13 Drug design: optimizing target interactions

In chapter 12, we looked at the various methods of discovering a lead compound. Once it *has* been discovered, the lead compound can be used as the starting point for drug design. There are various aims in drug design. The eventual drug should have a good selectivity and level of activity for its target, and have minimal side effects. It should be easily synthesized and be chemically stable. Finally, it should have acceptable pharmacokinetic properties and be non-toxic. In this chapter, we concentrate on the design strategies that can be used to optimize the interaction of the drug with its target, in other words its pharmacodynamic properties. In chapter 14, we look at the design strategies that can improve the drug's ability to reach its target and to have an acceptable lifetime, in other words its pharmacokinetic properties. Although these topics are in separate chapters, it would be wrong to think that they should be treated separately or in that order during a research programme. For example, it would be foolish to spend months or years perfecting a drug that interacts perfectly with its target, but has no chance of reaching that target because of adverse pharmacokinetic properties or metabolic instability. Pharmacodynamics and pharmacokinetics should have equal priority in influencing which strategies are used and which analogues are synthesized.

13.1 Structure–activity relationships

Once the structure of a lead compound is known, the medicinal chemist moves on to study its structure–activity relationships (SAR). The aim here is to discover which parts of the molecule are important to biological activity and which are not. If it is possible to crystallize the target with the drug bound to the binding site, the crystal structure of the complex could be solved by X-ray crystallography, then studied with molecular modelling software to identify important binding interactions. However, this may not be possible, either because the target structure cannot be crystallized or because the target structure has not been identified. If that is the case, it will be necessary to revert to the traditional method of synthesizing a selected number of compounds that vary slightly from the original structure, then studying what effect that has on the biological activity.

One can imagine the drug as a chemical knight entering the body in order to make battle with an affliction. The drug is armed with a variety of weapons and armour, but it may not be obvious which weapons are important to the drug's activity, or which armour is essential to its survival. We can only find this out by removing some of the weapons and armour to see if the drug is still effective. The weapons and armour involved are the various functional groups and alkyl groups present in the structure that can either bind to the drug's target (section 1.3), or assist and protect it on its journey through the body (chapter 14). Recognizing functional groups and the sort of intermolecular bonds that they can form is important in understanding how a drug might bind to a target.

Let us imagine that we have isolated a natural product with the structure shown in Fig. 13.1. We shall name it glipine. There are a variety of functional groups present in the structure, and the diagram shows the potential binding interactions that are possible with a target binding site.

It is unlikely that all of these interactions take place, so we have to identify those that do. By synthesizing compounds (such as the examples shown in Fig. 13.2) where one particular functional group of the molecule is removed or altered, it is possible to find out which groups are essential and which are not. This involves testing all the analogues for biological activity and comparing them with the original compound. If an analogue shows a significantly lowered activity, then the group that has been modified must have been important. If the activity remains similar, then the group is not essential.

The ease with which this task is carried out depends on how easily we can synthesize the necessary analogues. It may be possible to modify some lead compounds directly

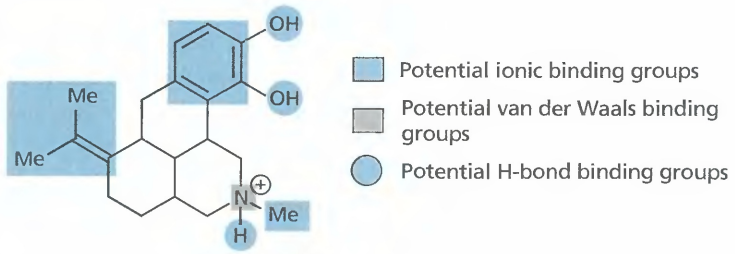


FIGURE 13.1 Glipine.

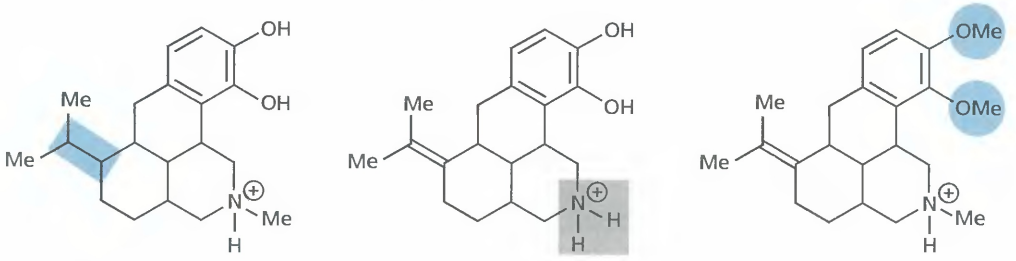


FIGURE 13.2 Modifications of glipine.

to the required analogues, whereas the analogues of other lead compounds may best be prepared by total synthesis. Let us consider the binding interactions that are possible for different functional groups and the analogues that could be synthesized to establish whether they are involved in binding or not.

13.1.1 Binding role of alcohols and phenols

Alcohols and phenols are functional groups which are common in many drugs and are often involved in hydrogen bonding. The oxygen can act as a hydrogen bond acceptor, and the hydrogen can act as a hydrogen bond donor (Fig. 13.3). The directional preference for hydrogen bonding is indicated by the arrows in the figure, but it is important to realize that slight deviations are possible (section 1.3.2). One or all of these interactions may be important in binding the drug to the binding site. Synthesizing a methyl ether or an ester analogue would be relevant in testing this, as it is highly likely that the bonding would be disrupted in either analogue. Let us consider the methyl ether first.

There are two reasons why the ether might hinder or prevent the hydrogen bonding of the original alcohol or phenol. The obvious explanation is that the proton of the original hydroxyl group is involved as a hydrogen bond donor and if it is removed, the hydrogen bond is lost (Frames 1 and 2 in Fig. 13.4). However, suppose the oxygen atom is acting as a hydrogen bond acceptor (Frame 3 in Fig. 13.4)? The oxygen is still present in the ether analogue, so could it still take part in hydrogen bonding? Well, it may, but possibly not to the same extent. The extra bulk of the methyl group should hinder the close approach that was previously attainable and should disrupt hydrogen bonding (Frame 4 in Fig. 13.4). The hydrogen bonding may not be completely prevented, but we could reasonably expect it to be weakened.

An ester analogue cannot act as a hydrogen bond donor either. There is still the possibility of it acting as a hydrogen bond acceptor, but the extra bulk of the acyl group is

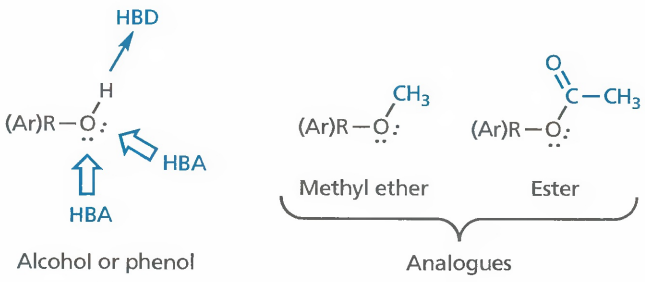


FIGURE 13.3 Possible hydrogen bonding interactions for an alcohol or phenol.

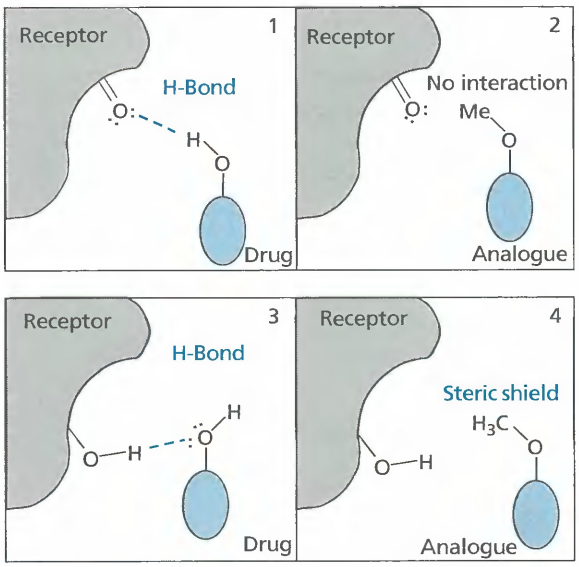


FIGURE 13.4 Possible hydrogen bond interactions for an alcohol/phenol in comparison with an ether analogue.

even greater than the methyl group of the ether, and this too should hinder the original hydrogen bonding interaction. There is also a difference between the electronic properties of an ester and an alcohol. The carboxyl group has a weak pull on the electrons from the neighbouring oxygen, giving the resonance structure shown in Fig. 13.5. Because the lone pair is involved in such an interaction, it will be less effective as a hydrogen bond acceptor. Of course, one could argue that the carbonyl oxygen may act as an alternative hydrogen bond acceptor, but it is in a different position relative to the rest of the molecule and it is unlikely that it would form a hydrogen bond to the same region of the binding site as the original oxygen.

It is relatively easy to acetylate alcohols and phenols to the corresponding esters and this was one of the early reactions that was carried out on natural products such as morphine (sections 24.3 and 24.5).

Both phenols and alcohols can be converted to ethers and esters to see what effect that has on binding, but it should be remembered that phenols consist of an OH group linked to an aromatic ring. We have seen how an OH group can interact with a binding site, but it is also possible for the aromatic ring to take part in intermolecular interactions. This is covered in the next section.

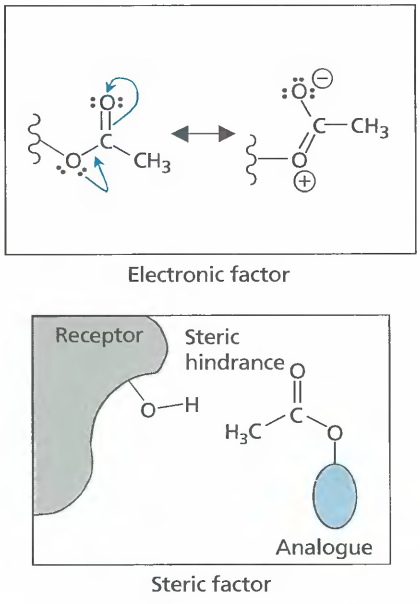


FIGURE 13.5 Factors by which an ester group can disrupt the hydrogen bonding of the original hydroxyl group.

13.1.2 Binding role of aromatic rings

Aromatic rings are planar, hydrophobic structures, commonly involved in van der Waals and hydrophobic interactions with flat hydrophobic regions of the binding site. An analogue containing a cyclohexane ring in place of the aromatic ring is less likely to bind so well, as the ring is no longer flat. The axial protons can interact weakly, but they also serve as buffers to keep the rest of the cyclohexane ring at a distance (Fig. 13.6). The binding region for the aromatic ring may also be a narrow slot rather than a planar surface. In that scenario, the cyclohexane ring would be incapable of fitting into it, as it is a bulkier structure.

Although there are methods of converting aromatic rings to cyclohexane rings, they are unlikely to be successful with most lead compounds, and so such analogues would normally be prepared using a full synthesis.

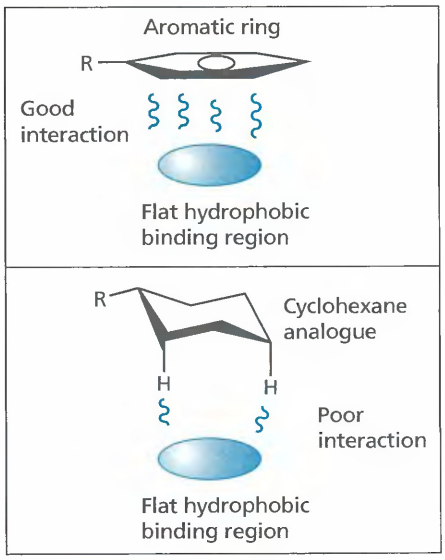


FIGURE 13.6 Binding comparison of an aromatic ring with a cyclohexyl ring.

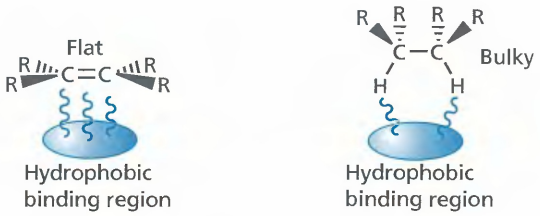


FIGURE 13.7 Binding comparison of an alkene with an alkane.

Aromatic rings could also interact with an ammonium ion through induced dipole interactions or hydrogen bonding (sections 1.3.4 and 1.3.2). Such interactions would not be possible for the cyclohexyl analogue.

13.1.3 Binding role of alkenes

Like aromatic rings, alkenes are planar and hydrophobic so they too can interact with hydrophobic regions of the binding site through van der Waals and hydrophobic interactions. The activity of the equivalent saturated analogue would be worth testing, since the saturated alkyl region is bulkier and cannot approach the relevant region of the binding site so closely (Fig. 13.7). Alkenes are generally easier to reduce than aromatic rings, so it may be possible to prepare the saturated analogue directly from the lead compound.

13.1.4 Binding role of ketones and aldehydes

A ketone group is not uncommon in many of the structures studied in medicinal chemistry. It is a planar group that can interact with a binding site through hydrogen bonding where the carbonyl oxygen acts as a hydrogen bond acceptor (Fig. 13.8). Two such interactions are possible, as two lone pairs of electrons are available on the carbonyl oxygen. The lone pairs are in sp^2 hybridized orbitals which are in the same plane as the functional group. The carbonyl group also has a significant dipole moment and so a dipole–dipole interaction with the binding site is also possible.

It is relatively easy to reduce a ketone to an alcohol and it may be possible to carry out this reaction directly on the lead compound. This significantly changes the geometry of the functional group from planar to tetrahedral. Such an alteration in geometry may well weaken any existing hydrogen bonding interactions and will certainly weaken any dipole–dipole interactions, as both the magnitude



FIGURE 13.8 Binding interactions that are possible for a carbonyl group.

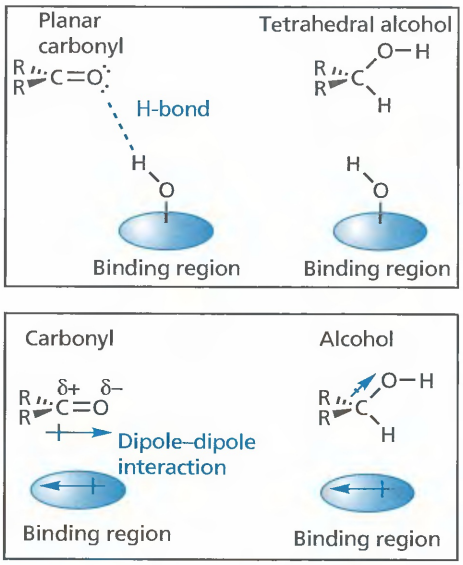


FIGURE 13.9 Effect on binding interactions following the reduction of a ketone or aldehyde.

and orientation of the dipole moment will be altered (Fig. 13.9). If it was suspected that the oxygen present in the alcohol analogue might still be acting as a hydrogen bond acceptor then the ether or ester analogues could be studied as described above. Reactions are available that can reduce a ketone completely to an alkane and remove the oxygen, but they are unlikely to be practical for many of the lead compounds studied in medicinal chemistry.

Aldehydes are less common in drugs since they are more reactive and are susceptible to oxidation to carboxylic acids. However, they could interact in the same way as ketones, and similar analogues could be studied.

