Y also occurs. In this depiction, X is referred to as the *hydrogen bond donor* and Y is the *hydrogen bond acceptor*. When X and Y are equivalent in electronegativity and degree of ionization, the proton can be shared equally between the two groups, i.e. –X···H···Y–, referred to as a *low-barrier hydrogen bond*.[20] On average, the hydrogen bond between a carbonyl oxygen and an alcohol proton is 2.75 Å long and that between a carbonyl and an NH proton is 2.90 Å.[21]

The hydrogen bond is unique to hydrogen because it is the only atom that can carry a positive charge at physiological pH while remaining covalently bonded in a molecule, and which also is small enough to allow close approach of a second electronegative atom. The strength of the hydrogen bond is related to the Hammett σ constants.[22]

There are *intramolecular* and *intermolecular* hydrogen bonds; the former are stronger (see salicylic acid used in wart removal remedies, in Figure 3.3). Intramolecular hydrogen bonding is an important property of molecules that may have a significant effect on lead modification approaches.[23] As discussed in Chapter 2 (Sections 2.1.2.3.4 and 2.2.4.4), the bioactive conformation of a molecule is the optimal conformation of the molecule when bound to its receptor. When there are hydrogen bond donor and acceptor groups in a compound that have the possibility of interacting to form a five- to seven-membered intramolecular ring, those interactions will produce a stable conformation that may or may not approximate the bioactive conformation. The order of stability for intramolecular hydrogen bond rings is six-membered ring >> five-membered ring > seven-membered ring with acceptor strength (carbonyl > heterocyclic N acceptor > sulfoxide > alkoxyl[24]) enhancing the probability of the intramolecular hydrogen bond. Intramolecular hydrogen bonding becomes increasingly important if a bioisosteric replacement of an oxygen atom in an ether (capable of forming strong hydrogen bonds) is replaced by a sulfur atom in a thioether (which forms very weak or no hydrogen bonds); this could have a major impact on the potency and even activity of the compound if an intramolecular hydrogen bond changes the conformation of the molecule. This same difference between oxygen or nitrogen and sulfur also becomes important in intermolecular bonding between the drug molecule and the receptor. Intramolecular hydrogen bonding also may mask the binding of a pharmacophoric group. For example, methyl salicylate (**3.3**, wintergreen oil), an active ingredient in many muscle pain remedies and antiseptics, is a weak antibacterial agent. The corresponding para-isomer, methyl *p*-hydroxybenzoate (**3.4**), however, is considerably more potent as an antibacterial agent and is used as a food preservative. It is believed that the antibacterial activity of **3.4** is derived from the phenolic hydroxyl group. In **3.3,** this group is masked by intramolecular hydrogen bonding.[25]



Methyl salicylate
3.3



3.4

Intramolecular hydrogen bonding produces structures that can be thought of as bioisosteres of bicyclic compounds, a type of scaffold hopping (see Chapter 2, Section 2.2.6.3) (Figure 3.4).[26] Some intramolecular hydrogen bonds are strong enough to persist in water.[27]

Because intramolecular hydrogen bonding removes one donor and one acceptor moiety from the molecule, it increases its lipophilicity and membrane permeability and decreases its aqueous solubility. This can have a significant impact on pharmacokinetics. For example, the increased brain penetration and pharmacological activity of neurokinin 1 receptor antagonists were attributed to increased

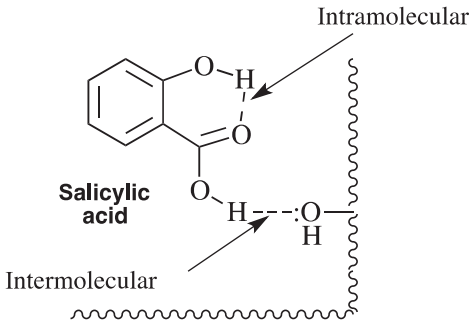


FIGURE 3.3 **Examples of hydrogen bonds.** Wavy line represents the receptor cavity.

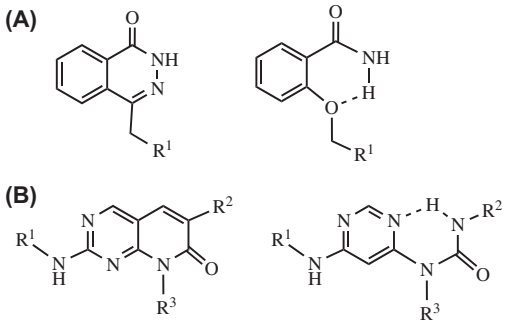


FIGURE 3.4 Two examples (A and B) of how intramolecular hydrogen bonding can mimic a bioisosteric heterocycle.

lipophilicity resulting from intramolecular hydrogen bonding.[28] Also, CLog *P* calculations (see Chapter 2, Sections 2.2.5.2.2 and 2.2.5.2.3) typically underestimate the lipophilicity of molecules that can undergo intramolecular hydrogen bonding; on average, the CLog *P* values should be increased by 0.4 for each intramolecular hydrogen bond in the molecule.[29]

Hydrogen bonds are essential to maintain the structural integrity of α-helix (**3.5**) and β-sheet (**3.6**) conformations of peptides and proteins and the double helix of DNA (**3.7**) (Figure 3.5). As is discussed in Chapter 6, many antitumor agents act by alkylation of the DNA bases, thereby preventing hydrogen bonding. This disrupts the double helix and destroys the DNA.

The ΔG^0 for hydrogen bonding can be between −1 and −7 kcal/mol, but usually is in the range −3 to −5 kcal/mol. Binding affinities increase by about one order of magnitude per hydrogen bond.

3.2.2.5. Charge–Transfer Complexes

When a molecule (or group) that is a good electron donor comes into contact with a molecule (or group) that is a good electron acceptor, the donor may transfer some of its charge

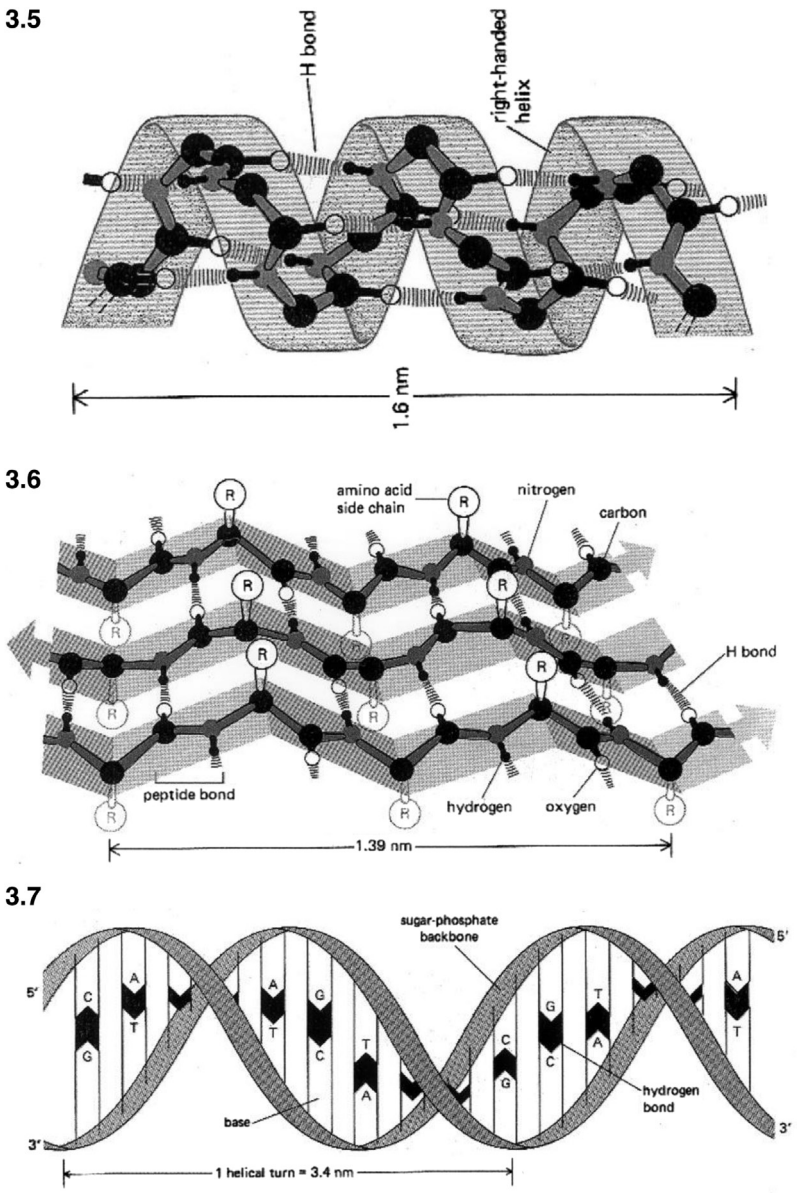
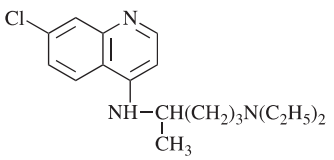


FIGURE 3.5 **3.5** is an example of an α-helix in a protein—Copyright 2007 from Molecular Biology of the Cell, Fifth Edition by Alberts, et al. Reproduced by permission of Garland Science/Taylor & Francis LLC. **3.6** is an example of a β-sheet in a protein—Copyright 2007 from Molecular Biology of the Cell, Fifth Edition by Alberts, et al. Reproduced by permission of Garland Science/Taylor & Francis LLC. **3.7** is an example of a double helix in DNA—Copyright 2007 from Molecular Biology of the Cell, Fifth Edition by Alberts, et al. *Reproduced by permission of Garland Science/Taylor & Francis LLC.*

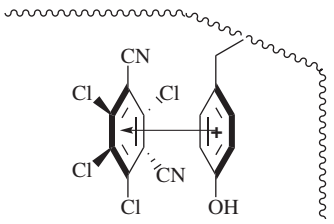
to the acceptor. This forms a *charge-transfer complex*, which, in effect, is a molecular dipole–dipole interaction. The potential energy of this interaction is proportional to the difference between the ionization potential of the donor and the electron affinity of the acceptor.

Donor groups contain π-electrons, such as alkenes, alkynes, and aromatic moieties with electron-donating substituents, or groups that contain a pair of nonbonded electrons, such as oxygen, nitrogen, and sulfur moieties. Acceptor groups contain electron-deficient π-orbitals, such as alkenes, alkynes, and aromatic moieties having electron-withdrawing substituents, and weakly acidic protons. There are groups on receptors that can act as electron donors, such as the aromatic ring of tyrosine or the carboxylate group of aspartate.

Charge-transfer interactions are believed to provide the energy for intercalation of certain planar aromatic antimalarial drugs, such as chloroquine (**3.8,** Aralen), into parasitic DNA (see Chapter 6). The fungicide, chlorothalonil (Bravo), is used in Figure 3.6 as a hypothetical example for a charge-transfer interaction with a tyrosine. The ΔG^o for charge-transfer interactions also can range from −1 to −7 kcal/mol.



Chloroquine
3.8



Chlorothalonil

FIGURE 3.6 **Example of a charge-transfer interaction.** Wavy line represents the receptor cavity.

3.2.2.6. Hydrophobic Interactions

In the presence of a nonpolar molecule or region of a molecule, the surrounding water molecules orient themselves and, therefore, are in a higher energy state than when only other water molecules are around. When two nonpolar groups, such as a lipophilic group on a drug and a nonpolar receptor group, each surrounded by ordered water molecules, approach each other, these water molecules become disordered in an attempt to associate with each other. This increase in entropy, therefore, results in a decrease in the free energy ($\Delta G = \Delta H - T\Delta S$), which stabilizes the drug–receptor complex. This stabilization is known as a *hydrophobic interaction* (see Figure 3.7). Consequently, this is not an attractive force of two nonpolar groups "dissolving" in one another, but, rather, is the decreased free energy of the nonpolar group because of the increased entropy of the surrounding water molecules. Jencks[30] has suggested that hydrophobic forces may be the most important single factor responsible for noncovalent intermolecular interactions in aqueous solution. Hildebrand,[31] on the other hand, is convinced that there is no hydrophobia between water and alkanes; instead, he believes that there just is not enough hydrophilicity to break the hydrogen bonds of water and allow alkanes to go into solution without the assistance of other polar groups. Addition of a single methyl group that can occupy a receptor-binding pocket improves binding by −1.5 kcal/mol or by about a factor of 12.[32] This hydrophobic interaction has been referred to as a "magic methyl" interaction.[33] In Figure 3.8 the topical anesthetic butamben is depicted in a hypothetical hydrophobic interaction with an isoleucine group.

Another type of hydrophobic interaction, called a π–π interaction, involves two aryl groups.[34] The more common π–π interactions involve a parallel arrangement of aromatic rings,[35] in which the π-electrons interact in a face-to-face arrangement, known as π-stacking.[36] In Figure 3.9, the phenyl ring of the anticonvulsant drug lacosamide (Vimpat) is shown in a hypothetical π-stacking interaction with a receptor phenylalanine. Alternatively, a T-shaped arrangement (edge-to-face interaction) is possible, in which the edge of one aromatic ring forms a T-shape with the face

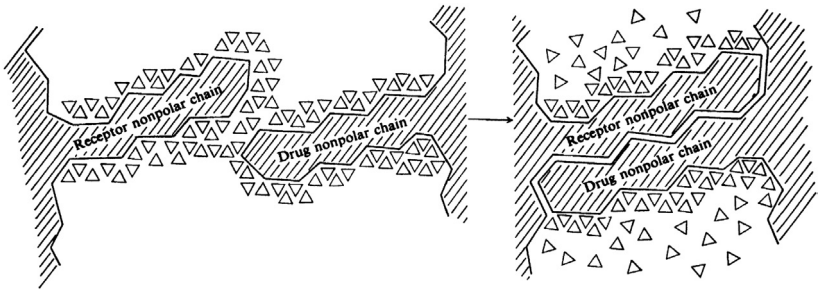


FIGURE 3.7 Formation of hydrophobic interactions. *From Korolkovas, A. (1970).* Essentials of Molecular Pharmacology, *p. 172. Wiley, New York. This material is reproduced with permission of John Wiley & Sons, Inc. and by permission of Kopple, K. D. 1966.* Peptides and Amino Acids. *Addison-Wesley, Reading, MA.*

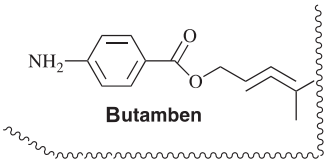


FIGURE 3.8 Example of hydrophobic interactions. The wavy line represents the receptor cavity.

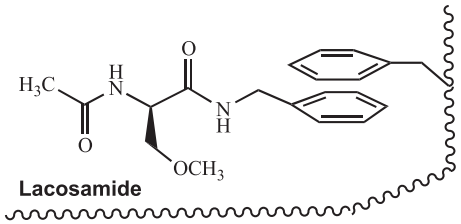


FIGURE 3.9 Example of π–π stacking. The wavy line represents the receptor cavity.

of the other aromatic ring. When one ring is electron deficient and the other is electron rich, then charge-transfer processes become important as well.

3.2.2.7. Cation–π Interaction

These interactions are very common in protein structure and also can be exploited for drug–receptor interactions.[37] In proteins, the most common aromatic group involved in a cation–π interaction is tryptophan (although phenylalanine, tyrosine, and histidine also participate), and the most common cation is arginine (although lysine is also important). A cationic group on a drug can undergo a cation–π interaction with an aromatic group on the receptor, or vice versa. Figure 3.10 is an example of a hypothetical cation–π interaction between the ammonium ion of lisdexamfetamine (Vyvanse), a drug for attention-deficit hyperactivity disorder, and a tryptophan residue. The ΔG^0 for cation–π interactions can be between −0.5 and −7 kcal/mol, but usually is in the range −1 to −5 kcal/mol.

3.2.2.8. Halogen Bonding

It has now been well established that a covalently bonded halogen atom can act as an electron acceptor (Lewis acid) to undergo *halogen bonding* with an electron-rich donor atom, such as O, N, or S.[38] On the basis of crystal structure and quantum mechanics/molecular mechanics data, it was found that many of the halogen to oxygen (or nitrogen) bond distances were equal to or less than the sum of the respective van der Waals radii, indicating the formation of a halogen bond (similar to a hydrogen bond).[39] The strength of these interactions is in the order H≈I>Br>Cl>>F. The interaction is caused by anisotropy of electron density on the halogen, resulting from the σ-hole, a positively charged region on the back side of the halogen atom along the R–X bond axis. These interactions can govern the conformation

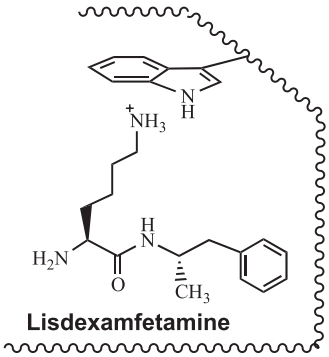


FIGURE 3.10 Example of a cation–π interaction. The wavy line represents the receptor cavity.

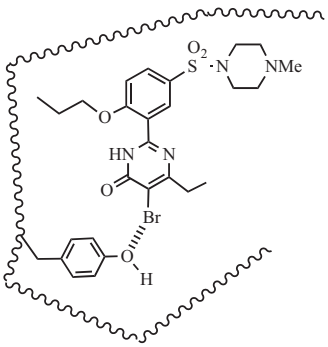


FIGURE 3.11 Example of halogen bonding. A compound bound into phosphodiesterase 5. The wavy line represents the enzyme cavity.

of molecules in the binding site of proteins. A series of phosphodiesterase type 5 inhibitors was designed with F, Cl, Br, and I atoms incorporated.[40] The potential halogen bond strengths were calculated, the molecules were synthesized, and then they were assayed; a good correlation was observed between the calculated binding energies and the activity of the molecules. The predicted interactions between the halogen atom and the phenolic oxygen atom of Tyr-612 were validated by X-ray crystallography, as shown in Figure 3.11. The ΔG^0 for halogen bonding can be between −1 and −15 kcal/mol,[41] but usually is in the range −1 to −5 kcal/mol.

