# 6 Nucleic acids: structure and function

In this chapter we discuss the structure and function of nucleic acids. Drug action at nucleic acids is discussed in Chapter 9 and in other chapters throughout the text. Although most drugs act on protein structures, there are several examples of important drugs which act directly on nucleic acids. There are two types of nucleic acid—**DNA** (deoxyribonucleic acid) and **RNA** (ribonucleic acid). We first consider the structure of DNA.

## 6.1 Structure of DNA

Like proteins, DNA has a primary, secondary, and tertiary structure.

### 6.1.1 The primary structure of DNA

The primary structure of DNA is the way in which the DNA building blocks are linked together. Whereas proteins have over 20 building blocks to choose from, DNA has only four—the **nucleosides deoxyadenosine**, **deoxyguanosine**, **deoxycytidine**, and **deoxythymidine** (Fig. 6.1). Each nucleoside is constructed from two components—a **deoxyribose** sugar and a base. The sugar is the same in all four nucleosides and only the base is different. The four possible bases are two bicyclic purines (**adenine** and **guanine**) and two smaller pyrimidine structures (**cytosine** and **thymine**) (Fig. 6.2).

The nucleoside building blocks are joined together through phosphate groups which link the 5′-hydroxyl group of one nucleoside unit to the 3′-hydroxyl group of the next (Fig. 6.3). With only four types of building block available, the primary structure of DNA is far less varied than the primary structure of proteins. As a result, it was long thought that DNA had only a minor role to play in cell biochemistry, as it was hard to see how such an apparently simple molecule could have anything to do with the mysteries of the genetic code. The solution to this mystery lies in the secondary structure of DNA.

### 6.1.2 The secondary structure of DNA

Watson and Crick solved the secondary structure of DNA by building a model that fitted all the known experimental results. The structure consists of two DNA chains arranged together in a double helix of constant diameter (Fig. 6.4). The double helix has a major groove and a minor groove, which are of some importance to the action of several anticancer agents acting as intercalators (section 9.1).

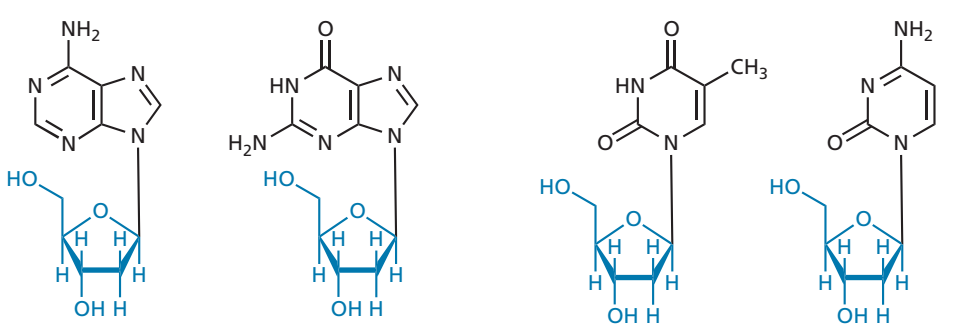



**FIGURE 6.1** Nucleosides—the building blocks of DNA.

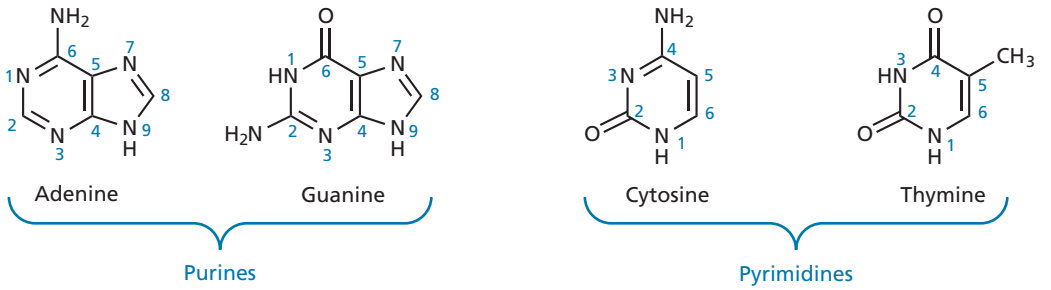



**FIGURE 6.2** The nucleic acid bases for DNA.

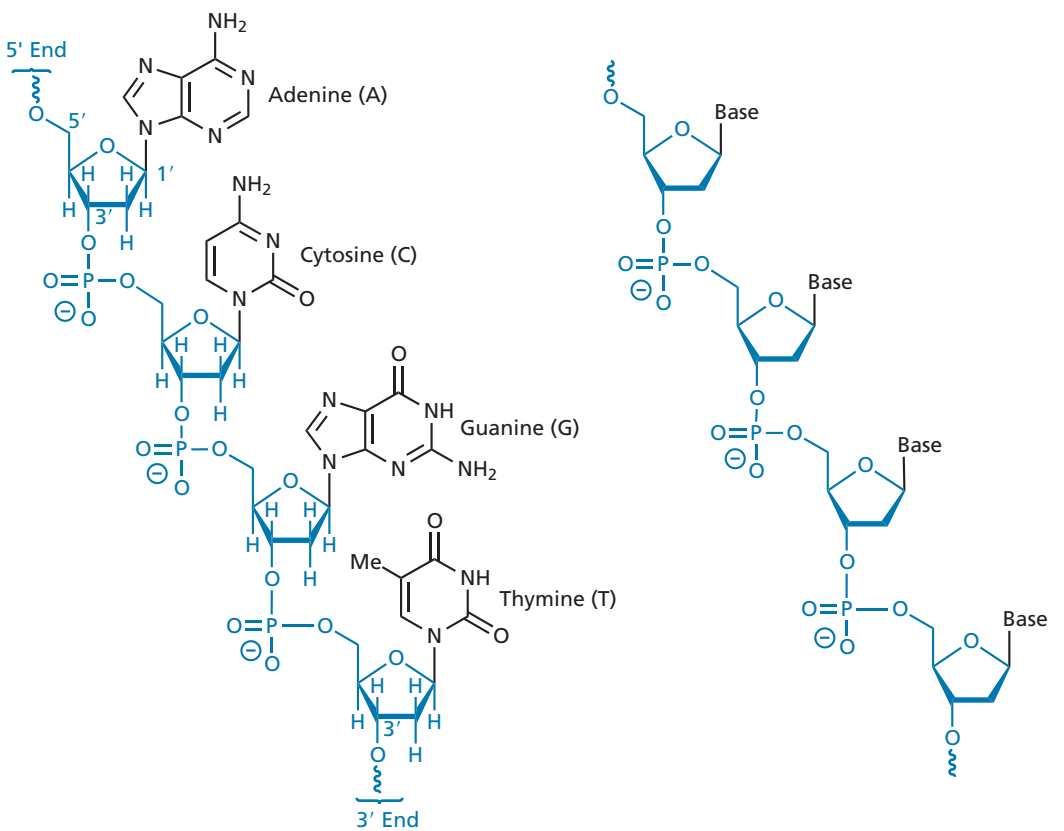



**FIGURE 6.3** Linkage of nucleosides through phosphate groups.

The structure relies crucially on the pairing up of nucleic acid bases between the two chains. Adenine pairs with thymine via two hydrogen bonds, whereas guanine pairs with cytosine via three hydrogen bonds. Thus, a bicyclic purine base is always linked with a smaller monocyclic pyrimidine base to allow the constant diameter of the double helix. The double helix is further stabilized by the fact that the base pairs are stacked one on top of each other, allowing hydrophobic interactions between the faces of the heterocyclic rings. The polar sugar–phosphate backbone is placed to the outside of the structure and can form favourable polar interactions with water.

The fact that adenine always binds to thymine and cytosine always binds to guanine means that the chains are complementary to each other. It is now possible to see how **replication** (the copying of genetic information) is feasible. If the double helix unravels, a new chain can be constructed on each of the original chains (Fig. 6.5). In other words, each of the original chains acts as a template for the construction of a new and identical double helix. The mechanism by which this

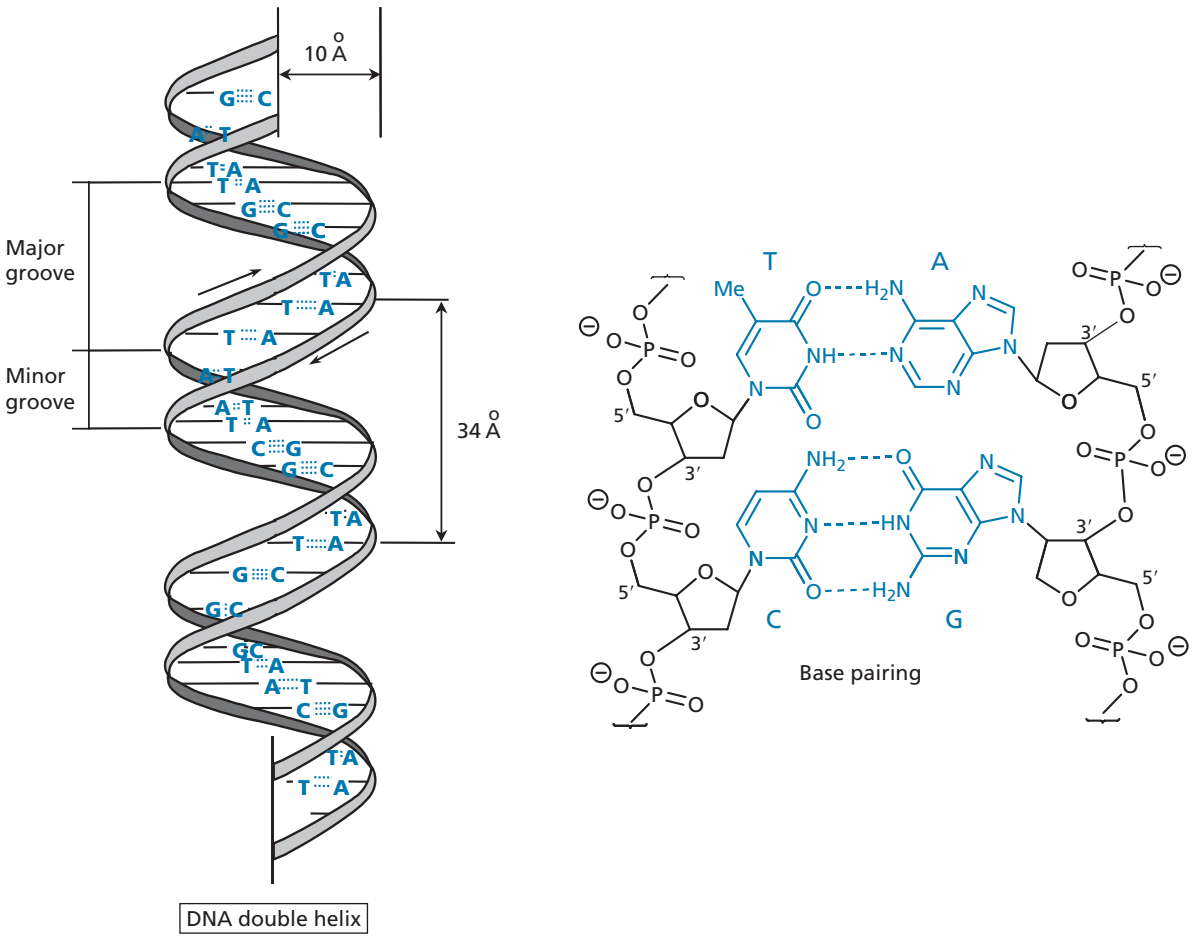



**FIGURE 6.4** The secondary structure of DNA.

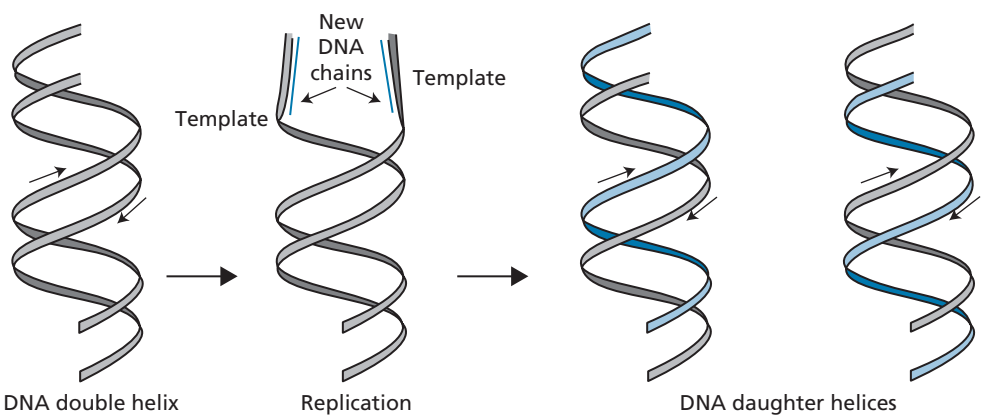



**FIGURE 6.5** Replication of DNA chains.

takes place is shown in Figs 6.6 and 6.7. The template chain has exposed bases which can base-pair by hydrogen bonding with individual **nucleotides** in the form of triphosphates. Once a nucleotide has base-paired, an enzyme-catalysed reaction takes place where the new nucleotide is spliced on to the growing complementary chain with the loss of a diphosphate group—the latter acting as a good leaving group. Note that the process involves each new nucleotide reacting with the 3′ end of the growing chain.

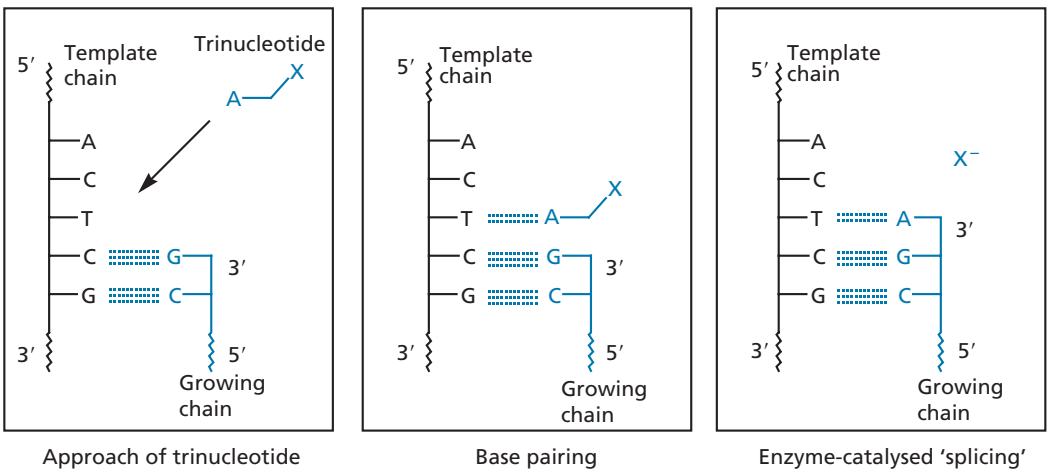



**FIGURE 6.6** Base pairing of a trinucleotide and extension of the growing DNA chain.

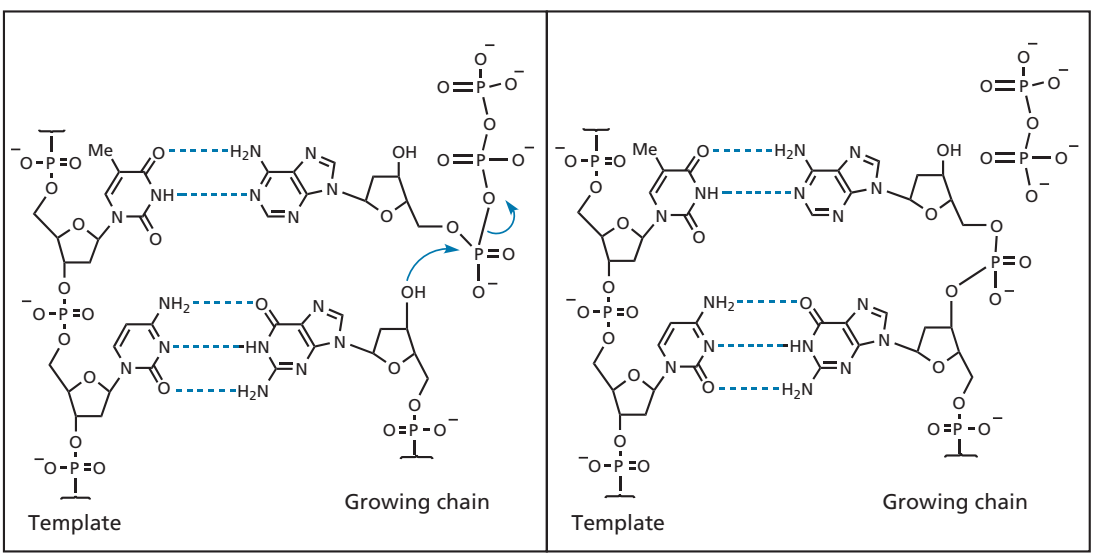



**FIGURE 6.7** Mechanism by which a nucleotide is linked to the growing DNA chain.

We can now see how genetic information is passed on from generation to generation, but it is less obvious how DNA codes for proteins. How can only four nucleotides code for over 20 amino acids? The answer lies in the **triplet code**. In other words, an amino acid is coded not by one nucleotide, but by a set of three. There are 64 ($4^3$) ways in which four nucleotides can be arranged in sets of three—more than enough for the task required. Appendix 2 shows the standard genetic code for the various triplets. We shall look at how this code is interpreted to produce a protein in section 6.2.

## 6.1.3 The tertiary structure of DNA

The tertiary structure of DNA is often neglected or ignored, but it is important to the action of the quinolone group of antibacterial agents (section 9.2) and to several anticancer agents (sections 9.1 and 9.2). DNA is an extremely long molecule: so long, in fact, that it would not fit into the nucleus of the cell if it existed as a linear molecule. It has to be coiled into a more compact three-dimensional shape which *can* fit into the nucleus—a process known as **supercoiling**. This process requires the action of a family of enzymes called **topoisomerases**, which can catalyse the seemingly impossible act of passing one stretch of DNA helix across another stretch. They do this by temporarily cleaving one, or both, strands of the DNA helix to create a temporary gap, then resealing the strand(s) once the crossover has taken place. Supercoiling allows the efficient storage of DNA, but the DNA has to be uncoiled again if replication and transcription (section 6.2.2) are to take place. If uncoiling did not take place, the

unwinding process (catalysed by **helicase** enzymes) that takes place during replication and transcription would lead to increased tension due to increased supercoiling of the remaining DNA double helix. You can demonstrate the principle of this by pulling apart the strands of rope or sisal. The same topoisomerase enzymes are responsible for catalysing the uncoiling process, so inhibition of these enzymes would effectively block transcription and replication.

**Topoisomerase II** is a mammalian enzyme that is crucial to the effective replication of DNA. The enzyme binds to parts of DNA where two regions of the double helix are in near proximity (Fig. 6.8). The enzyme binds to one of these DNA double helices and tyrosine residues are used to nick both strands of the DNA (Fig. 6.9). This results in a temporary covalent bond between the enzyme and the resulting 5′ end of each strand, thus stabilizing the DNA. The strands are now pulled in opposite directions to form a gap through which the intact DNA region can be passed. The enzyme then reseals the strands and departs.