13.1.5 Binding role of amines

Amines are extremely important functional groups in medicinal chemistry and are present in many drugs. They may be involved in hydrogen bonding either as a hydrogen bond acceptor or a hydrogen bond donor (Fig. 13.10). The nitrogen atom has one lone pair of electrons and can act as a hydrogen bond acceptor for one hydrogen bond. Primary and secondary amines have N–H groups and can act as hydrogen bond donors. Aromatic and heteroaromatic amines act only as hydrogen bond donors, because the lone pair interacts with the aromatic or heteroaromatic ring.

In many cases, the amine may be protonated when it interacts with its target binding site, which means that it is ionized and cannot act as a hydrogen bond acceptor. However, it can still act as a hydrogen bond donor and will form a stronger hydrogen bond than if it was not ionized (Fig. 13.11). Alternatively, a strong ionic interaction may take place with a carboxylate ion in the binding site (Fig. 13.12).

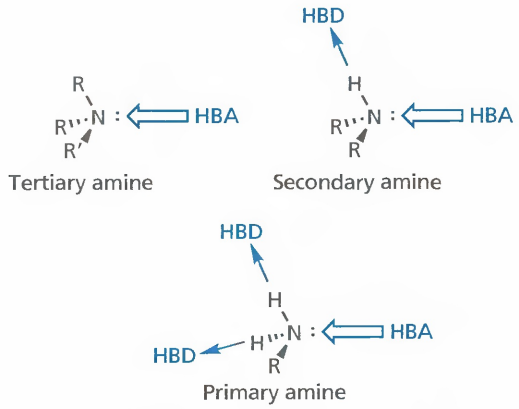


FIGURE 13.10 Possible binding interactions for amines.

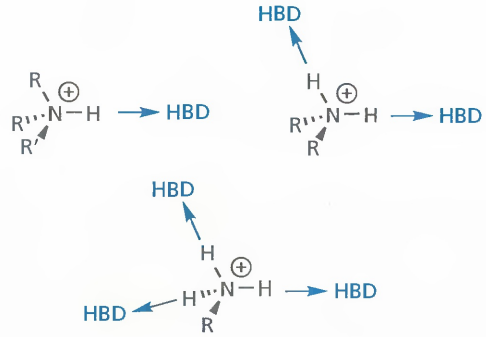


FIGURE 13.11 Possible hydrogen bonding interactions for ionized amines.

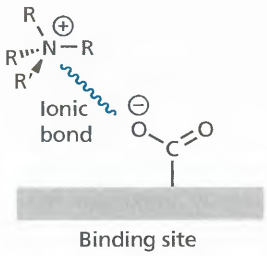


FIGURE 13.12 Ionic interaction between an ionized amine and a carboxylate ion.

To test whether ionic or hydrogen bonding interactions are taking place, an amide analogue could be studied. This will prevent the nitrogen acting as a hydrogen bond acceptor, as the nitrogen's lone pair will interact with the neighbouring carbonyl group instead (Fig. 13.13). This interaction also prevents protonation of the nitrogen and rules out the possibility of ionic interactions. You might argue that the right-hand structure in Fig. 13.13 has a positive charge on the nitrogen and could still take part in an ionic interaction. However, this structure is not present as a distinct entity and the figure is only used to represent the fact that the lone pair can interact with the carbonyl group to produce double-bond character in the amide group. Any positive charge is extremely weak in comparison to that present in an ionized amine.

It is relatively easy to form secondary and tertiary amides from primary and secondary amines respectively, and it may be possible to carry out this reaction directly on the lead compound. A tertiary amide lacks the N–H group of the original secondary amine and would test whether this is involved as a hydrogen bond donor. The secondary amide formed from a primary amine still has an N–H group present, but the steric bulk of the acyl group should hinder it acting as a hydrogen bond donor.

Tertiary amines cannot be converted directly to amides, but if one of the alkyl groups is a methyl group it is often possible to remove it with vinyloxycarbonyl chloride (VOC-Cl) to form a secondary amine which could then be converted to the amide (Fig. 13.14). This demethylation reaction is extremely useful and has been used to good effect in the synthesis of morphine analogues (see Box 24.2 for the reaction mechanism).

13.1.6 Binding role of amides

Many of the lead compounds currently studied in medicinal chemistry are peptides or polypeptides, consisting of

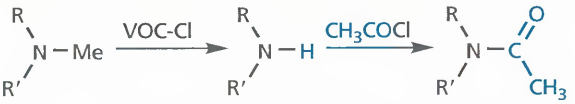


FIGURE 13.14 Dealkylation of a tertiary amine and formation of a secondary amide.

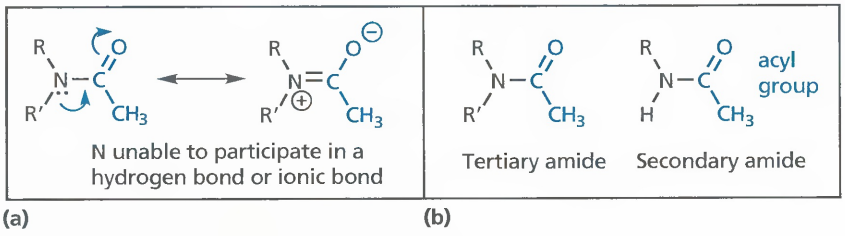


FIGURE 13.13 (a) Interaction of the nitrogen lone pair with the neighbouring carbonyl group in amides. (b) Secondary and tertiary amides.

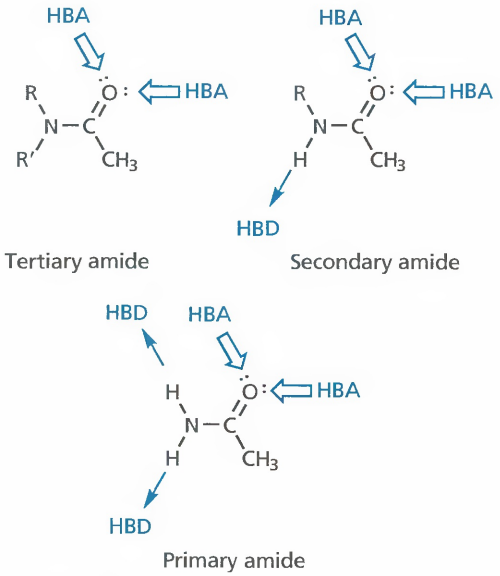


FIGURE 13.15 Possible hydrogen bonding interactions for amides.

amino acids linked together by peptide or amide bonds (section 2.1). Amides are likely to interact with binding sites through hydrogen bonding (Fig. 13.15). The carbonyl oxygen atom can act as a hydrogen bond acceptor and has the potential to form two hydrogen bonds. Both the lone pairs involved are in sp^2 hybridized orbitals which are located in the same plane as the amide group. The nitrogen cannot act as a hydrogen bond acceptor because the lone pair interacts with the neighbouring carbonyl group, as described above. Primary and secondary amides have an N–H group, which allows the possibility of this group acting as a hydrogen bond donor.

The most common type of amide in peptide lead compounds is the secondary amide. Suitable analogues that could be prepared to test out possible binding interactions are shown in Fig. 13.16. All the analogues apart from the primary and secondary amines could be used to check whether the amide is acting as a hydrogen bond donor, and the alkenes and amines could be tested to see whether the amide is acting as a hydrogen bond acceptor. However, there are traps for the unwary. The amide group is planar and does not rotate, because of its partial double bond character. The ketone, the secondary amine and the tertiary amine analogues have a single bond at the equivalent position, which *can* rotate. This would alter the relative positions of any binding groups on either side of the amide group and lead to a loss of binding even if the amide itself was not involved in binding. Therefore, a loss of activity would not necessarily mean that the amide is important as a binding group. With these groups, it would only be safe to say that the amide group is not essential if activity is retained. Similarly, the primary amine and carboxylic acid may be found to have no activity, but this might be due to the loss of important binding groups in one half of the molecule. These particular analogues would only be worth considering if the amide group is peripheral to the molecule (e.g. R–NHCOMe or R–CONHMe) and not part of the main skeleton.

The alkene would be a particularly useful analogue to test, since it is planar, cannot rotate, and cannot act as a hydrogen bond donor or hydrogen bond acceptor. However, the synthesis of this analogue may not be simple. In fact, it is likely that all the analogues described would have to be prepared using a full synthesis. Amides are relatively stable functional groups and although several of the analogues described might be attainable from the lead compound directly, it is more likely that the lead compound would not survive the forcing conditions required.

Amides which are within a ring system are called lactams. They too can form intermolecular hydrogen bonds, as described above. However, if the ring is small and suffers ring strain, the lactam can undergo a chemical reaction with the target leading to the formation of a covalent bond. The best examples of this are the penicillins which contain a four-membered β-lactam ring. This acts as an acylating agent and irreversibly inhibits a bacterial enzyme by acylating a serine residue in the active site (Fig. 13.17) (section 19.5.1.4).

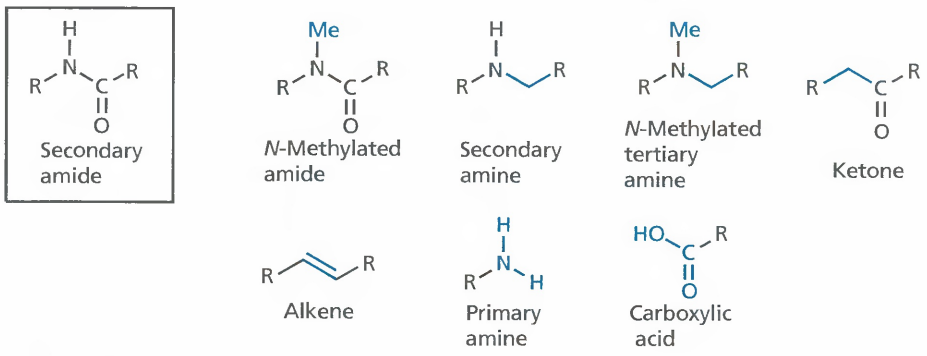


FIGURE 13.16 Possible analogues to test the binding interactions of a secondary amide.

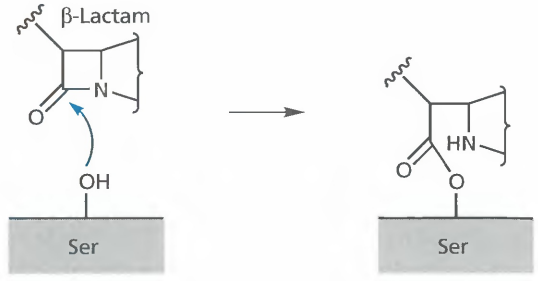


FIGURE 13.17 β-lactam ring acting as an acylating agent.

13.1.7 Binding role of quaternary ammonium salts

Quaternary ammonium salts are ionized and can interact with carboxylate groups by ionic interactions (Fig. 13.18). Another possibility is an **induced dipole interaction** between the quaternary ammonium ion and any aromatic rings in the binding site. The positively charged nitrogen can distort the π electrons of the aromatic ring such that a dipole is induced, whereby the face of the ring is slightly negative and the edges are slightly positive. This allows an interaction between the slightly negative faces of the aromatic rings and the positive charge of the quaternary ammonium ion.

The importance of these interactions could be tested by synthesizing an analogue that has a tertiary amine group rather than the quaternary ammonium group. Of course, it is possible that such a group could become ionized by being protonated, and interact in the same way. Converting the amine to an amide would prevent this possibility. The neurotransmitter **acetylcholine** has a quaternary ammonium group which is thought to bind to the binding site of its target receptor by ionic bonding and/or induced dipole interactions (section 22.7).

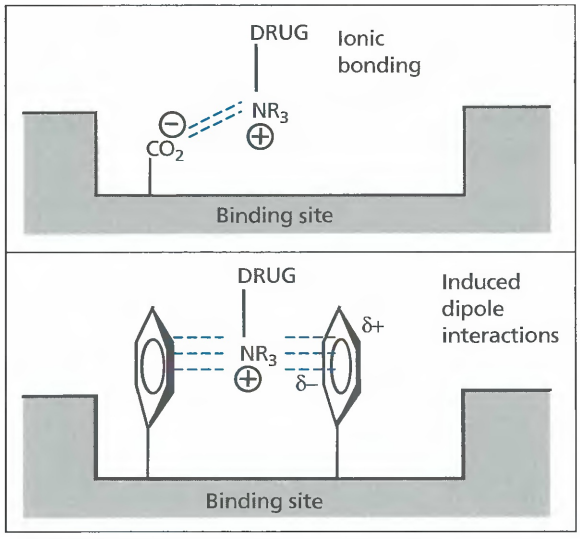


FIGURE 13.18 Possible binding interactions of a quaternary ammonium ion.

13.1.8 Binding role of carboxylic acids

The carboxylic acid group is reasonably common in drugs. It can act as a hydrogen bond acceptor in various ways, or as a hydrogen bond donor (Fig. 13.19). Alternatively, it may exist as the carboxylate ion. This allows the possibility of an ionic interaction and/or a strong hydrogen bond where the carboxylate ion acts as the hydrogen bond acceptor. The carboxylate ion has also been found to be a good ligand for zinc ions, which are present as cofactors in enzymes known as zinc metalloproteinases (section 21.7.1 and Case Study 2).

In order to test the possibility of such interactions, analogues such as esters, primary amides, primary alcohols, and ketones could be synthesized and tested (Fig. 13.20). None of these functional groups can ionize, so a loss of activity could imply that an ionic bond is important. The primary alcohol could shed light on whether the carbonyl oxygen is involved in hydrogen bonding, whereas the ester and ketone could indicate whether the hydroxyl group of the carboxylic acid is involved in hydrogen bonding. It may be possible to synthesize the ester and amide analogues directly from the lead compound, but the reduction of a carboxylic acid to a primary alcohol requires harsher conditions and this sort of analogue would normally be prepared by a full synthesis. The ketone would also have to be prepared by a full synthesis.

13.1.9 Binding role of esters

An ester functional group has the potential to interact with a binding site as a hydrogen bond acceptor only (Fig. 13.21). The carbonyl oxygen is more likely to act as the hydrogen bond acceptor than the alkoxy oxygen, as it

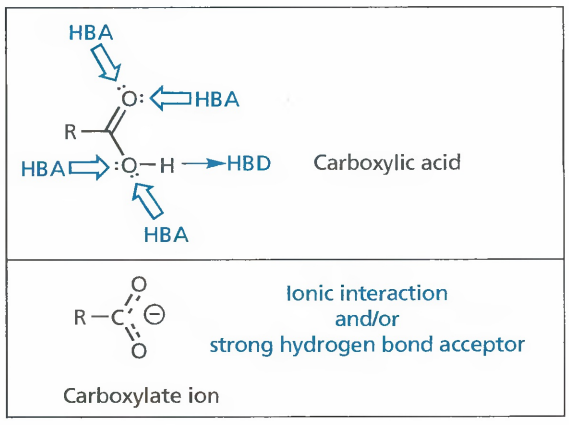


FIGURE 13.19 Possible binding interactions for a carboxylic acid and carboxylate ion.

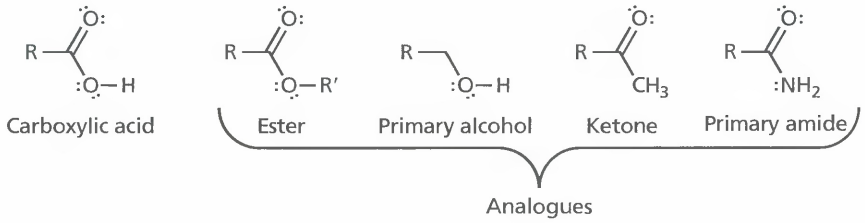


FIGURE 13.20 Analogues to test the binding interactions for a carboxylic acid.