3.2.2.9. van der Waals or London Dispersion Forces

Atoms in nonpolar molecules may have a temporary nonsymmetrical distribution of electron density, which results in the generation of a temporary dipole. As atoms from different molecules (such as a drug and a receptor) approach each other, the temporary dipoles of one molecule induce opposite dipoles in the approaching molecule. Consequently, intermolecular attractions, known as *van der Waals forces*, result. These weak universal forces only become significant when there is a close surface contact of the atoms; however, when there is molecular complementarity,

numerous atomic interactions result (each interaction contributing about –0.5 kcal/mol to the ΔG^0), which can add up to a significant overall drug–receptor binding component. Other weak interactions may contribute to receptor–ligand binding as well.[42]

3.2.2.10. Conclusion

Because noncovalent interactions are generally weak, cooperativity by several types of interactions is critical. Once the first interaction has taken place, translational entropy is lost. This results in a much lower entropy loss in the formation of the second interaction. The effect of this cooperativity is that several rather weak interactions may combine to produce a strong interaction. This phenomenon is the basis for why the SAR by NMR approach to lead modification (see Chapter 2, Section 2.1.2.3.6) can produce such high-affinity ligands from two moderate- or poor-affinity ligands. Because several different types of interactions are involved, selectivity in drug–receptor interactions can result. In Figure 3.12, the local anesthetic dibucaine is used as an example to show the variety of interactions that are possible.

The binding constants for 200 drugs and potent enzyme inhibitors were used to calculate the average strength of noncovalent bonds (i.e., the binding energy) associated with 10 common functional groups in an average drug–receptor environment.[43] As suggested above, charged groups bind more strongly than polar groups, which bind more tightly than nonpolar groups; ammonium ions form the best electrostatic interactions (11.5 kcal/mol), then phosphate (10.0 kcal/mol), and then carboxylate (8.2 kcal/mol). For loss of rotational and translational entropy, 14 kcal/mol of binding energy has to be subtracted and 0.7 kcal/mol of energy is subtracted for each degree of conformational freedom restricted.[44] Compounds that bind to a receptor exceptionally well have measured binding energies that exceed the calculated average binding energy, and those whose binding energy is less than the average calculated value fit poorly into the receptor.

3.2.3. Determination of Drug–Receptor Interactions

Hormones and neurotransmitters are important endogenous molecules that are responsible for the regulation of a myriad of physiological functions. These molecules interact with a specific receptor in a tissue and elicit a specific characteristic response. For example, the activation of a muscle by the CNS is mediated by release of the excitatory neurotransmitter acetylcholine (ACh; **3.9**). If the logarithm of the concentration of ACh added to a muscle tissue preparation is plotted against the percentage of total muscle contraction, the graph shown in Figure 3.13 may result. This is known as a *dose–response* or *concentration–response curve*. The low concentration part of the curve results from too few neurotransmitter molecules available for collision with the receptor. As the concentration increases, it reaches a point where a linear relationship is observed between the logarithm of the neurotransmitter concentration and the biological response. As most of the receptors become occupied, the probability of additional drug-receptor interactions diminishes, and the curve deviates from linearity (the high concentration end). Concentration–response curves are a means of measuring drug–receptor interactions by showing the relationship between drug concentration, usually plotted on the *X*-axis, and a biological response, usually plotted on the *Y*-axis. As shown in Figure 3.13, key parameters, such as K_d (the concentration of test compound that gives half-maximal binding) or EC_{50}, (the [effective] concentration of drug that elicits 50% of the total biological response) can be determined from the concentration–response data. EC_{50} is a common standard measure for comparing potencies of compounds that interact with a receptor and elicit a particular biological response. When the experiment is conducted in a whole animal, then *dose* (rather than concentration) is the variable normally plotted on the *X*-axis; in analogy to the terminology discussed above, the plot is called a *dose–*response curve, and ED_{50} (the [effective] *dose* that elicits 50% of the total response) is the parameter that is typically used to compare potencies across compounds.

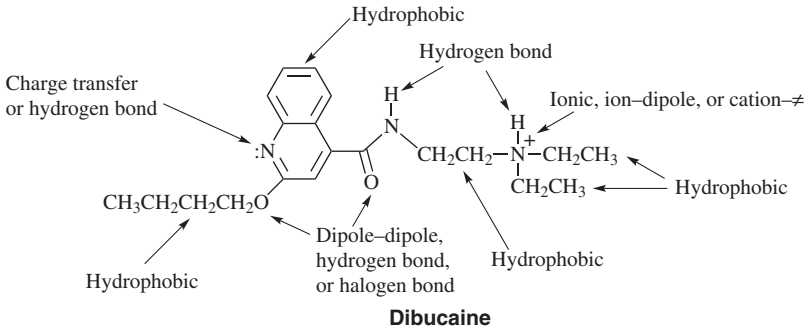


FIGURE 3.12 **Example of potential multiple drug–receptor interactions. The van der Waals interactions are excluded.**

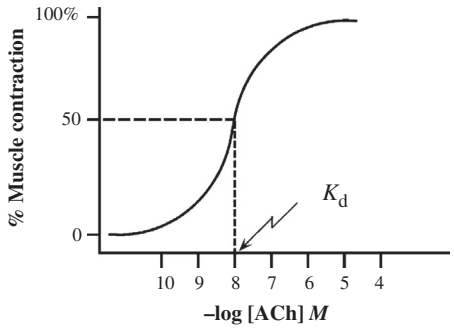
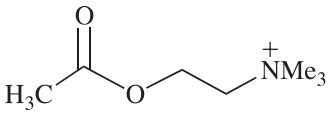


FIGURE 3.13 Effect of increasing the concentration of a neurotransmitter (ACh) on muscle contraction. The K_d is measured as the concentration of neurotransmitter that gives 50% of the maximal activity.



3.9

If another compound (W) is added in increasing amounts to the same tissue preparation, and the curve shown in Figure 3.14 results, the compound, which produces the same maximal response as the neurotransmitter, is called a *full agonist*.

A second compound (X) added to the tissue preparation shows no response at all (Figure 3.15A); however, if it is added to the neurotransmitter, and the effect of the neurotransmitter is blocked until a higher concentration of the neurotransmitter is added (Figure 3.15B), compound X is called a *competitive antagonist*. There are two general types of antagonists, competitive antagonists and noncompetitive antagonists. The former, which is the larger category, is one in which the degree of antagonism depends on the relative concentrations of the agonist and the antagonist; both bind to the same site on the receptor, or, at least, the antagonist directly interferes with the binding of the agonist. The most common assessment of the potency of competitive antagonists to establish a SAR is by determination of their IC_{50} values (the concentration of compound that inhibits the response of a given agonist by 50%) (Figure 3.15C). This allows for a direct comparison of different antagonists. The degree of blocking of a *noncompetitive antagonist* (X′) is independent of the amount of agonist present, so the EC_{50} does not change with increasing neurotransmitter (Figure 3.15D). Two different binding sites may be involved; when the noncompetitive antagonist binds to its *allosteric* binding site, a site to which the endogenous ligand normally does not bind, it may cause a conformational change in the protein, which affects binding of the endogenous molecule. Only competitive antagonists will be discussed further in this text.

If a compound Y is added to the tissue preparation and some response is elicited, but not a full response, regardless of how high a concentration of Y is used, then Y is called

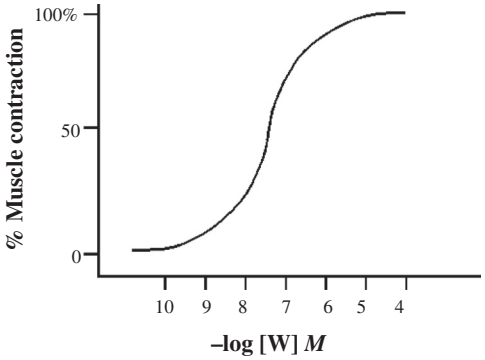
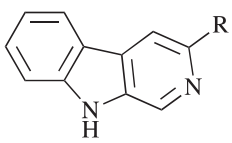


FIGURE 3.14 Dose–response curve for a full agonist (W).

a *partial agonist* (see Figure 3.16A). A partial agonist has properties of both an agonist and an antagonist.

When Y is added to low concentrations of a neurotransmitter sufficient to give a response less than the maximal response of the partial agonist (for example, 15%, as shown in Figure 3.16B), additive effects are observed as Y is increased, but the maximum response does not exceed that produced by Y alone. Under these conditions, the partial agonist has an agonistic effect. However, if Y is added to high concentrations of a neurotransmitter sufficient to give full response of the neurotransmitter, then antagonistic effects are observed; as Y increases, the response decreases to the point of maximum response of the partial agonist (Figure 3.16C). If this same experiment is done starting with higher concentrations of the neurotransmitter, the same results are obtained except that the dose–response curves shift to the right resembling the situation of adding an antagonist to the neurotransmitter (Figure 3.16C).

In a hypothetical situation, compound Z is added to the tissue preparation and muscle relaxation occurs (the opposite effect of the agonist). This would be a *full inverse agonist*, a compound that binds to the receptor, but displays an effect opposite to that of the natural ligand (Figure 3.17A). Valium (see **1.17**), for example, binds to a γ-aminobutyric acid (GABA) receptor and has an anticonvulsant effect, similar to that of the natural ligand GABA, and is thus an agonist; β-carbolines (**3.10**) bind to the same receptor, but act as convulsants, and are inverse agonists.[45] Just as an antagonist can displace an agonist or natural ligand (Figure 3.15B), it also can displace an inverse agonist (Figure 3.17B). A *partial inverse agonist* (Z′) is one that, at any concentration, does not give 100% of the effect of a full inverse agonist (Figure 3.17C).



3.10

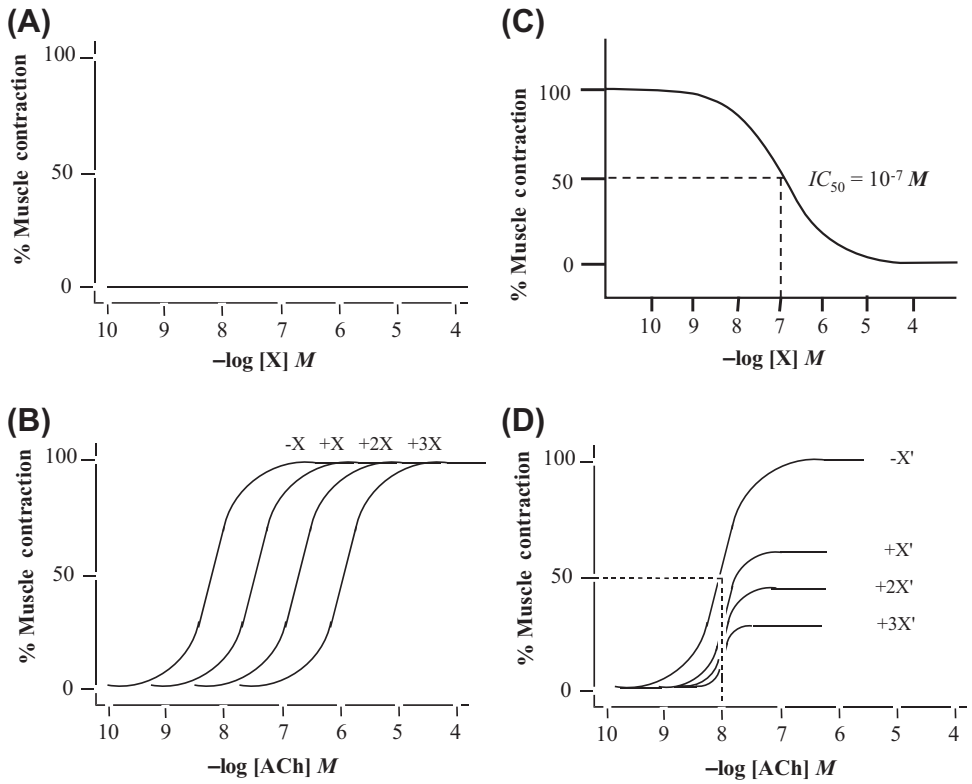


FIGURE 3.15 (A) Dose-response curve for an antagonist (X); (B) effect of a competitive antagonist (X) on the response of a neurotransmitter (acetylcholine; ACh); (C) effect of varying concentration of a competitive antagonist X in the presence of a fixed, maximally effective concentration of agonist (ACh); and (D) effect of various concentrations of a noncompetitive antagonist (X') on the response of the neurotransmitter (ACh).

On the basis of the above discussion, if you need a drug to effect a certain response of a receptor, an agonist would be desired; if you need a drug to prevent a particular response of a natural ligand, an antagonist would be required; if you need a drug that causes the opposite effect of the natural ligand, then an inverse agonist is what you want.

Sometimes, there are great structural similarities among a series of agonists, but little structural similarity in a series of competitive antagonists. For example, Table 3.1 shows some agonists and antagonists for histamine and epinephrine; a more detailed list of agonists and antagonists for specific receptors has been reported.[46] The differences in the structures of the antagonists is not surprising because a receptor can be blocked by an antagonist simply by its binding to a site near enough to the binding site for the agonist that it physically blocks the agonist from reaching its binding site. This may explain why antagonists are frequently much more bulky than the corresponding agonists. It is easier to design a molecule that blocks a receptor site than one that interacts with it in the specific way required to elicit a response. An agonist can be transformed into an antagonist by appropriate structural modifications, sometimes by relatively minor modifications. For example, both **3.11a** and **3.11b** (Table 3.1) bind to the progesterone receptor; however, **3.11a** is an antagonist (IC_{50} 5.0 nM) and **3.11b** is an agonist (EC_{50} 1.3 nM). Compound **3.11a** exhibited contraceptive activity in rats and monkeys.[47]

How is it possible for an antagonist to bind to the same site as an agonist and not elicit a biological response? There are several ways that this may occur. In Figure 3.18, panel A shows an agonist with appropriate groups interacting with three receptor-binding sites and eliciting a response. In panel B of Figure 3.18, the compound has two groups that can interact with the receptor, but one essential group is missing. In the case of enantiomers (panel C shows the enantiomer of the compound in panel A), only two groups are able to interact with the proper receptor sites. If appropriate groups must interact with all three binding sites in order for a response to be elicited, then the compounds depicted by panels B and C would be antagonists.

There are two general categories of compounds that interact with receptors: (1) compounds that occur naturally within the body, such as hormones, neurotransmitters, and other agents that modify cellular activity (*autocoids*) and (2) *xenobiotics*, compounds that are foreign to the body. Receptor selectivity is very important, but often difficult to attain because receptor structures are often unknown. Many current drugs are pharmacologically active at multiple receptors, some of which are not associated with the illness that is being treated. This can lead to side effects. For example, the clinical effect of neuroleptics (an early class of antipsychotic drugs with tranquilizing properties) is believed to result from their antagonism of dopamine receptors.[48] In general, this class of drugs also blocks cholinergic and

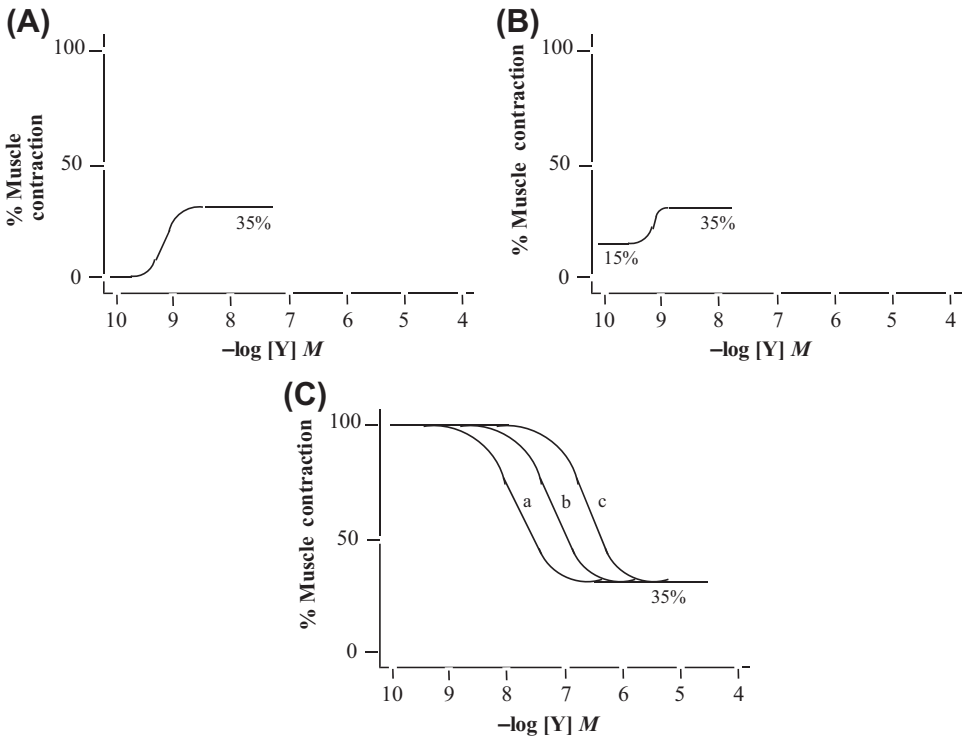


FIGURE 3.16 (A) Dose–response curve for a partial agonist (Y); (B) effect of a low concentration of neurotransmitter on the response of a partial agonist (Y); and (C) effect of a high concentration of neurotransmitter on the response of a partial agonist (Y). In (C), the concentration of the neurotransmitter (*a*,*b*,*c*) is $c > b > a$.

α-adrenergic receptors, and this results in side effects such as sedation and hypotension.

3.2.4. Theories for Drug–Receptor Interactions

Over the years a number of hypotheses have been proposed to account for the ability of a drug to interact with a receptor and elicit a biological response. Several of the more important proposals are discussed here, starting from the earliest hypothesis (the occupancy theory) to the current one (the multistate model).

3.2.4.1. Occupancy Theory

The *occupancy theory* of Gaddum[49] and Clark[50] states that the intensity of the pharmacological effect is directly proportional to the number of receptors occupied by the drug. The response ceases when the drug–receptor complex dissociates. However, as discussed in Section 3.2.3, not all agonists produce a maximal response. Therefore, this theory does not rationalize partial agonists, and it does not explain inverse agonists.

