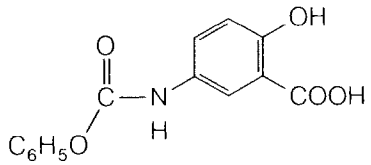
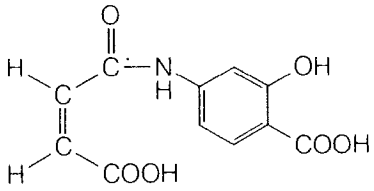
has supported this concept. Compounds studied possess appropriate structural features for reversible and highly selective association with an enzyme. If, in addition, the compounds carry reactive groups capable of forming covalent bonds, the substrate may be irreversibly bound to the drug–receptor complex by covalent bond formation with reactive groups adjacent to the active site. In studies with reversibly binding antimetabolites that carried additional alkylating and acylating groups of varying reactivities, selective irreversible binding by the related enzymes, lactic dehydrogenase and glutamic dehydrogenase, has been demonstrated. The selectivity of response has been attributed to the formation of a covalent bond between the carbophenoxyamino substituent of 5-(carbophenoxyamino)salicylic acid and a primary amino group in glutamic dehydrogenase[18] and between the maleamyl substituent of 4-(maleamyl)salicylic acid and a sulfhydryl group in lactic dehydrogenase.[19] Assignments of covalent bond formation with specific groups in the enzymes are based on the fact that the α,β-unsaturated carbonyl system of maleamyl groups reacts most rapidly with sulfhydryl groups, much more slowly with amino groups, and extremely slowly with hydroxyl groups. In contrast, the carbophenoxy group will react only with a primary amino group on a protein. The diuretic drug, ethacrynic acid (see Chap. 13), is an α,β-unsaturated ketone, thought to act by covalent bond formation with sulfhydryl groups of ion transport systems in the renal tubules.



5-(Carbophenoxyamino)salicylic Acid



4-(Maleamyl)salicylic Acid

Other examples of covalent bond formation between drug and biologic receptor site include the reaction of arsenicals and mercurials with essential sulfhydryl groups, the acylation of bacterial cell wall constituents by penicillin, and the inhibition of cholinesterase by the organic phosphates.

It is desirable that most drug effects be reversible. For this to occur, relatively weak forces must be involved in the drug–receptor complex, yet be strong enough that other binding sites will not competitively deplete the site of action. Compounds with a high degree of structural specificity may orient several weak-binding groups, such that the summation of their interactions with specifically oriented complementary groups on the receptor will provide a total bond strength sufficient for a stable combination.

Consequently, for drugs acting by virtue of their structural specificity, binding to the receptor site will be carried out by hydrogen bonds, ionic bonds, ion–dipole and dipole–dipole interactions, van der Waals and hydrophobic forces. Ionization at physiologic *p*H would normally occur with the carboxyl, sulfonamido, and aliphatic amino groups, as well as the quaternary ammonium group at any *p*H. These sources of potential ionic bonds are frequently found in active drugs. Differences in electronegativity between carbon and other atoms, such as oxygen and nitrogen, lead to an unsymmetric distribution of electrons (dipoles) that are also capable of forming weak bonds with regions of high or low electron density, such as ions or other dipoles. Carbonyl, ester, amide, ether, nitrile, and related groups that contain such dipolar functions are frequently found in equivalent locations in structurally specific drugs. Many examples may be found among the potent analgesics, the cholinergic-blocking agents, and local anesthetics.

The relative importance of the *hydrogen bond* in the formation of a drug–receptor complex is difficult to assess. Many drugs possess groups, such as carbonyl, hydroxyl, amino, and imino, with the structural capabilities of acting as acceptors or donors in the formation of hydrogen bonds. However, such groups would usually be solvated by water, as would the corresponding groups on a biologic receptor. Relatively little net change in free energy would be expected in exchanging a hydrogen bond with a water molecule for one between drug and receptor. However, in a drug–receptor combination, several forces could be involved, including the hydrogen bond, which would contribute to the stability of the interaction. Where multiple hydrogen bonds may be formed, the total effect may be sizeable, such as that demonstrated by the stability of the protein α-helix, and by the stabilizing influence of hydrogen bonds between specific base pairs in the double helical structure of DNA.