**Topoisomerase I** is similar to topoisomerase II in that it relieves the torsional stress of supercoiled DNA during replication, transcription, and the repair of DNA. The difference is that it cleaves only one strand of DNA, whereas topoisomerase II cleaves both strands. The enzyme catalyses a reversible transesterification reaction similar to that shown in Fig. 6.9, but where the tyrosine residue of the enzyme is linked to the 3′ phosphate end of the DNA strand rather than the 5′ end. This

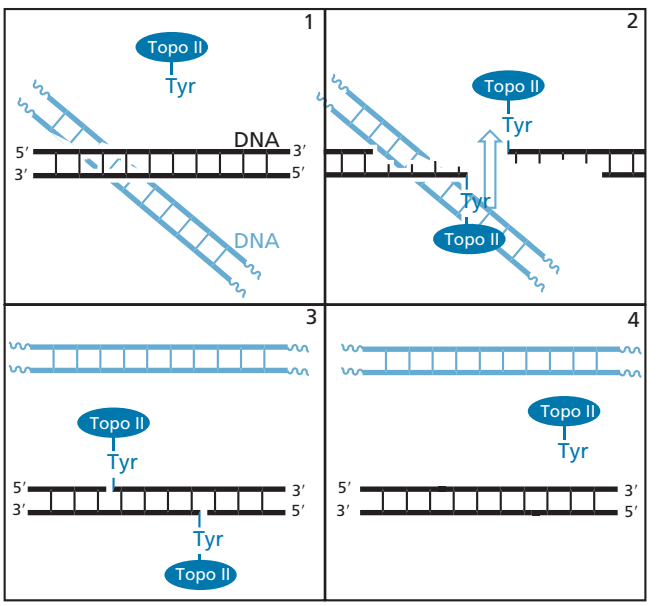



**FIGURE 6.8** Method by which topoisomerase II catalyses the crossover of DNA strands. Note that the same enzyme bonds covalently to each DNA strand.

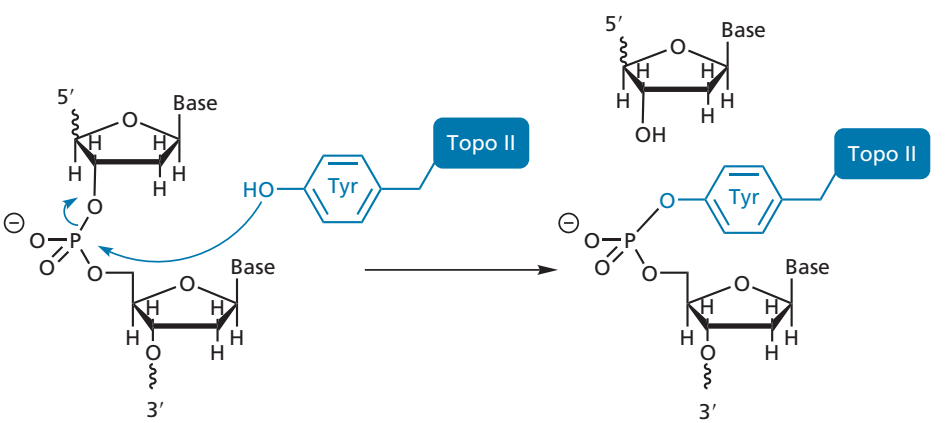



**FIGURE 6.9** Mechanism by which topoisomerase II splits a DNA chain.

creates a 'cleavable complex' with a single-strand break. Relaxation of torsional strain takes place either by allowing the intact strand to pass through the nick or by free rotation of the DNA about the uncleaved strand. Once the torsional strain has been relieved, the enzyme rejoins the cleaved strand of DNA and departs.

**Topoisomerase IV** is a bacterial enzyme that carries out the same process as the mammalian enzyme topoisomerase II and is an important target for the fluoroquinolone antibacterial agents (section 9.2).

### 6.1.4 Chromatins

So far, we have focused on the structure of DNA. However, DNA is not an isolated macromolecule within the nucleus of the cell. It is associated with a variety of proteins, such as histones, in a structure called a chromatin (Fig. 21.5). The histones and associated DNA form a structure called a **nucleosome**, which occurs regularly along the length of the chromatin and plays a crucial role in the regulation of DNA transcription (section 21.7.3).

### 6.1.5 Genetic polymorphism and personalized medicine

The process of replication is not 100% perfect and, occasionally, a mutation can occur. If the mutation does not prove fatal, it will be carried on from generation to generation. This leads to different individuals having subtly different gene sequences. On average, there is a difference of one base pair in every thousand base pairs between individuals. This is known as **genetic polymorphism**. As the nucleic acid bases act as the code for amino acids in proteins, a difference at this level results in a different amino acid being introduced into a protein, which may or may not have an effect on that protein's activity or function (sections 3.5.6 and 4.11). Genetic polymorphism has important consequences with respect to the susceptibility of individuals to disease and also to the kinds of drug therapies that are best suited for individuals. A detailed knowledge of a patient's genome opens up the possibility of predicting and preventing disease, as well as choosing the ideal drug therapy for that patient should a disease occur. This is known as **personalized medicine** (see also sections 15.1.4.4 and 21.1.11).

**KEY POINTS**

- The primary structure of DNA consists of a sugar phosphate backbone with nucleic acid bases attached to each sugar moiety. The sugar is deoxyribose and the bases are adenine, thymine, cytosine, and guanine.
- The secondary structure of DNA is a double helix where the nucleic acid bases are stacked in the centre and paired up such that adenine pairs with thymine, and cytosine pairs with guanine. Hydrogen bonding is responsible for the base-pairing and there are van der Waals interactions between the stacks of bases. Polar interactions occur between the sugar phosphate backbone and surrounding water.
- The DNA double helix is coiled up into a tertiary structure. The coiling and uncoiling of the double helix requires topoisomerase enzymes.
- The copying of DNA from one generation to the next is known as replication. Each strand of a parent DNA molecule acts as the template for a new daughter DNA molecule.
- The genetic code consists of nucleic acid bases, which are read in sets of three during the synthesis of a protein. Each triplet of bases codes for a specific amino acid.
- Knowing a patient's genome opens up the possibility of predicting disease and identifying the best therapies for that individual. This is known as personalized medicine.

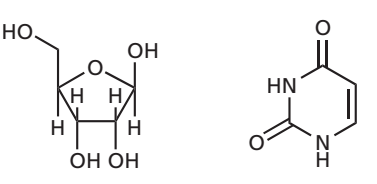
## 6.2 Ribonucleic acid and protein synthesis

### 6.2.1 Structure of RNA

The primary structure of RNA is the same as that of DNA, with two exceptions: **ribose** (Fig. 6.10) is the sugar component rather than **deoxyribose**, and **uracil** (Fig. 6.10) replaces thymine as one of the bases.

Base-pairing between nucleic acid bases can occur in RNA, with adenine pairing to uracil, and cytosine pairing to guanine. However, the pairing is between bases within the same chain and it does not occur for the whole length of the molecule (e.g. Fig. 6.11). Therefore, RNA is not a double helix, but it does have regions of helical secondary structure.

Because the secondary structure is not uniform along the length of the RNA chain, more variety is allowed in RNA tertiary structure. There are three main types of RNA molecules with different cellular functions: **messenger RNA** (mRNA), **transfer RNA** (tRNA), and **ribosomal RNA** (rRNA). These three molecules are crucial to the process by which protein synthesis takes place. Although DNA contains the genetic code for proteins, it cannot produce these proteins directly. Instead, RNA




Ribose Uracil

**FIGURE 6.10** Ribose and uracil.

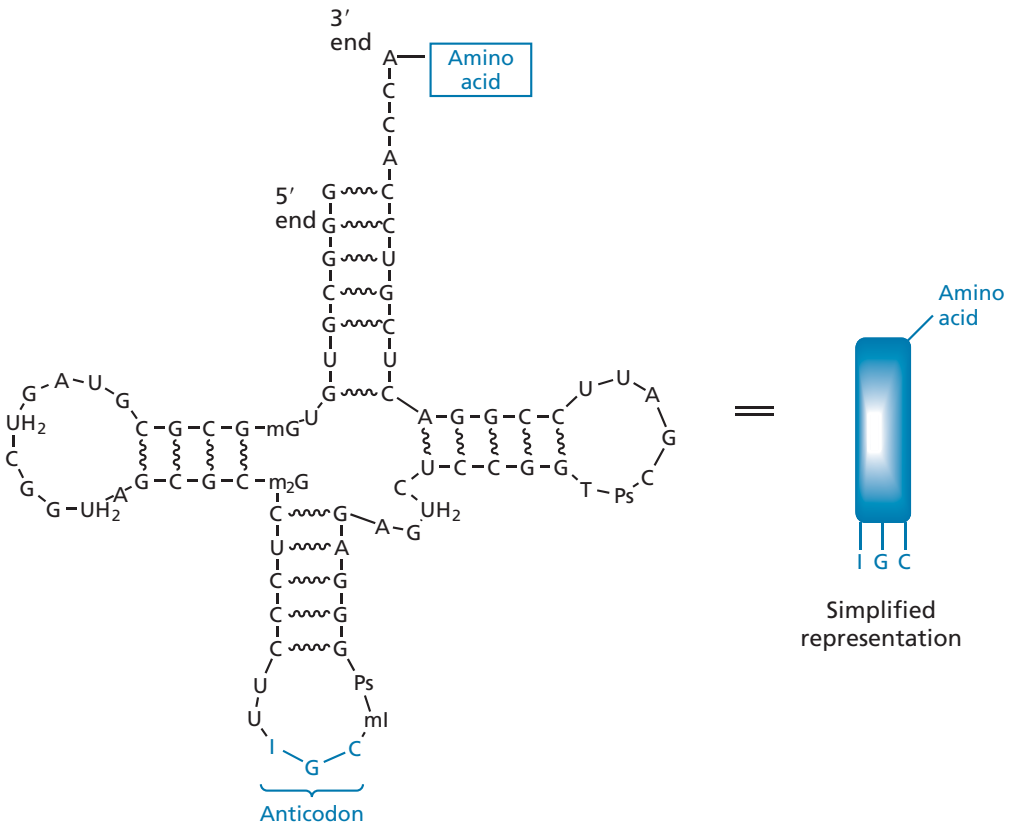



**FIGURE 6.11** Yeast alanine transfer RNA. The wiggly lines indicate base pairing (mI = methylinosine, $UH_2$ = dihydrouridine, T = ribothymidine, Ps = pseudouridine, mG = methylguanosine, $m_2G$ = dimethylguanosine).

takes on that role, acting as the crucial 'middle man' between DNA and proteins. This has been termed the **central dogma** of molecular biology.

The bases adenine, cytosine, guanine, and uracil are found in mRNA and are predominant in rRNA and tRNA. However, tRNA also contains a number of less common nucleic acids—see, for example, Fig. 6.11.

## 6.2.2 Transcription and translation

A molecule of mRNA represents a copy of the genetic information required to synthesize a single protein. Its role is to carry the required code out of the nucleus to a cellular organelle called the **endoplasmic reticulum**. This is where protein production takes place on bodies called **ribosomes**. The segment of DNA which is copied is called a gene and the process involved is called **transcription**. The DNA double helix unravels and the stretch that is exposed acts as a template on which the mRNA can be built (Fig. 6.12). Once complete, the mRNA departs the nucleus to seek out a ribosome, while the DNA re-forms its double helix.

Ribosomal RNA is the most abundant of the three types of RNA and is the major component of ribosomes. These can be looked upon as the production sites for protein synthesis—a process known as **translation.** The ribosome binds to one end of the mRNA molecule, then travels along it to the other end, allowing the triplet code to be read, and catalysing the construction of the protein molecule one amino acid at a time (Fig. 6.13). There are two segments to the mammalian ribosome, known as the 60S and 40S subunits. These combine to form an 80S ribosome. In bacterial cells, the ribosomes are smaller and consist of 50S and 30S subunits combining to form a 70S ribosome. The terms 50S, etc. refer to the sedimentation properties of the various structures. These are related qualitatively to size and mass, but not quantitatively—that is why a 60S and a 40S subunit can combine to form an 80S ribosome.

rRNA is the major component of each subunit, making up two thirds of the ribosome's mass. The 40S subunit contains one large rRNA molecule along with several proteins, whereas the 60S subunit contains three different sized rRNAs; again, with accompanying proteins. The secondary structure of rRNA includes extensive stretches of base pairing (**duplex regions**), resulting in a well-defined tertiary structure. It was thought at one time that rRNA only played a structural role and that the proteins were acting as enzymes to catalyse translation. The rRNA molecules certainly do have a crucial structural role, but

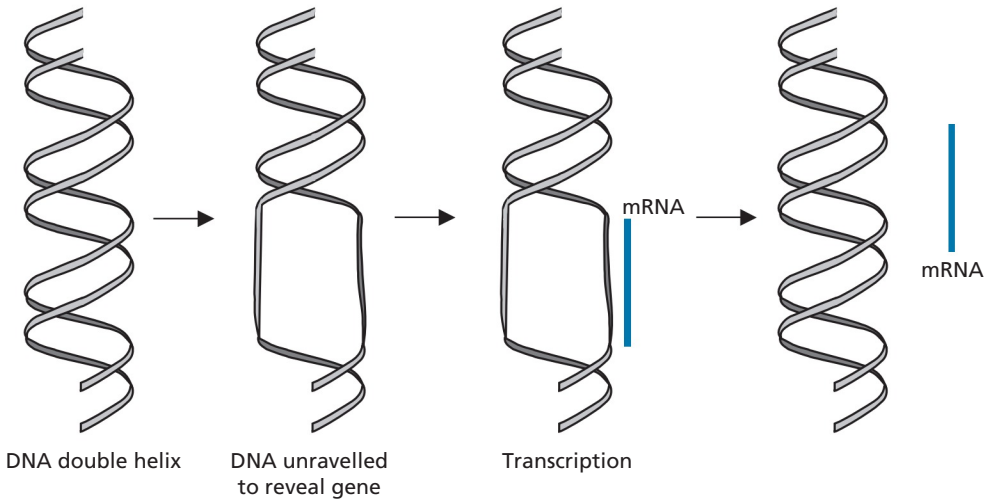



**FIGURE 6.12** Formation of mRNA.

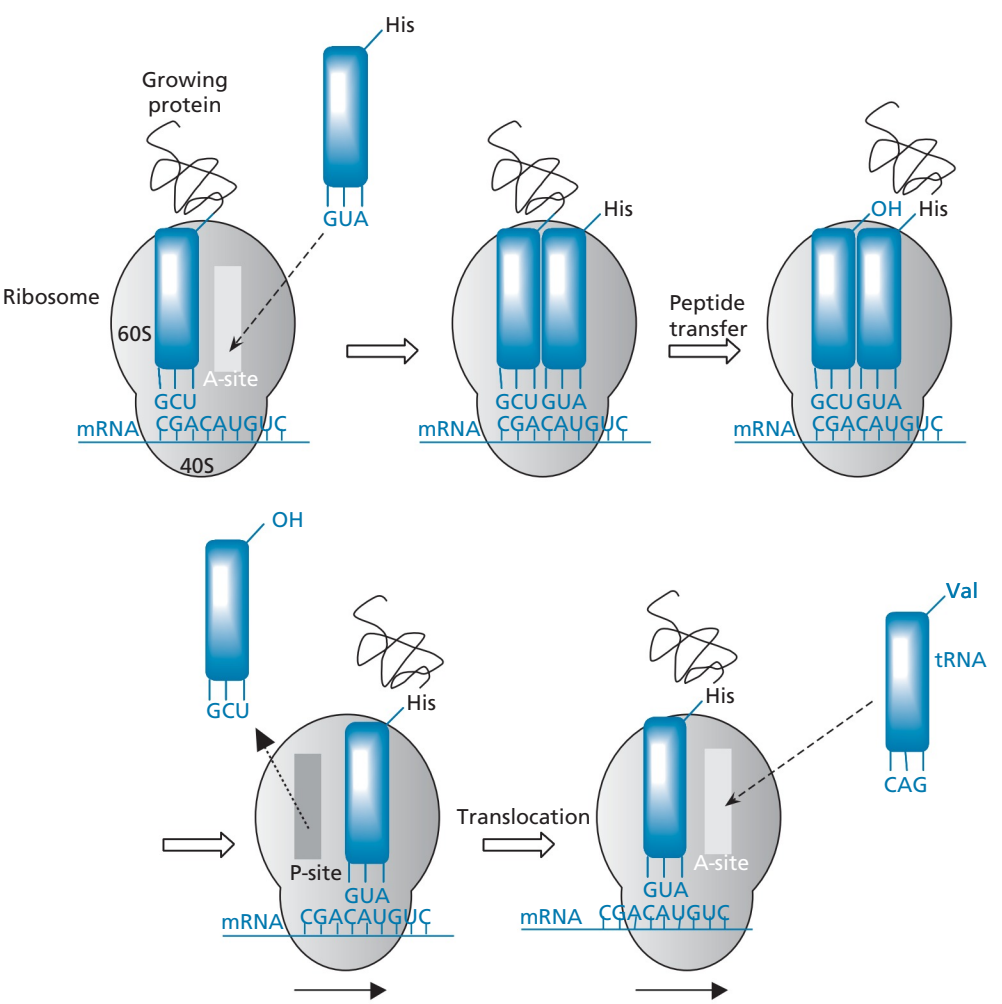



**FIGURE 6.13** Protein synthesis—translation.

it is now known that they, rather than the ribosomal proteins, have the major catalytic role. Indeed, the key sites in the ribosome where translation takes place are made up almost entirely of rRNA. The proteins are elongated structures which meander through the ribosome structure and are thought to have a fine-tuning effect on the translation process.

Transfer RNA is the crucial adaptor unit which links the triplet code on mRNA to a specific amino acid. This means there has to be a different tRNA for each amino acid. All the tRNAs are clover-leaf in shape, with two different binding regions at opposite ends of the molecule (see Fig. 6.11). One binding region is for the amino acid, where a specific amino acid is covalently linked to a terminal adenosyl residue. The other is a set of three nucleic acid bases **(anticodon)** which will base-pair with a complementary triplet on the mRNA molecule. A tRNA having a particular anticodon will always have the same amino acid attached to it.

Let us now look at how translation takes place in more detail. As rRNA travels along mRNA, it reveals the triplet codes on mRNA one by one. For example, in Fig. 6.13 the triplet code CAU is revealed along with an associated binding site called the A site. The A stands for aminoacyl and refers to the attached amino acid on the incoming tRNA. Any tRNA molecule can enter this site but it is accepted only if it has the necessary anticodon capable of base-pairing with the exposed triplet on mRNA. In this case, tRNA having the anticodon GUA is accepted and brings with it the amino acid histidine. The peptide chain that has been created so far is attached to a tRNA molecule which is bound to the P binding site (standing for peptidyl). A grafting process then takes place, catalysed by rRNA, where the peptide chain is transferred to histidine (Fig. 6.14). The tRNA occupying the P binding site now departs and the ribosome shifts along mRNA to reveal the next triplet (a process called **translocation**), and so the process continues until the whole strand is read. The new protein is then released from the ribosome, which is now available to start the process again. The overall process of transcription and translation is summarized in Fig. 6.15.

### 6.2.3 Small nuclear RNA

After transcription, mRNA molecules are frequently modified before translation takes place. This involves a splicing operation where the middle section of mRNA (the **intron**) is excised and the ends of the mRNA molecule (the **exons**) are spliced together (Fig. 6.16).

Splicing requires the aid of an RNA-protein complex called a **spliceosome**. The RNA molecules involved in this complex are called **small nuclear RNAs** (snRNAs). As the name indicates, these are small RNA molecules with fewer than 300 nucleotides that occur in the nucleus of the cell. The role of the snRNAs in the spliceosome is to base-pair with particular segments of mRNA such that the mRNA can be manipulated and aligned properly for the splicing process. Splice sites are recognized by their nucleotide sequences, but, on occasion, a mutation in DNA may introduce a new splice site somewhere else on mRNA. This results in faulty splicing, an altered mRNA, and a defective protein. About 15% of genetic diseases are thought to be due to mutations that result in defective splicing.