Ariëns[51] and Stephenson[52] modified the occupancy theory to account for partial agonists, a term coined by Stephenson. These authors utilized the original Langley concept of a receptor, namely, that drug–receptor interactions involve two stages: first, there is a complexation of the drug with the receptor, which they both termed the *affinity*; second, there is an initiation of a biological effect, which Ariëns termed the *intrinsic activity* and Stephenson called the *efficacy*. Affinity, then, is a measure of the capacity of a drug to bind to the receptor, and depends on the molecular complementarity of the drug and the receptor. Intrinsic activity (α) now refers to the maximum response induced by a compound relative to that of a given reference compound, and efficacy is the property of a compound that produces the maximum response or the ability of the drug–receptor complex to initiate a response.[53] Because of the slight change in definitions, we will use the term efficacy to refer to the ability of a compound to initiate a biological response. In the original theory, this latter property was considered to be constant. Examples of affinity and efficacy are given in Figure 3.19. Figure 3.19A shows the theoretical dose–response curves for five drugs with the same affinity for the receptor ($pK_d = 8$), but having efficacies varying from 100% of the maximum to 20% of the maximum. The drug with 100% efficacy is a full agonist; the others are partial agonists. Figure 3.19B shows dose–response curves for four drugs with the same efficacy (all full agonists), but having different affinities varying from a pK_d of 9 to 6.

Antagonists can bind tightly to a receptor (great affinity), but be devoid of activity (no efficacy). Potent agonists may have less affinity for their receptors than partial

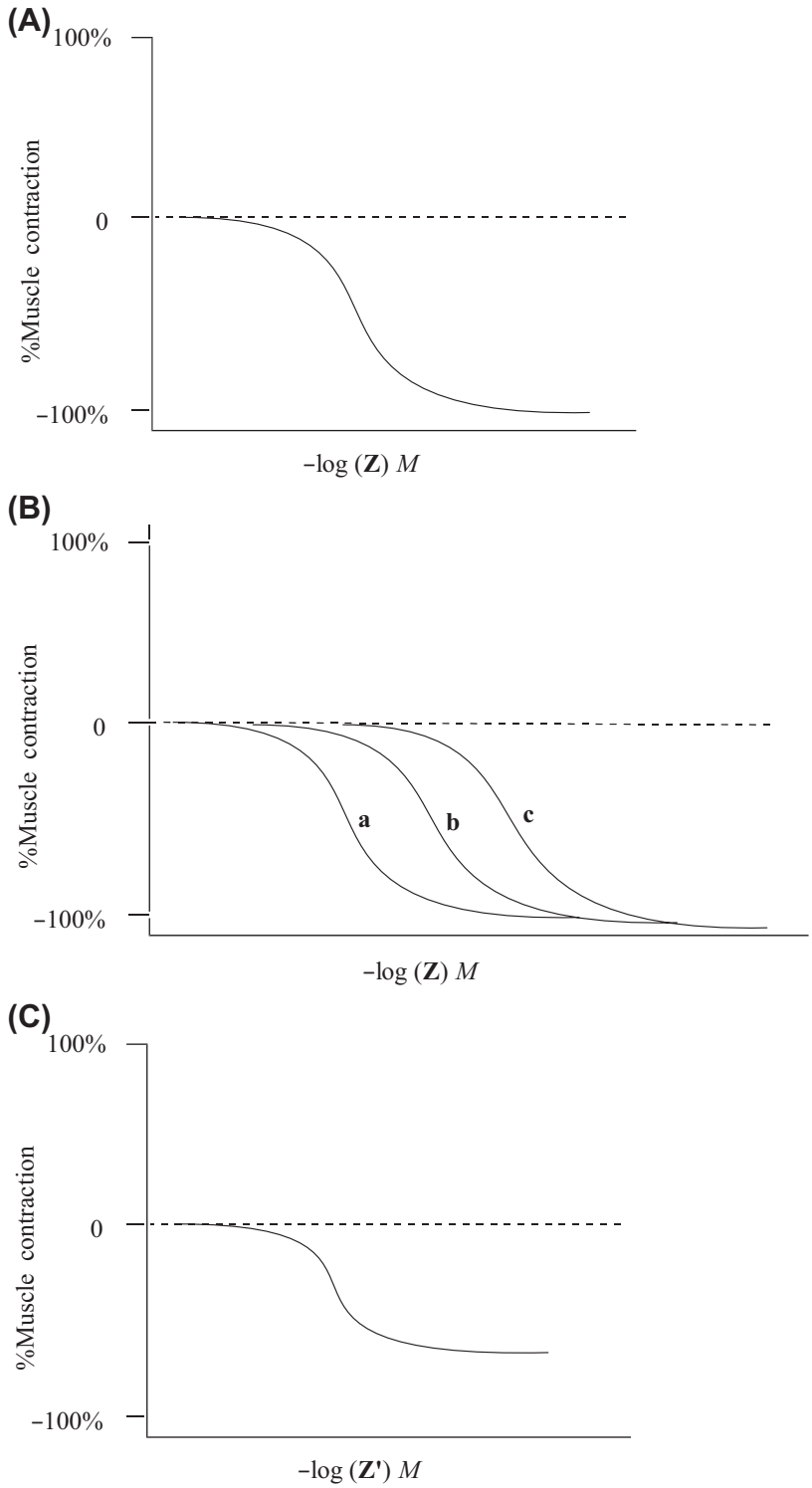
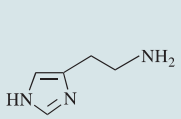
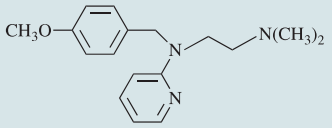
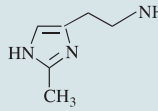
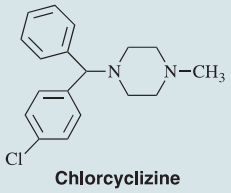
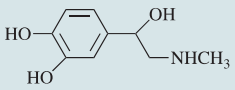
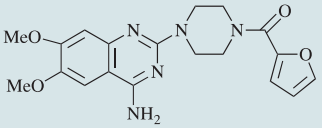
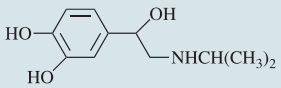
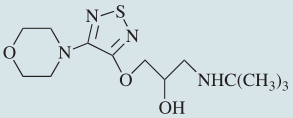
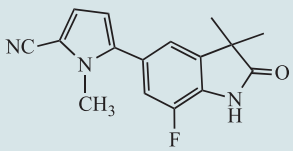
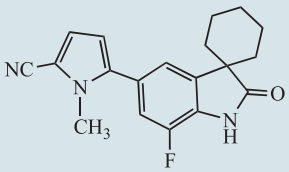


FIGURE 3.17 (A) Dose–response curve for a full inverse agonist (Z); (B) effect of a competitive antagonist on the response of a full inverse agonist (a, b, and c represent increasing concentrations of the added antagonist or natural ligand to Z); and (C) dose–response curve for a partial inverse agonist (Z′).

TABLE 3.1 Agonists and Antagonists

Neurotransmitter	Agonists	Antagonists
	 Histamine	 Pyrilamine
		 Chlorcyclizine
	 Epinephrine	 Prazosin
		 Timolol
	 3.11a Antagonist IC_{50} 5.0 nM	 3.11b Agonist EC_{50} 1.3 nM

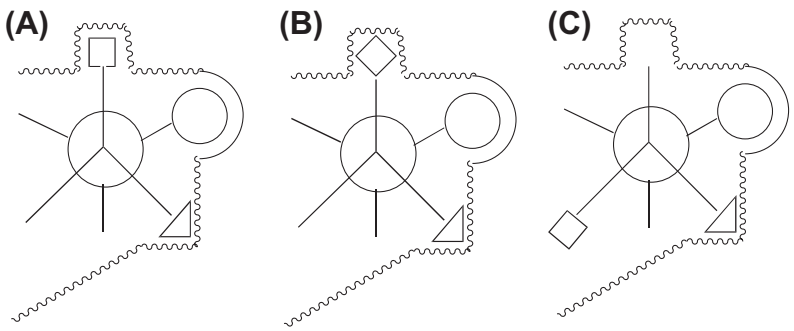


FIGURE 3.18 Inability of an antagonist to elicit a biological response. The wavy line is the receptor cavity. *Adapted with permission from W. O. Foye (Ed.), 1989 "Principles of Medicinal Chemistry," 3rd ed., p. 63. Copyright © 1989 Lea & Febiger, Philadelphia, Pennsylvania (Lippincott Williams & Wilkins/Wolters Kluwer).*

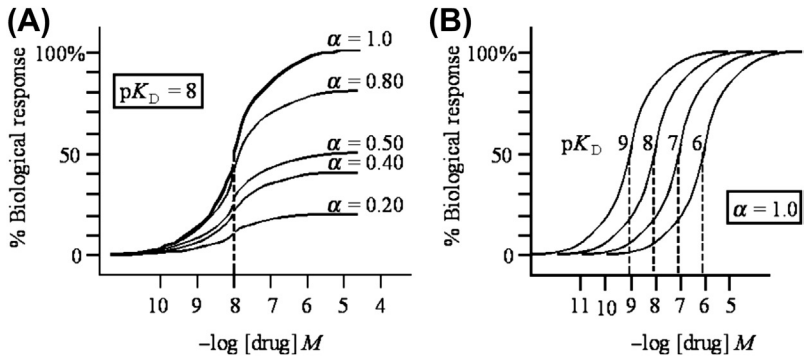


FIGURE 3.19 Theoretical dose–response curves illustrate (A) drugs with equal affinities and different efficacies (the top compound is a full agonist, and the others are partial agonists) and (B) drugs with equal efficacies (all full agonists) but different affinities.

agonists or antagonists. Therefore, these two properties, affinity and efficacy, are uncoupled. Also, the terms agonist, partial agonist, antagonist, and inverse agonist are biological system dependent and not necessarily properties of drugs. A compound that is an agonist for one receptor may be an antagonist or inverse agonist for another receptor. A particular receptor is considered to have an intrinsic *maximum response*; this is the largest magnitude of response that the receptor is capable of producing by any ligand. A compound that elicits the maximum response is a full agonist; a particular compound may be capable of exceeding the maximum response of a tissue, but the observed response can only be the maximum response of that particular tissue. A drug that is not capable of eliciting the maximum response of the tissue, which depends on the structure of the drug, is a partial agonist. A full agonist or partial agonist is said to display *positive efficacy*, an antagonist displays zero efficacy, and a full or partial inverse agonist displays *negative efficacy* (depresses basal tissue response).

The modified occupancy theory accounts for the existence of partial agonists and antagonists, but it does not account for why two drugs that can occupy the same receptor can act differently, i.e., one as an agonist, the other as an antagonist.

3.2.4.2. Rate Theory

As an alternative to the occupancy theory, Paton[54] proposed that the activation of receptors is proportional to the total number of encounters of the drug with its receptor per unit time. Therefore, the *rate theory* suggests that the pharmacological activity is a function of the rate of association and dissociation of the drug with the receptor and not the number of occupied receptors. Each association would produce a quantum of stimulus. In the case of agonists, the rates of both association and dissociation would be fast (the latter faster than the former). The rate of association of an antagonist with a receptor would be fast, but the dissociation would be slow. Partial agonists would have intermediate drug–receptor complex dissociation rates. At equilibrium, the occupancy and rate theories are mathematically equivalent. As in the case of the occupancy theory, the rate theory does not rationalize why the different types of compounds exhibit the characteristics that they do.

3.2.4.3. Induced-Fit Theory

The *induced-fit theory* of Koshland[55] was originally proposed for the action of substrates with enzymes, but it could apply to drug–receptor interactions as well. According to this theory, the receptor need not necessarily exist in the appropriate conformation required to bind the drug. As the drug approaches the receptor, a *conformational change* is induced, which orients the essential binding sites (Figure 3.20). The conformational change in the receptor could be responsible for the initiation of the biological response (movement of residues to interact with the substrate). The receptor (enzyme) was suggested to be elastic, and could return to its original conformation after the drug (product) was released. The conformational change need not occur only in the receptor (enzyme); the drug (substrate) also could undergo deformation, even if this resulted in strain in the drug (substrate). According to this theory, an agonist would induce a conformational change and elicit a response, an antagonist would bind without a conformational change, and a partial agonist would cause a partial conformational change. The induced-fit theory can be adapted to the rate theory. An agonist would induce a conformational change in the receptor, resulting in a conformation to which the agonist binds less tightly and from which it can dissociate more easily. If drug–receptor complexation does not cause a conformational change in the receptor, then the drug–receptor complex will be stable, and an antagonist will result.

Other theories evolved from the induced-fit theory, such as the macromolecular perturbation theory, the activation–aggregation theory, and multistate models.

3.2.4.4. Macromolecular Perturbation Theory

Having considered the conformational flexibility of receptors, Belleau[56] suggested that in the interaction of a drug with a receptor two general types of *macromolecular*

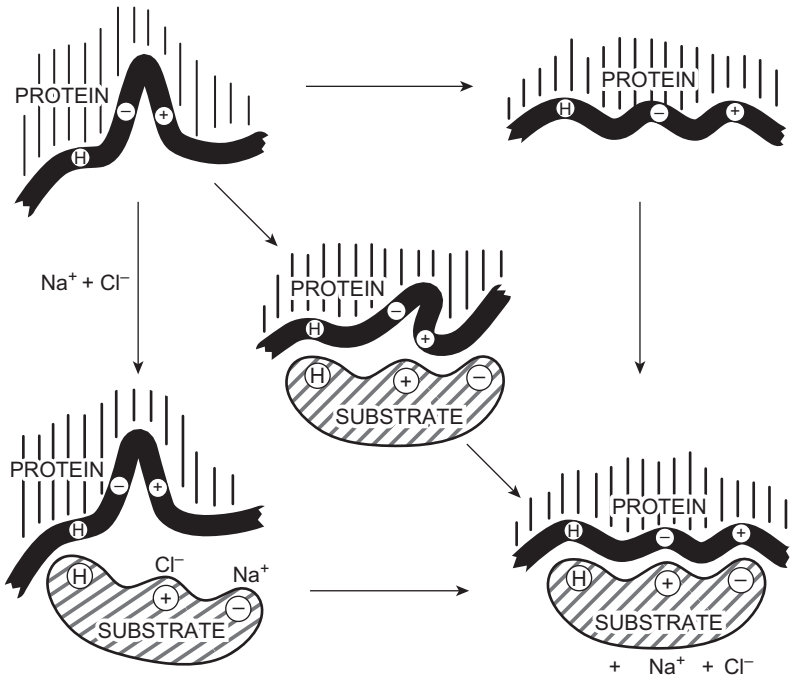


FIGURE 3.20 Schematic of the induced-fit theory. *Koshland, Jr., D. E., and Neet, K. E., Annu. Rev. Biochem., Vol. 37, 1968. Annual Review of Biochemistry by Annual Reviews. Reproduced with permission of Annual Reviews via Copyright Clearance Center, 2013.*

perturbations could result: *a specific conformational perturbation* makes possible the binding of certain molecules that produce a biological response (an agonist) and *a nonspecific conformational perturbation* accommodates other types of molecules that do not elicit a response (e.g., an antagonist). If the drug contributes to both macromolecular perturbations, a mixture of two complexes will result (a partial agonist). This theory offers a physicochemical basis for the rationalization of molecular phenomena that involve receptors, but does not address the concept of inverse agonism.

3.2.4.5. Activation–Aggregation Theory

An extension of the macromolecular perturbation theory (which also is based on the induced-fit theory) is the *activation–aggregation theory* of Monad, Wyman, and Changeux[57] and Karlin.[58] According to this theory, even in the absence of drugs, a receptor is in a state of dynamic equilibrium between an activated form (R_o), which is responsible for the biological response, and an inactive form (T_o). Using this theory, agonists bind to the R_o form and shift the equilibrium to the activated form, antagonists bind to the inactive form (T_o), and partial agonists bind to both conformations. In this model, the agonist binding site in the R_o conformation can be different from the antagonist binding site in the T_o conformation. If there are two different binding sites and conformations, then this could account for the structural differences in these classes of compounds and could rationalize why an agonist elicits a biological response but an antagonist does not. This theory can explain the ability of partial agonists to possess both the agonistic and antagonistic properties as depicted in Figure 3.16. In Figure 3.16B, as the partial agonist interacts with the remaining unoccupied receptors, there is an increase in the response up to the maximal response for the partial agonist interaction. In Figure 3.16C, the partial agonist competes with the neurotransmitter for the receptor sites. As the partial agonist displaces the neurotransmitter, it changes the amount of R_o and T_o receptor forms (T_o increases and, therefore, the response decreases) until all the receptors have the partial agonist bound. This theory, however, also does not address inverse agonists.[59]

3.2.4.6. The Two-State (Multistate) Model of Receptor Activation

The concept of a conformational change in a receptor inducing a change in its activity has been viable for many years.[60] The Monod–Wyman–Changeux idea described above involves a two-state model of receptor activation, but it does not go far enough. This model was revised based mostly on observations with GPCRs (see Section 3.1).[61]

The revised *two-state model of receptor activation* proposes that, in the absence of the natural ligand or agonist, receptors exist in equilibrium (defined by equilibrium constant *L*; Figure 3.21) between an active state (R^*), which is able to initiate a biological response, and a resting state (R), which cannot. In the absence of a natural ligand or agonist,

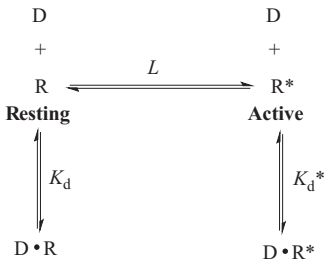


FIGURE 3.21 Two-state model of receptor activation. D is the drug, R is the receptor, and *L* is the equilibrium between the resting (R) and the active (R^*) state of the receptor.

the equilibrium between R^* and R defines the basal activity of the receptor. A drug can bind to one or both of these conformational states, according to equilibrium constants K_d and K_d^* for formation of the drug–receptor complex with the resting (D·R) and active ($D{\cdot}R^*$) states, respectively. Full agonists alter the equilibrium fully to the active state by binding to the active state and causing maximum response; partial agonists preferentially bind to the active state, but not to the extent that a full agonist does, so maximum response is not attained; full inverse agonists alter the equilibrium fully to the resting state by binding to the resting state, causing a negative efficacy (a decrease in the basal activity); partial inverse agonists preferentially bind to the resting state, but not to the extent that a full inverse agonist does; and antagonists have equal affinities for both states (i.e., have no effect on the equilibrium or basal activity, and, therefore, exhibit neither positive nor negative efficacy).[62] A competitive antagonist is able to displace either an agonist or an inverse agonist from the receptor.