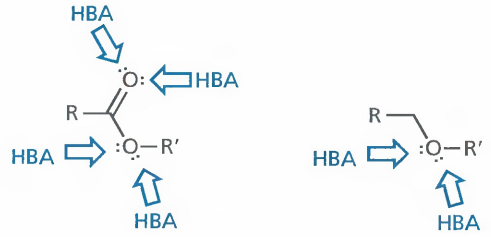


FIGURE 13.21 Possible binding interactions for an ester and an ether.

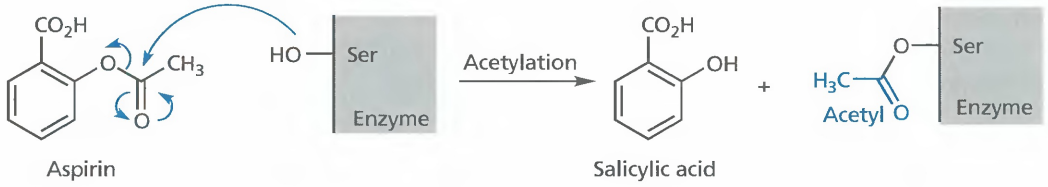


FIGURE 13.22 The disputed theory of aspirin acting as an acylating agent.

is sterically less hindered and has a greater electron density. The importance or otherwise of the carbonyl group could be judged by testing an equivalent ether, which would require a full synthesis.

Esters are susceptible to hydrolysis *in vivo* by metabolic enzymes called **esterases**. This may pose a problem if the lead compound contains an ester that is important to binding, as it means the drug might have a short lifetime *in vivo*. Having said that, there are several drugs that *do* contain esters and are relatively stable to metabolism, thanks to either electronic factors which stabilize the ester or steric factors that protect it.

Esters that are susceptible to metabolic hydrolysis are sometimes used deliberately to mask a polar functional group such as a carboxylic acid, alcohol or phenol in order to achieve better absorption from the gastrointestinal tract. Once in the blood supply, the ester is hydrolysed to release the active drug. This is known as a **prodrug** strategy (section 14.6).

Special mention should be made of the ester group in aspirin. Aspirin has an anti-inflammatory action resulting from its ability to inhibit an enzyme called **cyclooxygenase** (COX) which is required for **prostaglandin** synthesis. It is often stated that aspirin acts as an acylating agent, and that its acetyl group is covalently attached to a serine residue in the active site of COX (Fig. 13.22). However, this theory has been disputed, and it is stated that aspirin acts instead as a prodrug (section 14.6) to generate salicylic acid, which then inhibits the enzyme through non-covalent interactions.

13.1.10 Binding role of alkyl and aryl halides

Alkyl halides involving chlorine, bromine or iodine tend to be chemically reactive, since the halide ion is a good leaving group. As a result, a drug containing an alkyl halide is likely to react with any nucleophilic group that it encounters and become permanently linked to that group by a covalent bond—an alkylation reaction (Fig. 13.23). This poses a problem, as the drug is likely to alkylate a large variety of macromolecules which have nucleophilic groups, especially amine groups in proteins and nucleic acids. It is possible to moderate the reactivity to some extent, but selectivity is still a problem and leads to severe side effects. These drugs are therefore reserved for life-threatening diseases such as cancer (sections 9.3 and 21.2.3). Alkyl fluorides, on the other hand, are not alkylating agents, because the C–F bond is a strong one and is not easily broken. Fluorine is commonly used to replace a proton as it is approximately the same size, but



FIGURE 13.23 Alkylation of macromolecular targets by alkyl halides.

has different electronic properties. It may also protect the molecule from metabolism (sections 13.3.7 and 14.2.4).

Aryl halides do not act as alkylating agents and pose less of a problem in that respect. As the halogen substituents are electron-withdrawing groups, they affect the electron density of the aromatic ring and this may have an influence on the binding of the aromatic ring. The halogen substituents chlorine and bromine are hydrophobic in nature and may interact favourably with hydrophobic pockets in a binding site. Hydrogen bonding is not important. Although halide ions are strong hydrogen bond acceptors, halogen substituents are poor hydrogen bond acceptors.

Aliphatic and aromatic analogues lacking the halogen substituent could be prepared by a full synthesis to test whether the halogen has any importance in the activity of the lead compound.

13.1.11 Binding role of thiols and ethers

The thiol group (S–H) is known to be a good ligand for a zinc ion and has been incorporated into several drugs designed to inhibit enzymes containing a zinc cofactor. Such enzymes are known as zinc metalloproteinases (section 21.7.1 and Case Study 2). If the lead compound has a thiol group, the corresponding alcohol could be tested. This would have a far weaker interaction with a transition metal such as zinc.

An ether group (ArOr or ROR) might act as a hydrogen bond acceptor through the oxygen atom (Fig. 13.21). This could be tested by increasing the size of the neighbouring alkyl group to see whether it diminishes the ability of the group to take part in hydrogen bonding. Analogues where the oxygen is replaced with a methylene (CH_2) isostere should show significantly decreased binding affinity.

13.1.12 Binding role of other functional groups

A wide variety of other functional groups may be present in lead compounds that have no direct binding role, but could be important in other respects. Some may influence the electronic properties of the molecule (e.g. nitro groups or nitriles). Others may restrict the shape or conformation of a molecule (e.g. alkynes) (Box 13.3). Functional groups may also act as metabolic blockers (e.g. aryl halides) (section 14.2.4).

13.1.13 Binding role of alkyl groups and the carbon skeleton

The alkyl substituents and carbon skeleton of a lead compound are hydrophobic and may bind with hydrophobic regions of the binding site through van der Waals and hydrophobic interactions. The relevance of an alkyl substituent to binding can be determined by synthesizing an analogue which lacks the substituent. Such analogues generally have to be synthesized using a full synthesis if they are attached to the carbon skeleton of the molecule. However, if the alkyl group is attached to nitrogen or oxygen, it may be possible to remove the group from the lead compound as shown in Fig. 13.24. The analogues obtained may then be expected to have less activity if the alkyl group was involved in important hydrophobic interactions.

13.1.14 Binding role of heterocycles

There is a large diversity of heterocycles to be found in lead compounds. Heterocycles are cyclic structures that contain one or more heteroatoms such as oxygen, nitrogen, or sulfur. Nitrogen-containing heterocycles are particularly prevalent. The heterocycles can be aliphatic or aromatic in character, and have the potential to interact with binding sites through a variety of bonding forces. For example, the overall heterocycle can interact through van der Waals and hydrophobic interactions, while the individual heteroatoms present in the structure could interact by hydrogen bonding or ionic bonding.

As far as hydrogen bonding is concerned, there is an important directional aspect. The position of the

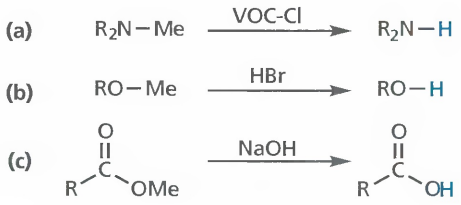


FIGURE 13.24 **(a)** *N*-Demethylation of a tertiary amine with vinyloxycarbonyl chloride (see Box 24.2 for mechanism). **(b)** Demethylation of a methyl ether using hydrogen bromide where nucleophilic substitution leads to an alcohol (or phenol) plus bromomethane. **(c)** Hydrolysis of an ester using sodium hydroxide where OH replaces OMe.

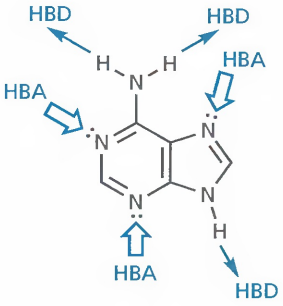


FIGURE 13.25 Possible hydrogen bonding interactions for adenine.

heteroatom in the ring and the orientation of the ring in the binding site can be crucial in determining whether or not a good interaction takes place. For example, a purine ring can take part in six hydrogen bonding interactions, three as a hydrogen bond donor and three as a hydrogen bond acceptor. The ideal directions for these interactions are shown in Fig. 13.25. Van der Waals interactions are also possible, to regions of the binding site above and below the plane of the ring system.

Heterocycles can be involved in quite intricate hydrogen bonding networks within a binding site. For example, the anticancer drug **methotrexate** contains a pteridine ring system that interacts with its binding site as shown in Fig. 13.26.

If the lead compound contains a heterocyclic ring, it is worth synthesizing analogues containing an aromatic ring or different heterocyclic rings to explore whether all the heteroatoms present are really necessary.

A complication with heterocycles is the possibility of **tautomers**. This played an important role in determining the structure of DNA (section 6.1.2). The structure of DNA consists of a double helix with base pairing between two sets of heterocyclic nucleic acid bases. Base pairing involves three hydrogen bonds between the base pair guanine and cytosine, and two hydrogen bonds between the base pair adenine and thymine (Fig. 13.27). The rings involved in the base pairing are coplanar, allowing the optimum orientation for the hydrogen bond donors and hydrogen bond acceptors. This in turn means that the base pairs are stacked above each other, allowing van der Waals interactions between the faces of each base pair. However, when Watson and Crick originally tried to devise a model for DNA, they incorrectly assumed that the preferred tautomers for the nucleic acid bases were as shown in the right-hand part of Fig. 13.27. With these tautomers, the required hydrogen bonding is not possible and would not explain the base pairing observed in the structure of DNA.

In a similar vein, knowing the preferred tautomers of heterocycles can be important in understanding how drugs interact with their binding sites. This is amply illustrated in the design of the anti-ulcer agent cimetidine (section 25.2).

With heterocyclic compounds, it is possible for a hydrogen bond donor and a hydrogen bond acceptor to be part of a conjugated system. Polarization of the electrons in the conjugated system permits **π-bond cooperativity**, where the strength of the hydrogen bond donor is enhanced by the hydrogen bond acceptor and *vice versa*. This has also been called **resonance-assisted hydrogen bonding**. This type of hydrogen bonding is possible for the hydrogen bond donors and acceptors for the nucleic acid base pairs (Fig. 13.28).

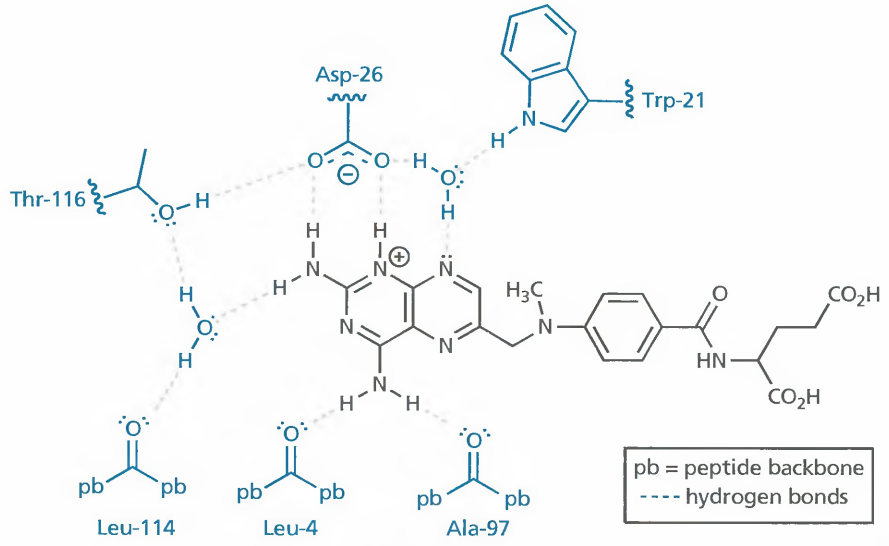


FIGURE 13.26 Binding interactions for the purine ring of methotrexate in its binding site.

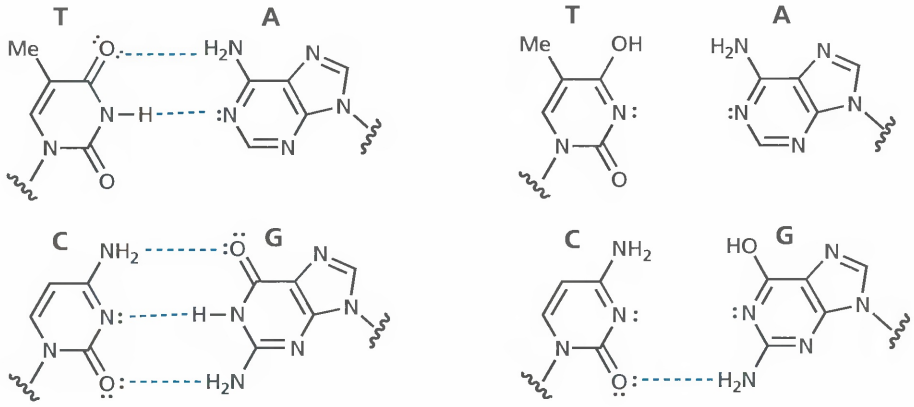


FIGURE 13.27 Base pairing in DNA and the importance of tautomers.

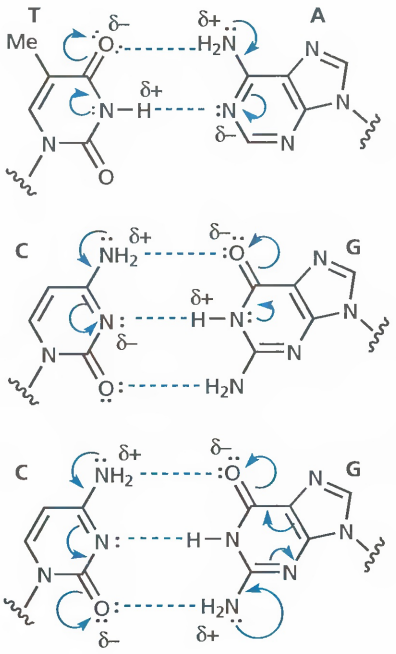


FIGURE 13.28 π-Bond cooperativity in hydrogen bonding.

13.1.15 Isosteres

Isosteres are atoms or groups of atoms which have the same valency (or number of outer shell electrons), and which have chemical or physical similarities (Fig. 13.29).

For example, SH, NH_2, and CH_3 are isosteres of OH, whereas S, NH, and CH_2 are isosteres of O. Isosteres can be used to determine whether a particular group is an important binding group or not, by altering the character of the molecule in as controlled a way as possible. Replacing O with CH_2, for example, will make little difference to the size of the analogue, but will have a marked effect on its polarity, electronic distribution, and bonding. Replacing OH with the larger SH may not have such an influence on the electronic character, but steric factors become more significant.

Isosteric groups could be used to determine whether a particular group is involved in hydrogen bonding. For example, replacing OH with CH_3 would completely eliminate hydrogen bonding, whereas replacing OH with NH_2 would not.

The β-blocker propranolol has an ether linkage (Fig. 13.30). Replacement of the OCH_2 segment with the isosteres CH=CH, SCH_2, or CH_2CH_2 eliminates activity, whereas replacement with $NHCH_2$ retains activity (though reduced). These results show that the ether oxygen is important to the activity of the drug and suggests that it is involved in hydrogen bonding with the receptor.

The use of isosteres in drug design is described in section 13.3.7.

13.1.16 Testing procedures

When investigating structure–activity relationships for drug–target binding interactions, biological testing should involve *in vitro* tests; for example inhibition studies on isolated enzymes or binding studies on membrane-bound receptors in whole cells. The results then show conclusively which binding groups are important in drug–target interactions. If *in vivo* testing is carried out, the results are less clear-cut because loss of activity may be due to the inability of the drug to reach its target rather than reduced drug–target interactions. On the other hand, *in vivo* testing may reveal functional groups that are important in protecting or assisting the drug in its passage through the body. This would not be revealed by *in vitro* testing.