## 6.3 Genetic illnesses

A number of genetic illnesses are due to genetic abnormalities that result in the non-expression of particular proteins or the expression of defective proteins. For example, **albinism** is a condition where the skin, hair, and eyes lack pigment; it is associated with a deficiency of an enzyme called **tyrosinase**. This is a copper-containing enzyme that catalyses the first two stages in the synthesis

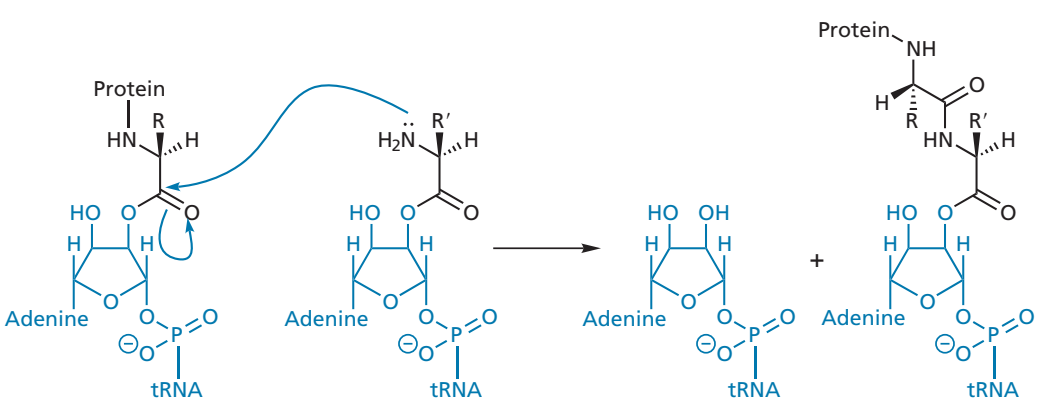



**FIGURE 6.14** Mechanism by which a growing protein is transferred to the next amino acid.

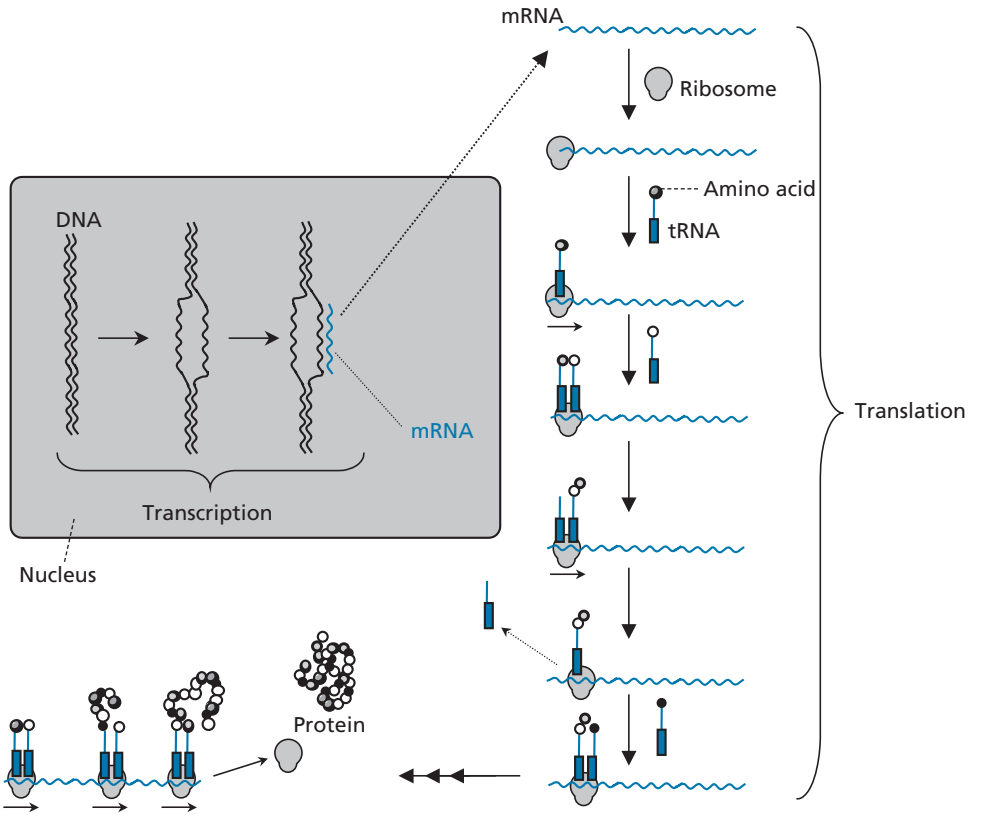



**FIGURE 6.15** Transcription and translation.

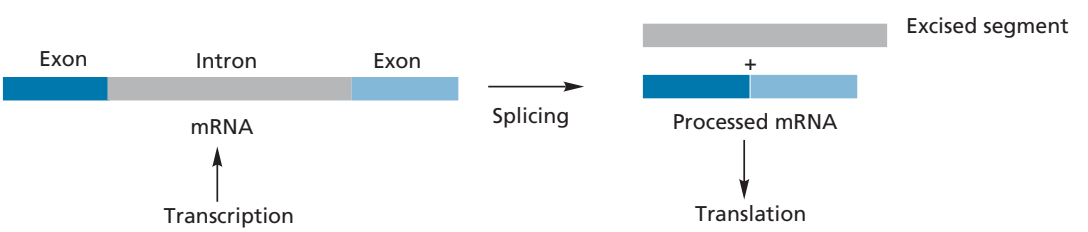



**FIGURE 6.16** Splicing messenger RNA (mRNA).

of the pigment **melanin**. Over 90 mutations of the tyrosinase gene have been identified which lead to the expression of inactive enzyme. Mutations in the triplet code result in one or more amino acids being altered in the resulting protein, and if these amino acids are important to the activity of the enzyme, activity is lost. Mutations which alter amino acids in the active site are the ones most likely to result in loss of activity.

**Phenylketonuria** is a genetic disease caused by the absence or deficiency of an enzyme called **phenylalanine hydroxylase**. This enzyme normally converts phenylalanine to tyrosine. In its absence the blood levels of phenylalanine rise substantially, along with alternative metabolic products, such as phenylpyruvate. If left untreated, this disease results in severe mental retardation.

**Haemophilias** are inherited genetic diseases in which one of the blood coagulation factors is deficient. This results in uncontrolled bleeding after an injury. In the past, people with this disease were likely to die in their youth. Nowadays, with the proper treatment, affected individuals should have a normal life expectancy. Treatment in severe cases involves regular intravenous infusion with the relevant coagulation factor. In less severe cases, transfusions can be used when an injury has taken place. The coagulation factors used to be typically derived from blood plasma, but this meant that people with haemophilia were susceptible to infection from infected blood samples. For example, during the period 1979–1985 more than 1200 people in the UK were infected with HIV as a result of receiving infected blood

products. For the same reason, they were also prone to viral infections caused by hepatitis B and C. During the 1990s, recombinant DNA technology (section 6.4) successfully produced blood coagulation factors and these are now the agents of choice as they eliminate the risk of infection. Unfortunately, some patients produce an immune response to the infused factor, which can preclude their use. At present, clinical trials are under way to test whether gene therapy can be used as a treatment. This involves the introduction of a gene which will code for the normal coagulation factor so that it can be produced naturally in the body (section 6.4).

**Muscular dystrophy** is another genetic disease that affects 1 in every 3500 males and is characterized by the absence of a protein called **dystrophin**. This has an important structural role in cells and its absence results in muscle deterioration. Gene therapy is also being considered for this disease.

Many cancers are associated with genetic defects which result in molecular signalling defects in the cell. This is covered more fully in Chapter 21.

## 6.4 Molecular biology and genetic engineering

Over the last few years, rapid advances in molecular biology and genetic engineering have had important repercussions for medicinal chemistry. It is now possible to clone specific genes and to include these genes into the DNA of fast-growing cells such that the proteins encoded by these genes are expressed in the modified cell. As the cells are fast-growing this leads to a significant quantity of the desired protein, which permits its isolation, purification, and structural determination. Before these techniques became available, it was extremely difficult to isolate and purify many proteins from their parent cells owing to the small quantities present. Even if one was successful, the low yields inherent in the process made an analysis of the protein's structure and mechanism of action very difficult. Advances in molecular biology and recombinant DNA techniques have changed all that.

**Recombinant DNA technology** allows scientists to manipulate DNA sequences to produce modified DNA or completely novel DNA. The technology makes use of natural enzymes called **restriction enzymes** and **ligases** (Fig. 6.17). The restriction enzymes recognize a particular sequence of bases in each DNA molecule and split a specific sugar phosphate bond in each strand of the double helix. With some restriction enzymes, the break is not a clean one; there is an overlap between the two chains resulting in a tail of unpaired bases on each side of the break. The bases on each tail are complementary and can still recognize each other, so they are described as 'sticky' ends. The same process is carried out on a different molecule of DNA and the molecules from both processes are mixed together. As these different molecules have the same sticky ends, they recognize each other such that base pairing takes place in a process called **annealing**. Treatment with the ligase enzyme then repairs the sugar phosphate backbone and a new DNA molecule is formed.

If the DNA molecule of interest does not have the required sequence recognized by the restriction enzyme, a synthetic DNA **linker** that *does* contain the sequence can be added to either end of the molecule using a ligase enzyme. This is then treated with the restriction enzyme as before (Fig. 6.18).

There are many applications for this technology, one of which is the ability to amplify and express the gene for a particular human protein in bacterial cells. In order to do this it is necessary to introduce the gene to the bacterial cell. This is done by using a suitable **vector** which will carry the gene into the cell. There are two suitable

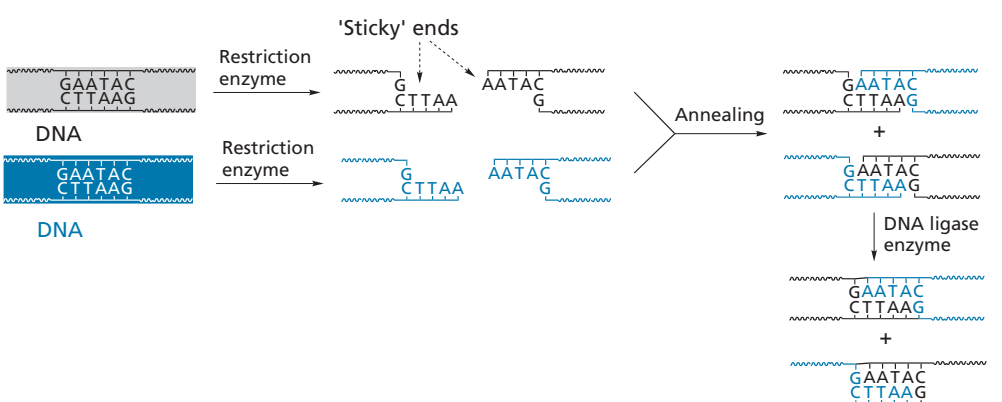



**FIGURE 6.17** Recombinant DNA technology.

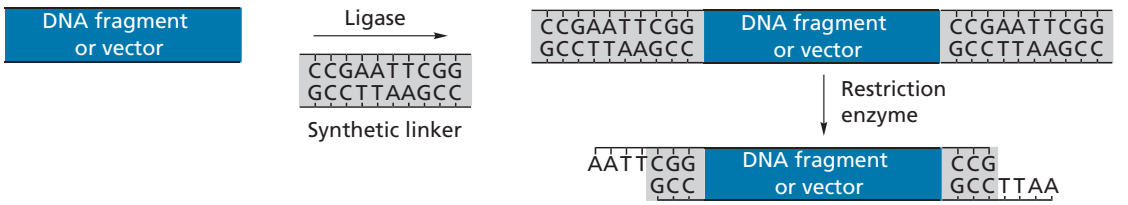



**FIGURE 6.18** Attaching sequences recognized by restriction enzymes.

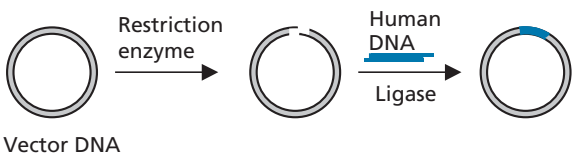



**FIGURE 6.19** Inserting a human gene into a plasmid by recombinant DNA technology.

vectors—**plasmids** and **bacteriophages**. Plasmids are segments of circular DNA that are transferred naturally between bacterial cells and allow the sharing of genetic information. Because the DNA is circular, the DNA representing a human gene can be inserted into the vector's DNA by the same methods described above (Fig. 6.19). Bacteriophages (phages for short) are viruses that infect bacterial cells. There are a variety of these, but the same recombinant DNA techniques can be used to insert human DNA into viral DNA.

Whichever vector is used, the modified DNA is introduced into the bacterial cell where it is cloned and amplified (Fig. 6.20). For example, once a phage containing modified nucleic acid infects a bacterial cell, the phage takes over the cell's biochemical machinery to produce multiple copies of itself and its nucleic acid.

Human genes can be introduced to bacterial cells such that the gene is incorporated into bacterial DNA and expressed as if it were the bacteria's own. This allows the production of human proteins in much greater quantity than would be possible by any other means. Such proteins could then be used for medicinal purposes, as described in the following sections. Modified genes can also be introduced and expressed to produce modified proteins to see what effect a mutation would have on the structure and function of a protein.

The following are some of the applications of genetic engineering to the medical field.

**Harvesting important proteins** The genes for important hormones or growth factors, such as **insulin** and **human growth factor**, have been included in fast-growing unicellular organisms. This allows the harvesting of these proteins in sufficient quantities that they can be marketed and administered to patients who are deficient in these important hormones. Genetic engineering has also been crucial in the production of monoclonal antibodies (section 14.8.3).

**Genomics and the identification of new protein drug targets** Nowadays, it is relatively easy to isolate and identify a range of signalling proteins, enzymes, and receptors by **cloning** techniques. This has led to the identification of a growing number of isozymes and receptor subtypes which offer potential drug targets for the future. The **Human Genome Project** involved the mapping of human DNA (completed in 2000) and has led to the discovery of previously unsuspected new proteins. These, too, may offer potential drug targets. The study of the structure and function of new proteins discovered from genomics is called **proteomics** (section 2.6).

**Study of the molecular mechanism of target proteins** Genetic engineering allows the controlled

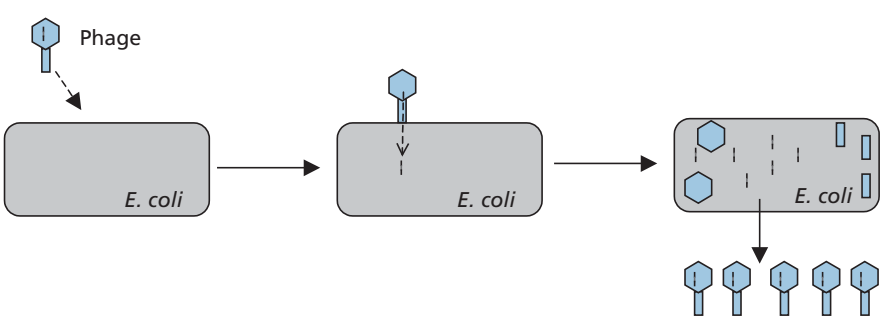



**FIGURE 6.20** Infecting *Escherichia coli* with a phage.

mutation of proteins such that specific amino acids are altered. This allows researchers to identify which amino acids are important to enzyme activity or to receptor binding. In turn, this leads to a greater understanding of how enzymes and receptors operate at the molecular level.

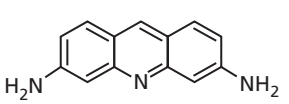
**Somatic gene therapy** involves the use of a carrier virus to smuggle a healthy gene into cells in the body where the corresponding gene is defective. Once the virus has infected the cell, the healthy gene is inserted into the host DNA where it undergoes transcription and translation. This approach has great therapeutic potential for cancers, AIDS, and genetic abnormalities, such as cystic fibrosis. However, the approach is still confined to research laboratories and there is still a long way to go before it is used clinically. There are several problems still to be tackled, such as how to target the viruses specifically to the defective cells, how to insert the gene into DNA in a controlled manner, how to regulate gene expression once it is in DNA, and how to avoid immune responses to the carrier virus. Progress in this field was set back significantly in 1999 as a result of the death of a teenage volunteer during a clinical trial in the USA. This was attributed to an over-reactive immune response to the carrier virus used in the trial. Consequently, there are now studies looking into the use of artificial viruses which would be less likely to cause an immune response. Non-viral delivery systems are also being studied involving caged molecules called **cyclodextrins**. In addition, lipids, polyaminoesters, glycine polymers, and carbon buckyballs are being investigated as carriers.

## KEY POINTS

- The primary structure of RNA is similar to that of DNA, but it contains ribose instead of deoxyribose. Uracil is used as a base in place of thymine, and other bases may be present in smaller quantities.
- Base pairing and sections of helical secondary structure are possible within the structure of RNA.
- There are three main types of RNA—messenger RNA, transfer RNA, and ribosomal RNA.
- Transcription is the process by which a segment of DNA is copied as mRNA. mRNA carries the genetic information required for the synthesis of a protein from the nucleus to the endoplasmic reticulum.
- rRNA is the main constituent of ribosomes where protein synthesis takes place. A ribosome moves along mRNA revealing each triplet of the genetic code in turn.
- tRNA interprets the coded message in mRNA. It contains an anticodon of three nucleic acid bases which binds to a complementary triplet on mRNA. Each tRNA carries a specific amino acid, the nature of which is determined by the anticodon.
- The process of protein synthesis is called translation. The growing protein chain is transferred from one tRNA to the amino acid on the next tRNA and is only released once the complete protein molecule has been synthesized.
- Genetic engineering has been used in the production of important hormones for medicinal purposes, the identification of novel drug targets, the study of protein structure and function, and gene therapy.

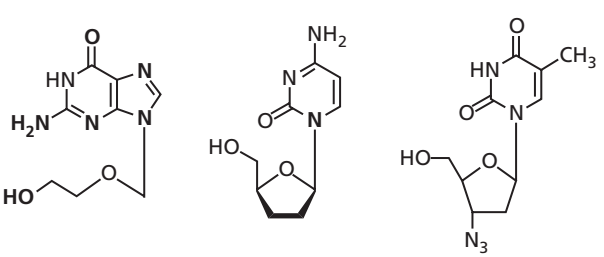
# QUESTIONS

1. Proflavine is a topical antibacterial agent which intercalates bacterial DNA and was used to treat wounded soldiers in the Far East during World War II. What role (if any) is played by the tricyclic ring and the primary amino groups? The drug cannot be used systemically. Suggest why this is the case.



Proflavine

2. The following compounds are antiviral drugs that mimic natural nucleosides. What nucleosides do they mimic?




3. Adenine is an important component of several important biochemicals. It has been proposed that adenine was synthesized early on in the evolution of life when the Earth's atmosphere consisted of gases, such as hydrogen cyanide and methane. It has also been possible to synthesize adenine from hydrogen cyanide. Consider the structure of adenine and identify how cyanide molecules might act as the building blocks for this molecule.

4. The genetic code involves three nucleic acid bases coding for a single amino acid (the triplet code). Therefore, a mutation to a particular triplet should result in a different amino acid. However, this is not always the case. For any triplet represented by XYZ, which mutation is least likely to result in a change in amino acid—X, Y, or Z?

5. The amino acids serine, glutamate, and phenylalanine were found to be important binding groups in a receptor binding site (see Appendix 1 for structures). The triplet codes for these amino acids in the

mRNA for this receptor were AGU, GAA, and UUU respectively. Explain what effect the following mutations might have, if any:

AGU to ACU; AGU to GGU; AGU to AGC
GAAto GAU; GAA to AAA; GAA to GUA
UUU to UUC; UUU to UAU; UUU to AUU

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# 7 Enzymes as drug targets

Many important drugs act as enzyme inhibitors. In other words, they hinder or prevent enzymes acting as catalysts for a particular reaction. We covered the structure and function of enzymes in Chapter 3. In this chapter, we concentrate on how drugs target enzymes and inhibit their action.

## 7.1 Inhibitors acting at the active site of an enzyme

### 7.1.1 Reversible inhibitors

In Chapter 3 we emphasized the importance of binding interactions between an enzyme and its substrate. If there are no interactions holding a substrate to the active site, then the substrate will drift in and back out again before there is a chance for it to react. Therefore, the more binding interactions there are, the stronger the substrate will bind, and the better the chance of reaction. But, there is a catch! What happens if a strongly bound substrate gives a product that also binds strongly to the active site (Fig. 7.1)?