van der Waals forces are attractive forces created by the polarizability of molecules and are exerted when any two uncharged atoms approach very closely. Their strength is inversely proportional to

the seventh power of the distance. Although individually weak, the summation of their forces provides a significant bonding factor in higher molecular weight compounds. For example, it is not possible to distill normal alkanes with more than 80 carbon atoms, because the energy of about 80 kcal/mol required to separate the molecules is approximately equal to the energy required to break a carbon–carbon covalent bond. Flat structures, such as aromatic rings, permit close approach of atoms. With van der Waals force approximately 0.5 to 1.0 kcal/mol for each atom, about six carbons (a benzene ring) would be necessary to match the strength of a hydrogen bond. The aromatic ring is frequently found in active drugs, and a reasonable explanation for its requirement for many types of biologic activity may be derived from the contributions of this flat surface to van der Waals binding to a correspondingly flat receptor area.

The *hydrophobic bond* is a concept used to explain attractive interactions between nonpolar regions of the receptor and the drug. Explanations such as the "isopropyl moiety of the drug fits into a hydrophobic cleft on the receptor composed of the hydrocarbon side chains of the amino acids valine, isoleucine, and leucine" are commonly used to explain why a nonpolar substituent at a particular position on the drug molecule is important for activity. Over the years, the concept of hydrophobic bonds has developed. There has been considerable controversy over whether or not the bond actually exists. Thermodynamic arguments on the gain in entropy (decrease in ordered state) when hydrophobic groups cause a partial collapse of the ordered water structure on the surface of the receptor have been proposed to validate a hydrophobic bonding model. There are two problems with this concept. First, the term *hydrophobic* implies repulsion. The term for attraction is *hydrophilicity*. Second and, perhaps, more important, there is no truly water-free region on the receptor. This is true, even in the areas populated by the nonpolar side amino acid side chains. An alternate approach is to consider only the concept of hydrophilicity and lipophilicity. The predominating water molecules solvate polar moieties, effectively squeezing the nonpolar residues toward each other.

Isosterism

The term *isosterism* has been widely used to describe the selection of structural components, the steric, electronic, and solubility characteristics of which make them interchangeable in drugs of the same pharmacologic class. The concept of isosterism has evolved and changed significantly in the years since its introduction by Langmuir[20] in 1919. Langmuir, while seeking a correlation that would explain similarities in physical properties for nonisomeric molecules, defined *isosteres* as compounds or groups of atoms having the same number and arrangement of electrons. Those isosteres that were isoelectric (i.e., with the same total charge as well as same number of electrons) would possess similar physical properties. For example, the molecules N_2 and CO, both possessing 14 total electrons and no charge, show similar physical properties. Related examples described by Langmuir were CO_2 and N_2O, and N_3^- and NCO^-.

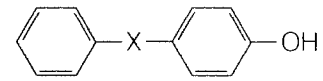
With increased understanding of the structures of molecules, less emphasis has been placed on the number of electrons involved, for variations in hybridization during bond formation may lead to considerable differences in the angles, the lengths, and the polarities of bonds formed by atoms with the same number of peripheral electrons. Even the same atom may vary widely in its structural and electronic characteristics when it forms a part of a different functional group. Thus, nitrogen is part of a planar structure in the nitro group, but forms the apex of a pyramidal structure in ammonia and the amines.

Groups of atoms that impart similar physical or chemical properties to a molecule, because of similarities in size, electronegativity, or stereochemistry, are now frequently referred to under the general term of *isostere*. The early recognition that benzene and thiophene were alike in many of their properties led to the term "ring equivalents" for the vinylene group (—CH=CH—) and divalent sulfur (—S—). This concept has led to replacement of the sulfur atom in the phenothiazine ring system of tranquilizing agents with the vinylene group to produce the dibenzazepine class of antidepressant drugs (see Chap. 10). The vinylene group in an aromatic ring system may be replaced by other atoms isosteric to sulfur, such as oxygen (furan) or NH (pyrrole); however, in such cases, aromatic character is significantly decreased.