NMR spectroscopy can also be used to test structure-activity relationships as described in section 12.4.10.

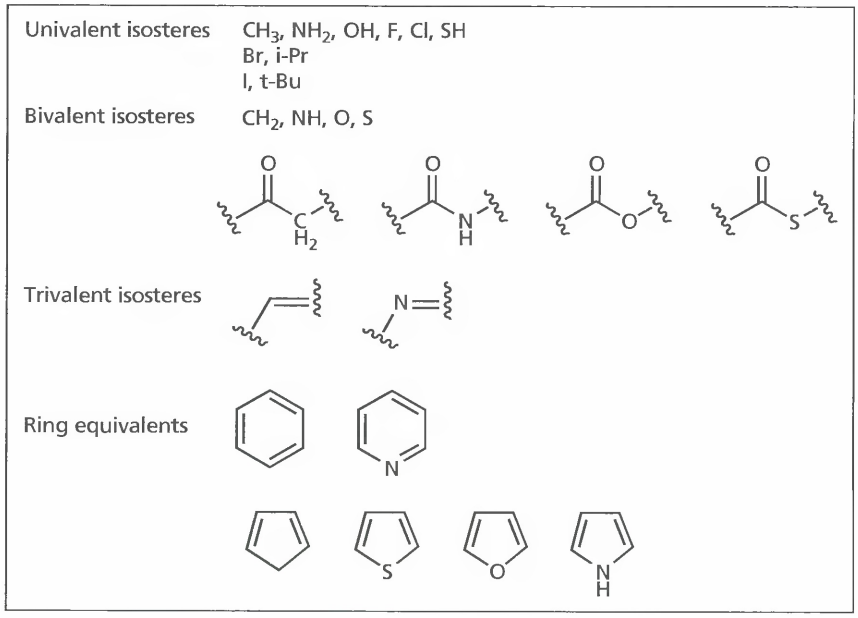


FIGURE 13.29 Examples of classic isosteres.

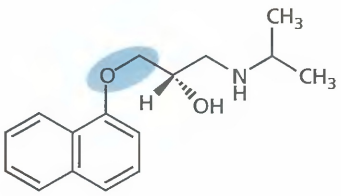


FIGURE 13.30 Propranolol.

13.2 Identification of a pharmacophore

Once it is established which groups are important for a drug's activity, it is possible to move on to the next stage—the identification of the pharmacophore. The pharmacophore summarizes the important binding groups which are required for activity, and their relative positions in space with respect to each other. For example, if we discover that the important binding groups for our hypothetical drug glipine are the two phenol groups, the aromatic ring, and the nitrogen atom, then the pharmacophore is as shown in Fig. 13.31. Structure I shows the two-dimensional (2D) pharmacophore and structure II shows the three-dimensional (3D) pharmacophore. The latter specifies the relative positions of the important groups in space. In this case, the nitrogen atom is 5.063 Å from the centre of the phenolic ring and lies at an angle of 18° from the plane of the ring. Note that it is not necessary to show the specific skeleton connecting the important groups. Indeed, there are benefits in not doing so, since it is easier to compare the 3D pharmacophores from different structural classes of compound to see if they share a common pharmacophore. 3D pharmacophores can be defined using molecular modelling (section 17.11) which allows the definition of 'dummy bonds' such as the one in Fig. 13.31 between nitrogen and the centre of the aromatic ring. The centre of the ring can be defined by a dummy atom called a **centroid** (not shown).

An even more general type of 3D pharmacophore is the one shown as structure III (Fig. 13.31)—a bonding-type pharmacophore. Here the bonding characteristics of each functional group are defined rather than the group itself. Note also that the groups are defined as points in space. This includes the aromatic ring, which is defined by a point at the centre of the ring—the centroid. All the points are connected by pharmacophoric triangles to define their positions. This allows the comparison of molecules which may have the same pharmacophore and binding interactions, but which use different functional groups to achieve these interactions. In this case, the phenol groups can act as hydrogen bond donors or acceptors, the aromatic ring can participate in van der Waals interactions, and the amine can act as a hydrogen bond acceptor or as an ionic centre if it is protonated. We shall return to the concept and use of 3D pharmacophores in sections 18.10 and 17.11.

Identifying 3D pharmacophores is relatively easy for rigid cyclic structures such as the hypothetical glipine. With more flexible structures, it is not so straightforward because the molecule can adopt a large number of shapes or conformations which place the important

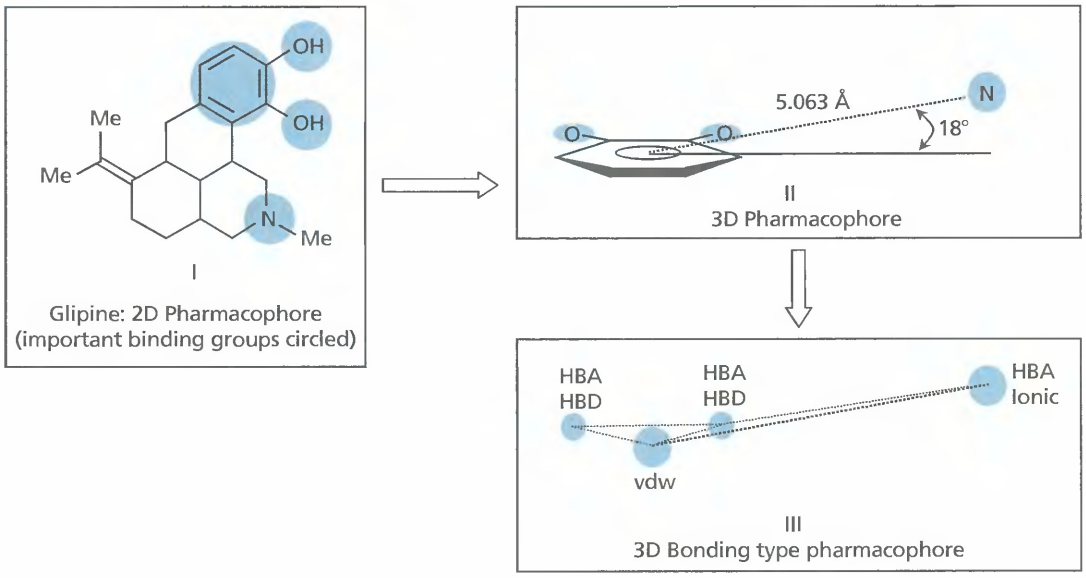


FIGURE 13.31 Pharmacophore for the fictitious structure glipine.

binding groups in different positions relative to each other. Normally, only one of these conformations is recognized and bound by the binding site. This conformation is known as the **active conformation**. In order to identify the 3D pharmacophore, it is necessary to know the active conformation. There are various ways in which this might be done. Rigid analogues of the flexible compound could be synthesized and tested to see whether activity is retained (section 13.3.9). Alternatively, it may be possible to crystallize the target with the compound bound to the binding site. X-ray crystallography could then be used to identify the structure of the complex as well as the active conformation of the bound ligand (section 17.10). Finally, progress has been made in using NMR spectroscopy to solve the active conformation of isotopically labelled molecules bound to their binding sites.

We finish this section with a warning! A drawback with pharmacophores is their unavoidable emphasis on functional groups as the crucial binding groups. In many situations this is certainly true, but in other situations it is not. It is not uncommon to find compounds that have the correct pharmacophore but nevertheless show disappointing activity and poor binding. It is important to realize that the overall skeleton of the molecule is involved in interactions with the binding site through van der Waals and hydrophobic interactions. The strength of these interactions can sometimes be crucial in whether a drug binds effectively or not, and the 3D pharmacophore does not take this into account.

KEY POINTS

- SARs define the functional groups or regions of a lead compound which are important to its biological activity.
- Functional groups such as alcohols, amines, esters, amides, carboxylic acids, phenols, and ketones can interact with binding sites by means of hydrogen bonding.
- Functional groups such as amines, quaternary ammonium salts, and carboxylic acids can interact with binding sites by ionic bonding.
- Functional groups such as alkenes and aromatic rings can interact with binding sites by means of van der Waals and hydrophobic interactions.
- Alkyl substituents and the carbon skeleton of the lead compound can interact with hydrophobic regions of binding sites by means of van der Waals and hydrophobic interactions.
- Interactions involving dipole moments or induced dipole moments may play a role in binding a lead compound to a binding site.
- Reactive functional groups such as alkyl halides may lead to irreversible covalent bonds being formed between a lead compound and its target.
- The relevance of a functional group to binding can be determined by preparing analogues where the functional group is modified or removed in order to see whether activity is affected by such a change.
- Some functional groups can be important to the activity of a lead compound for reasons other than target binding. They

may play a role in the electronic or stereochemical properties of the compound, or they may have an important pharmacokinetic role.

- Replacing a group in the lead compound with an isostere (a group having the same valency) makes it easier to determine whether a particular property such as hydrogen bonding is important.
- *In vitro* testing procedures should be used to determine the SAR for target binding.
- The pharmacophore summarizes the groups, which are important in binding a lead compound to its target, as well as their relative positions in three dimensions.

13.3 Drug optimization: strategies in drug design

Once the important binding groups and pharmacophore of the lead compound have been identified, it is possible to synthesize analogues that contain the same pharmacophore. But why is this necessary? If the lead compound has useful biological activity, why bother making analogues? The answer is that very few lead compounds are ideal. Most are likely to have low activity, poor selectivity, and significant side effects. They may also be difficult to synthesize, so there is an advantage in finding analogues with improved properties. We look now at strategies that can be used to optimize the interactions of a drug with its target in order to gain higher activity and selectivity.

13.3.1 Variation of substituents

Varying easily accessible substituents is a common method of fine tuning the binding interactions of a drug.

13.3.1.1 Alkyl substituents

Certain alkyl substituents can be varied more easily than others. For example, the alkyl substituents of ethers, amines, esters, and amides are easily varied as shown in Fig. 13.32. In these cases, the alkyl substituent already present can be removed and replaced by another substituent. Alkyl substituents which are part of the carbon skeleton of the molecule are not easily removed, and it is usually necessary to carry out a full synthesis in order to vary them.

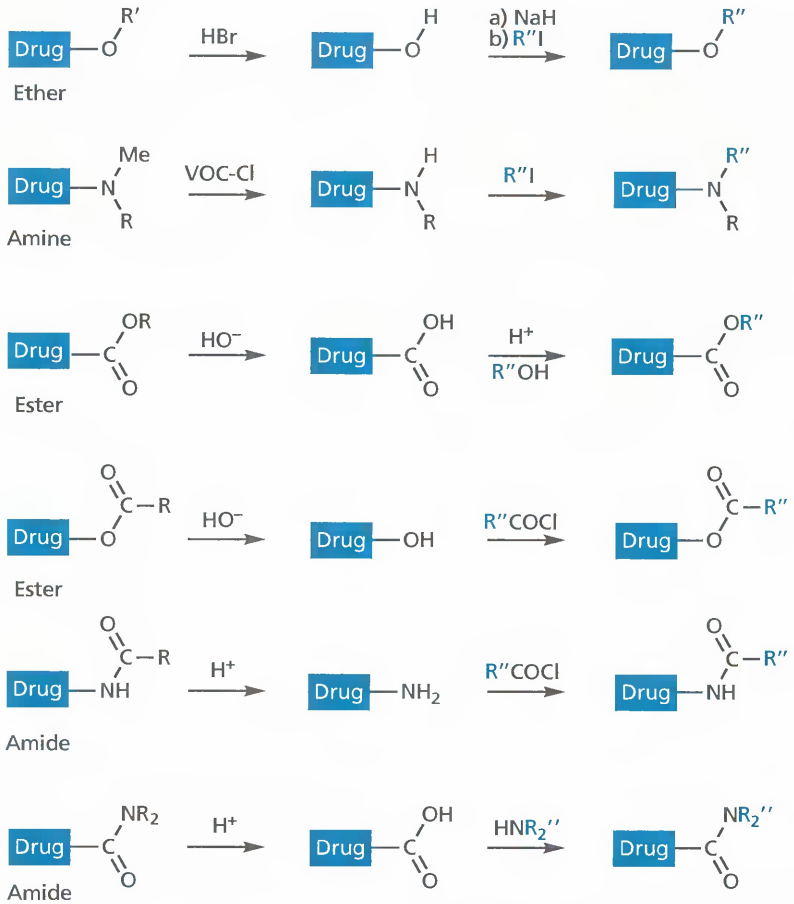


FIGURE 13.32 Methods of modifying an alkyl group.

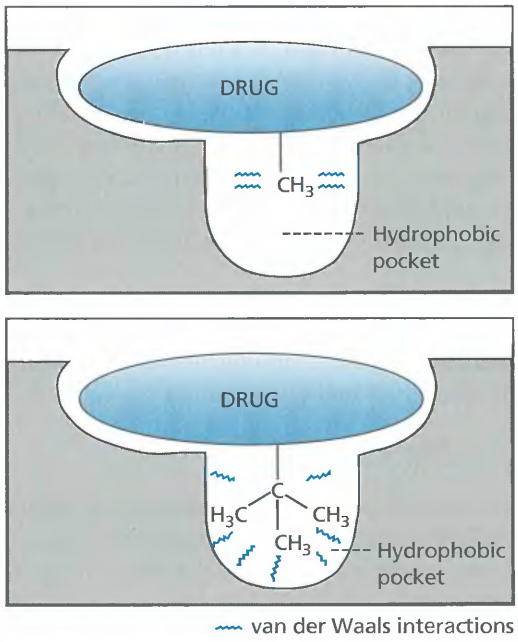


FIGURE 13.33 Variation of alkyl chain to fill a hydrophobic pocket.

If alkyl groups are interacting with a hydrophobic pocket in the binding site, then varying the length and bulk of the alkyl group (e.g. methyl, ethyl, propyl, butyl, isopropyl, isobutyl, or *t*-butyl) allows one to probe the depth and width of the pocket. Choosing a substituent that will fill the pocket will then increase the binding interaction (Fig. 13.33).

Larger alkyl groups may also confer selectivity on the drug. For example, in the case of a compound that interacts with two different receptors, a bulkier alkyl substituent may prevent the drug from binding to one of those receptors and so cut down side-effects (Fig. 13.34).

Isoprenaline (Fig. 13.35) is an analogue of **adrenaline** where a methyl group was replaced by an isopropyl group, resulting in selectivity for adrenergic β-receptors over adrenergic α-receptors (section 23.11.3).

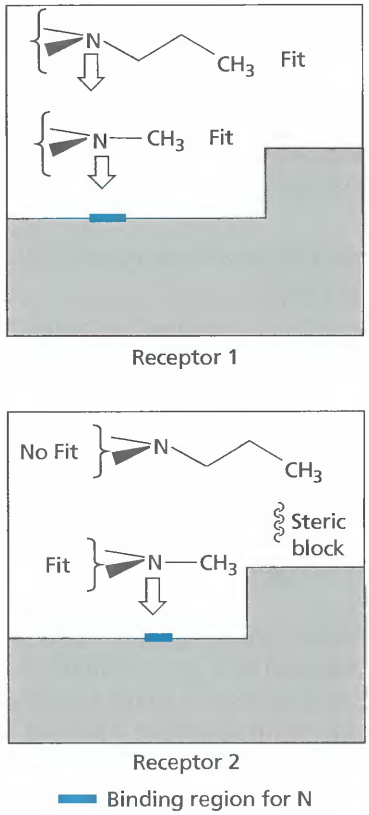


FIGURE 13.34 Use of a larger alkyl group to confer selectivity on a drug.