Leff and coworkers further extended the two-state receptor model to a *three-state receptor model*.[63] In this model, there are two active conformations (this becomes a multistate model by extension to more than two active conformations) and an inactive conformation. This accommodates experimental findings regarding variable agonist and inverse agonist behavior (both affinities and efficacies) in different systems containing the same receptor type (called *receptor promiscuity*). According to this hypothesis, the basis for differential agonist efficacies among different agonists is their different affinities for the different active states.

3.2.5. Topographical and Stereochemical Considerations

Up to this point in our discussion of drug–receptor interactions, we have been concerned with what stabilizes a drug–receptor complex, how drug–receptor interactions are measured, and possible ways that the drug–receptor complex may form. In this section, we turn our attention to molecular aspects and examine the topography and stereochemistry of drug–receptor complexes.

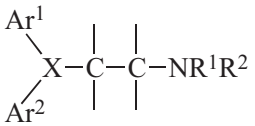


FIGURE 3.22 General structure of antihistamines

3.2.5.1. Spatial Arrangement of Atoms

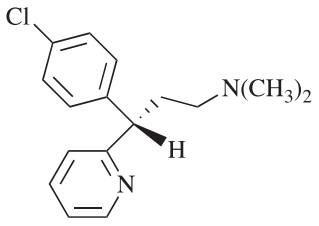
It was indicated in the discussion of bioisosterism (Chapter 2, Section 2.2.4.3) and from SAR studies that many antihistamines have a common pharmacophore (Figure 3.22).[64] In Figure 3.22, Ar^1 is aryl, such as phenyl, substituted phenyl, or heteroaryl (2-pyridyl or thienyl) and Ar^2 is aryl or arylmethyl. The two aryl groups can also be connected through a bridge (as in phenothiazines, **2.75**), and the C-C-N moiety can be part of another ring (as in chlorcyclizine (Di-Paralene), Table 3.1). X is CH–O–, N–, or CH–; C–C is a short carbon chain (two or three atoms), which may be saturated, branched, contain a double bond, or be part of a ring system. These compounds are called *antihistamines* because they are antagonists of a histamine receptor known as the *H_1 histamine receptor.* When a sensitized person is exposed to an allergen, an antibody is produced, an antigen–antibody reaction occurs, and histamine is released. Histamine binding to the H_1 receptor can cause stimulation of smooth muscle and produce allergic and hypersensitivity reactions such as hay fever, pruritus (itching), contact and atopic dermatitis, drug rashes, urticaria (edematous patches of skin), and anaphylactic shock. Antihistamines are used widely to treat these symptoms. Unlike histamine (see Table 3.1 for structure), most H_1 antagonists contain tertiary amino groups, usually a dimethylamino or pyrrolidino group. At physiological pH, then, this group will be protonated, and it is believed that an ionic interaction with the receptor is a key binding contributor. The commonality of structures of antihistamines suggests that there are specific binding sites on the H_1 histamine receptor that have an appropriate topography for interaction with certain groups on the antihistamine, which are arranged in a similar configuration (see Section 3.2.2). It must be reiterated, however, that although the antihistamines are competitive antagonists of histamine for the H_1 receptor, the same set of atoms on the receptor need not interact with both histamine and the antagonists.[65] Consequently, it is difficult to make conclusions regarding the receptor structure on the basis of antihistamine structure–activity relationships. Because of the essentiality of various parts of antihistamine molecules, it is likely that the minimum binding requirements include a negative charge or π system on the receptor to interact with the ammonium cation (electrostatic or cation–π, respectively) and hydrophobic (van der Waals) interactions with the aryl groups. Obviously, many other interactions are involved.

From the very simplistic view of drug–receptor interactions discussed above, it is not possible to rationalize

the fact that enantiomers, i.e., compounds that are nonsuperimposable mirror images of each other, can have quite different binding properties to receptors. This phenomenon is discussed in more detail in the next section.

3.2.5.2. Drug and Receptor Chirality

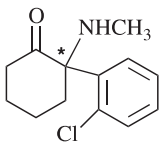
Histamine is an achiral molecule, but many of the H_1 receptor antagonists are chiral molecules. Proteins are polyamino acid macromolecules, and amino acids are chiral molecules (in the case of mammalian proteins, they are almost all L isomers); consequently, proteins (receptors) also are chiral substances. Complexes formed between a receptor and two enantiomers are diastereomers, not enantiomers, and, as a result, they have different energies and chemical properties. This suggests that dissociation constants for drug–receptor complexes of enantiomeric drugs may differ and may even involve different binding sites. The chiral antihistamine dexchlorpheniramine (**3.12**, Polaramine) is highly stereoselective (one stereoisomer is more potent than the other); the *S*-(+)-isomer is about 200 times more potent than the *R*-(−)-isomer.[66] According to the nomenclature of Ariëns,[67] when there is isomeric stereoselectivity, the more potent isomer is termed the *eutomer* and the less potent isomer is the *distomer*. The ratio of the potency of the more potent (higher affinity) enantiomer to the potency of the less potent enantiomer is termed the *eudismic ratio*. The in vivo eudismic ratio (−/+) for etorphine (Immobilon), a highly potent analgesic agent used to immobilize large nondomestic animals (see **2.63**, R = CH_3, R′ = C_3H_7), is greater than 6666.[68]



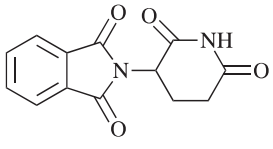
***S*-(+)-Dexchlorpheniramine**
3.12

High-potency antagonists are those having a high degree of complementarity with the receptor. When the antagonist contains a stereogenic center in the pharmacophore (see Chapter 2, Section 2.2.1), a high eudismic ratio is generally observed for the stereoisomers because the receptor complementarity would not be retained for the distomer. This increase in eudismic ratio with an increase in potency of the eutomer is *Pfeiffer's rule*.[69] Small eudismic ratios are typically observed when the stereogenic center lies outside of the region critically involved in receptor binding, i.e., is not part of the pharmacophore, or when both the eutomer and distomer have low affinity for the receptor (poor molecular complementarity).

The distomer actually should be considered as an impurity in the mixture, or, in the terminology of Ariëns, the *isomeric ballast*. It, however, may contribute to undesirable side effects and toxicity; in that case, the distomer for the biological activity may be the eutomer for the side effects. For example, *d*-ketamine (**3.13**; the asterisk marks the chiral carbon) is a hypnotic and analgesic agent; the *l*-isomer is responsible for the undesired side effects[70] (note that *d* is synonymous with (+) and *l* is synonymous with (−)). Probably the most horrendous example of toxicity by a distomer is that of thalidomide (**3.14**, Contergan), a drug used in the late 1950s and the early 1960s as a sedative and to prevent morning sickness during pregnancy, which was shown to cause severe fetal limb abnormalities (phocomelia, shortening of limbs, and amelia, absence of limbs) when taken in the first trimester of pregnancy. This tragedy led to the development of three phases of clinical trials and the requirement for Food and Drug Administration (FDA) approval of drugs (see Chapter 1, Section 1.3.6.2 and 1.3.6.3). Later, it was thought that the teratogenicity (birth defect) of thalidomide was caused by the (*S*)-isomer only[71]; however, then it was found that the (*R*)-isomer was converted into the (*S*)-isomer in vivo.[72] Despite the potential danger of this drug, it is back on the market (as the racemate, Thalomid) for the treatment of moderate or severe erythema nodosum leprosum in leprosy patients and for the treatment of multiple myeloma, but it is not administered to pregnant women and preferably only to those women beyond child-bearing age. The target for thalidomide seems to be the protein cereblon (CRBN), which is involved in limb outgrowth.[73]

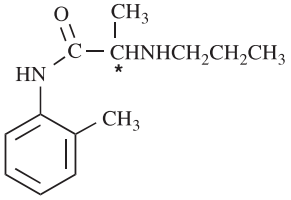


3.13



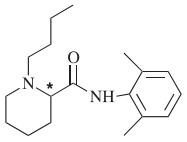
Thalidomide
3.14

It also is possible that both isomers are biologically active, but only one contributes to the toxicity, such as the local anesthetic prilocaine (**3.15**, Citanest).[74]

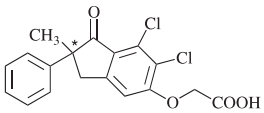


Prilocaine
3.15

In some cases it is desirable to have both isomers present. Both isomers of bupivacaine (**3.16**, Sensorcaine) are local anesthetics, but only the *l*-isomer shows vasoconstrictive activity.[75] The experimental diuretic (increases water excretion) drug indacrinone (**3.17**) has a uric acid retention side effect. The *d*-isomer of **3.17** is responsible (i.e., is the eutomer) for both the diuretic activity and the uric acid retention side effect. Interestingly, however, the *l*-isomer acts as a uricosuric agent (reduces uric acid levels). Unfortunately, the ratio that gives the optimal therapeutic index (see Chapter 2; Section 2.2.4) is 1:8 (*d*:*l*), not 1:1 as is present in the racemic mixture.[76]

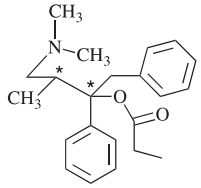


Bupivacaine
3.16

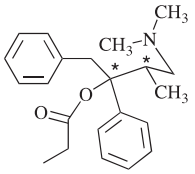


Indacrinone
3.17

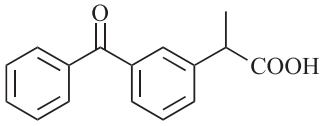
Enantiomers may have different therapeutic activities as well.[77] Darvon (**3.18**), 2*R*,3*S*-(+)-dextropropoxyphene, is an analgesic drug and its enantiomer, Novrad (**3.19**), 2*S*,3*R*-(−)-levopropoxyphene, is an antitussive (anticough) agent, an activity that is not compatible with analgesic action. Consequently, these enantiomers are marketed separately. You may have noticed that the trade names are enantiomeric as well! The (*S*)-(+)-enantiomer of the antiinflammatory/analgesic drug ketoprofen (**3.20**, Orudis) is the eutomer; the (*R*)-(−)-isomer shows activity against bone loss in periodontal disease.



Dextropropoxyphene
3.18

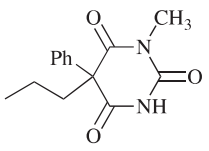


Levopropoxyphene
3.19



Ketoprofen
3.20

It, also, is possible for enantiomers to have opposite effects.[78] The (*R*)-(−)-enantiomer of 1-methyl-5-phenyl-5-propylbarbituric acid (**3.21**) is a narcotic, and the (*S*)-(+)-enantiomer is a convulsant![79] The (+)-isomer of the experimental narcotic analgesic picenadol (**3.22**) is an opiate agonist, the (−)-isomer is a narcotic antagonist, and the racemate is a partial agonist.[80] This suggests a potential danger in studying racemic mixtures; one enantiomer may antagonize the other, and no effect will be observed. For example, the racemate of UH-301 (**3.23**) exhibits no serotonergic activity; (*R*)-UH-301 is an agonist of the 5-hydroxytryptamine 1A (5-HT_{1A}) receptor, but (*S*)-UH-301 is an antagonist of the same receptor.[81] Consequently, no activity is observed with the racemate.



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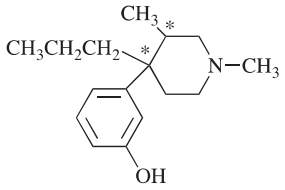

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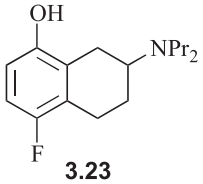
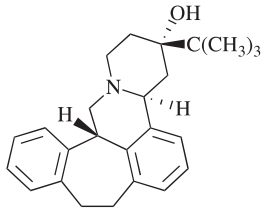
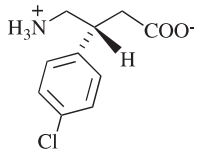


3.23

It is quite common for chiral compounds to show stereoselectivity with receptor action, and the stereoselectivity of one compound can vary for different receptors. For example, (+)-butaclamol (**3.24**) is a potent antipsychotic, but the (−)-isomer is essentially inactive; the eudismic ratio (+/−) is 1250 for the D_2-dopaminergic, 160 for the D_1-dopaminergic, and 73 for the α-adrenergic receptors. (−)-Baclofen (**3.25**, Lioresal) is a muscle relaxant that binds to the $GABA_B$ receptor; the eudismic ratio (−/+) is 800.[82]



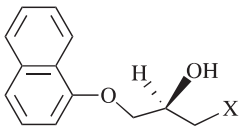
Butaclamol
(+)-3.24



Baclofen
(-)-3.25

Remember that the (+)- and (−)-nomenclature refers to the effect of the compound on the direction of rotation of plane polarized light and has nothing to do with the stereochemical configuration of the molecule. The stereochemistry about a stereogenic carbon atom is noted by the *R,S* convention of Cahn et al.[83] Because the *R,S*

convention is determined by the atomic numbers of the substituents about the stereogenic center, two compounds having the same stereochemistry, but a different substituent can have opposite chiral nomenclatures. For example, the eutomer of the antihypertensive agent, propranolol (Inderal) is the *S*-(−)-isomer (**3.26**, X = $NHCH(CH_3)_2$).[84] If X is varied so that the attached atom has an atomic number greater than that of oxygen, such as F, Cl, Br, or S, then the nomenclature rules dictate that the molecule is designated as an *R* isomer, even though there is no change in the stereochemistry. Note, however, that even though the absolute configuration about the stereogenic carbon remains unchanged after variation of the X group in **3.26**, the effect on plane polarized light cannot necessarily be predicted; the compound with a different substituent X can be either + or −. The most common examples of this phenomenon in nature are some of the amino acids. (*S*)-Alanine, for example, is the (+)-isomer and (*S*)-serine (same absolute stereochemistry) is the (−)-isomer; the only difference is a CH_3 group for alanine and a CH_2OH group for serine.



Propranolol
(-)-3.26

Propranolol (**3.26**, Inderal, X = $NHCH(CH_3)_2$), the first member of a family of drugs known as *β-blockers* (Sir James W. Black shared a Nobel Prize in Medicine in 1988 for this discovery), is a competitive antagonist (blocker) of the β-adrenergic receptor, which triggers a decrease in blood pressure and regulates cardiac rhythm and oxygen consumption for those with cardiovascular disease. The β_1- and β_2-adrenergic receptors are important to cardiac and bronchial vasodilation, respectively; propranolol is nonselective in its antagonism for these two receptors. The eudismic ratio (−/+) for propranolol is about 100; however, propranolol also exhibits local anesthetic activity for which the eudismic ratio is 1. The latter activity apparently is derived from some mechanism other than β-adrenergic receptor blockade. A compound of this type that has two separate mechanisms of action and, therefore, different therapeutic activities has been called a *hybrid drug* by Ariëns.[85] (+)-Butaclamol (**3.24**), which interacts with a variety of receptors, is another hybrid drug. However, butaclamol has three chiral centers and, therefore, has eight possible isomeric forms. When multiple isomeric forms are involved in the biological activity, the drug is called a *pseudohybrid drug*. Another important example of this type of drug is the antihypertensive agent, labetalol (Figure 3.23, Normodyne), which has two stereogenic centers and therefore exists in four stereoisomeric forms (two diastereomeric pairs of enantiomers), having the stereochemistries (*RR*), (*SS*), (*RS*), and (*SR*). In the drug form, labetalol contains a mixture of all four stereoisomers and has α- and β-adrenergic blocking properties (note that although labetalol has two stereogenic centers, all four isomers do not have to be included in the formulation, but they are in this case). The (*RR*)-isomer is predominantly the β-blocker (the eutomer for β-adrenergic blocking action), and the (*SR*)-isomer is mostly the α-blocker (the eutomer for α-adrenergic blocking); the other 50% of the isomers, the (*SS*)- and (*RS*)-isomers, are almost inactive (the isomeric ballast). Labetalol, then, is a pseudohybrid drug, a mixture of isomers having different receptor-binding properties.

Labetalol also is an example of how relatively minor structural modifications of an agonist can lead to transformation into an antagonist. *l*-Epinephrine (**3.27**) is a natural hybrid molecule that induces both α- and β-adrenergic effects. Introduction of the phenylalkyl substituent on the nitrogen transforms the α-adrenergic activity of the agonist *l*-epinephrine into the α-adrenergic antagonist labetalol. The modification of one of the catechol hydroxyl groups of *l*-epinephrine to a carbamyl group of labetalol changes the β-adrenergic action (agonist) to a β-adrenergic blocking action (antagonist).

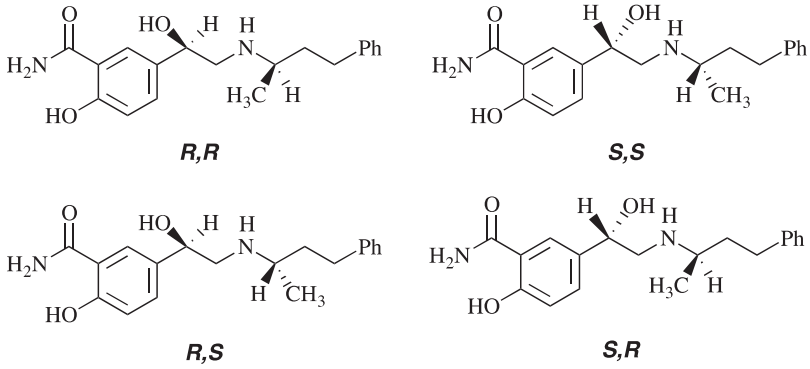
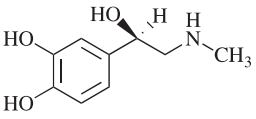


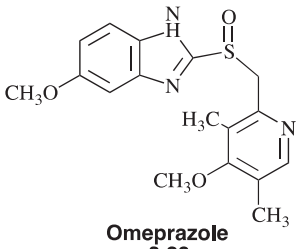
FIGURE 3.23 Four stereoisomers of labetalol



l-epinephrine
3.27

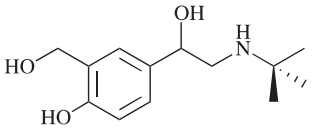
As pointed out by Ariëns[86] and by Simonyi,[87] it is quite common for mixtures of isomers, particularly racemates, to be marketed as a single drug, even though at least half of the mixture not only may be inactive for the desired biological activity but also may, in fact, be responsible for various side effects. In the early 1980s, only 58 of the 1200 drugs available were single enantiomers;[88] however, this has changed dramatically. In 2004, for the first time, all new chiral drugs introduced in the market (13 of them) were single enantiomers. This was motivated by guidelines issued by various regulatory agencies in the late 1980s and early 1990s (the FDA in 1992[89]), which allowed drug companies to choose whether to develop chiral drugs as racemates or single enantiomers, but required applicants with racemic drugs to submit rigorous scientific evidence why the racemate was developed rather than a single enantiomer.[90] Racemates were developed if it was discovered that the single enantiomer racemized easily in vitro and/or in vivo, if the enantiomers had similar pharmacological and toxicological profiles, or when the use of racemates resulted in synergistic effects (see Chapter 7), leading to better pharmacological or toxicological properties. The challenges, and sometimes prohibitive expense, in the synthesis of single enantiomer drugs have been assuaged by advancements in asymmetric synthesis and chiral separation technologies.