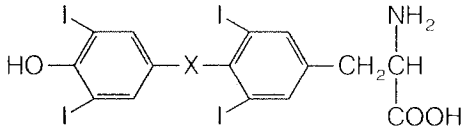
Examples of isosteric pairs that possess similar steric and electronic configurations are the carboxylate (COO^-) and sulfonamido (SO_2NR^-) ions, ketone (CO) and sulfone (SO_2) groups, chloride (Cl) and trifluoromethyl (CF_3) groups. Divalent ether (—O—), sulfide (—S—), amine (—NH—), and methylene ($—CH_2—$) groups, although dissimilar electronically, are sufficiently alike in their steric nature to be frequently interchangeable in drugs.

Compounds may be altered by isosteric replacements of atoms or groups, to develop analogues with select biologic effects, or to act as antagonists to normal metabolites. Each series of compounds

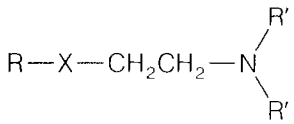
showing a specific biologic effect must be considered separately, for there are no general rules that will predict whether biologic activity will be increased or decreased. It appears that when isosteric replacement involves the bridge connecting groups necessary for a given response, a gradation of like effects results, with steric factors (bond angles) and relative polar character being important. Some examples of this type are as follows:



Antibacterial: X = S, Se, O, NH, CH_2

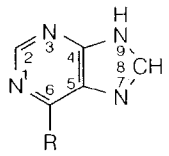


Thyroid Hormone Analogs: X = O, S, CH_2



Antihistamines: X = O, NH, CH_2
Cholinergic Blocking Agents: X = —COO—, —CONH—, —COS—

When a group is present in a part of a molecule in which it may be involved in an essential interaction or may influence the reactions of neighboring groups, isosteric replacement sometimes produces analogues that act as antagonists. Some examples from the field of cancer chemotherapy are:



	R	
Adenine	NH_2	Metabolites
Hypoxanthine	OH	
6-Mercaptopurine	SH	Antimetabolite

The 6-NH_2 and 6-OH groups appear to play essential roles in the hydrogen-bonding interactions of base pairs during nucleic acid replication in cells. The substitution of the significantly weaker hydrogen-bonding isosteric sulfhydryl groups results in a partial blockage of this interaction and a decrease in the rate of cellular synthesis.

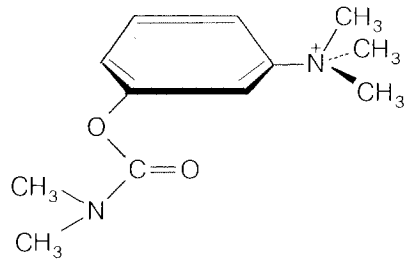
In a similar fashion, replacement of the hydroxyl group of pteroylglutamic acid (folic acid) by the amino group leads to methotrexate, an antagonist useful in the treatment of certain types of cancer.

As a better understanding develops of the nature of the interactions between drug, metabolizing enzymes, and biologic receptor, selection of isosteric groups with particular electronic, solubility, and steric properties should permit the rational preparation of more selectively acting drugs. But, in the meanwhile, results obtained by the systematic application of the principles of isosteric replacement are aiding in the understanding of the nature of these receptors.

Steric Features of Drugs

Regardless of the ultimate mechanism by which the drug and the receptor interact, the drug must approach the receptor and fit closely to its surface. Steric factors determined by the stereochemistry of the receptor site surface and that of the drug molecules are, therefore, of primary importance in determining the nature and the efficiency of the drug–receptor interaction. With the possible exception of the general anesthetics, such drugs must possess a high degree of structural specificity to initiate a response at a particular receptor.

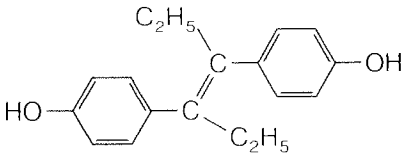
Some structural features contribute a high degree of structural rigidity to the molecule. For example, aromatic rings are planar, and the atoms attached directly to these rings are held in the plane of the aromatic ring. Hence, the quaternary nitrogen and carbamate oxygen attached directly to the benzene ring in the cholinesterase inhibitor, neostigmine, are restricted to the plane of the ring and, consequently, the spatial arrangement of at least these atoms is established.