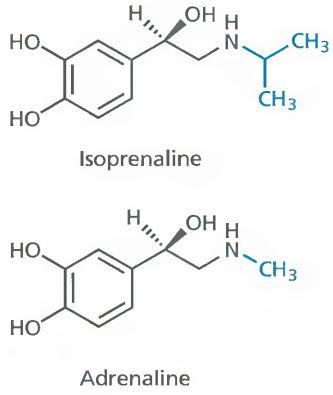


FIGURE 13.35 Isoprenaline and adrenaline.

13.3.1.2 Aromatic substituents

If a drug contains an aromatic ring, the position of substituents can be varied to find better binding interactions, resulting in increased activity (Fig. 13.36).

For example, the best anti-arrythmic activity for a series of benzopyrans was found when the sulfonamide substituent was at position 7 of the aromatic ring (Fig. 13.37).

Changing the position of one substituent may have an important effect on another. For example, an electron-withdrawing nitro group will affect the basicity of an aromatic amine more significantly if it is in the *para* position rather than the *meta* position (Figure 13.38). At the *para* position, the nitro group will make the amine a weaker base and less liable to protonate. This would decrease the amine's ability to interact with ionic binding groups in the binding site, and decrease activity.

If the substitution pattern is ideal, then we can try varying the substituents themselves. Substituents have different steric, hydrophobic, and electronic properties, and

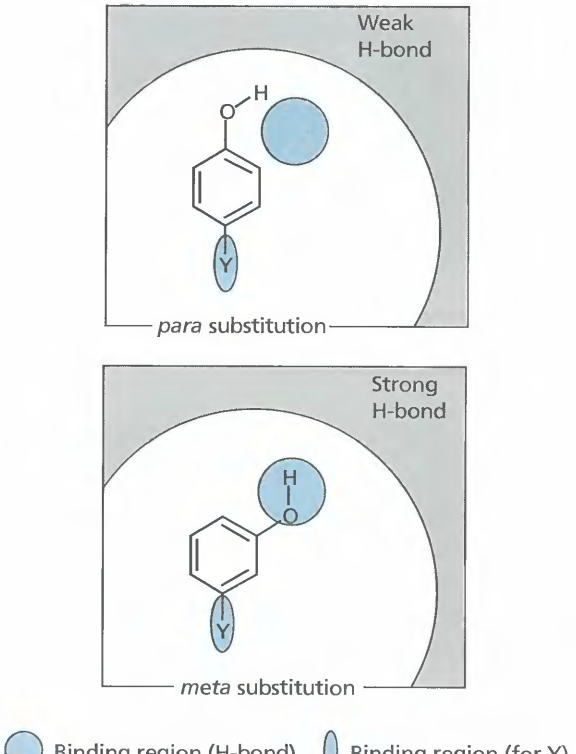


FIGURE 13.36 Varying the position of an aromatic substituent.

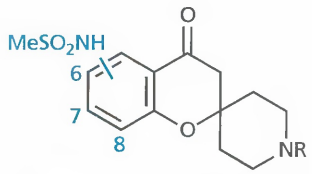


FIGURE 13.37 Benzopyrans.

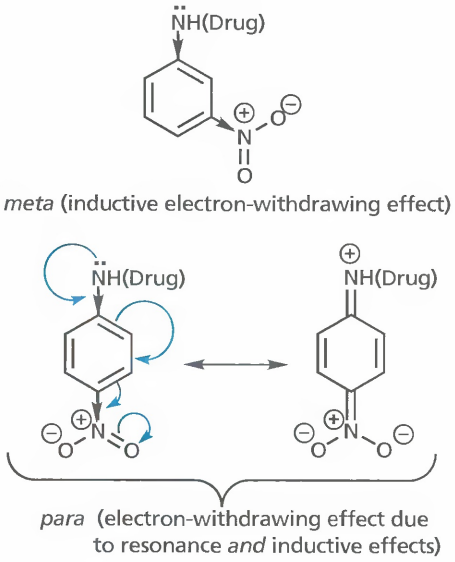


FIGURE 13.38 Electronic effects of different aromatic substitution patterns.

so varying these properties may have an effect on binding and activity. For example, activity might be improved by having a more electron-withdrawing substituent, in which case a chloro substituent might be tried in place of a methyl substituent.

The chemistry involved in these procedures is usually straightforward, so these analogues are made as a matter of course whenever a novel drug structure is developed. Furthermore, the variation of aromatic or aliphatic substituents is open to **quantitative structure–activity studies** (QSARs), as described in chapter 18.

13.3.2 Extension of the structure

The strategy of extension involves the addition of another functional group to the lead compound in order to probe for extra binding interactions with the target. Lead compounds are capable of fitting the binding site and have the necessary functional groups to interact with some of the important binding regions present. However, it is possible that they do not interact with all the binding regions available. For example, a lead compound may bind to three binding regions in the binding site but fail to use a fourth (Fig. 13.39). Therefore, why not add extra functional groups to probe for that fourth region?

Extension tactics are often used to find extra hydrophobic regions in a binding site, by adding various alkyl or arylalkyl groups (Box 13.1). Such groups could be added to functional groups such as alcohols, phenols, amines, and carboxylic acids should they be present in the drug. Other functional groups could be added to probe for extra hydrogen bonding interactions or ionic interactions.

Frequently, extension strategies are used to convert an agonist into an antagonist. This may happen because an extra binding interaction is found which is not used by the natural agonist or substrate. Since the overall binding interaction is different, antagonist activity results rather than agonist activity.

The extension tactic has been used successfully to produce more active analogues of morphine (sections 24.6.2 and 24.6.4) and more active adrenergic agents (sections 23.9–23.11). It was also used to improve the activity and selectivity of the protein kinase inhibitor, imatinib (section 21.6.2.2). Other examples of the extension strategy can be found in Case Studies 2, 5, 6, and 7, as well as sections 20.7.4 and 21.7.1.

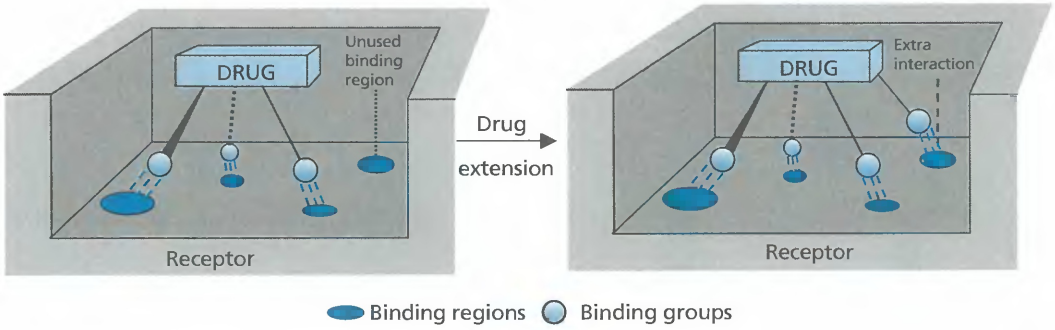


FIGURE 13.39 Extension of a drug to provide a fourth binding group.

BOX 13.1 Use of extension tactics

Extension tactics were used in the development of antihypertensive agents which inhibit an enzyme known as **angiotensin-converting enzyme** (ACE). Adding a phenylalkyl group to the lead compound (I) increased activity (*extension strategy*). Varying the length of the alkyl chain connecting the aromatic ring to (I) demonstrated that the phenethyl group was the best substituent (*chain extension strategy*). This structure showed a 1000-fold improvement in inhibition, demonstrating that the extra aromatic ring was binding to a hydrophobic pocket in the enzyme's active site (see also Case Study 2).

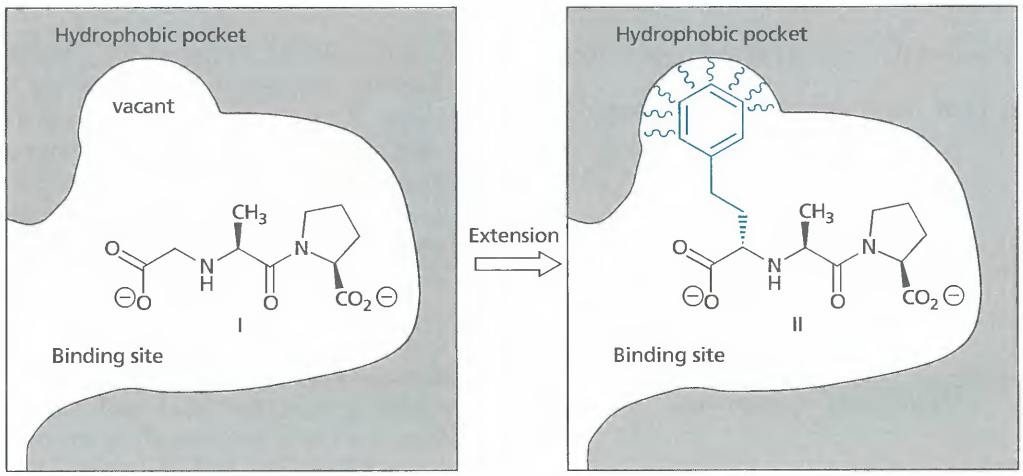


FIGURE 1 Ace inhibitors.

13.3.3 Chain extension/contraction

Some drugs have two important binding groups linked together by a chain, in which case it is possible that the chain length is not ideal for the best interaction. Therefore, shortening or lengthening the chain length is a useful tactic to try (Fig. 13.40, see also Box 13.1, section 24.6.2, and Case Study 2).

13.3.4 Ring expansion/contraction

If a drug has one or more rings that are important to binding, it is generally worth synthesizing analogues where one of these rings is expanded or contracted. The principle behind this approach is much the same as varying the substitution pattern of an aromatic ring. Expanding or contracting a ring may put other rings in different positions relative to each other, and may lead to better interactions with specific regions in the binding site (Fig. 13.41).

Varying the size of a ring can also bring substituents into a good position for binding. For example, during the development of the anti-hypertensive agent **cilazaprilat** (another ACE inhibitor), the bicyclic structure I showed promising activity (Fig. 13.42). The important binding groups were the two carboxylate groups and the amide

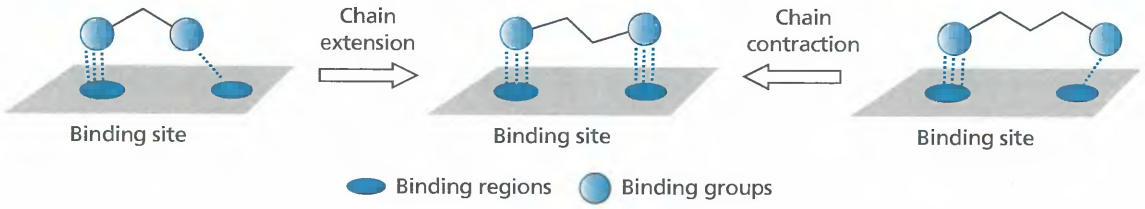


FIGURE 13.40 Chain contraction and chain extension.

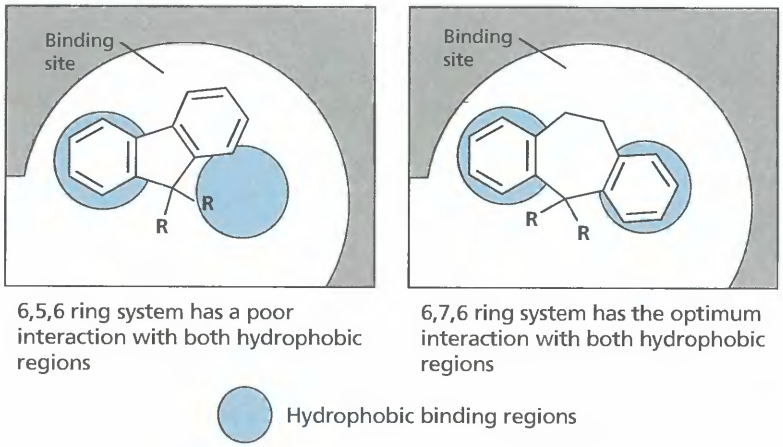


FIGURE 13.41 Ring expansion.

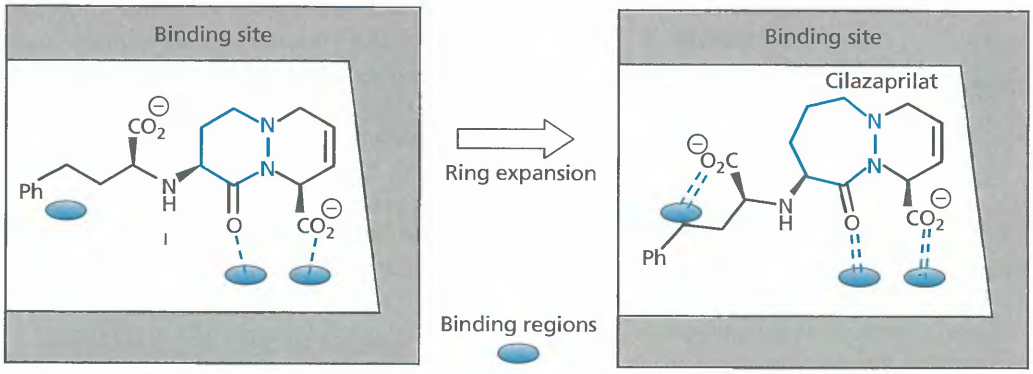


FIGURE 13.42 Development of cilazaprilat.

group. By carrying out various ring contractions and expansions, cilazaprilat was identified as the structure having the best interaction with the binding site.

13.3.5 Ring variations

A popular strategy used for compounds containing an aromatic or heteroaromatic ring is to replace the original ring with a range of other heteroaromatic rings of different ring size and heteroatom positions. For example, several non-steroidal anti-inflammatory agents (NSAIDs) have been reported, all consisting of a central ring with 1,2-biaryl substitution. Different pharmaceutical companies have varied the central ring to produce a range of active compounds (Fig. 13.43).

Admittedly, a lot of these changes are merely ways of avoiding patent restrictions (**'me too' drugs**), but there can often be significant improvements in activity, as well as increased selectivity and reduced side effects ('me-better' drugs). For example, the antifungal agent (I) (Fig. 13.44) acts against an enzyme present in both fungal and human cells. Replacing the imidazole ring of structure (I) with a 1,2,4-triazole ring to give UK 46245 resulted in better selectivity against the fungal form of the enzyme.

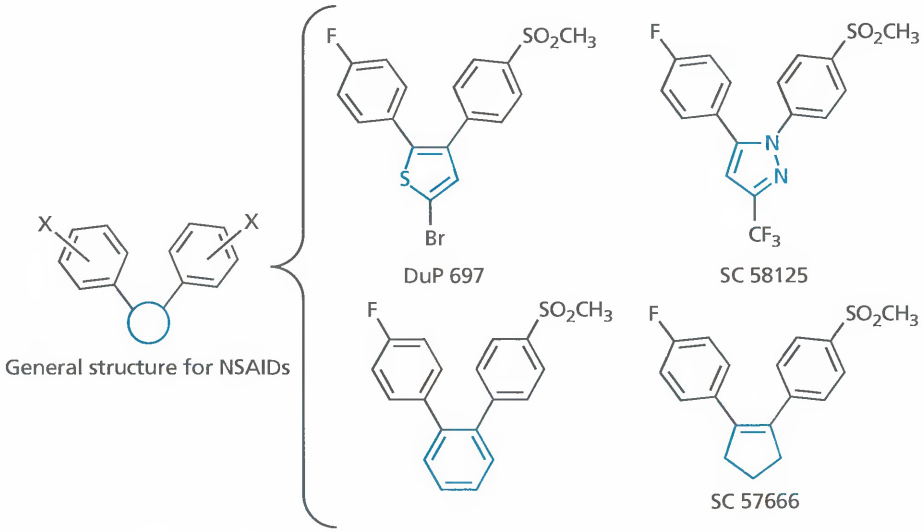


FIGURE 13.43 Non-steroidal anti-inflammatory drugs (NSAIDS).