To further encourage companies to prepare and market single-entity drugs, the concept of a *racemic switch* (also called *chiral switch*) was introduced. This is the redevelopment in single enantiomer form of a drug that is being marketed as a racemate (the racemate is switched for the eutomer). Even if the racemate is currently covered by an active patent, the patent office would allow a new patent to a second company for the eutomer of the racemate. Of course, the same company can be awarded a patent for a racemic switch as well, which is an interesting strategy to extend the life of exclusivity for a drug. For example, AstraZeneca markets the antiulcer drug omeprazole (**3.28**, Prilosec) as a racemate, but shortly before the patent expired, a new patent was issued to the same company for the active (*S*)-isomer, which was approved for marketing as esomeprazole (Nexium). Because the racemate had already been approved by the FDA, less testing was needed for the active enantiomer. Interestingly, the (*R*)-isomer is more potent than either the (*S*)-isomer or racemate in rats; the two enantiomers are equipotent in dogs, and the (*S*)-isomer is most potent in humans (apparently because of the higher bioavailability and consistent pharmacokinetics compared with the other enantiomer).[91]



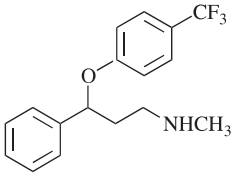
Omeprazole
3.28

The use of a single enantiomer is generally expected to lower side effects and toxicity. For example, the antiasthma drug albuterol (**3.29**, Ventolin/Proventil) is an agonist for β_2-adrenergic receptors on airway smooth muscle, leading to bronchodilation. The racemic switch, levalbuterol (the *R*-(−)-isomer, Xopenex), appears to be solely responsible for the therapeutic effect. The (*S*)-isomer seems to produce side effects such as pulse rate increases, tremors, and decreases in blood glucose and potassium levels. Because of this advantage, single isomer drug sales have been steadily increasing worldwide; in 1996, they accounted for 27% of the market, and in 2002, 39% of drug sales were of single enantiomers.[92]

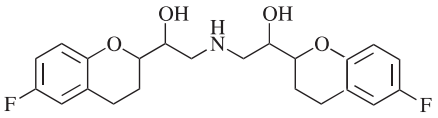


Albuterol
3.29

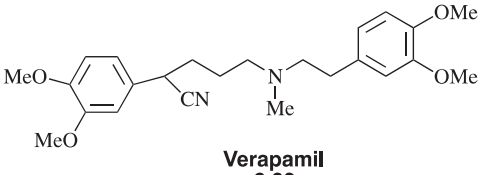
However, it is not always best to use the single enantiomer of the drug. The antidepressant drug fluoxetine (**3.30**, Prozac) is marketed as the racemate (in this case both isomers are active as selective serotonin reuptake inhibitors). Clinical trials with just the (*R*)-isomer at a higher dosage, however, produced a cardiac side effect. Another unusual problem associated with the use of a single enantiomer may occur if the two enantiomers have synergistic pharmacological activities. For example, the (+)-isomer of the antihypertensive drug nebivolol (**3.31**, Nebilet) is a β-blocker (see above); the (−)-isomer is not a β-blocker, but it is still a vasodilating agent (via the nitric oxide pathway), so the drug is sold as a racemate to take advantage of two different antihypertensive mechanisms. Sometimes an unexpected side benefit is associated with the use of a racemic mixture. The racemic calcium ion channel blocker (see Section 3.2.6) verapamil (**3.32**, Calan) has long been used as an antihypertensive drug. The (*S*)-isomer is the eutomer, but the (*R*)-isomer has been found to inhibit the resistance of cancer cells to anticancer drugs.[93]



Fluoxetine
3.30

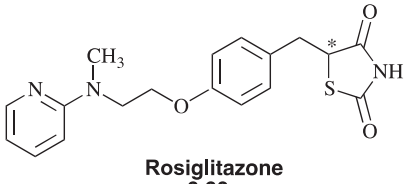


Nebivolol
3.31



Verapamil
3.32

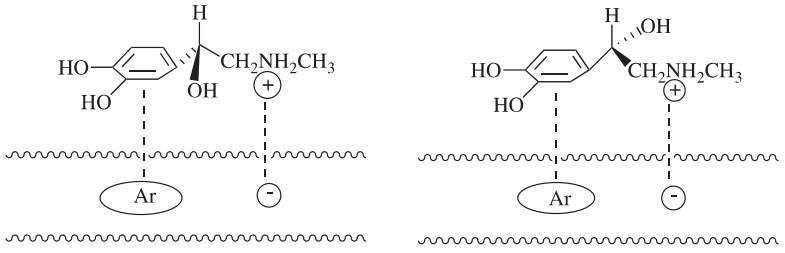
For cases in which the enantiomers are readily interconvertible in vivo, there is no reason to go to the expense of marketing a single enantiomer. Enantiomers of the antidiabetes drug rosiglitazone (**3.33**, Avandia) spontaneously racemize in solution, so it is sold as a racemate. Because of the reasons noted above for continuing the use of racemates, about 10% of annual drug approvals (13% for FDA and 9% worldwide) are still racemates.[94]



Rosiglitazone
3.33

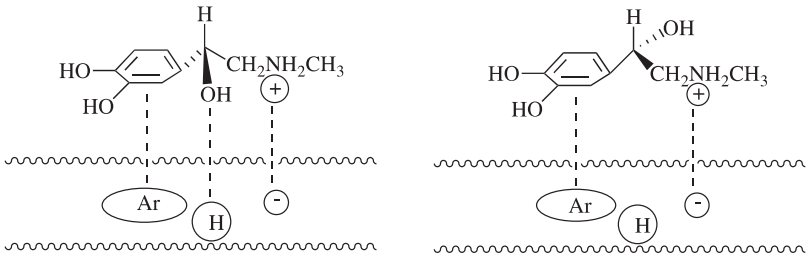
Because of the potential vast differences in activities of two enantiomers, caution should be used when applying quantitative structure-activity relationship (QSAR) methods such as Hansch analyses (see Chapter 2; Section 2.2.6.2.2.1) to racemic mixtures. These methods really should be applied to the separate isomers.[95]

It is quite apparent from the above discussion that receptors are capable of recognizing and selectively binding optical isomers. Cushny[96] was the first to suggest that enantiomers could have different biological activities because one isomer could fit into a receptor much better than the other. How are they able to accomplish this? If you consider two enantiomers, such as epinephrine, interacting with a receptor that has only two binding sites (Figure 3.24), it becomes apparent that the receptor cannot distinguish between them. However, if there are at least three binding sites (Figure 3.25), the receptor easily can differentiate them. The *R*-(−)-isomer has three points of interaction and is held in the conformation shown to maximize molecular complementarity. The *S*-(+)-isomer can have only two sites of interaction (the hydroxyl group cannot interact with the hydroxyl binding site and may even have an adverse steric interaction); consequently it has a lower binding energy. Easson and Stedman[97] were the first to recognize this *three-point attachment* concept: a receptor can differentiate enantiomers if there are as few as three binding sites. As in the case of the β-adrenergic receptors discussed above, the structure of α-adrenergic receptors to which epinephrine



***R*-(-)-Epinephrine** ***S*-(+)-Epinephrine**

FIGURE 3.24 Binding of epinephrine enantiomers to a two-site receptor. The wavy lines are the receptor surfaces.

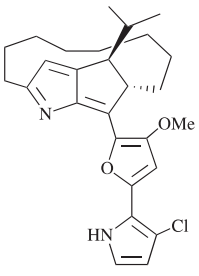


***R*-(-)-Epinephrine** ***S*-(+)-Epinephrine**

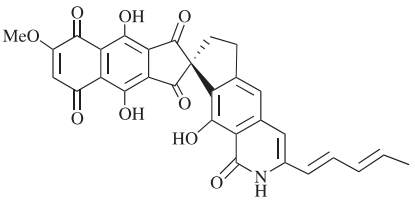
FIGURE 3.25 Binding of epinephrine enantiomers to a three-site receptor. The wavy lines are the receptor surfaces.

binds is unknown. α-Adrenergic receptors appear to mediate vasoconstrictive effects of catecholamines in bronchial, intestinal, and uterine smooth muscle. The eudismic ratio (*R/S*) for vasoconstrictor activity of epinephrine is only 12–20,[98] indicating that there is relatively little difference in binding energy for the two isomers to the α-adrenergic receptor. Although the above discussion was directed at the enantioselectivity of receptor interactions, it should be noted that there is also enantioselectivity with respect to pharmacokinetics, i.e., absorption, distribution, metabolism, and excretion, which will be discussed in Chapter 8.[99]

As noted in Chapter 2 (Section 2.1.2.3.1), a relatively large percentage of antiinfectives and antitumor compounds are natural products or are analogs of natural products. The above discussion would suggest that the chirality of natural products should be very important to their biological activities. This is true; however, the nonnatural enantiomer of the natural product could be even more potent than the natural product. For example, *ent*-(−)-roseophilin (**3.34**), the unnatural enantiomer of the natural antitumor antibiotic, is 2–10 times more potent than the natural (+)-isomer in cytotoxicity assays,[100] and *ent*-fredericamycin A (**3.35**) is as cytotoxic as its natural enantiomer.[101] Why should that be so? The organisms that produce these natural products may not be producing them for the purpose of protecting themselves from the disease state we have in mind for these compounds. After all, are these organisms really concerned with developing cancer? There are many possible mechanisms of action for antitumor agents, and the *ent*-natural product may bind to the relevant receptor better than the natural product does.



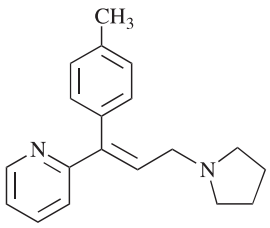
***Ent*-roseophilin**
3.34



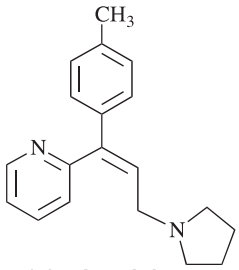
***Ent*-fredericamycin A**
3.35

3.2.5.3. Diastereomers

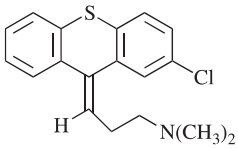
Two (or more) compounds having different spatial arrangements (i.e., are stereoisomers) that are not mirror images of each other (i.e., are not enantiomers of each other) are diastereomers. Geometric isomers (*E*- and *Z*-isomers[102]) are a special case of diastereomers. Epimers (a pair of compounds with multiple stereogenic centers that have opposite configuration at only one stereocenter) are another special case. Diastereomers are different compounds, having different energies and stabilities. As a result of their different configurations, receptor interactions will be different. Unlike enantiomers, which are relatively difficult to separate, diastereomers often can be easily separated by chromatography or recrystallization, so they should be tested separately. The antihistamine activity of *E*-triprolidine (**3.36a**, found in cold remedies, such as Actifed) was found to be 1000-fold greater than the corresponding *Z*-isomer (**3.35b**).[103] Likewise, the neuroleptic potency of the *Z*-isomer of the antipsychotic drug chlorprothixene (**3.37a**, Taractan) is more than 12 times greater than that of the corresponding *E*-isomer (**3.37b**).[104] On the other hand, the *E*-isomer of the anticancer drug diethylstilbestrol (**3.38a**) has 14 times greater estrogenic activity than the *Z*-isomer (**3.38b**), possibly because its overall structure and the interatomic distance between the two hydroxyls in the *E*-isomer are similar to that of estradiol (**3.39**).



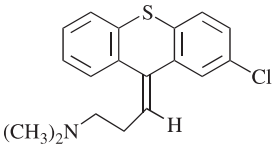
(*E*)-Triprolidine
3.36a



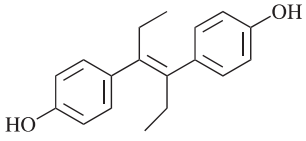
(*Z*)-Triprolidine
3.36b



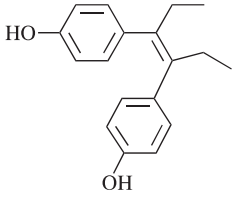
(*Z*)-Chlorprothixene
3.37a



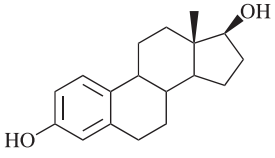
(*E*)-Chlorprothixene
3.37b



Diethylstilbesterol
3.38a



3.38b



3.39

Although, in some cases, the *cis*- and *trans*-nomenclature does correspond with *Z*- and *E*-, respectively, it should be kept in mind that these terminologies are based on different

conventions, so there may be confusion. The *Z,E* nomenclature is unambiguous, and should be used.

3.2.5.4. Conformational Isomers

Diastereomers and enantiomers can be separated, isolated, and screened individually. There are isomers, however, that typically cannot be separated, namely *conformational isomers* or *conformers* (isomers generated by a change in conformation). As a result of free rotation about single bonds in acyclic molecules and conformational flexibility in many cyclic compounds, a drug molecule can assume a variety of *conformations*, i.e., the location of the atoms in space without breakage of bonds. The pharmacophore of a molecule is defined not only by the configuration of a set of atoms but also by the bioactive conformation in relation to the receptor-binding site. A receptor may bind only one of the conformers. As was pointed out in Chapter 2 (Section 2.2.4.4), the conformer that binds to a receptor need *not* be the lowest energy conformer observed in the crystalline state, as determined by X-ray crystallography, or found in solution, as determined by NMR spectrometry, or determined theoretically by molecular mechanics calculations. The binding energy to the receptor may overcome the barrier to the formation of a higher energy conformation. In order for drug design to be efficient, it is extremely helpful to know the *bioactive conformation* (the active conformation when bound to the receptor) in the drug–receptor complex. Figure 3.26 is a crystal structure of the antidiabetic drug rosiglitazone (see **3.33**, Avandia) bound to the peroxisome proliferator-activated receptor gamma (PPARγ), a transcription factor.[105] Note that the bioactive conformation of the drug bound to the receptor is an inverted U shape, rather than an extended conformation. Compounds that cannot attain this inverted U structure will not be able to bind to that site.

If a lead compound has low potency, it may only be because the population of the active conformer in solution is low (higher in energy); for example, with PPARγ shown in Figure 3.26, an inverted U conformation of the compound is essential for high potency. The energy of a conformer will determine the relative population of that conformer in the equilibrium mixture of conformers. A higher energy conformer will be in lower concentration in the equilibrium mixture of conformers. Therefore, if the bioactive conformation is a high-energy conformer, the K_d for the molecule will appear high (poor affinity), not because the structure of the compound is incorrect, but because the population of the ideal conformation is so low. If the conformation of the ideal conformer were in higher concentration, the K_d would be much lower. To give you a simple example of conformational populations from organic chemistry, consider 1-*tert*-butylcyclohexane (Scheme 3.2). Cyclohexanes can exist in numerous conformations, including a chair form with the substituent in the equatorial position (**a**), a half-chair (**b**), a boat (**c**) (including twist-boat), a different half-chair (**d**), and a chair conformer

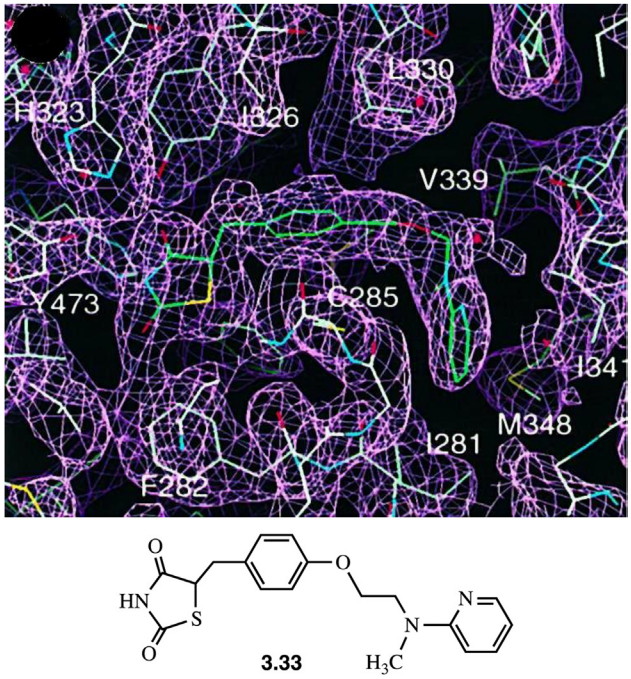
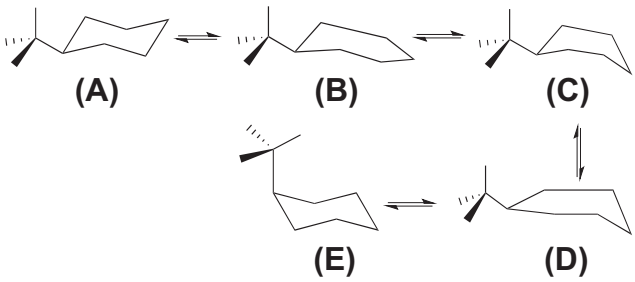


FIGURE 3.26 **An example of a bioactive conformation.** Rosiglitazone (**3.33**), an antidiabetic drug (green structure in the middle), bound to PPARγ. Note the sickle-shaped conformation in the binding site to accommodate the shape formed by the active site residues. *Reprinted from* Mol. Cell, *Vol. 5, "Asymmetry in the PPARγ/RXRα Crystal Structure Reveals the Molecular Basis of Heterodimerization among Nuclear Receptors", pp. 545–555. Reprinted with permission of Elsevier.*

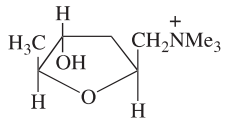


SCHEME 3.2 Cyclohexane conformations. **a**, chair (substituent equatorial); **b**, half-chair; **c**, boat; **d**, half-chair; **e**, chair (substituent axial).