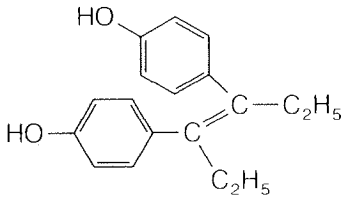
Neostigmine

The relative positions of atoms attached directly to multiple bonds are also fixed. For the double bond, *cis* and *trans* isomers result. For example, diethylstilbestrol exists in two fixed stereoisomeric forms. *trans*-Diethylstilbestrol is estrogenic, whereas

the *cis*-isomer is only 7% as active. In *trans*-diethylstilbestrol, resonance interactions and minimal steric interference tend to hold the two aromatic rings and connecting ethylene carbon atoms in the same plane.



trans-Diethylstilbestrol

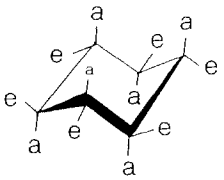


cis-Diethylstilbestrol

Geometric isomers, such as the *cis* and the *trans* isomers, hold structural features at different relative positions in space. These isomers also have significantly different physical and chemical properties. Therefore, their distributions in the biologic medium are different, as well as their capabilities for interacting with a biologic receptor in a structurally specific manner.

More subtle differences exist for *conformational* isomers. Similarly to geometric isomers, these exist as different arrangements in space for the atoms or groups in a single classic structure. Rotation about bonds allows interconversion of conformational isomers; however, an energy barrier between isomers is often sufficiently high for their independent existence and reaction. Differences in reactivity of functional groups, or interaction with biologic receptors, may be due to differences in steric requirements. In certain semirigid ring systems, such as the steroids, conformational isomers show significant differences in biologic activities (see Chap. 18). Methods for calculating these energy barriers will be discussed later.

The principles of conformational analysis have established some generalizations about the more stable structures for reduced (nonaromatic) ring systems. In the cyclohexane derivatives, bulky groups tend to be held approximately in the plane of the ring, the *equatorial* position. Substituents attached to bonds perpendicular to the general plane of the ring (*axial* position) are particularly susceptible to steric crowding. Thus, 1,3-diaxial substituents larger than hydrogen may repel each other, twisting the flexible ring and placing the substituents in the less crowded equatorial conformation.

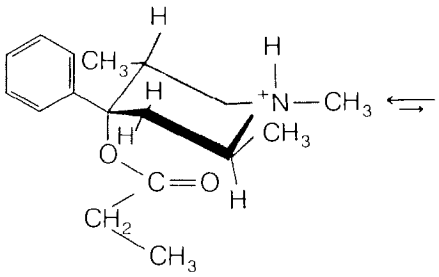


Equatorial (e) and *axial (a)* substitution in the chair form of cyclohexane.

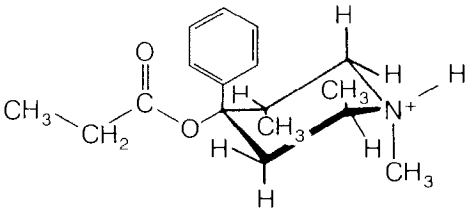
Similar calculations may be made for reduced heterocyclic ring systems, such as substituted piperidines. Generally, an equilibrium mixture of conformers may exist. For example, the potent analgesic trimeperidine (see Chap. 17) has been calculated to exist largely in the form in which the bulky phenyl group is in the equatorial position, this form being favored by 7 kcal/mol over the axial species. The ability of a molecule to produce potent analgesia has been related to the relative spatial positioning of a flat aromatic nucleus, a connecting aliphatic or alicyclic chain, and a nitrogen atom, which exists largely in the ionized form at physiologic pH.[21] It might be expected that one of the conformers would be responsible for the analgesic activity; however, here, it appears that both the axially and the equatorially oriented phenyl group may contribute. In structurally related isomers the conformations of which are fixed by the fusion of an additional ring, both compounds in which the phenyl group is the axial and those in which it is in the equatorial position have equal analgesic potency.[22]

In a related study of conformationally rigid diastereoisomeric analogues of meperidine, the *endo*-phenyl epimer was more potent than was the *exo*-isomer.[23] However, the *endo*-isomer penetrated brain tissue more effectively because of slight differences in pK_a values and partition coefficients between the isomers. This emphasizes the importance of considering differences in physical properties of closely related compounds before interpreting differences in biologic activities solely on steric grounds and relative spatial positioning of functional groups.