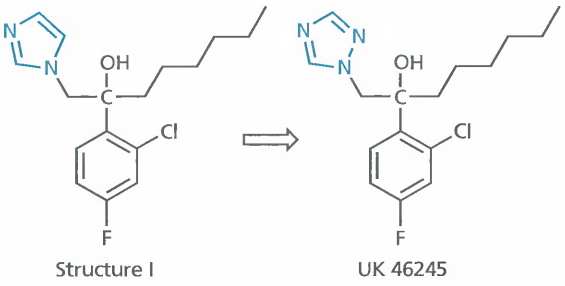


FIGURE 13.44 Development of UK 46245.

One advantage of altering an aromatic ring to a heteroaromatic ring is that it introduces the possibility of an extra hydrogen bonding interaction with the binding site, should a suitable binding region be available. For example, structure I (Fig. 13.45) was the lead compound for a project looking into novel antiviral agents. Replacing the aromatic ring with a pyridine ring resulted in an additional binding interaction with the target enzyme. Further development led eventually to the antiviral agent **nevirapine** (Fig. 13.45).

13.3.6 Ring fusions

Extending a ring by ring fusion can sometimes result in increased interactions or increased selectivity. One of the major advances in the development of the selective β-blockers was the replacement of the aromatic ring in **adrenaline** with a naphthalene ring system (**pronethalol**) (Fig. 13.46). This resulted in a compound that was able to distinguish between two very similar receptors, the α- and β-receptors for adrenaline. One possible explanation for this could be that the β-receptor has a larger van der Waals binding area for the aromatic system than the α-receptor and can interact more strongly with pronethalol than with adrenaline. Another possible explanation is that the naphthalene ring system is sterically too big for the α-receptor, but is just right for the β-receptor.

13.3.7 Isosteres and bioisosteres

Isosteres (section 13.1.15) have often been used in drug design to vary the character of the molecule in a rational way with respect to features such as size, polarity, electronic distribution, and bonding. Some isosteres can be

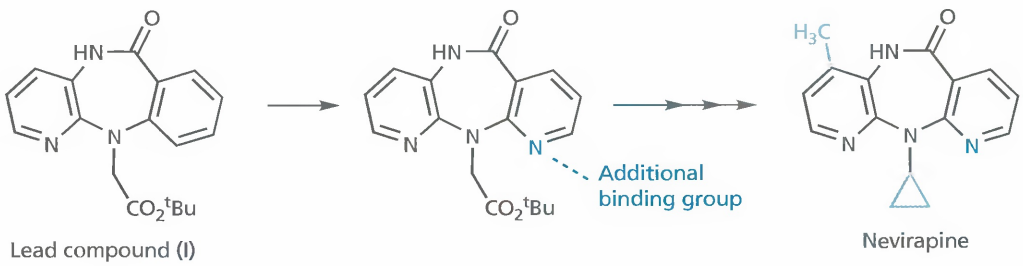
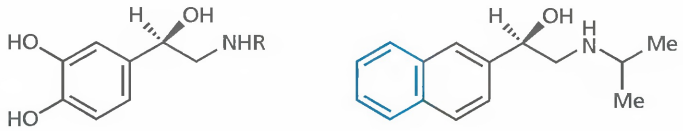


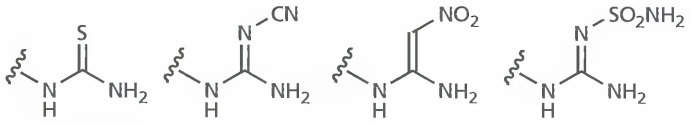
FIGURE 13.45 Development of nevirapine.



R = Me Adrenaline
R = H Noradrenaline

Pronethalol

FIGURE 13.46 Structures of adrenaline, noradrenaline and pronethalol.



Thiourea

FIGURE 13.47 Non-classic isosteres for a thiourea group.

used to determine the importance of size towards activity, whereas others can be used to determine the importance of electronic factors. For example, fluorine is often used as an isostere of hydrogen since it is virtually the same size. However, it is more electronegative and can be used to vary the electronic properties of the drug without having any steric effect.

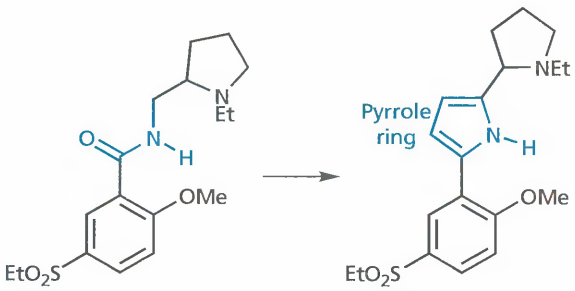
The presence of fluorine in place of an enzymatically labile hydrogen can also disrupt an enzymatic reaction, as C–F bonds are not easily broken. For example, the antitumour drug **5-fluorouracil** described in section 21.3.2 is accepted by its target enzyme because it appears little different from the normal substrate—**uracil**. However, the mechanism of the enzyme-catalysed reaction is totally disrupted, as the fluorine has replaced a hydrogen which is normally lost during the enzyme mechanism.

Several non-classic isosteres have been used in drug design as replacements for particular functional groups. Non-classic isosteres are groups which do not obey the steric and electronic rules used to define classic isosteres, but which have similar physical and chemical properties. For example, the structures shown in Fig. 13.47 are non-classic isosteres for a thiourea group. They are all planar groups of similar size and basicity.

The term bioisostere is used in drug design and includes both classic and non-classic isosteres. A bioisostere is a group that can be used to replace another group while retaining the desired biological activity. Bioisosteres are often used to replace a functional group that is important for target binding but is problematic in one way or another. For example, the thiourea group was present as an important binding group in early histamine antagonists, but was responsible for toxic side effects. Replacing it with bioisosteres allowed the important binding interactions to be retained for histamine antagonism but avoided the toxicity problems (section 25.2.6). Further examples of the use of bioisosteres are given in sections 14.1.5, 14.2.2, and 20.7.4. It is important to realize that bio-isosteres are specific for a particular group of compounds and their target. Replacing a functional group with a bioisostere is not guaranteed to retain activity for every drug at every target.

As stated above, bioisosteres are commonly used in drug design to replace a problematic group while retaining activity. In some situations, the use of a bioisostere can actually increase target interactions and/or selectivity. For example, a pyrrole ring has frequently been used as a bioisostere for an amide. Carrying out this replacement on the dopamine antagonist **sultopride** led to increased activity and selectivity towards the dopamine D_3-receptor over the dopamine D_2-receptor (Fig. 13.48). Such agents show promise as anti-psychotic agents which lack the side effects associated with the D_2-receptor.

Introducing a bioisostere to replace a problematic group often involves introducing further functional groups that might form extra binding interactions with the target binding site (section 13.3.2). For example, a



Sultopride DU 122290

FIGURE 13.48 Introducing a pyrrole ring as a bioisostere for an amide group.

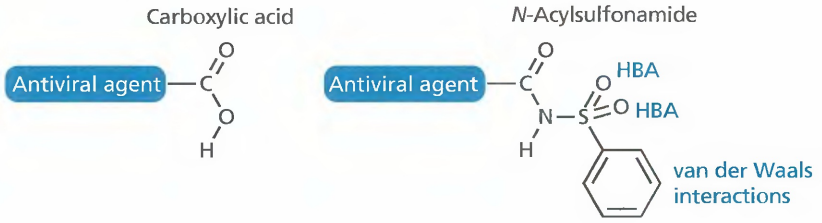


FIGURE 13.49 Extra binding interactions that might be possible when using an *N*-acylsulfonamide as a bioisostere for a carboxylic acid.

ten-fold increase in activity was observed for an antiviral agent when an *N*-acylsulfonamide was used as a bioisostere for a carboxylic acid (Fig. 13.49). The *N*-acylsulfonamide group introduces the possibility of further hydrogen bonding or van der Waals interactions with the binding site.

Transition-state isosteres are a special type of isostere used in the design of transition-state analogues. These are drugs that are used to inhibit enzymes (section 7.4). During an enzymatic reaction, a substrate goes through a transition state before it becomes product. It is proposed that the transition state is bound more strongly than either the substrate or the product, so it makes sense to design drugs based on the structure of the transition state rather than the structure of the substrate or the product. However, the transition state is inherently unstable and so transition-state isosteres are moieties that are used to mimic the crucial features of the transition state, but which are stable to the enzyme-catalysed reaction. For example, the transition state of an amide hydrolysis resembles the tetrahedral reaction intermediate shown in Fig. 13.50. This is a geminal diol, which is inherently unstable. The hydroxethylene moiety shown is a transition-state isostere since it shares the same tetrahedral geometry, retains one of the hydroxyl groups and is stable to hydrolysis. Further examples of the use of transition-state isosteres are given in sections 20.7.4, 20.8.3, and 21.3.4, and Case Studies 1 and 2.

13.3.8 Simplification of the structure

Simplification is a strategy which is commonly used on the often complex lead compounds arising from natural sources (Box 13.2). Once the essential groups of such a drug have been identified by SAR, it is often possible to discard the non-essential parts of the structure without losing activity. Consideration is given to removing functional groups which are not part of the pharmacophore, simplifying the carbon skeleton and removing asymmetric centres.

This strategy is best carried out in small stages. For example, consider our hypothetical natural product glipine (Fig. 13.51). The essential groups have been highlighted, and we might aim to synthesize simplified compounds in the order shown. These still retain the groups that we have identified as being essential—the pharmacophore.

Chiral drugs pose a particular problem. The easiest and cheapest method of synthesizing a chiral drug is to make the racemate. However, both enantiomers then have to be tested for their activity and side effects, doubling the number of tests that have to be carried out. This is because different enantiomers can have different activities. For example, compound **UH-301** (Fig. 13.52) is inactive as a racemate, whereas its enantiomers have opposing agonist and antagonist activity at the serotonin receptor (5-HT_{1A}). One enantiomer of thalidomide is teratogenic whereas the other is not.

The use of racemates is discouraged, and it is preferable to use a pure enantiomer. This could be obtained by separating the enantiomers of the racemic drug, or carrying out an asymmetric synthesis. Both options inevitably add to the cost of the synthesis, and so designing a structure that lacks some or all of the asymmetric centres, can be advantageous and represents a simplification of the structure. For example, the cholesterol-lowering agent **mevinolin** has eight asymmetric centres, but a second

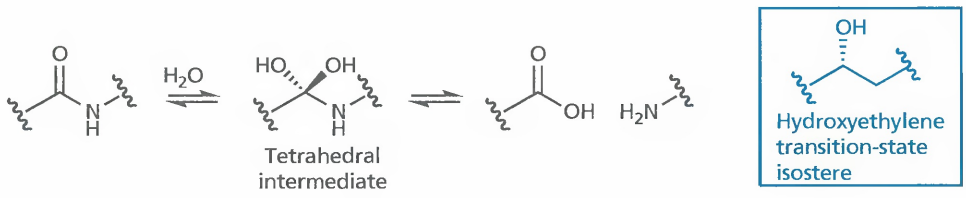


FIGURE 13.50 Example of a transition-state isostere designed to resemble a tetrahedral intermediate formed during an enzyme-catalysed reaction.

BOX 13.2 Simplification

Simplification tactics have been used successfully with the alkaloid **cocaine**. Cocaine has local anaesthetic properties, and its simplification led to the development of local anaesthetics which could be easily synthesized in the laboratory. One of the earliest was **procaine (Novocaine)**, discovered in 1909 (Fig. 1). Simplification tactics have also proved effective in the design of simpler morphine analogues (section 24.6.3).

More recently, the microbial metabolite **asperlicin** was simplified to **devazepide**, retaining the benzodiazepine and indole skeletons inherent in the structure (Fig. 2). Both asperlicin and devazepide act as antagonists of a neuropeptide chemical messenger called **cholecystokinin** (CCK) which has been implicated in causing panic attacks. Therefore, antagonists may be of use in treating such attacks.

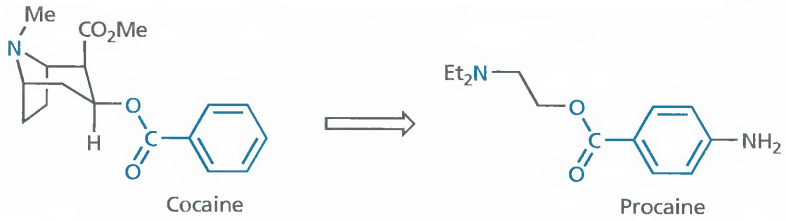


FIGURE 1 Simplification of cocaine (pharmacophore shown in colour).

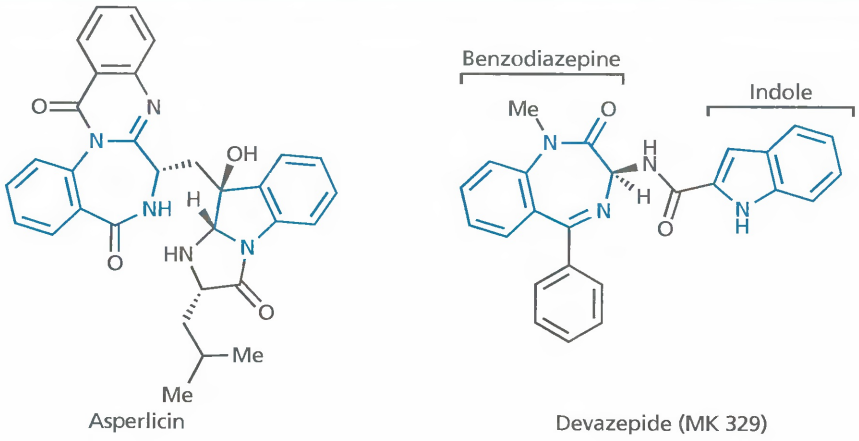


FIGURE 2 Simplification of asperlicin.

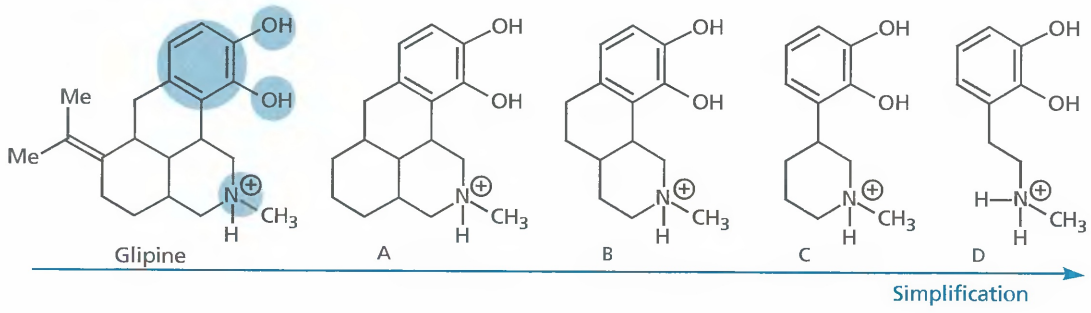


FIGURE 13.51 Glipine analogues.