The answer is that the enzyme becomes clogged up and is unable to accept any more substrate. Therefore, the binding interactions holding the substrate or the product to the enzyme must be properly balanced. They must be sufficiently strong to hold the substrate in the active site long enough for the reaction to occur, but weak enough to allow the product to leave. This bonding balancing act can be turned to great advantage if the medicinal chemist wishes to inhibit a particular enzyme or switch it off altogether. A molecule can be designed which is similar to the natural substrate or product, and can fit the active site, but which binds more strongly. It may not undergo any reaction when it is in the active site, but as long as it stays there it blocks access to the natural substrate and prevents the enzymatic reaction (Fig 7.2). This is known as **competitive inhibition**, as the drug is competing with the natural substrate for the active site. The longer the inhibitor is present in the active site, the greater the inhibition. Therefore, if a medicinal chemist knows the position and nature of different binding regions within an active site, it is possible to design molecules that will fit that active site, bind strongly, and act as inhibitors.

Competitive inhibitors bind to the active site through intermolecular bonds and so the binding is reversible, allowing an equilibrium to occur between bound drug and unbound drug—a kind of 'yoyo' effect where the drug binds to the active site, is released, then binds again. This means that the inhibition caused by the drug

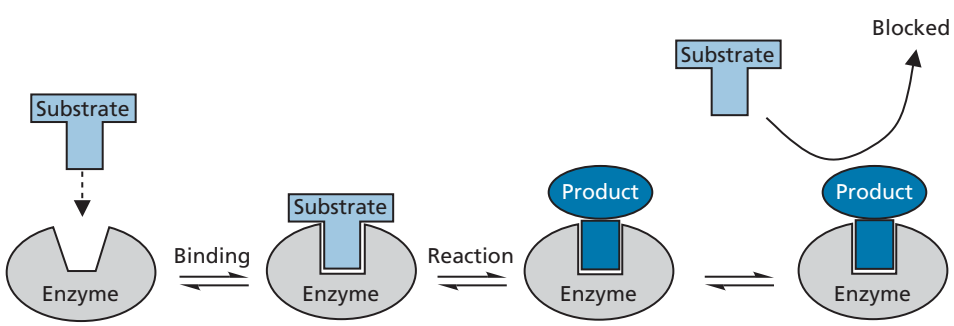



**FIGURE 7.1** Example of an enzyme being 'clogged up' if the product remains bound.

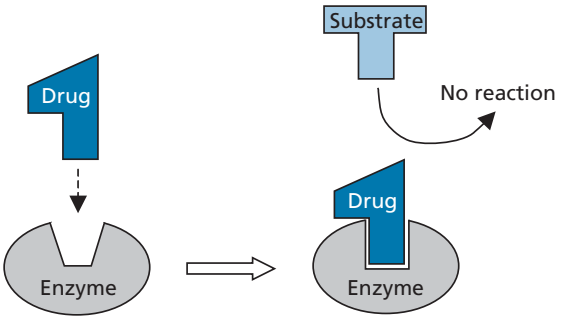



**FIGURE 7.2** Competitive inhibition.

is reversible. If the concentration of substrate increases, it competes more effectively with the drug for the active site, and so inhibition by the drug will be less effective (Box 7.1).

There are many examples of useful drugs that act as **competitive inhibitors**. For example, the **sulphonamides** act as antibacterial agents by inhibiting a bacterial enzyme in this fashion (section 19.4.1.5). Many diuretics used to control blood pressure are competitive inhibitors, as are some antidepressants (section 23.12.5). Other examples include the statins (Case study 1), angiotensin converting enzyme (ACE) inhibitors (Case study 2), and protease inhibitors (section 20.7.4). Indeed, the majority of clinically useful enzyme inhibitors are of this nature.

As stated above, competitive inhibitors frequently bear some resemblance to the natural substrate, allowing them to be recognized by the active site. Some of these inhibitors may have additional features which allow them to form extra binding interactions to regions of the active site that are not occupied by the substrate. This allows them to bind more strongly and to be more effective inhibitors. The statins described in Case study 1 are a good example of this.

Although competitive inhibitors often bear some resemblance to the substrate, this is not always the case. As long as the drug has the right shape to fit the active site and has functional groups that can interact with the binding regions available, it can still bind to the active site and inhibit the enzyme. Therefore, it is possible for drugs with a totally different skeleton to the substrate to act as competitive inhibitors. Such drugs may bind to a combination of binding regions within the active site, some of which are used by the substrate and some of which are not.

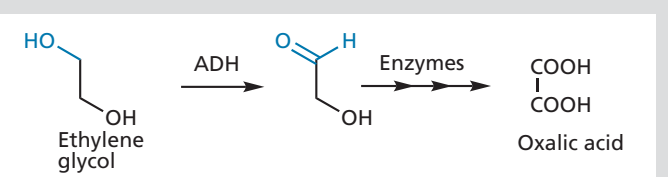
It should also be remembered that the product of an enzyme-catalysed reaction is bound to the active site before it is finally released, and so it is possible to have enzyme inhibitors which resemble the structure of the product more closely than the substrate. Other drugs are designed to mimic the transition state of the enzyme-catalysed reaction (section 7.4).

Finally, some competitive inhibitors bind to the active site, but do not compete with the substrate. How can this occur? The answer lies in the fact that the active sites of several enzymes bind a substrate *and* an enzyme **cofactor.** Therefore, it is possible to have competitive inhibitors that occupy the binding region normally occupied by the cofactor, and so the competition is with the cofactor rather than the substrate. The kinase inhibitors described in section 21.6.2 are a good example of this. Many of these agents compete with the cofactor ATP for the active site of kinase enzymes, and not the protein substrate. The competitive nature of the inhibition is illustrated in resistant

## BOX 7.1 A cure for antifreeze poisoning

Competitive inhibitors can generally be displaced by increasing the level of natural substrate. This feature has been useful in the treatment of accidental poisoning by antifreeze. The main constituent of antifreeze is **ethylene glycol**, which is oxidized in a series of enzymatic reactions to **oxalic acid**, which is toxic. Blocking the synthesis of oxalic acid leads to recovery.

The first step in this enzymatic process is the oxidation of ethylene glycol by **alcohol dehydrogenase (ADH)**. Ethylene glycol is acting here as a substrate, but we can view it as a competitive inhibitor because it is competing with the natural substrate for the enzyme. If the levels of natural substrate are increased, it will compete far better with ethylene glycol and prevent it from reacting. Toxic oxalic acid will no longer be formed and the unreacted ethylene glycol is eventually excreted from the body. The cure, then, is to administer high doses of the natural substrate—alcohol!



tumour cells, where a mutated enzyme shows greater affinity for ATP over the inhibitors (Box 21.11).

### 7.1.2 Irreversible inhibitors

Irreversible enzyme inhibitors can form a covalent bond to a key amino acid in the active site and permanently block the affected enzyme (Fig. 7.3). The most effective **irreversible inhibitors** are those that contain an electrophilic functional group (X) capable of reacting with a nucleophilic group present on an amino acid side chain. Invariably, the amino acid affected is either **serine** or **cysteine**, because these amino acids are often present in active sites and contain nucleophilic groups (OH and SH respectively) that are involved in the mechanism of many enzyme-catalysed reactions (section 3.5.3). Electrophilic functional groups used in irreversible inhibitors include alkyl halides, epoxides, α,β-unsaturated ketones, or strained lactones and lactams (Fig. 7.4). The highly toxic **nerve agents** (section 22.13.2.1) contain electrophilic fluorophosphonate groups and are irreversible inhibitors of mammalian enzymes.

Not all irreversible inhibitors are highly toxic, though, and several are used clinically. For example, **penicillins** (section 19.5.1) contain a **β-lactam** group that irreversibly inhibits an enzyme that is crucial to bacterial cell wall synthesis. **Disulfiram** (Antabuse) (Box 12.6) is an irreversible inhibitor of the enzyme alcohol dehydrogenase and is used to treat alcoholism. The **proton pump inhibitors** described in section 25.3 are irreversible inhibitors and are used as anti-ulcer agents. The anti-obesity drug **orlistat** is also an irreversible inhibitor (Box 7.2). Having said that, it is generally better to inhibit an enzyme with a reversible inhibitor rather than an irreversible inhibitor. As irreversible inhibitors have reactive functional groups, there is a risk that they might react with other proteins or nucleic acids and cause toxic side effects.

Irreversible enzyme inhibitors are not competitive inhibitors. Increasing the concentration of substrate will not reverse their inhibition as the inhibitors cannot be displaced from the active site. This can cause problems if the build up of a particular substrate leads to toxic side effects. For example, the **monoamine oxidase inhibitors** (MOAIs) block the metabolism of **noradrenaline** and have antidepressant activity (section 23.12.5). Unfortunately, the metabolism of substrates other than noradrenaline is also inhibited, leading to a build up of these compounds and serious side effects. More modern MAOIs have been designed to be reversible inhibitors in order to avoid this problem.

## 7.2 Inhibitors acting at allosteric binding sites

**Allosteric binding sites** were discussed in section 3.6 and are a means by which enzyme activity can be controlled by natural inhibitors. When an allosteric inhibitor binds to its binding site, the resulting induced fit also deforms the shape of the active site such that it becomes unrecognizable to the substrate. Drugs can be designed to mimic this natural control of the enzyme. If the drug binds through intermolecular interactions, the inhibition is reversible. If the drug contains a reactive group

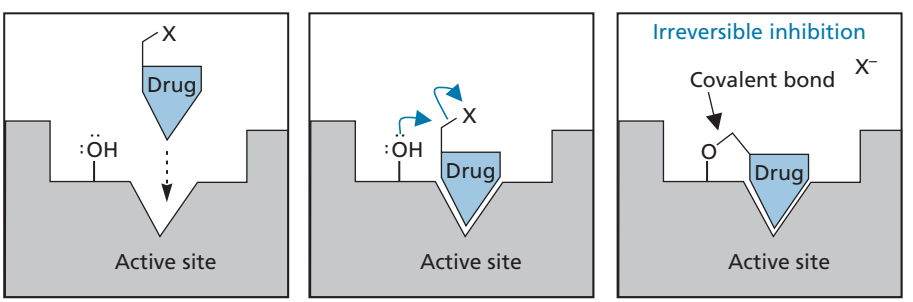
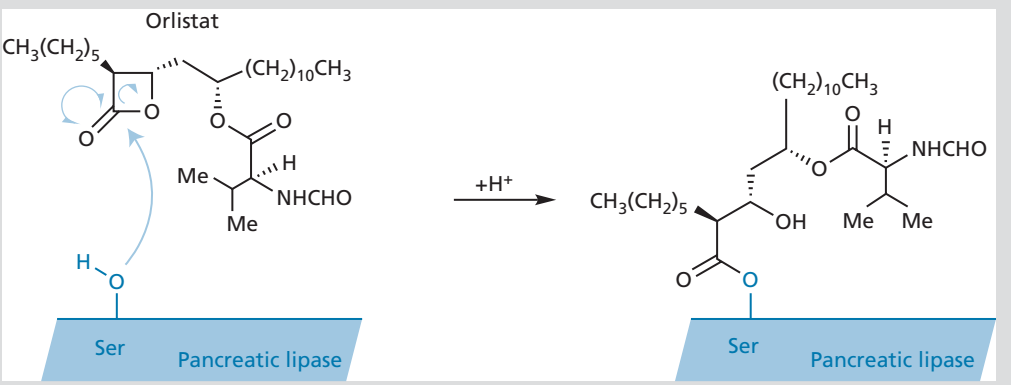



**FIGURE 7.3** Irreversible inhibition of an enzyme with an alkylating agent. (X = halogen leaving group).


**FIGURE 7.4** Examples of electrophilic functional groups.

## BOX 7.2 Irreversible inhibition for the treatment of obesity

Fat in the diet is composed mainly of triglycerides which are digested in the small intestine to fatty acids and 2-monoglycerides. The digestion products are then absorbed and act as the building blocks for fat biosynthesis in the body. The enzyme **pancreatic lipase** is responsible for catalysing the digestion of fats, and so inhibition of this enzyme will result in reduced absorption of glycerides and fatty acids from the gut. Consequently, less fat will be synthesized in the body. **Orlistat** is an anti-obesity drug that acts as an irreversible inhibitor of pancreatic lipase as a result of the presence of an electrophilic 4-membered lactone group. This acylates a serine residue in the active site, which is part of a catalytic triad of serine, histidine, and aspartic acid (compare section 3.5.3).



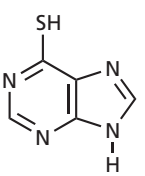




**FIGURE 7.5** 6-Mercaptopurine.

allowing it to form a covalent bond to the allosteric binding site, the inhibition is irreversible.

The drug **6-mercaptopurine** (Fig. 7.5), used in the treatment of leukaemia, is an example of an allosteric inhibitor. It inhibits the first enzyme involved in the synthesis of purines (section 6.1.1) and blocks purine synthesis. In turn, this blocks DNA synthesis.

## 7.3 Uncompetitive and non-competitive inhibitors

Uncompetitive inhibitors are inhibitors that bind reversibly to an enzyme when the substrate is already bound to the active site. In other words, the inhibitor binds to the enzyme–substrate complex. In this situation, increasing the substrate concentration will not overcome inhibition. Indeed, the level of inhibition is dependent on sufficient substrate being present to form the enzyme–substrate complex. Therefore, uncompetitive inhibitors are less effective at low substrate concentrations. Uncompetitive inhibitors are not very common.

In theory, a non-competitive inhibitor binds to an allosteric binding site and inhibits the enzyme-catalysed reaction without affecting the strength of substrate binding. This would occur if the induced fit arising from the binding of the allosteric inhibitor distorts the active site sufficiently to prevent the catalytic mechanism, but has no effect on the substrate binding process. In practice, this ideal situation is extremely rare, if it even occurs at all. It is almost inevitable that any active site distortion affecting the catalytic process will also affect substrate binding. Therefore, those inhibitors which inhibit the catalytic process, while still allowing substrates to bind, normally cause some inhibition of substrate binding. This is known as **mixed inhibition** as it is neither pure competitive inhibition nor pure non-competitive inhibition.

## 7.4 Transition-state analogues: renin inhibitors

An understanding of an enzyme mechanism can help medicinal chemists design more powerful inhibitors. For example, it is possible to design inhibitors which bind so strongly to the active site (using non-covalent forces)

that they are effectively irreversible inhibitors—a bit like inviting someone for dinner and finding that they have moved in on a permanent basis. One way of doing this is to design a drug that resembles the transition state for the catalysed reaction. Such a drug should bind more strongly than either the substrate or the product and be a strong inhibitor as a result. Such compounds are known as **transition-state analogues or inhibitors**.

The use of transition-state analogues has been particularly effective in the development of **renin inhibitors** (Fig. 7.6). Renin is a protease enzyme which is responsible for hydrolysing a specific peptide bond in the protein **angiotensinogen** to form **angiotensin I**. Angiotensin I is further converted to **angiotensin II** (see Case study 2), which acts to constrict blood vessels and retain fluid in the kidneys, both of which lead to a rise in blood pressure. Therefore, an inhibitor of renin should act as an antihypertensive agent (i.e. lower blood pressure) by preventing the first stage in this process.

Renin contains two aspartyl residues and a bridging water molecule in the active site which are crucial to the mechanism by which an amide bond in the substrate is hydrolysed (Fig. 7.7). In the first stage of this mechanism, a tetrahedral intermediate is formed. In order to form this intermediate, the reaction mechanism has to proceed through a high-energy transition state, and it is this transition state that we wish to mimic with a transition-state analogue. However, it is not possible to isolate such a high-energy species in order to study its structure; so how can one design a drug to mimic it? The answer is to base the design of the drug on the reaction intermediate. The rationale for this is as follows. As the intermediate is less stable than the substrate, it is presumed that it is closer in character to the transition state. This, in turn, implies that the transition state is more tetrahedral in character than planar. Therefore, drugs based on the structure of the tetrahedral intermediate are more likely to mimic the transition state.

The intermediate itself is reactive and easily cleaved. Therefore, an analogue has to be designed which binds just as strongly, but is stable to hydrolysis. This can be done by introducing a feature that mimics the tetrahedral structure of the intermediate, but has no leaving group for the second part of the reaction mechanism. A variety of mimics have been tried and a hydroxyethylene moiety has proved effective (e.g. **aliskiren**; Fig. 7.8). The hydroxyethylene group has the required tetrahedral geometry and one of the two hydroxyl groups required for good binding. It is also stable to hydrolysis because there is no leaving group present. Aliskiren was approved by the **United States Food and Drug Administration** (**FDA**) in 2007 for the treatment of hypertension.

Similar strategies have been used successfully to design antiviral agents which act as transition-state analogue inhibitors for the **HIV protease enzyme** (section 20.7.4). The **statins** can also be viewed as transition-state

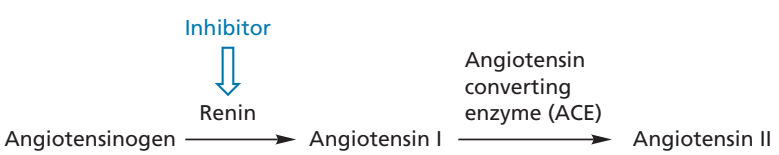



**FIGURE 7.6** Inhibition of renin to block the synthesis of angiotensin I and angiotensin II.

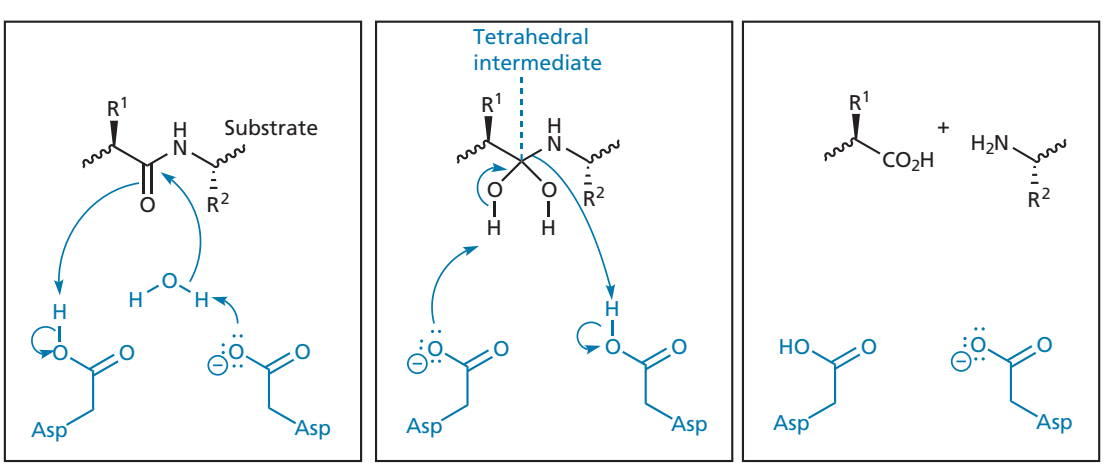



**FIGURE 7.7** Mechanism of renin-catalysed hydrolysis.

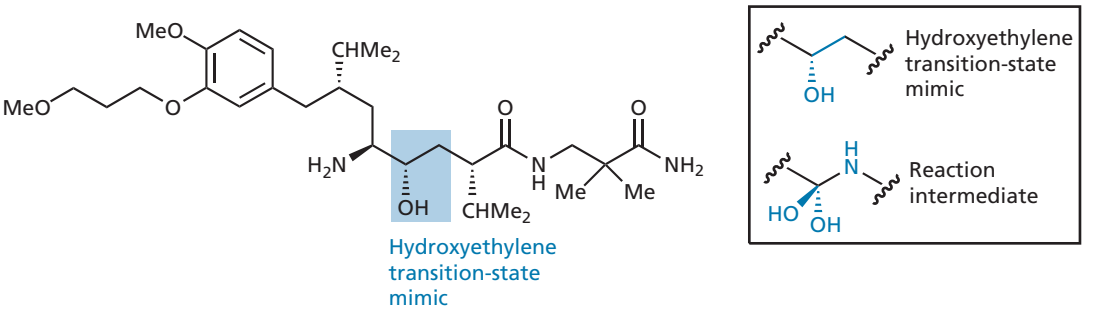



**FIGURE 7.8** Aliskiren.

analogues (Case study 1), as can some **ACE inhibitors** (Case study 2).