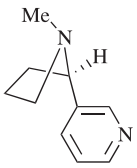
with the substituent axial (**e**). The difference in free energy for the two chair conformers with the *tert*-butyl group either equatorial (**a**) or axial (**e**), which in a receptor-binding site would make an enormous difference on binding effectiveness, is −5.4 kcal/mol; this translates into an equilibrium mixture ([equatorial]/[axial]) at 37 °C of 6619 ($\Delta G^0 = -RT \ln K$). If the axial conformer were the bioactive conformation, and the mixture were 1 μM in 1-*tert*-butylcyclohexane, it would only be 0.00015 μM (i.e., 150 pM) in the axial conformer of 1-*tert*-butylcyclohexane. This would lead to the conclusion that 1-*tert*-butylcyclohexane was inactive, whereas, if only the axial conformer existed in solution, it could be the most potent binder ever observed for that receptor.

A unique approach has been taken to determine the bioactive conformation of a drug molecule in the drug–receptor complex. This approach involves the synthesis of *conformationally rigid analogs* of flexible drug molecules. The potential pharmacophore becomes locked in various configurations by judicious incorporation of cyclic or unsaturated moieties into the drug molecule. These conformationally rigid analogs are, then, tested, and the analog with the optimal activity (or potency) can be used as the prototype for further structural modification. Conformationally rigid analogs are propitious because key functional groups, presumably part of the pharmacophore, are constrained in one position, thereby permitting the determination of the *pharmacophoric conformation*. The major drawback to this approach is that in order to construct a rigid analog of a flexible molecule, usually additional atoms and/or bonds must be attached to the original compound, and these can affect the chemical and physical properties. Consequently, it is imperative that the conformationally rigid analog and the drug molecule be as similar as possible in size, shape, and mass.

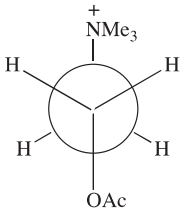
First we will look at the conformationally rigid analog approach to determine the bioactive conformation of a natural ligand (a neurotransmitter); then we will apply this methodology to lead modification. An example of the use of conformationally rigid analogs for the elucidation of receptor-binding site topography is the study of the interaction of the neurotransmitter, acetylcholine (ACh), with its receptors. There are at least two important receptors for ACh, one activated by the alkaloid muscarine (**3.40**) and the other by the alkaloid nicotine (**3.41**; presumably in the protonated pyrrolidine form); binding of nicotine to the ACh receptor is stabilized by a strong cation–π interaction between the ammonium ion of nicotine and a tryptophan residue in the receptor.[106] ACh has a myriad of conformations; four of the more stable possible conformers (groups staggered) are **3.42a**–**3.42d**. There are also conformers with groups eclipsed that are higher in energy. Four different *trans*-decalin stereoisomers were synthesized[107] (**3.43a–d**) corresponding to the four ACh conformers shown as **3.42**. All four isomers exhibited low muscarinic receptor activity; however, **3.43a** (which corresponds to the most stable conformer, the *anti*-conformer) was the most potent (0.06 times the potency of ACh). The low potency of **3.43a** is believed to be the result of the additional atoms present in the *trans*-decalin moiety. A comparison of *erythro*-(**3.44**) and *threo*-2,3-dimethylacetylcholine (**3.45**) gave the startling result that **3.44** was 14 times *more* potent than ACh and **3.45** was only 0.036 times as potent as ACh; in one case, the additional methyl groups enhanced the potency, and in the other case, they decreased the potency. Compound **3.43a** corresponds to *threo*-isomer **3.45**, and, therefore, is expected to have low potency. The corresponding *erythro* analog (**3.44**) does not have a *trans*-decalin analogy, so it could not be tested. To minimize the number of extra atoms added to ACh, *trans*-(**3.46**) and *cis*-1-acetoxy-2-trimethylammoniocyclopropanes (**3.47**) were synthesized and tested[108] for *cholinomimetic properties*, i.e., production of a response resembling that of ACh. The (+)-*trans*-isomer (shown in **3.46**)[109] has about the same muscarinic activity as does ACh, thus indicating the importance of minimizing additional atoms; the (−)-*trans*-isomer is about 1/500th the potency of ACh. This strongly supports the *anti*-conformer (**3.42a**) as the bioactive conformer. Unfortunately, the other conformers cannot be modeled by substituted cyclopropane analogs; the *cis*-isomer (**3.47**) models an eclipsed conformer of ACh. Nonetheless, the racemic *cis*-isomer has negligible activity. The (+)-*trans*-isomer has the same absolute configuration as the active enantiomers of the two muscarinic receptor agonists muscarine and acetyl β-methylcholine. These results suggest that ACh binds in an extended form (**3.42a**). However, both the *cis*- and the *trans*-cyclopropyl isomers, as well as all of the *trans*-decalin stereoisomers (**3.43a–d**), were only weakly active with the nicotinic cholinergic receptor. Because **3.46**, the lowest energy conformer, corresponding to ACh, is not active with the nicotinic ACh receptor, it can be supposed that a higher energy conformer is. This supports the concept that the lowest energy conformer does not have to be the one that binds to a receptor. In general, for this type of analysis to be convincing, it is best to identify a conformationally rigid analog that exhibits comparable or improved potency to that of the flexible compound being mimicked; otherwise, there could be many alternative interpretations for the results.



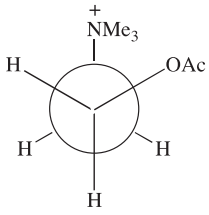
Muscarine
3.40



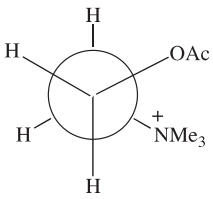
Nicotine
3.41



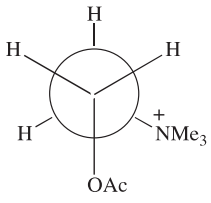
3.42a



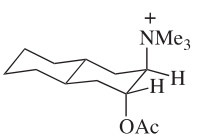
3.42b



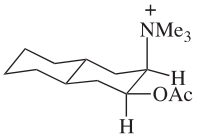
3.42c



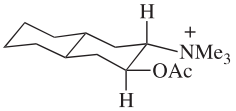
3.42d



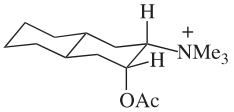
3.43a



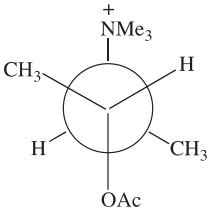
3.43b



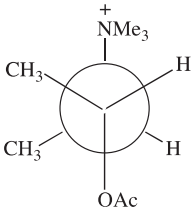
3.43c



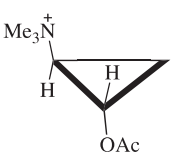
3.43d



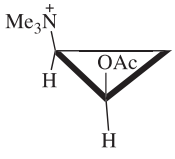
3.44



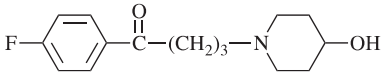
3.45



3.46

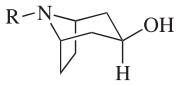


3.47

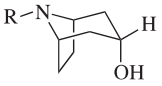


3.48

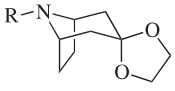
An example of the use of conformationally rigid analogs in drug design was reported by Li and Biel.[110] 4-(4-Hydroxypiperidino)-4′-fluorobutyrophenone (**3.48**) was found to have moderate tranquilizing activity in lower animals and man; however, unlike the majority of antipsychotic butyrophenone-type compounds, it only had minimal antiemetic (prevents vomiting) activity. The piperidino ring can exist in various conformations (**3.49a–d**, R = F-$C_6H_4CO(CH_2)_3$-), including two chair forms (**3.49a** and **3.49d**) and two twist-boat forms (**3.49b** and **3.49c**). The difference in free energy between the axial and equatorial hydroxyl conformers of the related compound, *N*-methyl-4-piperidinol (**3.49**, R = Me) is 0.94 ± 0.05 kcal/mol at 40 °C (the equatorial conformer is favored by a factor of 4.56 over the axial conformer).[111] Energies for the twist-boat conformers are about 6 kcal/mol higher, but because of hydrogen bonding, **3.49b** should be more stable than **3.49c**. On the assumption that the chair conformers are more likely, three conformationally rigid chair analogs, **3.50**–**3.52**, were synthesized to determine the effect on receptor binding of the hydroxyl group in the equatorial (**3.50**), axial (**3.51**), and both (**3.52**) positions. Of course, with **3.52**, it must be assumed that, if the hydroxyl group is involved in hydrogen bonding, it is as an acceptor, not as a donor. Also, for synthetic reasons, the conformationally rigid analog of **3.49d** could not be made; instead, the diastereomer (with the R group still equatorial, but the hydroxyl group axial) was synthesized. This study, then, provides data for the preference of the position of the hydroxyl group, not strictly for the conformer preference. When subjected to muscle relaxation tests, the order of potency was **3.51** > **3.52** > **3.50**, indicating again that the conformationally less stable compound with the axial hydroxyl group has better molecular complementarity with the receptor than does the more stable compound with the equatorial hydroxyl group. This suggests that further analogs should be prepared where the axial hydroxyl is the more stable conformer or where it can be held in that configuration.



3.50

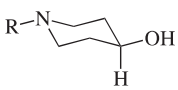


3.51

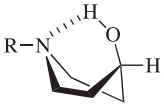


3.52

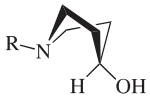
Another use of conformationally rigid analogs is to determine the appropriate orientation of pharmacophoric groups for binding to related receptors of unknown structure. The *N*-methyl-D-aspartate (NMDA) subclass of glutamate receptors is composed of an ion channel with multiple binding sites, including one for phencyclidine (PCP, **3.53**, Semylan, Figure 3.27). PCP analogs can bind to the PCP site of the NMDA receptor, the σ receptor, and the dopamine-D_2 receptor.[112] Neither the physical nature nor endogenous ligands for the σ receptor has been identified, but several structurally unrelated ligands are known.[113] PCP is a flexible molecule that can undergo conformational ring inversion of both the cyclohexyl and piperidinyl rings as well as rotation of the phenyl group. The various conformations place the ammonium ion and the phenyl ring in different spatial orientations, which may be responsible for binding to the various receptor sites. Conformationally rigid analogs of PCP were synthesized that fixed the orientation of the ammonium center of the PCP with



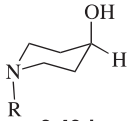
3.49a



3.49b



3.49c



3.49d

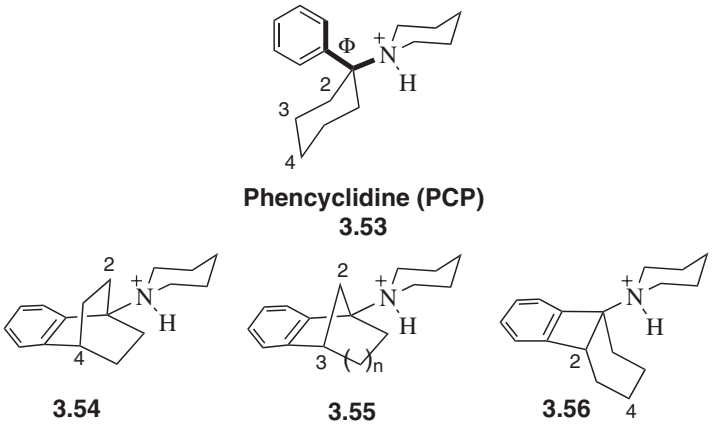


FIGURE 3.27 PCP, **3.53** and three conformationally rigid analogs of PCP

respect to the centrum of the phenyl ring to determine the importance of conformation on selectivity between the PCP and σ sites (Figure 3.27; ϕ is the angle defined by the darkened bonds in **3.53**).[114] The designed analogs incorporated a new bond connecting the *ortho* position of the phenyl ring to the 4-, 3-, or 2-position of the cyclohexane ring to give, respectively, **3.54**, **3.55** ($n=2$), and **3.56**; for synthetic reasons, **3.55** ($n=1$) was actually prepared and tested in place of **3.55** ($n=2$). In **3.54**, ϕ is 0°; in **3.55,** ϕ is 30°; and in **3.56,** ϕ is 60°. As the rigidity increases (**3.56**→**3.55**→**3.54**), the affinity for the PCP site of the NMDA receptor is diminished, and none binds well to this site (the best, **3.56**, only has 2% of the affinity of PCP). However, all three bind well to the σ site, almost twice as well as does PCP itself and fit a pharmacophore model for the σ receptor.[115]

As described in Chapter 2 (Section 2.2.4.5) peptides are unfavorable structures for drug discovery because they are too polar and flexible. Polarity, and some flexibility, can be handled with the use of peptidomimetics and bioisosteres (Chapter 2, Section 2.2.4.3). A conformationally rigid analog approach was taken to lock in the amide bond conformation, which generally favors the trans-conformation, and give either the cis- or trans-conformation using a 1,4- or 1,5-disubstituted 1,2,3-triazole, respectively, as an amide bond bioisostere (Figure 3.28).[116]

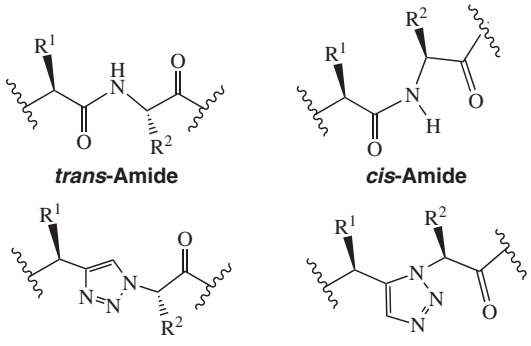


FIGURE 3.28 Use of a triazole as a conformationally rigid bioisostere to lock in an amide bond conformation

3.2.5.5. *Atropisomers*

Another type of conformational chirality, called *atropisomerism*, occurs when there is hindered rotation about a single bond as a result of steric or electronic constraints, causing slow interconversion of two conformers (a rule of thumb is having a half-life >1000 s) (Figure 3.29).[117] Slow interconversion can lead to two conformers, thereby giving a single chiral compound, a mixture of two chiral compounds (diastereomers), or a racemate, depending on whether there are stereogenic centers in the molecule and the rate of interconversion. This can be problematic in drug design, if you are assuming that the molecule exists as a single structure.[118] Energy barriers to rotation were calculated using quantum mechanics, and it was estimated that 20 kcal/mol was the minimum bond rotation energy to distinguish between atropisomers and nonatropisomers with a prediction accuracy of 86%.[119] Figure 3.30 shows an example of three analogs having different bond rotation energies that are (**a**) nonatropisomeric, (**b**) unstably atropisomeric, and (**c**) stably atropisomeric. The stable atropisomers can be separated and treated as two individual compounds, as in the case of enantiomers. The utility of this calculation is found in the prediction and validation of atropisomerism during the hit-to-lead (Chapter 2, Section 2.1.2.3.5) and lead modification (Chapter 2, Section 2.2) stages of drug discovery. Telenzepine (**3.57**, Figure 3.31), an anticholinergic compound, is atropisomeric, and the enantiomers have been resolved; the (+)-isomer is

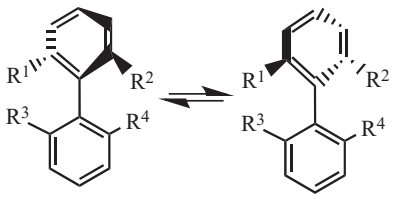


FIGURE 3.29 General example of atropisomerization

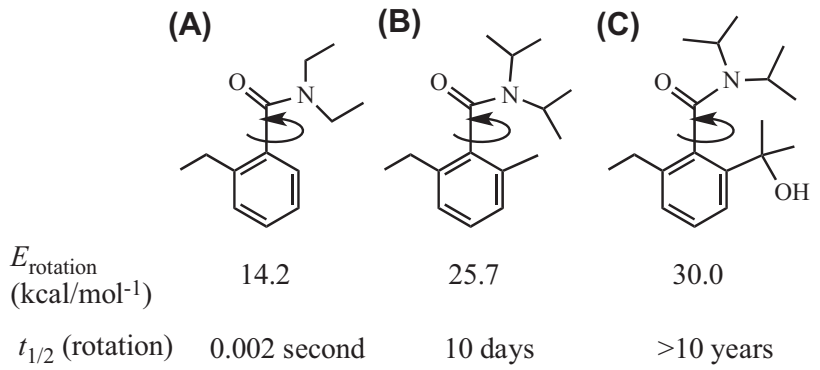
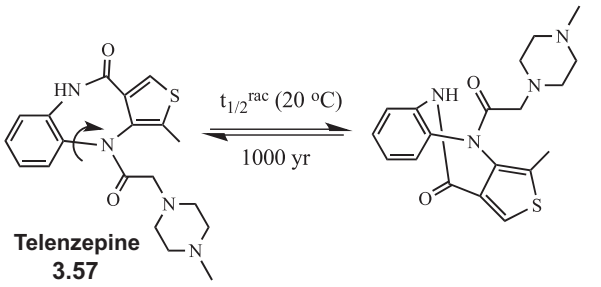


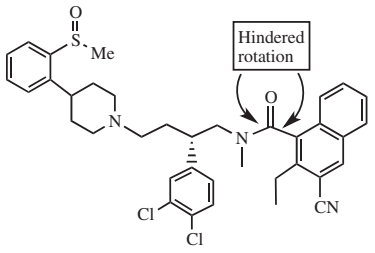
FIGURE 3.30 Example of a nonatropisomer, an unstable atropisomer, and a stable atropisomer



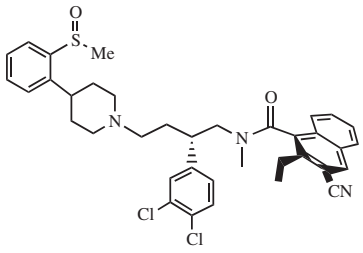
Telenzepine
3.57

FIGURE 3.31 Exceedingly slow isomerization of atropisomers of telenzepine (**3.57**)

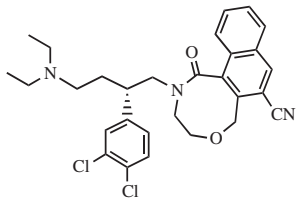
atropisomeric or nonatropisomeric. For example, a neurokinin 1 (NK_1) antagonist (**3.58**) was identified at AstraZeneca for the treatment of depression, but it was shown to be a composite of four atropisomers because of restriction about two amide single bonds.[120] The active atropisomer had conformation **3.59**. A conformationally rigid analog of **3.59** was designed (**3.60**), which, unfortunately, was found to exist as two diastereomeric atropisomers, but **3.61** existed as a single conformer (the additional methyl group hinders rotation), which had excellent potency in vitro and in vivo.