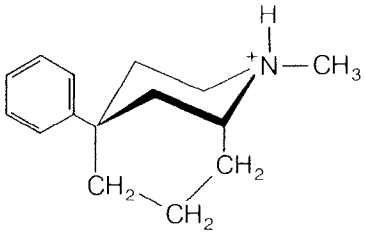
Open chains of atoms, which form an important part of many drug molecules, are not equally free to assume all possible conformations, there being some that are sterically preferred.[24] Energy barriers to free rotation of the chains are present, owing to interactions of nonbonded atoms. For example, the atoms tend to position themselves in space such that they occupy staggered positions, with no two atoms directly facing (eclipsed). Thus, for butane at 37°, the calculated relative probabilities for four possible conformations show that the maximally extended *trans* form is favored 2 : 1 over the two equivalent bent (skew) forms. The *cis* form, in which all of the atoms are facing or *eclipsed*, is much hindered, and



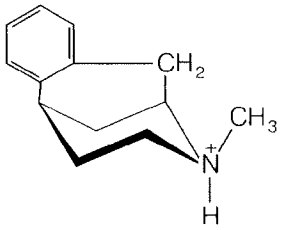
Trimeperidine (*equatorial*-phenyl)



Trimeperidine (*axial*-phenyl)



Equatorial-phenyl (analgesic ED_{50} 18.4 mg/kg)

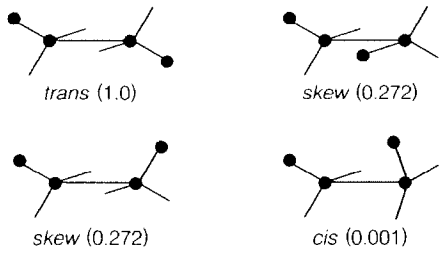


Axial-phenyl analgesic ED_{50} 18.7 mg/kg)

Ring-fused Analgesics

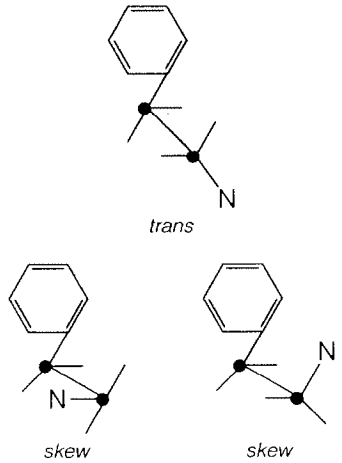
only about 1 : 1000 molecules may be expected to be in this conformation at normal temperatures.

Nonbonded interactions in polymethylene chains tend to favor the most extended *trans* conformations, although some of the partially extended *skew* conformations also exist. A branched methyl group reduces somewhat the preference for the *trans* form in that portion of the chain and, therefore, the probability distribution for the length of the chain is shifted toward the shorter distances. This situation is present in substituted chains that contain the elements of many drugs, such as the β-phenylethyl-



Relative probabilities for the existence of conformations of butane

amines. It should be noted that such amines are largely protonated at physiologic *p*H, and exist in a charged tetra-covalent form. Accordingly, their stereochemistry closely resembles that of carbon, although in the following diagrams, the hydrogen atoms attached to nitrogen are not shown. As may be expected, the fully extended *trans* form, with maximal separation of the phenyl ring and the nitrogen atom, is favored, and a smaller population of the two equivalent *skew* forms, in which the ring and the nitrogen are closer together, exists in solution. Introduction of an α-methyl group alters the favored position of the *trans* form, as positioning of the bulky methyl group away from the phenyl group (*skew* form 2) also results in a decrease in nonbonded interactions. Clearly, *skew* form 1 with both the methyl and the amine group close to phenyl is less favorable. The overall result is a reduction in the average distance between the aromatic group and the basic nitrogen atom in α-methyl-substituted β-arylethylamines. This steric factor influences the strength of the binding interaction with a biologic receptor required to produce a given pharmacologic effect. It is possible that the altered stereochemistry of α-methyl-β-arylethylamines may partially account for their slow rate of metabolic deamination (see Chap. 11).



Conformantions of β-phenylethylamines

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