## 7.5 Suicide substrates

Transition-state analogues can be viewed as *bona fide* visitors to an enzyme's active site that become stubborn squatters once they have arrived. Other, apparently harmless, visitors can turn into lethal assassins once they have bound to their target enzyme. Such agents are designed to undergo an enzyme-catalysed transformation which converts them into a highly reactive species that forms a covalent bond to the active site.

One example of a suicide substrate is **clavulanic acid**, which is used clinically in antibacterial medications (e.g. **Augmentin**) to inhibit the bacterial **β-lactamase** enzyme (section 19.5.4.1). This enzyme is responsible for the penicillin resistance observed in several bacterial strains because it catalyses the hydrolysis of the penicillin β-lactam ring. The mechanism involves a serine residue in the active site acting as a nucleophile to form an intermediate where serine is covalently linked via an ester group to the ring-opened penicillin. The ester group is then hydrolysed to release the inactivated penicillin and free up the active site, such that the catalytic process can be repeated (Fig. 7.9).

Clavulanic acid also fits the active site of β-lactamase, and the β-lactam ring is opened by the serine residue in the same manner. However, the acyl-enzyme intermediate then reacts further with another enzymatic nucleophilic group (possibly $NH_2$) to bind the drug irreversibly to the enzyme (Fig. 7.10). The mechanism requires the loss or gain of protons at various stages, and an amino acid, such as histidine, in the active site would be capable of acting as a proton donor/acceptor (compare sections 3.5.2 and 22.12.3.2).

Drugs that operate in this way are often called **mechanism-based inhibitors** or **suicide substrates** because the enzyme is committing suicide by reacting with them (see also Box 7.3). The great advantage of this approach is that the **alkylating agent** is generated at the site where it is meant to act and is, therefore, highly selective for the target enzyme. If the alkylating group had not been disguised in this way, the drug would have alkylated the first nucleophilic group it met in the body and would have shown little, or no, selectivity. The uses of alkylating agents and the problems associated with them are discussed in sections 9.3 and 21.2.3.

The main use for suicide substrates has been in labelling specific enzymes. The substrates can be labelled with radioactive isotopes and reacted with their target enzyme in order to locate the enzyme in tissue preparations. However, some clinically useful agents do act as

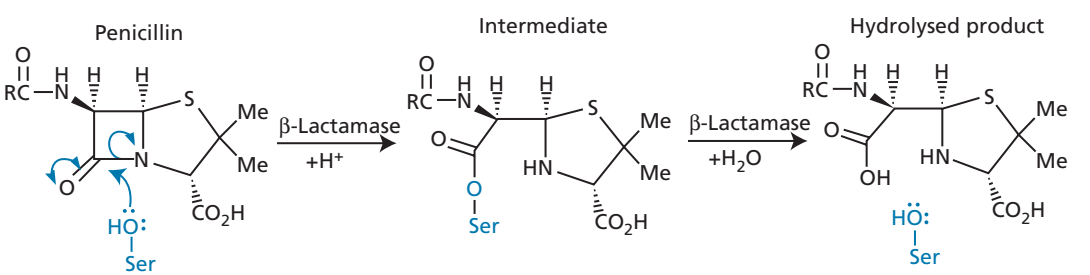



**FIGURE 7.9** Reaction catalysed by bacterial β-lactamase enzymes.

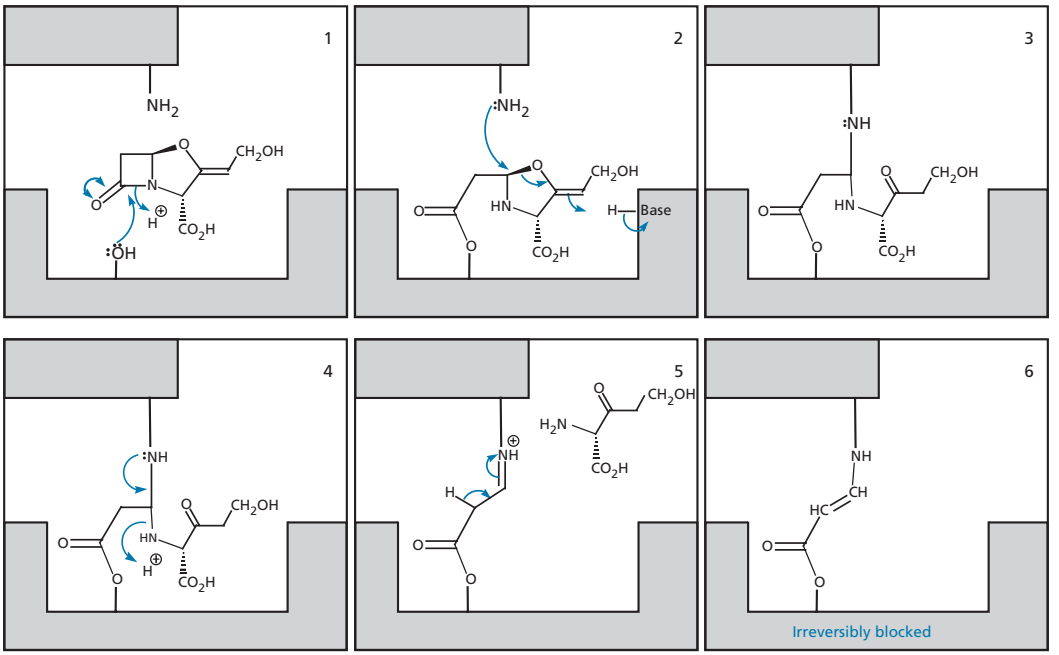



**FIGURE 7.10** Clavulanic acid acting as a suicide substrate.

suicide substrates, such as clavulanic acid (as described earlier). Some monoamine oxidase inhibitors are also thought to be suicide substrates (Box 7.4). Another interesting example of a suicide substrate is **5-fluorodeoxyuracil monophosphate** (5-FdUMP). The anticancer agent **5-fluorouracil** is used to treat cancers of the breast, liver, and skin, and is converted to 5-FdUMP in the body. This then acts as a suicide substrate for the enzyme thymidylate synthase (section 21.3.2). In this case, the covalent bond is formed between the suicide substrate and the enzyme cofactor, but the overall effect is the same.

## 7.6 Isozyme selectivity of inhibitors

Identification of isozymes that predominate in some tissues, but not others, allows the possibility of designing tissue-selective enzyme inhibitors (Box 7.4).

For example, the non-steroidal anti-inflammatory drug (NSAID) **indometacin** (Fig. 7.11) is used to treat inflammatory diseases, such as rheumatoid arthritis, and works by inhibiting the enzyme **cyclooxygenase**. This enzyme is involved in the biosynthesis of **prostaglandins**—agents which are responsible for the pain and inflammation of rheumatoid arthritis. Inhibiting the enzyme lowers prostaglandin levels and alleviates the symptoms of the disease. However, the drug also inhibits the synthesis of beneficial prostaglandins in the gastrointestinal tract and the kidney. It has been discovered that cyclooxygenase has two isozymes: COX-1 and COX-2. Both isozymes carry out the same reactions, but COX-1 is the isozyme that is active under normal healthy conditions. In rheumatoid arthritis, the normally dormant COX-2 becomes activated and produces excess inflammatory prostaglandins. Therefore, drugs such as **valdecoxib**, **rofecoxib**, and **celecoxib** have been developed to be selective for the COX-2 isozyme, so that only the production of inflammatory prostaglandins is reduced. Selectivity is possible by taking advantage of the fact that an isoleucine group is present in the binding site of COX-1, whereas the corresponding group in COX-2 is valine. Rofecoxib was authorized in 1999, but had to be withdrawn in 2004 as it was linked to an increased risk of heart attack and stroke when taken over a period of 18 months or so.

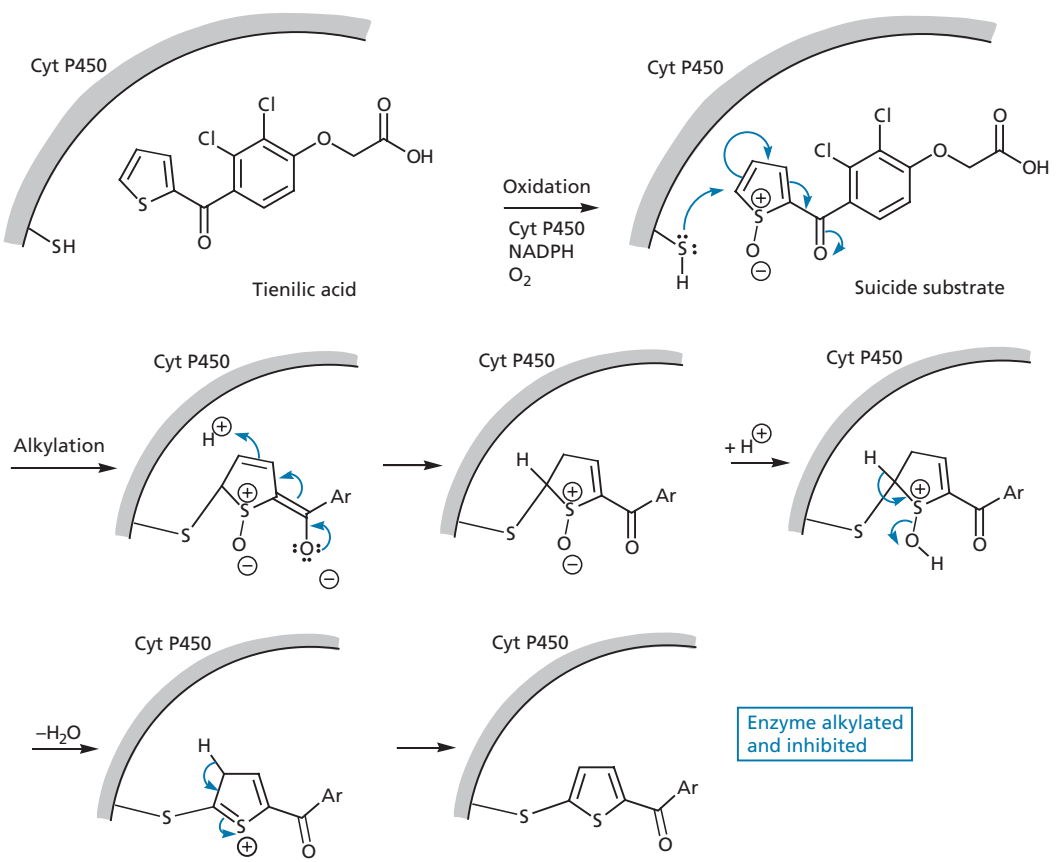
## 7.7 Medicinal uses of enzyme inhibitors

### 7.7.1 Enzyme inhibitors used against microorganisms

Inhibitors of enzymes have been extremely successful in the war against infection. If an enzyme is crucial to a microorganism, then switching it off will clearly kill the cell or prevent it from growing. Ideally, the enzyme chosen should be one that is not present in our own bodies. Fortunately, such enzymes exist because of the significant biochemical differences between bacterial cells

## **BOX 7.3** Suicide substrates

Suicide substrates are agents which are converted to highly reactive species when they undergo an enzyme-catalysed reaction. They form covalent bonds to the enzyme and inhibit it irreversibly. In some cases, this can cause toxicity. For example, the diuretic agent **tienilic acid** had to be withdrawn from the market because it was found to act as a suicide substrate for the cytochrome P450 enzymes involved in drug metabolism (section 11.5.2). Unfortunately, the metabolic reaction carried out by these enzymes converted tienilic acid to a thiophene sulphoxide, which proved highly electrophilic. This encouraged a Michael reaction leading to alkylation of a thiol group in the enzyme's active site. Loss of water from the thiophene sulphoxide restored the thiophene ring and resulted in the formation of a covalent link to the enzyme, thus inhibiting the enzyme irreversibly.




Irreversible inhibition of cytochrome P450 by tienilic acid.

and our own. Nature, of course, is well ahead in this game. For example, many fungal strains produce metabolites that act as inhibitors of bacterial enzymes, but have no effect on fungal enzymes. This gives fungi an advantage over their microbiological competitors when competing for nutrients. It has also provided medicine with important antibiotics such as **penicillin** and **cephalosporin C**.

Although it is preferable to target enzymes that are unique to the foreign invader, it is still possible to target enzymes that are present in both bacterial and mammalian cells, as long as there are significant differences between them. Such differences are perfectly feasible. Although the enzymes in both species may have derived from a common ancestral protein, they have evolved and mutated separately over several million years. Identifying these differences allows the medicinal chemist to design drugs that will bind and act selectively against the bacterial enzyme. Chapter 19 covers antibacterial agents such

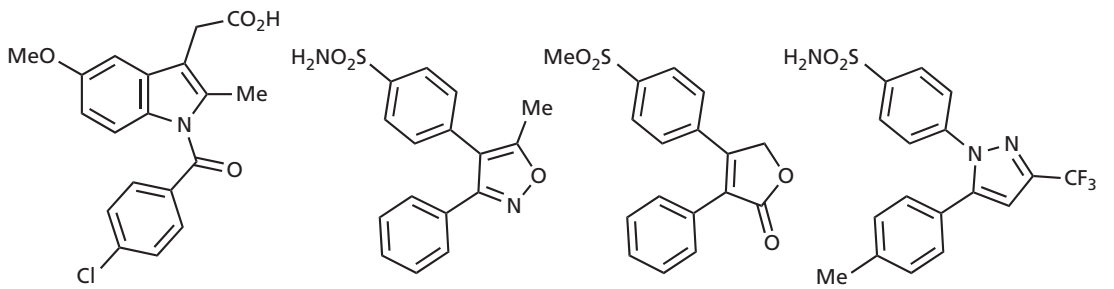
as the **sulphonamides**, **penicillins**, and **cephalosporins**, all of which act by inhibiting enzymes. Synthetic enzyme inhibitors, such as the **fluoroquinolones**, are also covered in this chapter.

### 7.7.2 Enzyme inhibitors used against viruses

Enzyme inhibitors are also extremely important in the battle against viral infections (e.g. herpesvirus and HIV). Successful antiviral drugs include **aciclovir** for herpes, and drugs such as **zidovudine** and **saquinavir** for HIV (see Chapter 20).

### 7.7.3 Enzyme inhibitors used against the body's own enzymes

Drugs that act on the body's own enzymes are important in medicine. There are many examples discussed elsewhere in this text and Table 7.1 indicates several of these, with a cross-reference to the relevant section in this textbook.




Indometacin Valdecoxib Rofecoxib Celecoxib

**FIGURE 7.11** Cyclooxygenase inhibitors.

## BOX 7.4 Designing drugs to be isozyme-selective

Designing drugs to be isozyme-selective means that they can be designed to act on different diseases, despite acting on the same enzyme. This is because isozymes differ in substrate specificity and are distributed differently in the body. **Monoamine oxidase (MAO)** is one of the enzymes responsible for the metabolism of important neurotransmitters such as **dopamine, noradrenaline**, and **serotonin** (section 4.2), and exists in two isozymic forms (MAO-A and MAO-B). These isozymes differ in substrate specificity, tissue distribution, and primary structure, but carry out the same reaction by the same mechanism (Fig. 1). MAO-A is selective for noradrenaline and serotonin whereas MAO-B is selective for dopamine. MAO-A inhibitors, such as **clorgiline**, are used clinically as antidepressants, while MAO-B inhibitors, such as **selegiline**, are administered with **levodopa** for the treatment of Parkinson's disease (Fig. 2). MAO-B inhibition protects levodopa from metabolism. Clorgiline and selegiline are thought to act as suicide substrates where they are converted by the enzyme to reactive species that react with the enzyme and form covalent bonds. The amine and alkyne functional groups present in both drugs are crucial to this process.

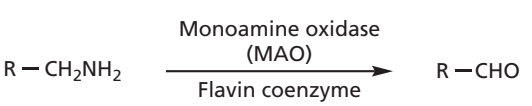
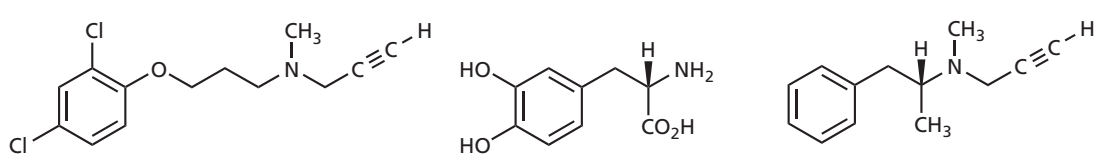



**FIGURE 1** Reaction catalysed by MAO.




Clorgiline Levodopa Selegiline (Deprenyl)

**FIGURE 2** Clorgiline, levodopa, and selegiline.

**TABLE 7.1** Enzyme inhibitors that act against enzymes in the body

| Drug | Target enzyme | Field of therapy | Relevant section |
|---|---|---|---|
| Aspirin | Cyclooxygenase | Anti-inflammatory | 13.1.9 |
| Captopril and enalapril | Angiotensin converting enzyme (ACE) | Antihypertension | Case study 2 |
| Simvastatin | HMG-CoA reductase | Lowering cholesterol levels | Case study 1 |
| Phenelzine | Monoamine oxidase | Antidepressant | 23.12.5 |
| Clorgiline, moclobemide | Monoamine oxidase-A | Antidepressant | Box 7.4, 23.12.5 |
| Selegiline | Monoamine oxidase-B | Parkinson's disease | Box 7.4 |
| Methotrexate, permetrexed, pralatrexate | Dihydrofolate reductase | Anticancer | 21.3.1 |
| 5-Fluorouracil, raltitrexid | Thymidylate synthase | Anticancer | 21.3.2 |
| Gefitinib, imatinib, etc. | Tyrosine kinases | Anticancer | 21.6.2 |
| Sildenafil | Phosphodiesterase enzyme (PDE5) | Treatment of male erectile dysfunction | 12.4.4.2 |
| Allopurinol | Xanthine oxidase | Treatment of gout | |
| Hydroxycarbamide | Ribonucleotide reductase | Anticancer | 21.3.3 |
| Pentostatin | Adenosine deaminase | Antileukaemia | 21.3.4 |
| Cytarabine, gemcitabine, fludarabine | DNA polymerases | Anticancer | 21.3.5 |
| Omeprazole, lansoprazole, pantoprazole, rabeprazole | Proton pump | Anti-ulcer | 25.3 |
| Physostigmine, donepezil, tacrine, organophosphates | Acetylcholinesterase | Myasthenia gravis, glaucoma, Alzheimer's disease | 22.12–22.15 |
| Various structures | Matrix metalloproteinase | Potential anticancer agents | 21.7.1 |
| Racecadotril | Enkephalinase | Treatment of diarrhoea | 24.8.4 |
| Zileutin | 5-Lipoxygenase | Anti-asthmatic | |
| Bortezomib | Proteasome | Anticancer | 21.7.2 |
| Vorinostat | Histone deacetylase | Anticancer | 21.7.3 |
| Lonafarnib | Farnesyl transferase | Anticancer | 21.6.1 |

## BOX 7.5 Action of toxins on enzymes

The toxicity of several poisons, toxins, and heavy metals result from their action on enzymes. Heavy metals, such as lead, cadmium, and mercury, have teratogenic effects leading to babies being born with malformed limbs. The worst case of mercury poisoning was in Japan, where a local population ate fish contaminated with methyl mercury ($MeHg^+$) that had been used as an agricultural fungicide. The compound inactivates enzymes by reacting with the thiol groups (R-SH) of cysteine residues to form covalent bonds (R-S-HgMe).

Mercury poisoning can also affect enzymes in the central nervous system leading to strange behaviour. For example, mercury nitrate was used by hat makers to soften and shape animal furs, and, inevitably, some of the chemical was absorbed through the skin. So many in the trade were poisoned in this way that their peculiar manner of behaviour led to the phrase 'mad as a hatter'.