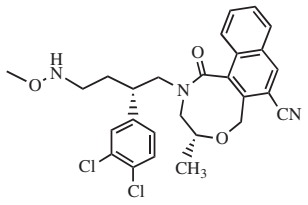
3.58



3.59



3.60

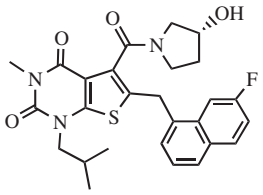


3.61

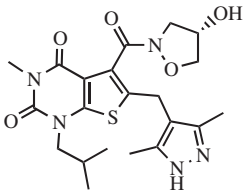
500 times more potent than the (−)-isomer at muscarinic receptors.

It is important to recognize when your compounds are potentially atropisomeric so you can either be sure that they are chiral or not and can ascertain how stable they are. If they are in the pseudo-atropisomer regime, you may need modification to make them either stably

Another way to deal with atropisomerism is to engineer the molecule so that it has faster bond rotation, making the conformers interconvertible. Compound **3.62** was an effective monocarboxylate transporter 1 antagonist having immunomodulatory activity but existed in four atropisomeric forms.[121] Modification to **3.63** allowed all conformers to readily interconvert.

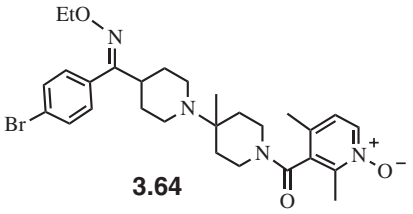


3.62

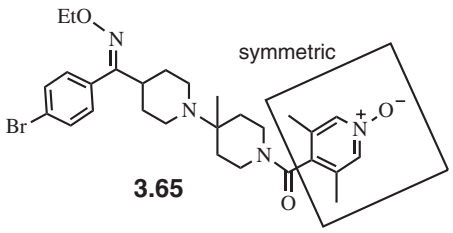


3.63

A third way to avoid atropisomerism is by symmetrization. A group at Schering-Plough (now Merck) was interested in developing a C–C chemokine receptor type 5 (CCR5) antagonist, which inhibits human immunodeficiency virus (HIV) entry into host cells.[122] A clinical candidate (**3.64**, SCH 351125) reduced levels of HIV-1 RNA in infected patients, but it existed in four atropisomeric forms (a pair of diastereomeric enantiomers). Symmetric isomer **3.65** eliminated the two diastereomeric conformers, and the two remaining enantiomeric conformers were rapidly interconverted.[123]



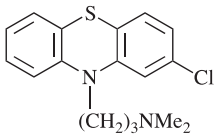
3.64



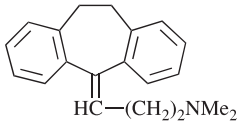
3.65

3.2.5.6. *Ring Topology*

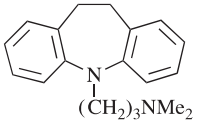
Tricyclic psychomimetic drugs show an almost continuous transition of activity in going from structures such as the tranquilizer chlorpromazine (**3.66**, Thorazine) through the antidepressant amitriptyline (**3.67**, Elavil), which has a tranquilizing side effect, to the pure antidepressant agent imipramine (**3.68**, Tofranil).[124] Stereoelectronic effects seem to be the key factor, even though tranquilizers and antidepressants have different molecular mechanisms. Three angles can be drawn to define the positions of the two aromatic rings in these compounds (Figure 3.32). The angle α (**3.69**) describes the bending of the ring planes; β (**3.70**) is the annellation angle of the ring axes that passes through carbon 1 and 4 of each aromatic ring; γ (**3.71**) is the torsional angle of the aromatic rings as viewed from the side of the molecule. In general, the tranquilizers have essentially only a bending angle α and little or no β and γ angles. The mixed tranquilizer–antidepressants have both bending (α) and annellation angles (β), but no γ angle. The pure antidepressants exhibit all three angles. The activities arise from the binding of the compounds to different receptors; these angles determine the overall three-dimensional structure of the pharmacophoric groups of the compound, which dictate the binding affinities for various receptors.



Chlorpromazine
3.66



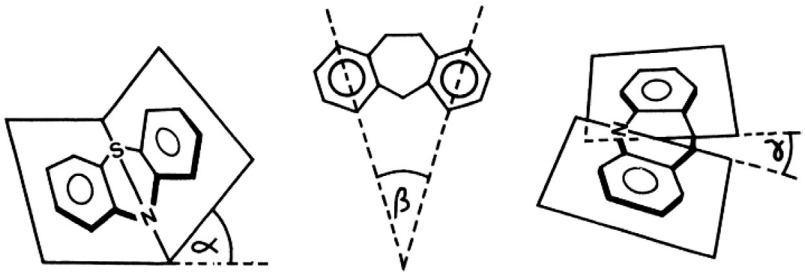
Amitriptyline
3.67



Imipramine
3.68

3.2.6. Case History of the Pharmacodynamically Driven Design of a Receptor Antagonist: Cimetidine

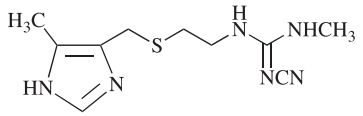
There are many drugs on the market that were discovered by rational design using the application of physical organic chemical principles. The antiulcer drug cimetidine (**3.72**, Tagamet) is a truly elegant early example of a pharmacodynamically driven approach in drug discovery, utilizing various lead modification methods discussed in Chapter 2, to uncover the first histamine H_2 receptor antagonist and an entirely new class of drugs. Cimetidine is one of the first drugs discovered by a rational approach, thanks to the valiant efforts of medicinal chemists C. Robin Ganellin and Graham Durant and pharmacologist James Black at Smith, Kline, & French Laboratories (now GlaxoSmithKline; Sir James W. Black shared the 1988 Nobel Prize in Physiology or Medicine for the discovery of propranolol and is also credited for the discovery of this drug; actually, the medicinal chemists would have made the discovery). This is a case, however, where neither QSAR nor molecular graphics approaches were utilized. As described in Section 3.2.5.1, histamine binds to the H_1 receptor and causes allergic and hypersensitivity reactions, which antihistamines antagonize. Black found that another action of histamine is the stimulation of gastric acid secretion.[125] However, antihistamines have no effect on this activity; consequently, it was suggested that there was a



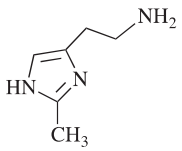
3.69 3.70 3.71

FIGURE 3.32 Ring topology of tricyclic psychomimetic drugs. *Reproduced with permission from Nogrady, T. (1985). In "*Medicinal Chemistry: A Biochemical Approach*," p. 29. Oxford University Press, New York. By permission of Oxford University Press, USA.*

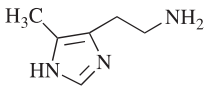
second histamine receptor, which was termed the *H_2 receptor*. The H_1 and H_2 receptors can be differentiated by agonists and antagonists. 2-Methylhistamine (**3.73**) preferentially elicits H_1 receptor responses, and 4-methylhistamine (**3.74**) has the corresponding preferential effect on H_2 receptors. An antagonist of the histamine H_2 receptor would be beneficial for the treatment of hypersecretory conditions such as duodenal and gastric ulcers (peptic ulcers). Consequently, in 1964, Smith, Kline & French Laboratories in England initiated a search for a lead compound that would antagonize the H_2 receptor.[126] Actually, now there are four different histamine receptors known, each one responsible for a different physiological function.[127] The critically important challenge in drug design is to get selectivity of action of molecules.



Cimetidine
3.72



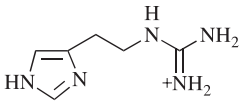
3.73



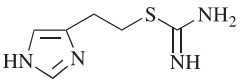
3.74

The first requirement for initiation of a lead discovery program for the H_2 receptor is an efficient bioassay (screen). Unfortunately, there were no high-throughput screens at that time. In fact, no in vitro screen was possible, so a tedious in vivo screen was developed: histamine was infused into anesthetized rats to stimulate gastric acid secretion, the stomach was perfused, and then the pH of the perfusate from the lumen of the stomach was measured before and after administration of the compound. Needless to say, this is a highly time-consuming and variable assay.

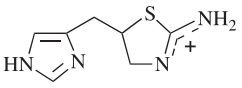
The lead discovery approach that was taken involved the use of the endogenous ligand of the receptor as the lead, as described in Chapter 2 (Section 2.1.2.1). Histamine analogs were synthesized on the assumption that the receptor would recognize that general backbone structure. However, the structure had to be sufficiently different so as not to stimulate a response (i.e., act as an agonist) and defeat the purpose. After 4 years, none of the 200 or so compounds made showed any H_2 receptor antagonistic activity. Then a new, more sensitive assay was developed, and some of the same compounds were retested, which identified the first lead compound, N^{α}-guanylhistamine (**3.75**). This compound was only very weakly active as an inhibitor of histamine stimulation; later it was determined to be a partial agonist, not an antagonist. An isostere, isothiourea **3.76**, was made, which was found to be more potent. The corresponding conformationally rigid analog **3.77** (a ring–chain transformation), however, was less potent than **3.76**; consequently, it was thought that flexibility in the side chain was important. Many additional compounds were synthesized, but they acted as partial agonists. They could block histamine binding, but they could not fully prevent acid secretion.



3.75



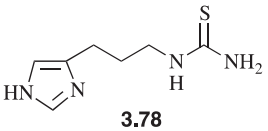
3.76



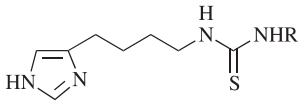
3.77

It, therefore, became necessary to separate the agonist and antagonist activities. The reason for their agonistic activity, apparently, was their structural similarity to histamine. Not only were these compounds imidazoles, but at physiological pH, the side chains were protonated and positively charged, just like histamine. Consequently, it was reasoned that the imidazole ring should be retained for receptor recognition, but the side chain could be modified to eliminate the positive charge. After numerous substitutions, the

neutral thiourea analog (**3.78**) was prepared, having weak antagonistic activity without stimulatory activity. Homologation of the side chain gave a purely competitive antagonist (**3.79**, R = H); no agonist effects were observed. Methylation



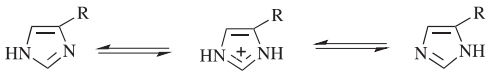
3.78



3.79

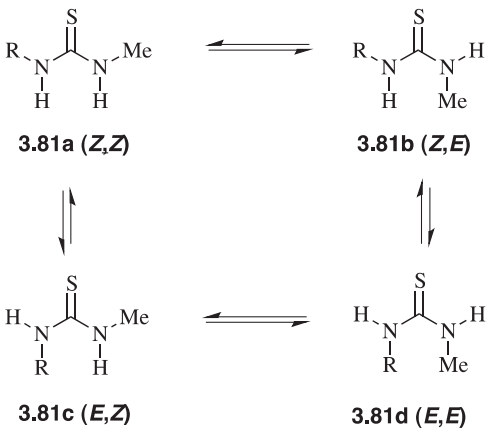
and further homologation on the thiourea nitrogen were carried out; the *N*-methyl analog (**3.79**, R = CH_3) called burimamide, was found to be highly specific as a competitive antagonist of histamine at the H_2 receptor. It was shown to be moderately effective in the inhibition of histamine-stimulated gastric acid secretion in rat, cat, dog, and man. Burimamide was the first H_2 receptor antagonist tested in humans,[128] but it lacked adequate oral activity, so the search for analogs with improved activity continued.

The poor oral potency of burimamide could be a pharmacokinetic problem (poor ability for the drug to reach its target) or a pharmacodynamic problem (suboptimal interaction of the drug with the target). The Smith, Kline, and French group decided to consider the latter. In aqueous solution at physiological pH, the imidazole ring can exist in three main forms (**3.80a**–**3.80c**, Figure 3.33; R is the rest of burimamide). The thioureido group can exist as four conformers (**3.81a**–**3.81d**, Figure 3.34; R is the remainder of burimamide). The side chain can exist in a myriad of conformers. Therefore, it is possible that only a very small fraction of the molecules in equilibrium would



3.80a **3.80b** **3.80c**

FIGURE 3.33 Three principal forms of 5-substituted imidazoles at physiological pH



3.81a (*Z*,*Z*) **3.81b (*Z*,*E*)**

3.81c (*E*,*Z*) **3.81d (*E*,*E*)**

FIGURE 3.34 Four conformers of the thioureido group

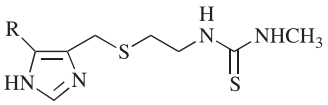
have the bioactive conformation, and this could account for the low potency.

One approach taken to increase the potency of burimamide was to compare the population of the imidazole form in burimamide at physiological pH to that in histamine.[129] The population can be estimated from the electronic influence of the side chain, which alters the electron densities at the ring nitrogen atoms, and, therefore, affects the proton acidity. This effect is more important at the nearer nitrogen atom, so if R is electron donating, it would make the adjacent nitrogen more basic, and **3.80c** (Figure 3.33) should predominate; if R is electron withdrawing, it would make the adjacent nitrogen less basic, and **3.80a** should be favored. The fraction present as **3.80b** can be determined from the ring pK_a and the pH of the solution. The electronic effect of R can be calculated from the measured ring pK_a with the use of the Hammett equation (Eqn (3.3)), where pK_a^R is the pK_a of the substituted imidazole, pK_a^H is that of

$$pK_a^R = pK_a^H + \rho\sigma_m \qquad (3.3)$$

imidazole (R = H), σ_m is the meta electronic substituent constant, and ρ is the reaction constant (see Chapter 2, Section 2.2.5.1). Imidazole has a pK_a of 6.80, and at physiological temperature and pH, 20% of the molecules are in the protonated form. The imidazole in histamine, under these conditions, has a pK_a of 5.90. This indicates that the side chain in histamine is electron withdrawing, thus favoring tautomer **3.80a** (to the extent of 80%), and only 3% of the molecules are in the cationic form (**3.80b**). The pK_a of the imidazole in burimamide, however, is 7.25, indicating an electron-donating side chain, which favors tautomer **3.80c**. The cation is one of the principal species, about 40% of the molecules. Therefore, even though the side chains in histamine and burimamide appear to be similar, they have opposite electronic effects on the imidazole ring. On the assumption that the desired form of the imidazole should resemble that in histamine, the Smith, Kline & French group decided to increase the electron-withdrawing effect of the side chain of burimamide; however, they did not want to make a major structural modification. Incorporation of an electron-withdrawing atom into the side chain near the imidazole ring was contemplated, and the isosteric replacement of a methylene by a sulfur atom to give thiaburimamide (**3.82**, R = H) was carried out. A comparison of the physical properties of the two compounds (**3.79**, R = CH_3 and **3.82**, R = H) shows that they have similar van der Waals radii and bond angles, although the C–S bond is slightly longer than the C–C bond and is more flexible. A sulfur atom is also slightly more hydrophilic than a methylene group; the log *P* for thiaburimamide is 0.16 and for burimamide is 0.39. The pK_a of the imidazole in thiaburimamide was determined to be 6.25, indicating that the electron-withdrawing effect of the side chain increased, and more of the favored tautomeric form was the same as that in histamine (**3.80a**).

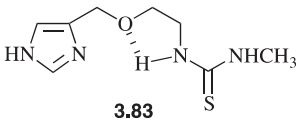
Thiaburimamide is about three times more potent as a histamine H_2 receptor antagonist in vitro than burimamide.



3.82

A second way to increase the population of tautomer **3.80a** would be to introduce an electron-donating substituent at the 4-position of the ring, because electron-donating groups increase the basicity of the adjacent nitrogen, which is chemically equivalent to putting an electron-withdrawing group at the side chain position in thiaburimamide. Because 4-methylhistamine (**3.74**) is a known H_2 receptor agonist, there should be no steric problem with a 4-methyl group. However, the addition of an electron-donating group should increase the pK_a of the ring, thereby increasing the population of the cation (**3.80b**). Although the increase in tautomer **3.80a** is somewhat offset by the decrease in the total uncharged population, the overall effect was favorable. Metiamide (**3.82**, R = CH_3) has a pK_a identical to that of imidazole, indicating that the effect of the electron-withdrawing side chain exactly balanced the effect of the electron-donating 4-methyl group; the percentage of molecules in the charged form was 20%. The important result, however, is that metiamide is eight to nine times more potent than burimamide.

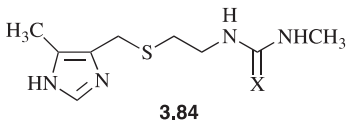
You would think that the tautomeric form would be shifted even more favorably toward **3.80a** by substitution of the side chain with a more electronegative oxygen atom instead of a sulfur atom. Theoretically, that should be the case. This compound, oxaburimamide, was synthesized, but it was *less* potent than burimamide! The explanation for this unexpected result is that intramolecular hydrogen bonding between the oxygen atom and the thiourea NH produces an unfavorable *conformationally restricted analog* (**3.83**). This is one of the problems associated with isosteric replacements; although CH_2, NH, O, and S can have similar biological activity, NH and O can participate in intramolecular (and intermolecular) hydrogen bonding, which changes the shape of the compound and may disfavor (although in other cases, it may favor) the bioactive conformation.



3.83

Metiamide was tested on 700 patients with duodenal ulcers and was found to produce a significant increase in the healing rate with marked symptomatic relief. However, a few cases of granulocytopenia (reduction of the number of white blood cells in the blood) developed. Even though this was a reversible side effect, it was undesirable (compromises the immune system), and it halted further clinical work with this compound.