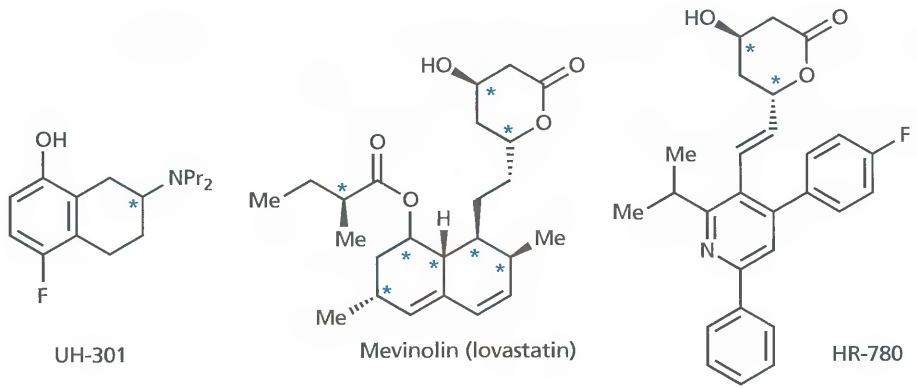


FIGURE 13.52 UH-301, mevinolin and HR-780.

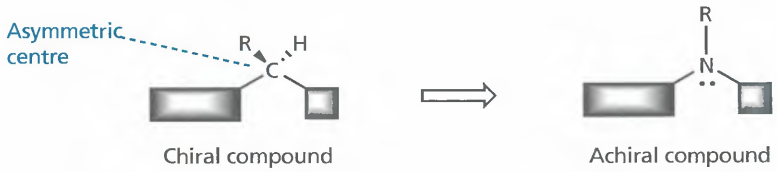


FIGURE 13.53 Replacing an asymmetric carbon with nitrogen.

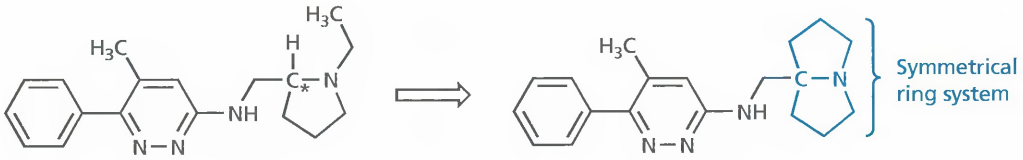


FIGURE 13.54 Introducing symmetry.

generation of cholesterol-lowering agents has been developed which contain far fewer (e.g. **HR 780**; Fig. 13.52; Case Study 1).

Various tactics can be used to remove asymmetric carbon centres. For example, replacing the carbon centre with nitrogen has been effective in many cases (Fig. 13.53). An illustration of this can be seen in the design of thymidylate synthase inhibitors described in Case Study 5. (It should be noted that the introduction of an amine in this way may well have significant effects on the pharmacokinetics of the drug in terms of log*P*, basicity, polarity, etc.; see chapters 11 and 14.) Another tactic is to introduce symmetry where originally there was none. For example, the muscarinic agonist (II) was developed from (I) in order to remove asymmetry, and has the same activity (Fig. 13.54).

The advantage of simpler structures is that they are easier, quicker, and cheaper to synthesize in the laboratory. Usually the complex lead compounds obtained from natural sources are impractical to synthesize and have to be extracted from the source material—a slow, tedious, and expensive business. Removing unnecessary functional groups can also be advantageous in removing side effects if these groups interact with other targets, or are chemically reactive. There are, however, potential disadvantages in oversimplifying molecules. Simpler molecules are often more flexible and can sometimes bind differently to their targets compared to the original lead compound, resulting in different effects. It is best to simplify in small stages, checking that the desired activity is retained at each stage. Oversimplification may also result in reduced activity, reduced selectivity, and increased side effects. We shall see why in the next section (section 13.3.9).

13.3.9 Rigidification of the structure

Rigidification has often been used to increase the activity of a drug or to reduce its side effects. In order to understand why this tactic can work, let us consider

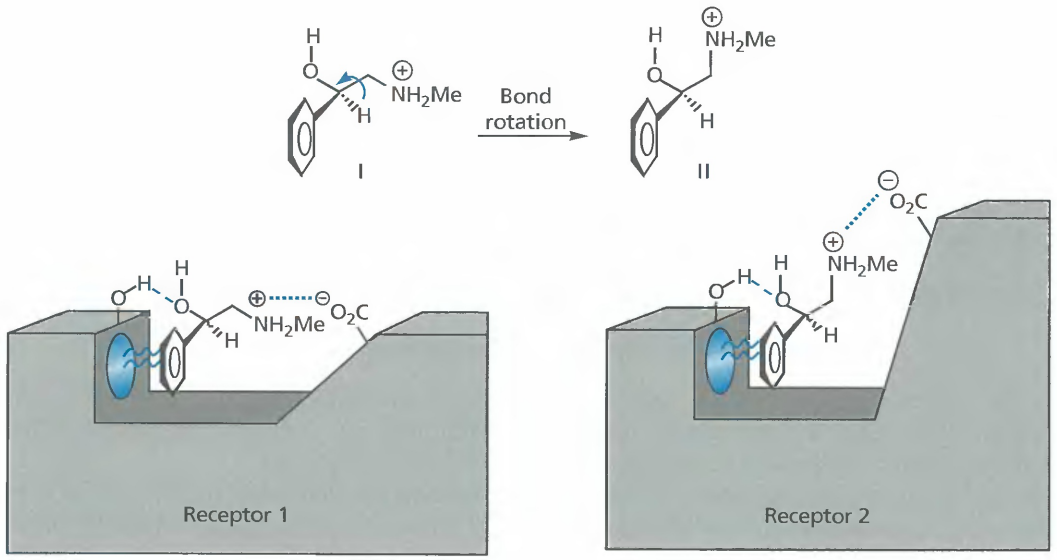


FIGURE 13.55 Two conformations of a neurotransmitter which are capable of binding with different receptors.

again our hypothetical neurotransmitter from chapter 5 (Fig. 13.55). This is quite a simple molecule and highly flexible. Bond rotation can lead to a large number of conformations or shapes. As seen in Fig. 13.55, conformation I is the conformation recognized by the receptor and represents the **active conformation**. Other conformations such as II have the ionized amino group too far away from the anionic centre to interact efficiently, so this is an inactive conformation for our model receptor binding site. However, it is quite possible that a different receptor exists which *is* capable of binding conformation II. If this is the case, then our model neurotransmitter could switch on two different receptors and give two different biological responses.

The body's own neurotransmitters are highly flexible molecules (section 4.2), but fortunately the body is efficient at releasing them close to their target receptors, then quickly inactivating them so that they do not make the journey to other receptors. This is not the case for drugs. They have to be sturdy enough to travel throughout the body and will interact with all the receptors that are prepared to accept them. The more flexible a drug molecule is, the more likely it will interact with more than one receptor and produce other biological responses (side effects). Too much flexibility is also bad for oral bioavailability (section 11.2).

The strategy of rigidification is to lock the molecule into a more rigid conformation so that it cannot take up these other shapes or conformations. Consequently, other receptor interactions and side effects are eliminated. This same strategy should also increase activity since, by locking the drug into the active conformation, the drug is ready to fit its target receptor site and does not need to adjust to the correct conformation. This is also important when it comes to the thermodynamics of binding. A flexible molecule has to adopt a single active conformation in order to bind to its target, which means that it has to become more ordered. This results in a decrease in entropy, and since the free energy of binding is related to entropy by the equation $\Delta G = \Delta H - T\Delta S$, any decrease in entropy will adversely affect ΔG. This in turn lowers the binding affinity (K_i), which is related to ΔG by the equation $\Delta G = -RT \ln K_i$. A totally rigid molecule, on the other hand, is already in its active conformation, and there is no loss of entropy involved in binding to the target. If the binding interactions (ΔH) are exactly the same as for the more flexible molecule, the rigid molecule will have the better overall binding affinity.

Incorporating the skeleton of a flexible drug into a ring is the usual way of locking a conformation, and for our model compound the analogue shown in Fig. 13.56 would be suitably rigid.

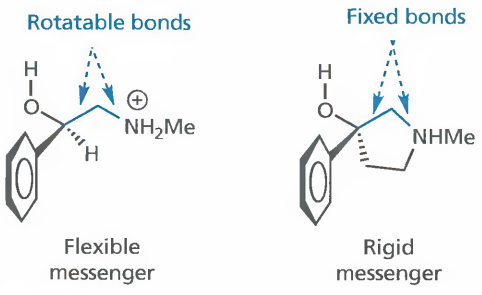
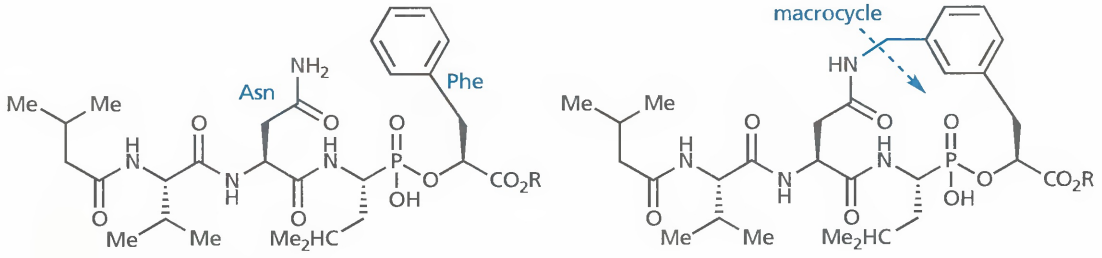


FIGURE 13.56 Rigidification of a molecule by locking bonds within a ring.



Acyclic pentapeptide (K_i 42 nM) Rigidified pentapeptide (K_i 0.1 nM)

FIGURE 13.57 Rigidification of an acyclic pentapeptide.

A ring was used to rigidify the acyclic pentapeptide shown in Fig. 13.57. This is a highly flexible molecule that acts as an inhibitor of a proteolytic enzyme. It was decided to rigidify the structure by linking the asparagine residue with the aromatic ring of the phenylalanine residue to form a macrocyclic ring. The resulting structure showed a 400-fold increase in activity.

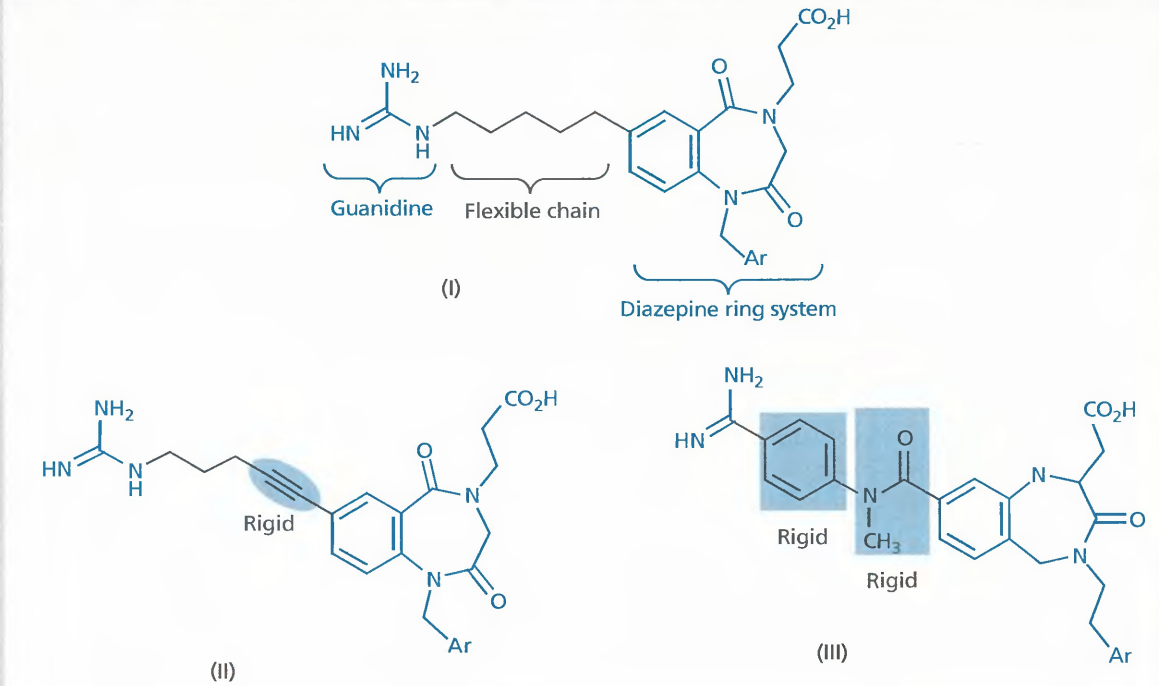
Similar rigidification tactics have been useful in the development of the anti-hypertensive agent **cilazapril** (Fig. 12.11) from captopril, and the development of the sedative **etorphine** (section 24.6.4). Other examples of rigidification can be seen in Case Study 6, as well as sections 21.7.1 and 25.2.8.1).

Locking a rotatable bond into a ring is not the only way a structure can be rigidified. A flexible side chain can be partially rigidified by incorporating a rigid functional group such as a double bond, alkyne, amide, or aromatic ring (Box 13.3).

Rigidification also has potential disadvantages. Rigidified structures may be more complicated to synthesize.

BOX 13.3 Rigidification tactics in drug design

The diazepine (I) is an inhibitor of platelet aggregation and binds to its target receptor by means of a guanidine functional group and a diazepine ring system. These binding groups are linked together by a highly flexible chain. Structures (II) and (III) are examples of active compounds where the connecting chain between the guanidine group and the bicyclic system has been partially rigidified by the introduction of rigid functional groups.



Rigidification of flexible chains.

There is also no guarantee that rigidification will retain the active conformation; it is perfectly possible that rigidification will lock the compound into an inactive conformation. Another disadvantage involves drugs acting on targets which are prone to mutation. If a mutation alters the shape of the binding site, then the drug may no longer be able to bind, whereas a more flexible drug may adopt a different conformation that *could* bind.

13.3.10 Conformational blockers

We have seen how rigidification tactics can restrict the number of possible conformations for a compound. Another tactic that has the same effect is the use of conformational blockers. In certain situations, a quite simple substituent can hinder the free rotation of a single bond. For example, introducing a methyl substituent to the dopamine (D_3) antagonist (I in Fig. 13.58) gives structure II and results in a dramatic reduction in affinity. The explanation lies in a bad steric clash between the new methyl group and an *ortho* proton on the neighbouring ring which prevents both rings being in the same plane. Free rotation around the bond between the two rings is no longer possible and so the structure adopts a conformation where the two rings are at an angle to each other. In structure I, free rotation around the connecting bond allows the molecule to adopt a conformation where the aromatic rings are coplanar—the active conformation for the receptor. In this case, a conformational blocker 'rejects' the active conformation. Examples of a conformational blocker favouring the active conformation can be seen with 4-methylhistamine (section 25.2.2.2), the design of a serotonin antagonist (Figs. CS6.21 and CS6.23), and the development of the anticancer agent imatinib (section 21.6.2.2). In the last case, conformational restraint not only increased activity, but also introduced selectivity between two similar target binding sites.

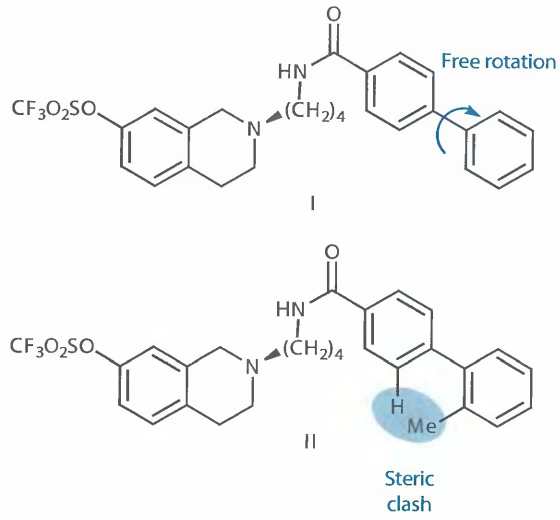


FIGURE 13.58 Introducing rigidity by conformational blocking.