The poison arsenite ($AsO_3^{3-}$) reacts with the thiol groups of an enzyme cofactor called dihydrolipoate, which is a prosthetic group (section 3.5.4) in some enzymes (Fig. 1). It is possible to reverse the poisoning by administering reagents with adjacent thiol groups that displace the arsenic from the cofactor. **2,3-Dimercaptopropanol** was developed after World War I as an antidote to an arsenic-based chemical weapon called **lewisite**.

*(Continued)*

**BOX 7.5** Action of toxins on enzymes (*Continued*)

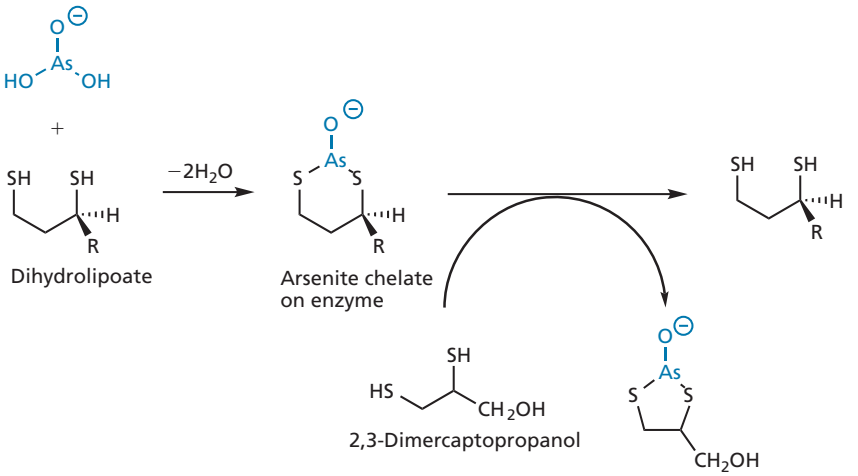



**FIGURE 1** Mechanism of arsenite poisoning and its treatment.

The search continues for new enzyme inhibitors, especially those that are selective for a specific isozyme, or act against recently discovered enzymes. Some current research projects include investigations into inhibitors of the **COX-2 isozyme** (section 7.6), **matrix metalloproteinases** (anti-arthritic and anticancer drugs; section 21.7.1), **aromatases** (anticancer agents; section 21.4.5), and **caspases**. The caspases are implicated in the processes leading to cell death and inhibitors of caspases may have potential in the treatment of stroke victims (Box 12.1). A vast amount of research is also taking place on **kinase inhibitors**. The kinase enzymes catalyse the phosphorylation of proteins and play an important role in signalling pathways within cells (see also Chapter 5 and section 21.6.2).

For additional material see Web article 1: steroids as novel anticancer agents.

**KEY POINTS**

- Enzyme inhibition is reversible if the drug binds through intermolecular interactions. Irreversible inhibition results if the drug reacts with the enzyme and forms a covalent bond.
- Competitive inhibitors bind to the active site and compete with either the substrate or the cofactor.
- Allosteric inhibitors bind to an allosteric binding site, which is different from the active site. They alter the shape of the enzyme such that the active site is no longer recognizable.
- Transition-state analogues are enzyme inhibitors designed to mimic the transition state of an enzyme-catalysed reaction mechanism. They bind more strongly than either the substrate or the product.
- Suicide substrates are molecules that act as substrates for a target enzyme, but which are converted into highly reactive species as a result of the enzyme-catalysed reaction mechanism. These species react with amino acid residues present in the active site to form covalent bonds and act as irreversible inhibitors.
- Drugs that selectively inhibit isozymes are less likely to have side effects, and will be more selective in their effect.
- Enzyme inhibitors are used in a wide variety of medicinal applications.

## 7.8 Enzyme kinetics

Studies of enzyme kinetics are extremely useful in determining the properties of an enzyme inhibitor. In this section, we will look at how Lineweaver-Burk plots are used to determinine what type of inhibition is occurring, as well as important quantitative measurements related to that inhibition.

### 7.8.1 Lineweaver-Burk plots

The Lineweaver-Burk plot (described in section 3.8.2) can be used to determine whether the inhibitor of an enzyme-catalysed reaction is competitive, uncompetitive, or non-competitive (Figs 7.12b and 7.14). The reciprocals of the reaction rate and the substrate concentration are plotted, with and without an inhibitor being present. This generates straight lines having the following equation,

where the slope (m) corresponds to $K_M$/rate$_{max,}$ and the intersection with the y-axis (c) corresponds to 1/rate$_{max}$:

$$\frac{1}{\text{rate}} = \frac{K_M}{\text{rate}_{max}} \cdot \frac{1}{[S]} + \frac{1}{\text{rate}_{max}} \qquad y = m.x + c$$

In the case of competitive inhibition, the lines cross the y-axis at the same point (i.e. the maximum rate of the enzyme-catalysed reaction is unaffected), but the slopes are different (i.e. the values of the **Michaelis constant** $K_M$ are different). The fact that the maximum rate is unaffected reflects the fact that the inhibitor and substrate are competing for the same active site and that increasing the substrate concentration sufficiently will overcome the inhibition. The increase in the slope that results from adding an inhibitor is a measure of how strongly the inhibitor binds to the enzyme and decreases the rate of the enzyme-catalysed reaction. In the presence of a competitive inhibitor, the apparent value of $K_M$ is increased by a constant α (the **degree of inhibition**) that depends on the concentration of inhibitor present:

$$K_M\,(\text{apparent}) = \alpha K_M$$

The degree of inhibition (α) can be determined by rearranging this equation as shown:

$$\alpha = \frac{K_M\,(\text{apparent})}{K_M}$$

A useful measure of inhibition is the apparent **inhibition constant** $K_i$. which is a measure of the equilibrium between the enzyme–inhibitor complex and the uncomplexed enzyme and inhibitor.

$$EI \rightleftharpoons E + I \qquad K_I = \frac{[E][I]}{[EI]}$$

We can write an expression linking the apparent inhibition constant $K_i$ to the inhibitor concentration [I] and the degree of inhibition α:

$$K_i = \frac{[I]}{\alpha - 1} \quad \text{where [I] is the inhibitor concentration}$$

Replacing α with $K_M$(app)/$K_M$, then rearranging the equation gives the straight line equation shown in Figure 7.13. A plot of this line will give the Michaelis constant $K_M$ as the intersect with the y-axis, while the slope corresponds to $K_M/K_i$. From this, one can get the value of $K_i$.

To create this plot, a series of Lineweaver-Burk plots is first created in order to get values of $K_M$ (apparent) at different inhibitor concentrations. The plot of $K_M$ (apparent) versus [I] is then drawn, allowing $K_i$ to be calculated from the slope of the line. The lower the value of $K_i$, the more potent the inhibitor.

In the case of an uncompetitive inhibitor (Fig. 7.14), the inhibitor binds to the enzyme–substrate complex rather than the free enzyme. Enzyme inhibition studies result in Lineweaver-Burk plots where the lines are parallel

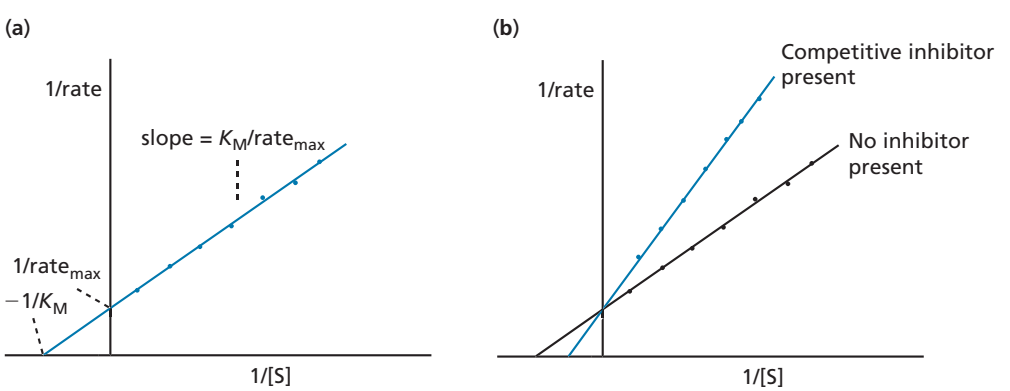



**FIGURE 7.12** (**a**) Lineweaver-Burk plot. (**b**) Lineweaver-Burk plots with and without a competitive inhibitor present.

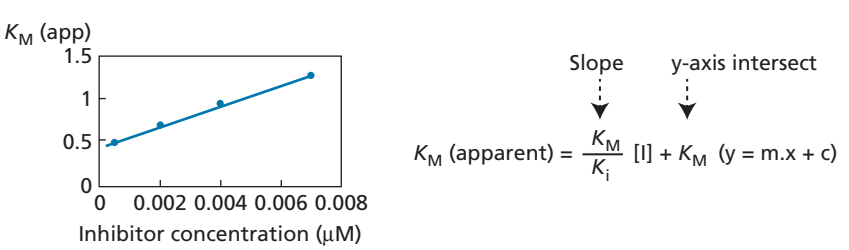



**FIGURE 7.13** Plot of $K_M$ (apparent) versus inhibitor concentration [I].

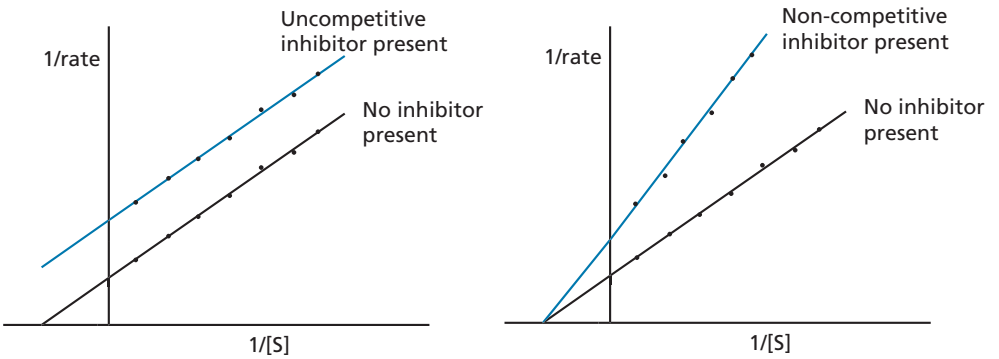



**FIGURE 7.14** Lineweaver-Burk plots with and without an uncompetitive inhibitor or non-competitive inhibitor.

and cross the y-axis at different points, indicating that the maximum rate for the enzyme has been reduced. For a reversible, non-competitive inhibitor, the lines have the same intercept point on the x-axis (i.e. $K_M$ is unaffected), but have different slopes and different intercepts on the y-axis. Therefore, the maximum rate for the enzyme has been reduced.

Lineweaver-Burk plots are extremely useful in determining the nature of inhibition, but they have their limitations and are not applicable to enzymes that are under allosteric control.

## 7.8.2 Comparison of inhibitors

When comparing the activity of enzyme inhibitors, the $IC_{50}$ value is often quoted. This is the concentration of inhibitor required to reduce the activity of the enzyme by 50%. Compounds with a high $IC_{50}$ are less powerful inhibitors than those with a low $IC_{50}$, as a higher concentration of the former is required to attain the same level of inhibition.

$K_i$ values are also reported in enzyme inhibition studies and it can be shown that $IC_{50} = K_i + [E]_{total}/2$. If the concentration of inhibitor required to inhibit the enzyme by 50% is much greater than the concentration of enzyme, then $K_i$ is much larger than $[E]_{total}$ and this equation approximates to $IC_{50} = K_i$.

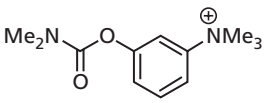
$IC_{50}$ and $K_i$ values are measured in assays involving isolated enzymes. However, it is often useful to carry out enzyme inhibition studies where the enzyme is present in whole cells or tissues. In these studies, a cellular effect resulting from enzyme activity is monitored. $EC_{50}$ values represent the concentration of inhibitor required to reduce that particular cellular effect by 50%. It should be noted that the effect being measured may be several stages downstream from the enzyme reaction concerned.

**KEY POINTS**

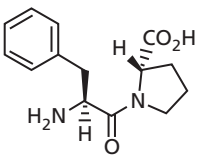
- The Michaelis-Menten equation relates the rate of an enzyme-catalysed reaction to substrate concentration.
- Lineweaver-Burk plots are derived from the Michaelis-Menten equation and are used to determine whether inhibition is competitive, uncompetitive, or non-competitive.
- The activity of different enzymes can be compared by measuring values of $EC_{50}$, $K_i$, or $IC_{50}$.

# QUESTIONS

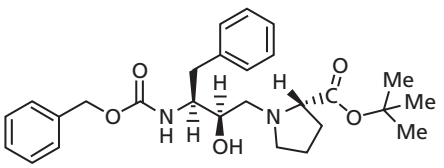
1. It is known that the amino acid at position 523 of the cyclooxygenase enzyme is part of the active site. In the isoenzyme COX-1, this amino acid is isoleucine, whereas in COX-2, it is valine. Suggest how such information could be used in the design of drugs that selectively inhibit COX-2.
2. Neostigmine is an inhibitor of acetylcholinesterase. The enzyme attempts to catalyse the same reaction on neostigmine as it does with acetylcholine. However, a stable intermediate is formed which prevents completion of the process and which results in a molecule being covalently linked to the active site. Identify the stable intermediate and explain why it is stable.
3. The human immunodeficiency virus contains a protease enzyme that is capable of hydrolysing the peptide bond of L-Phe-L-Pro. Structure I was designed as a transition-state inhibitor of the protease enzyme. What is a transition-state inhibitor and how does structure I fit the description of a transition-state inhibitor? What is meant by $IC_{50}$ 6500 nM?



Neostigmine



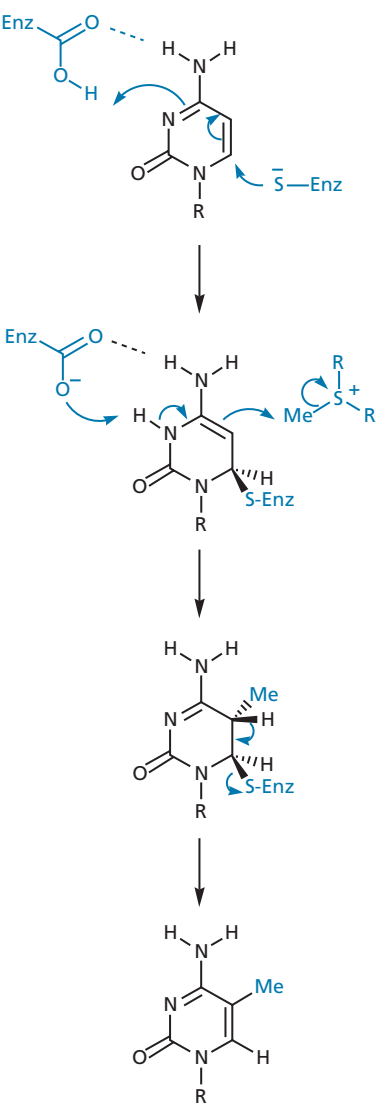
L-Phe-L-Pro



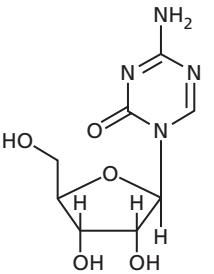
(I) $IC_{50}$ 6500 nM

**4.** Why should a transition state be bound more strongly to an enzyme than a substrate or a product?

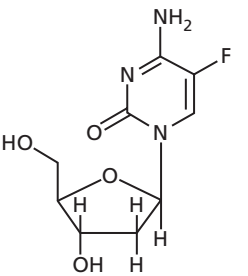
**5.** The methylation of cytosine residues in DNA plays a role in the regulation of transcription and is catalysed by the enzyme DNA methyltransferase. The mechanism is shown as follows.



5-Azacytidine and 5-fluoro-2'-deoxycytidine are mechanism-based inhibitors of DNA methyltransferase. Explain why.



5-Azacytidine

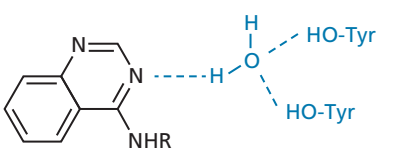


5-Fluoro-2′-deoxycytidine

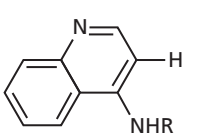
**6.** 17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) is an enzyme that catalyses the conversion of estrone to estradiol in the presence of the cofactor NADH. The initial rate data for the enzyme-catalysed reaction in the absence of an inhibitor is given in Question 5 of Chapter 3. EM-1745 is an inhibitor of the enzyme. The following data was determined with EM-1745 present at a concentration of 4nM. Using this and the data recorded in Chapter 3 determine whether the compound acts as a competitive, uncompetitive, or mixed inhibitor. Calculate the value of $K_i$.

| Substrate concentration ($10^{-2}$ mol dm$^{-3}$) | 5 | 10 | 20 | 40 | 83.3 |
|---|---|---|---|---|---|
| Initial rate (mol dm$^{-3}$ s$^{-1}$) | 1.0 | 2.0 | 3.45 | 6.25 | 10.0 |

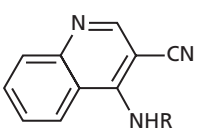
**7.** The quinazoline structure shown is an inhibitor of the enzyme scytalone dehydratase. One of the binding interactions between the inhibitor and the active site is a hydrogen bond to a water molecule, which acts as a hydrogen-bonding bridge to two tyrosine residues. Explain why analogue I is three times less active, whereas analogue II is 20 times more active.



Quinazoline



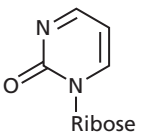
Analogue I



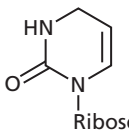
Analogue II

**8.** Cytidine deaminase is an enzyme that converts cytidine to uridine. Suggest a mechanism by which this reaction might occur, considering that a highly conserved water molecule is present in the active site. Zebularine is a natural product

that is converted into a highly potent transition-state (TS) inhibitor of the enzyme ($K_i$ = 1.2 pM) when it is in the active site. Suggest what the structure of the TS-inhibitor might be and why it is so effective. Explain why 3,4-dihydrozebularine has a binding affinity that is only 30 μM.



Zebularine



3,4-Dihydrozebularine

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# 8 Receptors as drug targets

## 8.1 Introduction

The structures and functions of various receptors were described in Chapter 4. Receptors and their chemical messengers are crucial to the communication systems of the body. Such communication is clearly essential to the normal workings of the body. When it goes wrong, a huge variety of ailments can arise, such as depression, heart problems, schizophrenia, and muscle fatigue, to name just a few. What sort of things *could* go wrong though?

One problem would be if too many chemical messengers were released. The target cell could (metaphorically) start to overheat. Alternatively, if too few messengers were sent out, the cell could become sluggish. It is at this point that drugs can play a role by either acting as replacement messengers or blocking receptors from receiving their natural messengers. Drugs that mimic the natural messengers and activate receptors are known as **agonists**. Drugs that block receptors are known as **antagonists**. The latter compounds still bind to the receptor, but they do not activate it. However, as they are bound, they prevent the natural messenger from binding.

What determines whether a drug acts as an agonist or an antagonist, and is it possible to predict whether a new drug will act as one or the other? To answer these questions, we have to move down to the molecular level and consider what happens when a small molecule, such as a drug or a neurotransmitter, interacts with a receptor protein.

In sections 4.4–4.5, we looked at a hypothetical receptor and neurotransmitter. We saw that a chemical messenger caused the receptor to change shape—a process known as an induced fit. It is this induced fit which activates the receptor and leads to the 'domino' effect of signal transduction—the method by which the message carried by the chemical messenger is transferred into the cell (Chapters 4 and 5).