The Smith, Kline & French group conjectured that the granulocytopenia associated with metiamide was caused by the thiourea group; consequently, alternative substituents were sought. An isosteric replacement approach was taken. The corresponding urea (**3.84**, X = O) and guanidino (**3.84**, X = NH) analogs were synthesized and found to be 20 times less potent than metiamide. Of course, the guanidino analog would be positively charged at physiological pH, and that could be the cause of the lower potency. Charton[130] found a Hammett relationship between the σ and pK_a values for *N*-substituted guanidines; consequently, if guanidino basicity were the problem, then substitution of the guanidino nitrogen with electron-withdrawing groups could lower the pK_a. In fact, cyanoguanidine and nitroguanidine have pK_a values of −0.4 and −0.9, respectively (compared with −1.2 for thiourea), a drop of about 14 pK_a units from that of guanidine. The corresponding cyanoguanidine (**3.84**, X = N–CN; cimetidine, Tagamet) and nitroguanidine (**3.84**, X = N–NO_2) were synthesized in 1972, and both were potent H_2 antagonists, comparable in potency to metiamide, but without the granulocytopenia (cimetidine was slightly more potent than **3.84**, X = NNO_2).



3.84

Because strong electron-withdrawing substituents on the guanidino group favor the imino tautomer, the cyanoguanidino and nitroguanidino groups correspond to the thiourea structure (**3.84**, X = NCN, NNO_2, and S, respectively). These three groups are actually bioisosteres; they are all planar structures of similar geometries, are weakly amphoteric (weakly basic and acidic); being unionized in the pH range 4–11, are very polar; and are hydrophilic. The crystal structures of metiamide (**3.82**, R = CH_3) and cimetidine (**3.84**, X = NCN) are almost identical. The major difference in the two groups is that whereas *N,N'*-disubstituted thioureas assume three stable conformers (see Figure 3.34; *Z,Z, Z,E,* and *E,Z*), *N,N'*-disubstituted cyanoguanidines appear to assume only two stable conformers (*Z,E* and *E,Z*). This suggests that the most stable conformer of metiamide, the *Z,Z* conformer, is not the bioactive conformation. An isocytosine analog (**3.85**) also was prepared (pK_a 4.0), which can exist only in the *Z,Z* and *E,Z* conformations. It was only about one-sixth as potent as cimetidine. However, the isocytosino group has a lower log *P* (more hydrophilic) than that of the *N*-methylcyanoguanidino group, and it was thought that lipophilicity may be an important physicochemical parameter. There was, indeed, a correlation found between the H_2 receptor antagonist activity in vitro and the octanol–water partition coefficient of the corresponding

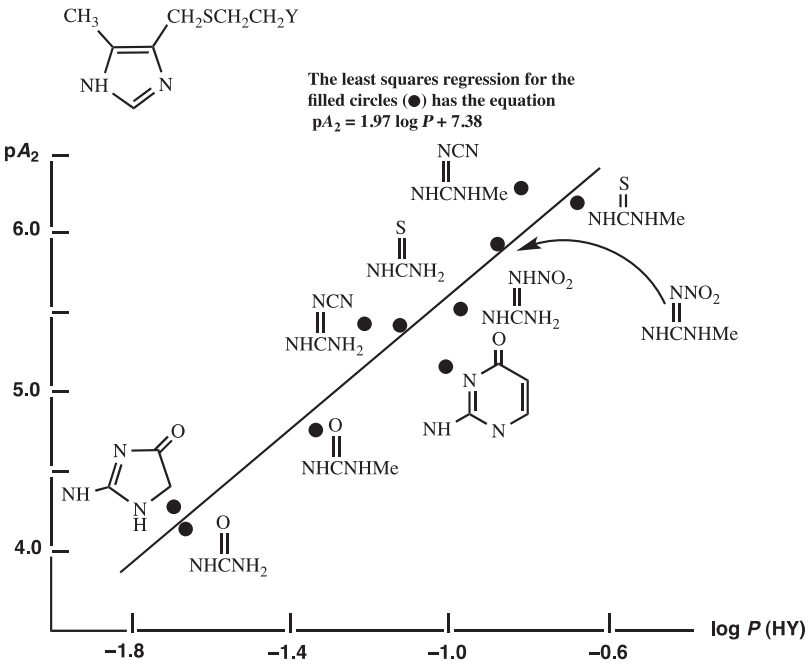
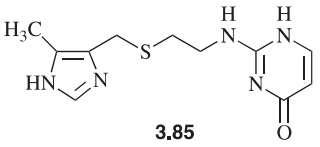


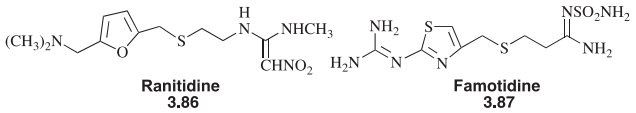
FIGURE 3.35 Linear free energy relationship between H_2 receptor antagonist activity (pA_2) and the partition coefficient. *Reprinted with Permission of Elsevier. This article was published in Pharmacology of Histamine Receptors, Ganellin, C. R., and Parsons, M. E. (1982), p. 83, Wright-PSG, Bristol.*

acid of the substituent Y (Figure 3.35). Although increased potency correlates with increased lipophilicity, all these compounds are fairly hydrophilic. Because the correlation was determined in an in vitro assay, membrane transport is not a concern; consequently, these results probably reflect a property involved with receptor interaction, not with transport. Therefore, it is not clear if the lower potency of the isocytosine analog is structure or hydrophilicity dependent.



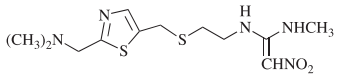
3.85

Cimetidine was first marketed in the United Kingdom in 1976; therefore, it took only 12 years from initiation of the H_2 receptor antagonist program to commercialization. Subsequent to the introduction of cimetidine onto the US drug market, three other H_2 receptor antagonists were approved, ranitidine (**3.86**, Zantac, Glaxo Laboratories), which rapidly became the largest selling drug worldwide, famotidine (**3.87**, Pepcid, Yamanouchi/Merck), and nizatidine (**3.88**, Axid, Eli Lilly); the only difference in structure between ranitidine and nizatidine is the heterocyclic ring incorporated. It is apparent that an imidazole ring is not essential for H_2 receptor recognition and that a positive charge near the heterocyclic ring (the Me_2N– of **3.86** and **3.88** and the guanidino group of **3.87** will be protonated at physiological pH) is not unfavorable.



Ranitidine
3.86

Famotidine
3.87



Nizatidine
3.88

Cimetidine became the first drug ever to achieve more than $1 billion a year in sales, thereby having the distinction of being the first *blockbuster drug*. The discovery of cimetidine is one of the many examples now of how the judicious use of physical organic chemistry can result in lead discovery, if not in drug discovery.

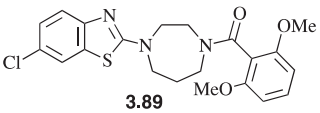
One approach for combination therapy with H_2 receptor antagonists stems from the discovery that a bacterium, *Helicobacter pylori*, is found in the stomach and is associated with peptic ulcers.[131] The organism protects itself from the acid in the stomach partly because it lives within the layer of mucus that the stomach secretes to protect itself against the acid and partly because the bacterium produces the enzyme urease, which converts urea in the blood into ammonia to neutralize the acid.[132] This bacterium was discovered in 1983 by Drs Barry J. Marshall and J. Robin Warren at the Royal Perth Hospital in Australia, who were trying to grow mysterious cells taken from the stomach. The culture was left much longer to grow than

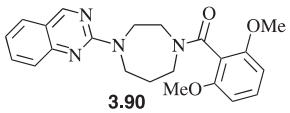
normal because of the four-day Easter weekend that year, and upon their return, they noticed the growth of a bacterium with spiral, helix-shaped cells, which they called *Helicobacter*. Although it took more than a decade to convince others that this bacterium was really living in the stomach and that could cause ulcers, it is now widely accepted. In fact, Marshall and Warren jointly received the 2005 Nobel Prize in Physiology or Medicine for this discovery. Because there are many people who have this bacterium, but do not have ulcers, there must be additional factors, such as stress, that are needed for ulcer formation.[133] Treatment with antibacterial agents can kill these bacteria, but generally other drugs that can lower stomach acid are needed in combination.[134]

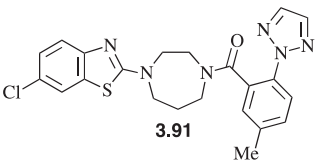
3.2.7. Case History of the Pharmacokinetically Driven Design of Suvorexant

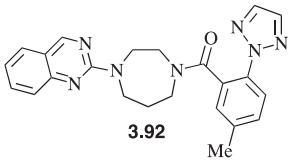
Over 50% of American adults polled reported at least one symptom of insomnia in the previous year, and one-third reported symptoms almost every night;[135] about one-fourth of American adults take sleep medication.[136] Orexins A and B are neuropeptides that have been shown to affect the sleep/wake cycle by binding to the orphan G protein-coupled receptors orexin receptor 1 (OX_1R) and orexin receptor 2 (OX_2R).[137] Mice deficient in these neuropeptides exhibit excessive sleepiness;[138] intracerebroventricular infusion of these neuropeptides into rat cerebrospinal fluid leads to an increase in wakefulness.[139] Therefore, an antagonist of these receptors could be an important new mechanism for the treatment of insomnia.

A high-throughput screen of the Merck sample collection revealed four active scaffolds, including *N,N*-disubstituted 1,4-diazepanes, such as **3.89**, having IC_{50} values for OX_1R and OX_2R of 630 and 98 nM, respectively.[140] The western heterocycle was optimized to **3.90**, and the eastern heterocycle to **3.91**; the composite structure (**3.92**) had IC_{50} values for OX_1R and OX_2R of 29 and 27 nM, respectively. Compound **3.92** promoted sleep in rats when orally dosed at 100 mg/kg. The problem with **3.92**, however, was its low oral bioavailability (16% in dogs; 2% in rats) and rapid oxidative metabolism (see Chapter 8) of all three rings. The most detrimental oxidation was that of the 1,4-diazepane ring adjacent to the eastern nitrogen (**3.93**, Scheme 3.3).

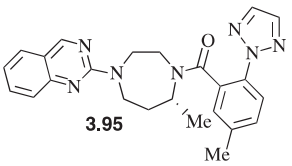


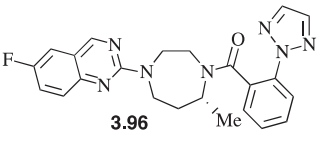


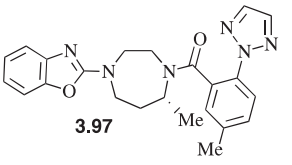


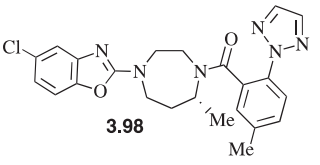


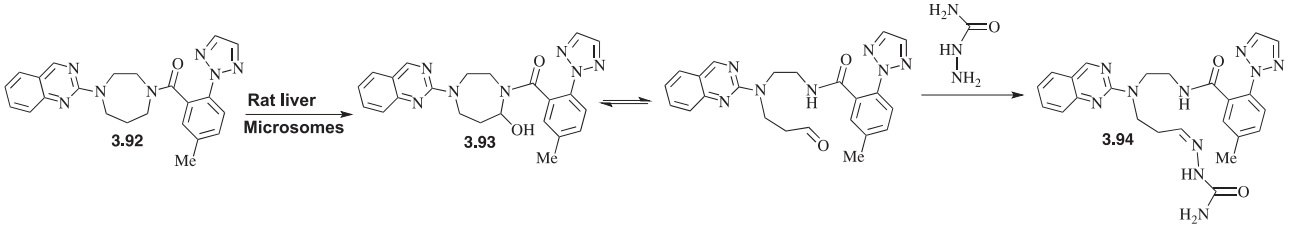
This is in equilibrium with the corresponding aldehyde, which was trapped by added semicarbazide to detect its presence (**3.94**), indicating its potential as an undesirable reactive metabolite. It was found that an effective way to block that metabolism was by methylation, and the (*R*)-antipode had superior potency (**3.95**). Although the clearance rate decreased, the oral bioavailability decreased until the western heterocycle was metabolically protected with a fluorine and the methyl group on the eastern heterocycle was removed (**3.96**), leading to comparable potency as **3.92** and an oral bioavailability of 37% in dogs, which is still only moderate. To further decrease the clearance rate and increase the potency and oral bioavailability, several additional modifications were made to the western heterocycle on the basis of earlier studies in dog, which identified benzoxazole as having reduced clearance properties. Installation of the unsubstituted benzoxazole gave **3.97**, which had the lowest clearance rate to that date, but the potency dropped 20-fold. From their SAR, they knew that increased lipophilicity on that heterocycle was highly beneficial for potency, and addition of a chlorine atom (**3.98**, suvorexant) increased the potency 10-fold with excellent brain penetration and an oral bioavailability of 56% in dogs. Suvorexant has completed Phase III clinical trials.





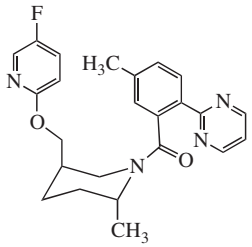






SCHEME 3.3 Oxidative metabolism of the 1,4-diazepane ring of **3.92**

A backup compound for suvorexant was designed from an understanding of the conformational properties believed to favor high orexin receptor binding. Conformational studies with 1,4-diazepane carboxamides were the rationale for the design of molecules with a piperidine core that could permit intramolecular aryl–aryl interactions. Methylation of the piperidine core adjacent to the amide nitrogen atom gave analogs with a piperidine in the chair conformation having a 2,5-*trans*-diaxial conformation (**3.99**), which promotes the desired aryl–aryl interaction. This compound was found to be more potent than suvorexant in vivo and is in Phase II clinical trials.



3.99

Next, we turn our attention to a special class of receptors called enzymes, which also are very important targets for drug design.

3.3. GENERAL REFERENCES

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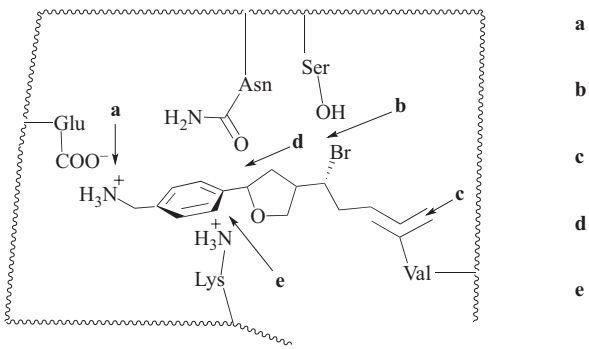
3.4. PROBLEMS (ANSWERS CAN BE FOUND IN THE APPENDIX AT THE END OF THE BOOK)

1. Indicate what drug–receptor interactions are involved at every arrow shown. More than one kind of interaction is possible for each letter.



2. A receptor has lysine and histidine residues important to binding, which do not interact with each other.

The pK_a of the lysine residue is 6.4 (pK_a in solution is 10.5), and the pK_a of the histidine residue is 9.4 (pK_a in solution is 6.5). On the basis of the discussion in Chapter 2 about pK_a variabilities as a result of the environment, what can you say about possible other residues in the binding site to rationalize these observations.

3. Draw a dose–response curve for:
- **a.** a full agonist
- **b.** a mixture of a full agonist and a competitive antagonist

4. Draw dose–response curves (place on same plot) for a series of three compounds with the following properties:

	K_d (*M*)	α
1	10^{-6}	1.0
2	10^{-9}	0.8
3	10^{-9}	0.4

5. A series of dopamine analogs was synthesized and assayed for their effect on the D_2 dopamine receptor. The results are shown in Table 3.2.

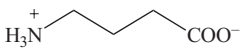
TABLE 3.2 Effect of dopamine analogs on D_2 dopamine receptor

Compound	K_d (nM)	% Change in Basal Activity	% Change When 100 μM Dopamine Added
1	180	−12.1	−6.2
2	37	0.1	0.8
3	0.46	19	19
4	14.9	12.2	18.4
Dopamine	1.9	19	

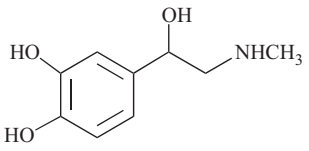
- **a.** Compare the affinities of **1–4** to that of dopamine.
- **b.** Compare the efficacies of **1–4** to dopamine.
- **c.** What type of effect is produced by **1–4**?

6.
- **a.** What problems are associated with administration of racemates?
- **b.** How can you increase the eudismic ratio?

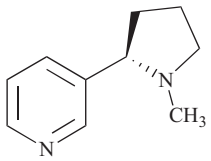
7. Design conformationally-rigid analogs for:
- **a.** 4-aminobutyric acid (GABA)



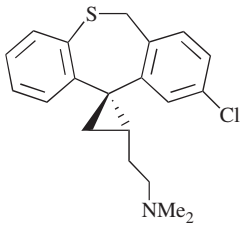
- **b.** Epinephrine



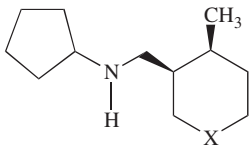
- **c.** Nicotine



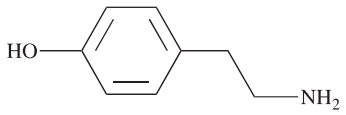
8. On the basis of generalizations about ring topology discussed in the chapter, would you expect the compound below to be a tranquilizer, have both antidepressant and tranquilizing properties, or be an antidepressant agent? Why?



9. An isosteric series of compounds shown below, where X=CH_2, NH, O, S, was synthesized. The order of potency was X=NH>O>S>CH_2. How can you rationalize these results (you need to consider the three-dimensional structure)?

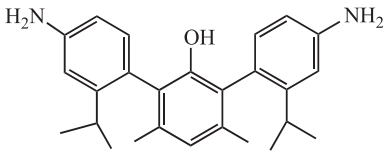


10. Tyramine binds to a receptor that triggers the release of norepinephrine, which can raise the blood pressure. If the tyramine receptor was isolated, and you wanted to design a new antihypertensive agent, discuss what you would do in terms of lead discovery and modification.



Tyramine

11. A receptor was isolated, a crystal structure was obtained with the natural ligand bound, and it was found that the binding site displayed C_2 symmetry. Computer modeling was done, and a C_2 symmetric antagonist (**5**) was designed. However, it exhibited very low potency. What could be the problem? Show it.



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