Rigidification is also possible through intramolecular hydrogen bonding which may help to stabilize particular conformations (Fig. 13.59). An example is the kinase inhibitor SU 5416 described in section 21.6.2.4.

13.3.11 Structure-based drug design and molecular modelling

So far we have discussed the traditional strategies of drug design. These were frequently carried out with no knowledge of the target structure, and the results obtained were useful in providing information about the target binding site. Clearly, if a drug has an important binding group, there must be a complementary binding region present in the binding site of the receptor or enzyme.

If the macromolecular target can be isolated and crystallized, then it may be possible to determine the structure using X-ray crystallography. Unfortunately, this does not reveal where the binding site is, and so it is better to crystallize the protein with a known inhibitor or antagonist (ligand) bound to the binding site. X-ray crystallography can then be used to determine the structure of the complex and this can be downloaded to a computer. Molecular modelling software is then used to identify where the ligand is, and thus identify the binding site. Moreover, by measuring the distances between the atoms of the ligand and neighbouring atoms in the binding site, it is possible to identify important binding interactions between the ligand and the binding site. Once this has been done, the ligand can be removed from the binding site *in silico*, and novel lead compounds can

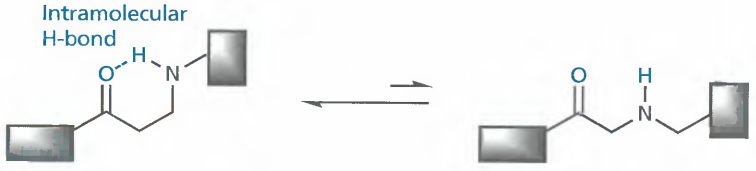


FIGURE 13.59 Rigidification involving an intramolecular hydrogen bond.

be inserted *in silico* to see how well they fit. (The term *in silico* indicates that the virtual process concerned is being carried out on a computer using molecular modelling software.) Regions in the binding site which are not occupied by the lead compound can be identified and used to guide the medicinal chemist as to what modifications and additions can be made to design a new drug that occupies more of the available space and binds more strongly. The drug can then be synthesized and tested for activity. If it proves active, the target protein can be crystallized with the new drug bound to the binding site, and then X-ray crystallography and molecular modelling can be used again to identify the structure of the complex and to see if binding took place as expected. This approach is known as structure based drug design. Examples of the use of structure-based drug design can be found in Case Studies 2, 5, and 6, as well as sections 14.9.1, 20.7.3.2, 20.9, 21.6.2, and 20.7.4.

A related process is known as *de novo* drug design (section 17.15). This involves the design of a novel drug structure, based on a knowledge of the binding site alone. This is quite a demanding exercise, but there are examples where *de novo* design has successfully led to a novel lead compound which can then be the start point for structure-based drug design (Case Study 5 and section 20.7.4.4).

Structure-based drug design cannot be used in all cases. Sometimes the target for a lead compound may not have been identified, and even if it has been identified, it may not be possible to crystallize it. This is particularly true for membrane-bound proteins. One way round this is to identify a protein, which is thought to be similar to the target protein, and which *has* been crystallized and studied by X-ray crystallography. The structural and mechanistic information obtained from that analogous protein can then be used to design drugs for the target protein (Case Studies 2 and 5, and section 21.6.2.1).

Molecular modelling can also be used to study different compounds which are thought to interact with the same target. The structures can be compared and the important pharmacophore identified (section 17.11), allowing the design of novel structures containing the same pharmacophore. Compound databanks can be searched for those pharmacophores to identify novel lead compounds (section 17.13).

There are many other applications of molecular modelling in medicinal chemistry, some of which are described in chapter 17. However, a warning! It is important to appreciate that molecular modelling studies usually tackle only one part of a much bigger problem—the design of an effective drug. True, one might design a compound that binds perfectly to a particular enzyme or receptor, but if the drug cannot be synthesized or never reaches the target protein in the body, it is useless.

13.3.12 Drug design by NMR

The use of NMR in designing lead compounds has already been discussed in section 12.4.10. This can also be seen as a method of drug design, since the focus is not only on designing a lead compound, but in designing a *potent* lead compound. Usually, drug design aims to optimize a lead compound once it has been discovered. In the NMR method, optimization of the component parts (epitopes) is carried out first to maximize binding interactions; then they are linked together to produce the final compound.

NMR is also being increasingly used to identify the structure of target proteins that cannot be crystallized and studied by X-ray crystallography. Once the structure has been identified, molecular modelling techniques can be used for drug design as described in section 13.3.11.

13.3.13 The elements of luck and inspiration

It is true to say that drug design has become more rational, but the role of chance or the need for hard-working, mentally alert medicinal chemists has not yet been eliminated. Most of the drugs still on the market were developed by a mixture of rational design, trial and error, hard graft, and pure luck. There are a growing number of drugs that were developed by rational design such as the ACE inhibitors (Case Study 2), thymidylate synthase inhibitors (Case Study 5), HIV protease inhibitors (section 20.7.4), neuraminidase inhibitors (section 20.8.3), **pralidoxime** (section 22.16), and **cimetidine** (section 25.2), but they are still in the minority.

Frequently, the development of drugs is helped by watching the literature to see what works on related compounds and what doesn't, then trying out similar alterations to one's own work. It is often a case of groping in the dark, with the chemist asking whether the addition of a group at a certain position will have a steric effect, an electronic effect, or a bonding effect. Even when drug design is carried out on rational lines, good fortune often has a role to play (Box 13.4).

Finally, there are some cases where the use of logical step by step modifications to a structure fails to result in significantly improved activity. In such cases, there may be some advantage in synthesising a large range of structures with different substituents or modifications in the hope of striking lucky. This is illustrated in the development of the anticancer agent **sorafenib** (Box 21.10). The breakthrough here was the discovery of an active structure which contained two substituents that were known to be bad for activity when only one or other were present. When both were present, however, there was a beneficial synergistic effect.

BOX 13.4 A slice of luck

The development of the β-blocker **propranolol** was aided by a slice of good fortune. Chemists at ICI were trying to improve on a drug called **pronethalol**, the first β-blocker to reach the market (section 23.11.3). It was known that the naphthalene ring and the ethanolamine segment were important to activity and selectivity, so these groups had to be retained. Therefore, it was decided to study what would happen if the distance between these two groups was extended (*chain extension*). Perhaps by doing so, the two groups would interact with their respective binding regions more efficiently.

Various segments were to be inserted, one of which was the OCH_2 moiety (Fig. 1a). The analogue that would have been obtained is shown as structure I. β-Naphthol was the starting material, but was not immediately available. Rather than waste the day, α-naphthol was used instead (Fig. 1b). The result was propranolol, which has proved a successful drug in the treatment of angina for many years. When the original target structure was eventually made, it showed little improvement over the original lead compound, pronethalol.

A further interesting point concerning this work is that propranolol had been synthesized some years earlier. However, the workers involved had not been searching for β-blocking activity and had not recognized the potential of the compound.

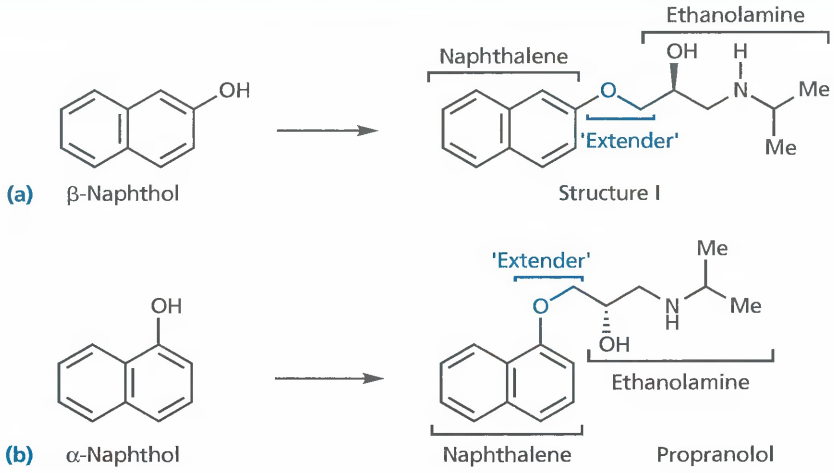


FIGURE 1 The development of the β-blocker propranolol.

KEY POINTS

- Drug optimization aims to maximize the interactions of a drug with its target binding site in order to improve activity and selectivity, and to minimize side effects. Designing a drug that can be synthesized efficiently and cheaply is another priority.
- The length and size of alkyl substituents can be modified to fill up hydrophobic pockets in the binding site or to introduce selectivity for one target over another. Alkyl groups attached to heteroatoms are most easily modified.
- Aromatic substituents can be varied in character and/or ring position.
- Extension is a strategy where extra functional groups are added to the lead compound in order to interact with extra binding regions in the binding site.
- Chains connecting two important binding groups can be modified in length in order to maximize the interactions of each group with the corresponding binding regions.
- Rings linking important binding groups can be expanded or contracted such that the binding groups bind efficiently with relevant binding regions.
- Rings acting as scaffolds for important binding groups can be varied in order to give novel classes of drugs which may have improved properties.
- Rings can be fused to existing rings in order to maximize binding interactions or to increase selectivity for one target over another.
- Classic and non-classic isosteres are frequently used in drug optimization.
- Simplification involves removing functional groups from the lead compound that are not part of the pharmacophore.

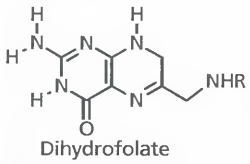
Unnecessary parts of the carbon skeleton or asymmetric centres can also be removed in order to design drugs that are easier and cheaper to synthesize. Oversimplification can result in molecules that are too flexible, resulting in decreased activity and selectivity.

- Rigidification is applicable to flexible lead compounds. The aim is to reduce the number of conformations available while retaining the active conformation. Locking rotatable rings into ring structures, or introducing rigid functional groups are common methods of rigidification.
- Conformational blockers are groups that are introduced into a lead compound to reduce the number of conformations that the molecule can adopt.
- Structure-based drug design makes use of X-ray crystallography and computer-based molecular modelling to study how a lead compound and its analogues bind to a target binding site.
- NMR studies can be used to determine protein structure and to design novel drugs.
- Serendipity plays a role in drug design and optimization.

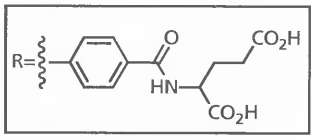
QUESTIONS

1. **DU 122290** was developed from **sultopride** (Fig. 13.48) and shows improved activity and selectivity. Suggest possible reasons for this.

2. Methotrexate inhibits the enzyme dihydrofolate reductase. The pteridine ring system of methotrexate binds to the binding site as shown in Fig. 13.26. Suggest how dihydrofolate (the natural substrate for the enzyme) might bind.

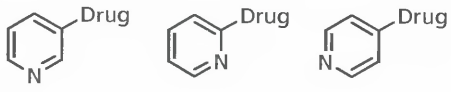


Dihydrofolate



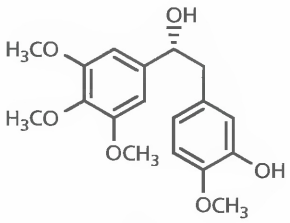
3. A lead compound containing a methyl ester was hydrolysed to give a carboxylic acid. An *in vivo* bioassay suggested that the ester was active and that the acid was inactive. However, an *in vitro* bioassay suggested that the ester was inactive and that the acid was active. Explain these contradictory results.

4. A lead compound contains an aromatic ring. The following structures were made as analogues. Structures I and II were similar in activity to the lead compound whereas structure III showed a marked increase in activity. Explain these results and describe the strategies involved.

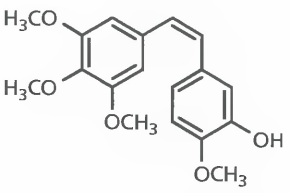


I II III

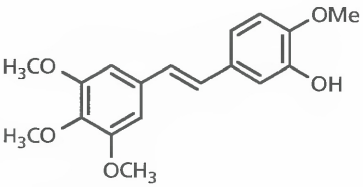
5. The pharmacophore of cocaine is shown in Box 13.2. Identify possible cyclic analogues that are simpler



Combretastatin



V



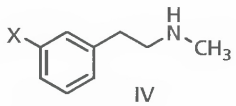
VI

than cocaine and that would be expected to retain activity.

6. Procaine (Box 13.2) has been a highly successful local anaesthetic and yet there are three bonds between the important ester and amine binding groups, compared to four in cocaine. This might suggest that these groups are too close together in procaine. In fact, this is not the case. Suggest why not.

7. The aromatic amine on procaine is not present in cocaine. Comment on its possible role.

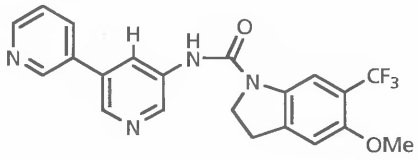
8. Explain how you would apply the principles of rigidification to structure IV below in order to improve its pharmacological properties. Give two specific examples of rigidified structures.



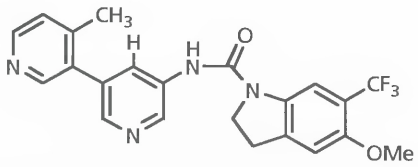
IV

9. Combretastatin is an anticancer agent discovered from an African plant. Analogue V is more active than combretastatin whereas analogue VI is less active. What strategy was used in designing analogues V and VI? Why is analogue V more active than combretastatin, and analogue VI less active?

10. Structure VII is a serotonin antagonist. A methyl group has been introduced into analogue VIII, resulting in increased activity. What role does the methyl group play and what is the term used for such a group? Explain why increased activity arises.



VII



VIII

FURTHER READING

Acharya, K. R., *et al.* (2003) ACE revisited: a new target for structure-based drug design. *Nature Reviews Drug Discovery*, **2**, 891–902.

Bolin, J. T., Filman, D. J., Matthews, D. A., Hamlin, R. C., and Kraut, J. (1982) Crystal structures of *Escherichia coli* and *Lactobacillus casei* dihydrofolate reductase refined at 1.7 Å resolution. I. General features and binding of methotrexate. *Journal of Biological Chemistry*, **257**, 13650–13662 (methotrexate binding).

Hruby, V. J. (2002) Designing peptide receptor agonists and antagonists. *Nature Reviews Drug Discovery*, **1**, 847–858.

Jeffrey, G. A. (1991) *Hydrogen bonding in biological structures.* Springer-Verlag, New York.

Khan, A. R., *et al.* (1998) Lowering the entropic barrier for binding conformationally flexible inhibitors to enzymes. *Biochemistry*, **37**, 16839–16845.

Luca, S., *et al.* (2003) The conformation of neurotensin bound to its G protein-coupled receptor. *Proceedings of the National Academy of Sciences of the USA*, **100**, 10706–10711 (active conformation by NMR).

Meyer, E. G., *et al.* (1995) Backward binding and other structural surprises. *Perspectives in Drug Discovery and Design*, **3**, 168–195.

Pellecchia, M., Sem, D. S., and Wuthrich, K. (2002) NMR in drug discovery. *Nature Reviews Drug Discovery*, **1**, 211–219.

Rees, D. C., *et al.* (2004) *Fragment-based Lead Discovery*, **3**, 660–672

Titles for general further reading are listed on p. 725