## 8.2 The design of agonists

We are now at the stage of understanding how drugs might be designed in such a way that they mimic the natural chemical messengers. Assuming that we know what binding regions are present in the receptor site and where they are located, we can design drugs to interact with the receptor in the same way. Let us look at this more closely and consider the following requirements in turn:

- the drug must have the correct binding groups;
- the drug must have these binding groups correctly positioned;
- the drug must be the right size for the binding site.

### 8.2.1 Binding groups

If we know the structure of the natural chemical messenger and can identify the functional groups that form important interactions with the binding site, then we might reasonably predict which of a series of molecules would interact in the same way. For example, consider the hypothetical neurotransmitter shown in Fig 8.1. The important binding groups are indicated in blue—an

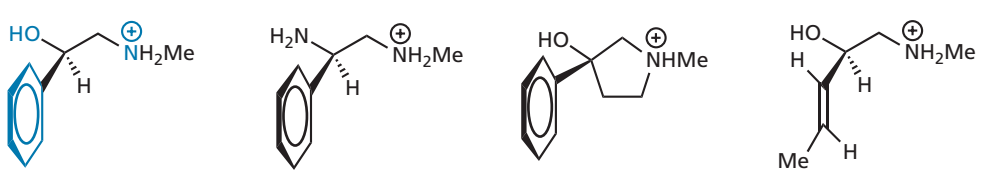



**FIGURE 8.1** A hypothetical neurotransmitter and possible agonists (binding groups shown in blue).

aromatic ring, alcohol, and aminium ion. These interact with the binding site through van der Waals interactions, hydrogen bonding, and ionic bonding respectively (Fig. 8.2a). Consider now the other structures in Fig. 8.1. They all look different, but they all contain functional groups which could interact in the same way. Therefore, they may well be potential agonists that will activate the receptor.

What about the structures in Fig. 8.3? They lack one or more of the required binding groups and should, therefore, have poor activity. We would expect them to drift into the binding site, then drift back out again binding only weakly, if at all.

Of course, we are making an assumption here, namely that all three binding groups are essential. It might be argued that a compound such as structure II in Fig. 8.3 might be effective even though it lacks a suitable hydrogen bonding group. Why, for example, could it not bind initially by van der Waals interactions alone and then alter the shape of the receptor protein via ionic bonding?

In fact, this seems unlikely when we consider that neurotransmitters appear to bind, pass on their message, and then leave the binding site relatively quickly. In order to do that, there must be a fine balance in the binding interactions between the receptor and the neurotransmitter. They must be strong enough to bind the neurotransmitter effectively such that the receptor changes shape. However, the binding interactions cannot be too strong or else the neurotransmitter would not be able to leave and the receptor would not be able to return to its original shape. Therefore, it is reasonable to assume that

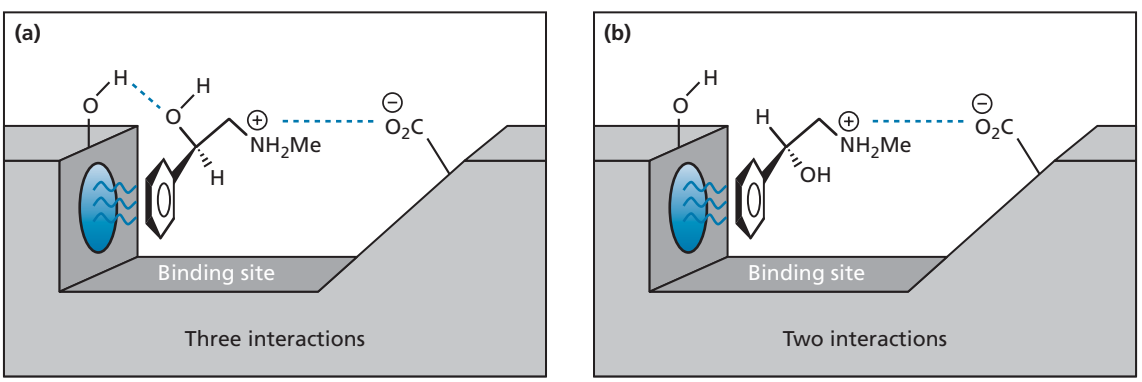



**FIGURE 8.2** A comparison of interactions involving (**a**) the hypothetical neurotransmitter and (**b**) its mirror image with a hypothetical binding site.

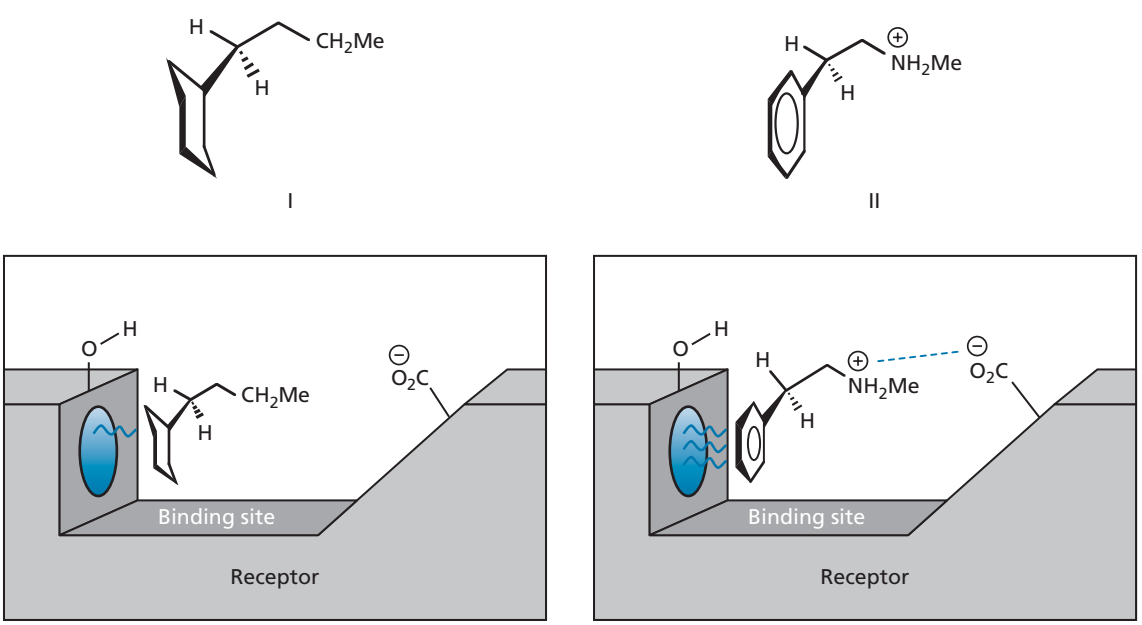



**FIGURE 8.3** Weaker binding to the hypothetical receptor by structures that possess fewer than the required binding groups.

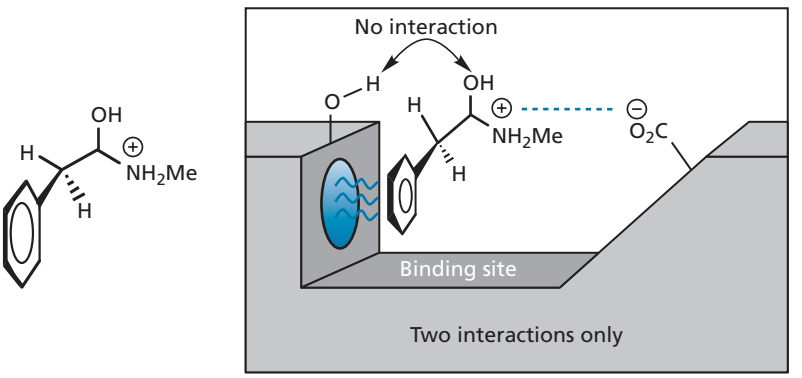



**FIGURE 8.4** Weaker binding to the hypothetical receptor by a molecule containing binding groups in incorrect positions.

a neurotransmitter needs all of its binding interactions to be effective. The lack of even one of these interactions would lead to a significant loss in activity.

## 8.2.2 Position of the binding groups

The molecule may have the correct binding groups, but if they are in the wrong relative positions they will not be able to form bonds at the same time. As a result, bonding would be too weak to be effective.

A molecule such as the one shown in Fig. 8.4 obviously has one of its binding groups (the hydroxyl group) in the wrong position, but there are more subtle examples of molecules that do not have the correct arrangement of binding groups. For example, the mirror image of our hypothetical neurotransmitter would not bind strongly to the binding site (Fig. 8.5). The structure has the same formula and the same constitutional structure as our original structure. It will have the same physical properties and undergo the same chemical reactions, but it is not the same shape. It is a non-superimposable mirror image and it cannot interact with all the binding regions of the receptor binding site at the same time (Fig. 8.2b).

Compounds which exist as non-superimposable mirror images are termed **chiral** or **asymmetric**. There are only two detectable differences between the two mirror images (or **enantiomers)** of a chiral compound. They rotate plane-polarized light in opposite directions and they interact differently with other chiral systems, such as enzymes and receptors. This has very important consequences for the pharmaceutical industry.

Pharmaceutical agents are usually synthesized from simple starting materials using simple achiral (symmetrical) chemical reagents. These reagents are incapable of distinguishing between the two mirror images of a chiral compound. As a result, most chiral drugs used to be synthesized as a mixture of both mirror images—a

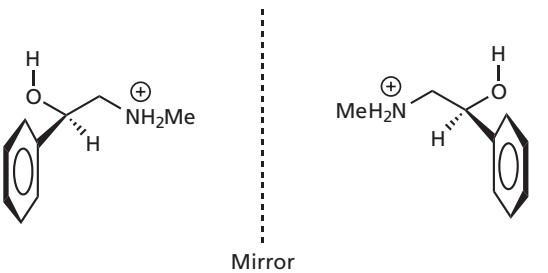



**FIGURE 8.5** Mirror image of a hypothetical neurotransmitter.

**racemate**. However, we have seen from our own simple example that only one of these enantiomers is going to interact properly with a target receptor. What happens to the other enantiomer?

At best, it floats about in the body doing nothing. At worst, it interacts with a totally different target and results in an undesired side effect. Even if the 'wrong' enantiomer does not do any harm, it seems a great waste of time, money, and effort to synthesize drugs that are only going to be 50% efficient. That is why one of the biggest areas of chemical research in recent years has been in **asymmetric synthesis**—the selective synthesis of a single enantiomer of a chiral compound.

Of course, nature has been at it for millions of years. Nature* has chosen to work predominantly with the 'left-handed' enantiomer of amino acids, so enzymes (made

* Naturally occurring asymmetric amino acids exist in mammals as the one enantiomer, termed the L-enantiomer. This terminology is historical and defines the absolute configuration of the asymmetric carbon present at the head-group of the amino acid. The current terminology for **asymmetric centres** is to define them as *R* or *S* according to a set of rules known as the Cahn-Ingold-Prelog rules. The L-amino acids exist as the (*S*)-configuration (except for cysteine, which is *R*), but the older terminology still dominates here. Experimentally, the L-amino acids are found to rotate plane-polarized light anticlockwise or to the left. It should be noted that D-amino acids can occur naturally in bacteria (see, for example, section 19.5.5).

up of left-handed amino acids) are also present as a single mirror image and therefore catalyse **enantiospecific** reactions—reactions which give only one enantiomer. Moreover, the enantiomers of asymmetric enzyme inhibitors can be distinguished by the target enzyme, which means that one enantiomer is more potent than the other.

The importance of having binding groups in the correct position has led medicinal chemists to design drugs based on what is considered to be the important **pharmacophore** of the messenger molecule. In this approach, it is assumed that the correct positioning of the binding groups is what decides whether the drug will act as a messenger or not and that the rest of the molecule serves as a scaffold to hold the groups in those positions. Therefore, the activity of apparently disparate structures at a receptor can be explained if they all contain the correct binding groups at the correct positions. Totally novel structures or molecular frameworks could then be designed to obey this rule, leading to a new series of drugs. There is, however, a limiting factor to this, which will now be discussed.

## 8.2.3 Size and shape

It is possible for a compound to have the correct binding groups in the correct positions and yet fail to interact effectively if it has the wrong size or shape. As an example, consider the structure shown in Fig. 8.6 as a possible ligand for our hypothetical receptor.

The structure has a *meta*-methyl group on the aromatic ring and a long alkyl chain attached to the nitrogen atom. Both of these features would prevent this molecule from binding effectively to the binding site shown.

The *meta*-methyl group acts as a **steric shield** and prevents the structure from sinking deep enough into the binding site for effective binding. Similarly, the long alkyl chain on the nitrogen atom makes that part of the molecule too long for the space available to it. A thorough understanding of the space available in the binding site is therefore necessary when designing drugs to fit it. Having said that, it is important to appreciate that there is a level of flexibility in the binding site. A potential agonist may appear too large, but a slightly different induced fit might occur which allows the molecule to fit and bind, yet still activate the receptor. In exceptional cases, there can be quite significant alterations in the induced fit (Box 8.1).

## 8.2.4 Other design strategies

The discussion in previous sections in this chapter describes how agonists can be designed from a knowledge of the structure, shape, and binding interactions of the natural messenger. However, there are several

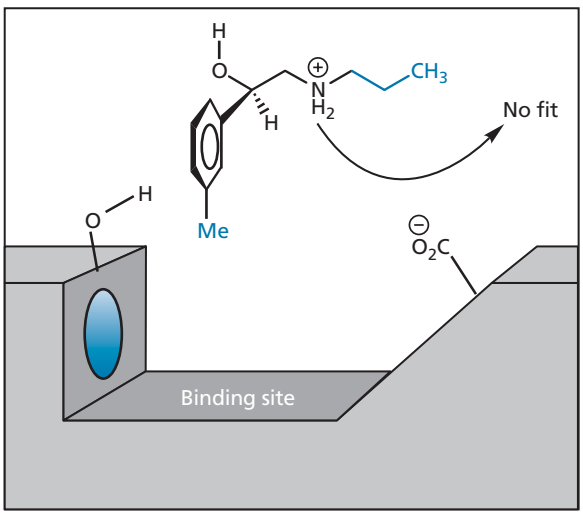



**FIGURE 8.6** Failed interaction of a structure with a binding site because of steric factors.

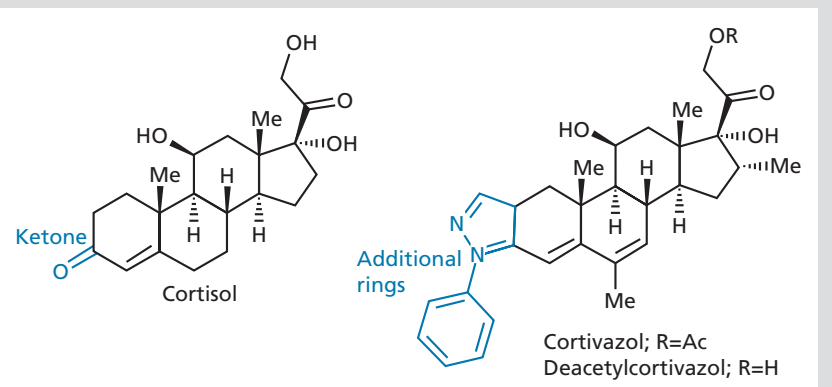
agonists that are quite different in structure from the natural messenger. How are these designed? It has to be remembered that the binding site is bristling with amino acid residues and peptide links, all of which might be capable of interacting with a visiting molecule by different types of intermolecular bonds. In other words, there may be other binding regions present than just those used by the natural messenger (Fig. 8.7). A drug that has the same three binding interactions described earlier, as well as an extra binding interaction, would be expected to bind more strongly and be a more potent agonist if the correct induced fit still takes place for receptor activation. Moreover, the loss of one of the key binding groups required by the natural messenger could be compensated by the presence of other binding groups capable of interacting with different binding regions.

## 8.2.5 Pharmacodynamics and pharmacokinetics

The study of how molecules interact with targets such as receptors or enzymes to produce a pharmacological effect is called pharmacodynamics. Such studies can typically be carried out on the pure target protein, or on isolated cells or tissues which bear the target protein (*in vitro* **studies**), but it is important to appreciate that designing a drug to interact effectively with a protein *in vitro* does not guarantee a clinically useful drug. Studies should also be carried out concurrently to ensure that promising-looking drugs are active in whole organisms (*in vivo* **studies**). This is a field known as pharmacokinetics and is covered in Chapters 11 and 14. It is also important to identify at an early stage whether the structures being studied might be prone to toxic or unacceptable side effects, so that time is

## BOX 8.1 An unexpected agonist

Glucocorticoid steroids such as **cortisol** are used clinically as anti-inflammatory agents and act as agonists at the glucocorticoid receptor (Case study 6). These have the correct size, shape, and binding groups to fit the binding site, and produce the required induced fit for receptor activation. Recently, it has been discovered that **cortivazol** acts as an agonist, yet it lacks one of the important binding groups (the ketone) and has two extra rings that should make it too big for the binding site. A crystal structure of the receptor/ligand complex was studied and it was found that a different induced fit had occurred from normal. This had resulted in a new channel being opened up in the binding site that could accommodate the extra rings. Moreover, extra interactions with the rings compensated for the loss of the usually crucial ketone group. Normally, a different induced fit would be expected to result in antagonist activity, but, in this case, the receptor was still activated (see also Box 17.6).



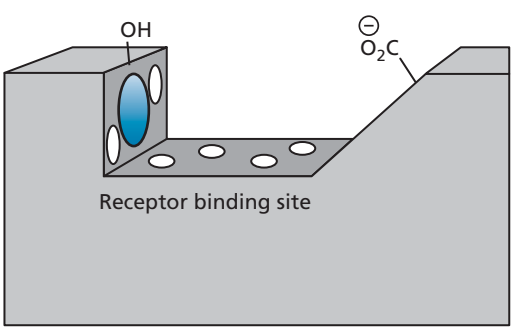




**FIGURE 8.7** The hypothetical binding site showing extra binding regions (in white) that are not used by the natural chemical messenger.

not wasted taking a candidate drug all the way to clinical trials only for it to be rejected (Box 12.3)

### 8.2.6 Examples of agonists

There are numerous examples of drugs that act as agonists at various target receptors. In this textbook, you will find a description of cholinergic agonists which are used in the treatment of glaucoma and myasthenia gravis (section 22.8). There is a description of the adrenergic agonists used as anti-asthmatic agents in section 23.10.3, while Chapter 24 covers the opioid analgesics which act as agonists. The glucocorticoids that are used as anti-inflammatory agents also act as agonists (Case study 6; see also Box 8.1).

Other examples of agonists used in the clinic are dopamine agonists used in the treatment of Parkinson's disease and serotonin agonists used in the treatment of migraines. Agonists designed to act on the estrogen receptor are used as contraceptives. There are many more examples.

### 8.2.7 Allosteric modulators

Some drugs have an indirect agonist effect by acting as allosteric modulators. By binding to an allosteric site on a target receptor they mimic the action of endogenous modulators and enhance the action of the natural or endogenous chemical messenger (section 4.10). For example, the **benzodiazepines** used as sleep medicines target the allosteric binding site of the **$GABA_A$ receptor**. **Cinacalcet** (Fig. 8.8) is used to treat thyroid problems and is an allosteric modulator for a G-protein-coupled receptor known as the **calcium-sensing receptor**. **Galantamine** acts as an enzyme inhibitor in the treatment of Alzheimer's disease (section 22.15), but is also an allosteric modulator of the **nicotinic receptor**.

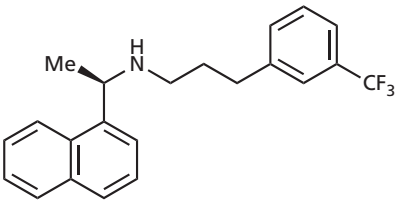



**FIGURE 8.8** Cinacalcet.

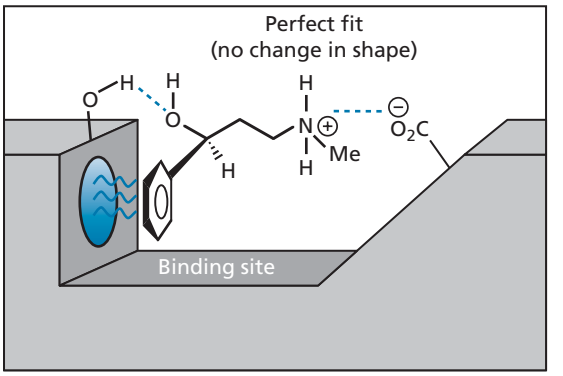



**FIGURE 8.9** Compound acting as an antagonist at the binding site.

## 8.3 The design of antagonists

### 8.3.1 Antagonists acting at the binding site

We have seen how it might be possible to design drugs (agonists) to mimic natural chemical messengers and how these would be useful in treating a shortage of the natural ligand. However, suppose that we have too many messengers operating in the body. How could a drug counteract that? The answer would be to design a drug (an antagonist) that will bind to the binding site, but will not activate the receptor. Since it is bound, it will prevent the normal ligand from binding and activating the receptor.

There are several strategies in designing antagonists, but one way is to design a drug that is the right shape to bind to the receptor binding site, but which either fails to change the shape of the binding site or distorts it in the wrong way. Consider the following scenario.

The compound shown in Fig. 8.9 fits the binding site perfectly and, as a result, does not cause any change of shape. Therefore, there is no biological effect and the binding site is blocked to the natural neurotransmitter.

Another strategy is to find different binding regions within the binding site that are not used by the natural chemical messenger (Fig. 8.7). Drugs could be designed to interact with some of these extra binding regions such that the resultant binding produces a quite different induced fit from that obtained when the natural messenger binds—an induced fit that fails to activate the receptor.

Extra binding regions do not necessarily have to be within the part of the binding site occupied by the natural messenger. It is quite common to find antagonists that are larger than the natural messenger and which access extra binding regions beyond the reach of the usual messenger. Many antagonists are capable of binding to both the normal binding site and these neighbouring regions.

To illustrate this, we will once more consider our hypothetical neurotransmitter and its receptor, but this time we will represent the binding site in a different way, as if we were looking at it from above and drawing a map of where the binding regions are located (Fig. 8.10). This

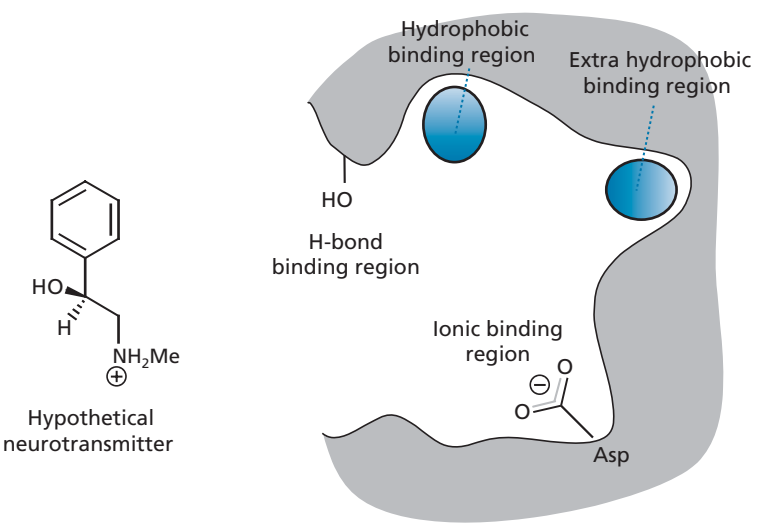



**FIGURE 8.10** 'Map' of the hypothetical binding site.

kind of representation is used frequently in order to simplify binding site diagrams, but it is important to appreciate that the binding site is a three-dimensional shape and that the interactions involved are also three-dimensional.

The three important binding regions are still present, but our 'map' shows an extra hydrophobic region which could act as a potential binding region.

Binding of the hypothetical neurotransmitter results in the correct induced fit required for receptor activation (Fig. 8.11). Note that the extra binding region is not within the range of the messenger molecule.

We could now design a molecule which would bind to all four of these binding regions (Fig. 8.12). This molecule will bind more strongly than the natural messenger owing to the extra binding interaction. If the binding produces the same induced fit then we have designed a more potent agonist, but, in this case, the induced fit is significantly different and so the receptor is not activated. Therefore, the molecule acts as an antagonist; it binds to the receptor, but fails to activate it. Moreover, by occupying the binding site it prevents the normal messenger from binding.

Antagonists that bind strongly to a target binding site are often used to label receptors. Such antagonists are synthesized with a radioactive isotope incorporated into their structure, allowing them to be detected more easily.

For additional material see Web article 2: antagonists as molecular labels.

To sum up, if we know the shape and characteristics of a receptor binding site then we should be able to design

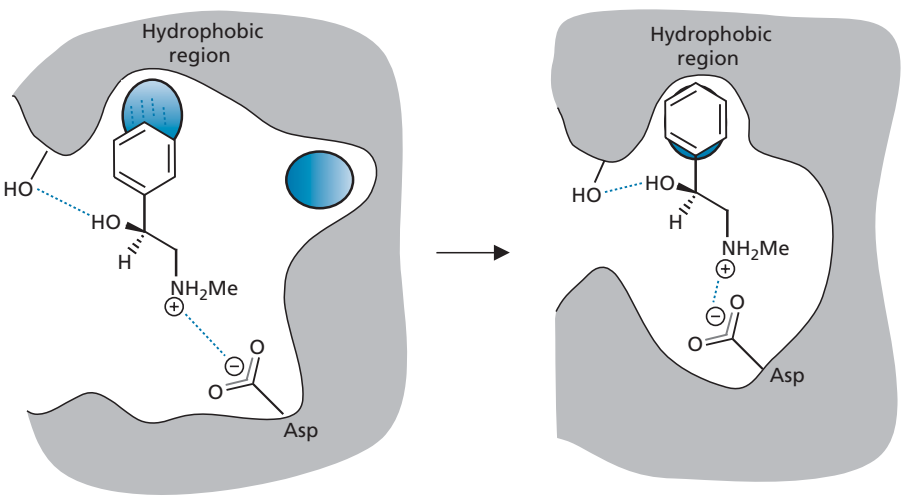



**FIGURE 8.11** Binding of the natural chemical messenger resulting in an induced fit that activates the receptor.

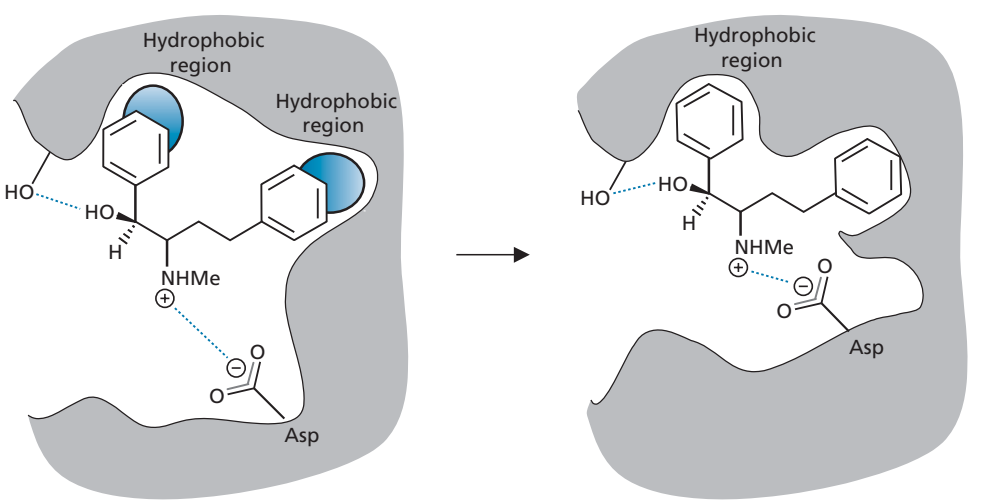



**FIGURE 8.12** Binding of an antagonist leading to a different induced fit.

drugs to act as agonists *or* antagonists. Unfortunately, determining the layout of a receptor binding site is not as straightforward as it sounds. For many years, the only feasible approach was to synthesize a large number of compounds, identify those that fitted the binding site and those that did not, then propose what the binding site might look like from those results—a bit like a three-dimensional jigsaw. Nowadays, the use of genetic engineering, X-ray crystallography of protein targets, and computer-based molecular modelling allows a more accurate representation of proteins and their binding sites (Chapter 17). This has heralded new approaches to developing new drugs, such as *de novo* **drug design** and **structure-based drug design** (see also Box 8.2). Some of these studies can reveal surprising results, where the binding of a particular drug causes a different kind of induced fit from normal, resulting in the exposure of new potential binding regions (Box 8.1).

There are many examples in this book of antagonists that act at the binding site of a receptor. These include the histamine $H_2$ antagonists used for the treatment of ulcers (Chapter 25), the adrenergic antagonists used in cardiovascular medicine (section 23.11.3), serotonin antagonists as potential central nervous system-active drugs (Case study 7) and the cholinergic antagonists used as neuromuscular blockers (section 22.10.2). Another example is **raloxifene**, which acts as an antagonist of the **estrogen receptor** (Box 8.2). This compound is an example of an antagonist that binds to the same binding regions as the natural ligand, as well as an extra binding region.

## BOX 8.2 Estradiol and the estrogen receptor

17β-Estradiol is a steroid hormone that affects the growth and development of a number of tissues. It does so by crossing cell membranes and interacting with the binding site of an estrogen intracellular receptor. Estradiol uses its alcohol and phenol groups to form hydrogen bonds with three amino acids in the binding site, while the hydrophobic skeleton of the molecule forms van der Waals and hydrophobic interactions with other regions (Fig. 1). The binding pocket is hydrophobic in nature and quite spacious, except for the region where the phenol ring binds. This is a narrow slot and will only accept a planar aromatic ring. Owing to these constraints, the binding of estradiol's phenolic ring determines the orientation for the rest of the molecule.

The binding of estradiol induces a conformational change in the receptor which sees a helical section known as H12 folding across the binding site like a lid (Fig. 2). This not only seals estradiol into its binding site, it also exposes a hydrophobic region called the activating function (AF-2) region which acts as a binding site for a co-activator protein. As dimerization has also taken place, there are two of these regions available and the co-activator binds to both to complete the nuclear transcription factor. This now binds to a specific region of DNA and switches on the transcription of a gene, resulting in the synthesis of a protein.

**Raloxifene** (Fig. 3) is an antagonist of the estrogen receptor and is used for the treatment of hormone-dependent breast cancer. It is a synthetic agent which binds to the binding site without activating the receptor and prevents estradiol from binding. The molecule has two phenol groups that mimic the phenol and alcohol group of estradiol. The skeleton is also hydrophobic and matches the hydrophobic character of estradiol. So why does raloxifene not act as an agonist?

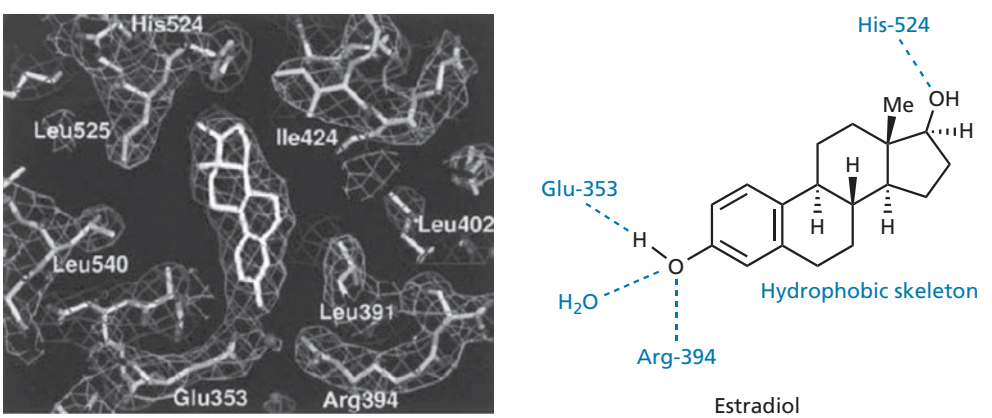



**FIGURE 1** Binding mode of estradiol with the estrogen receptor.

*(Continued)*

## BOX 8.2 Estradiol and the estrogen receptor (*Continued*)

The answer lies in a side chain. This side chain contains an amino group which is protonated and forms a hydrogen bond to Asp-351—an interaction that does not take place with estradiol. In doing so, the side chain protrudes from the binding pocket and prevents the receptor helix H12 folding over as a lid. As a result, the AF-2 binding region is not exposed, the co-activator cannot bind, and the transcription factor cannot be formed. Hence, the side chain is crucial to antagonism. It must contain an amine group of the correct basicity such that it ionizes and forms the interaction with Asp-351, and it must be of the correct length and flexibility to place the amine in the correct position for binding.

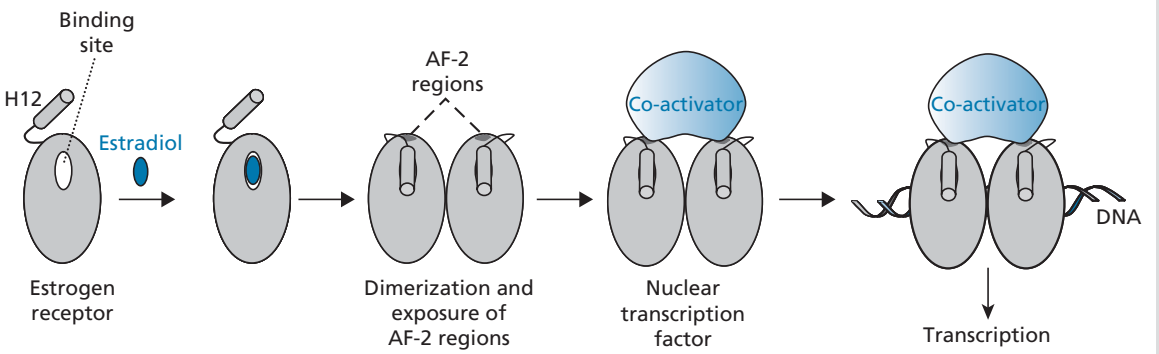



**FIGURE 2** Control of transcription by the estrogen receptor.

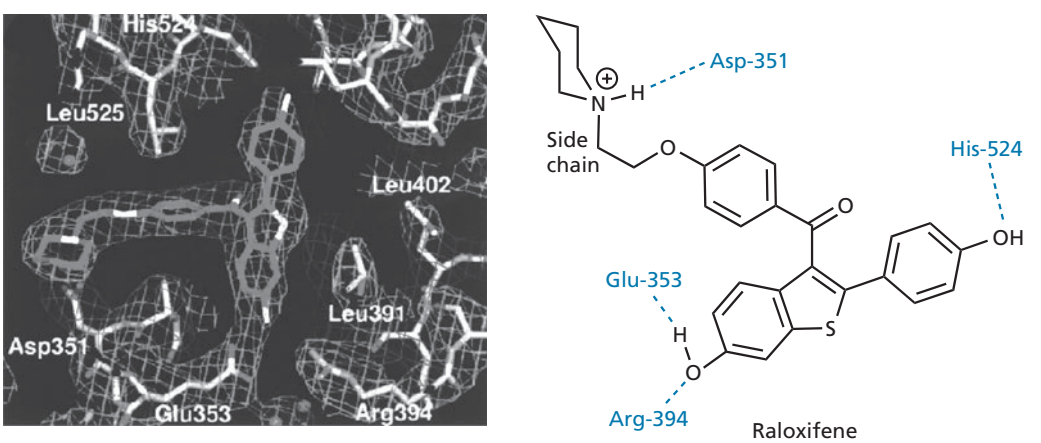



**FIGURE 3** Binding mode of raloxifene with the estrogen receptor.

## 8.3.2 Antagonists acting out with the binding site

There are examples of antagonists which do not bind to the binding site used by the natural chemical messenger. How do these antagonists work? There are two possible explanations.

**Allosteric modulators** Some receptors have allosteric binding sites. These are binding sites which are located on a different part of the receptor surface from the binding site, and which bind natural molecules called **modulators** that 'modulate' the activity of receptors by either enhancing it (section 8.2.7) or diminishing it. If activity is diminished, the modulator is acting indirectly as an antagonist. The mechanism by which this takes place could be viewed in a similar way to the allosteric inhibition of enzymes (section 3.6). The modulator binds to the allosteric binding site and causes it to change shape—an

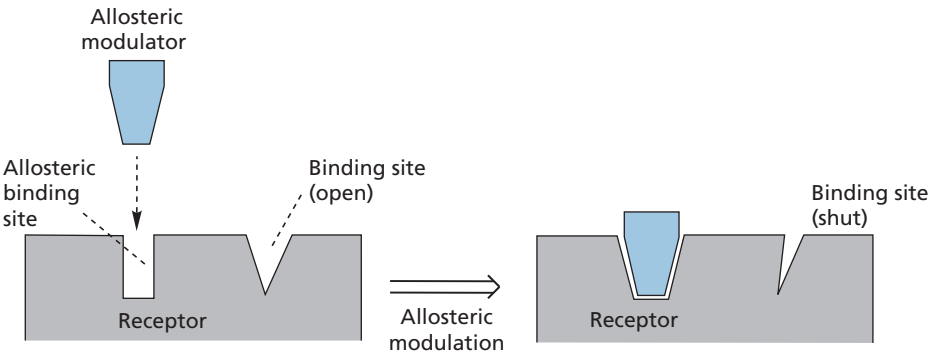



**FIGURE 8.13** Principle by which an allosteric antagonist distorts a binding site.

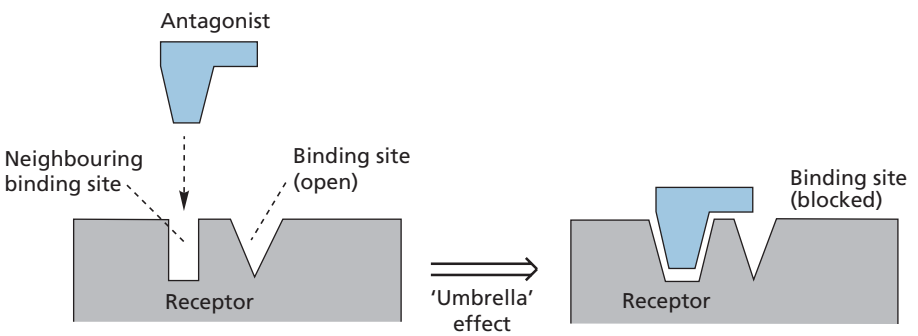



**FIGURE 8.14** Antagonism by the 'umbrella effect'.

induced fit. This has a 'knock-on' effect which alters the shape of the normal binding site. If the site becomes too distorted, then it is no longer able to bind the normal chemical messenger or binds it less effectively. Therefore, it is possible to design an antagonist that will bind to the allosteric binding site rather than to the normal binding site (Fig. 8.13).

**Antagonism by the 'umbrella' effect** Some antagonists are thought to bind to regions of the receptor which are close to the normal binding site. Although they do not bind directly to the binding site, the molecule acts as a 'shield' or as an 'umbrella', preventing the normal messenger from accessing the binding site (Fig. 8.14).

## 8.4 Partial agonists

Frequently, a drug is discovered which cannot be defined either as a pure antagonist or a pure agonist. The compound acts as an agonist and produces a biological effect, but that effect is not as great as one would get with a full agonist. Therefore, the compound is called a **partial agonist**. There are several possible explanations for this.

- A partial agonist must, obviously, bind to a receptor in order to have an agonist effect. However, it may be binding in such a way that the conformational change induced is not ideal and the subsequent effects of receptor activation are decreased. For example, a receptor may be responsible for the opening of an ion channel. The normal chemical messenger causes an induced fit that results in the ion channel fully opening up. A partial agonist, however, binds to the receptor and causes a less significant induced fit which results in only a slight distortion of the receptor. As a result, the ion channel is only partially opened (Fig. 8.15).
- The partial agonist may be capable of binding to a receptor in two different ways by using different binding regions in the binding site. One method of binding activates the receptor (an agonist effect), but the other does not (an antagonist effect). The balance of agonism versus antagonism would then depend on the relative proportions of molecules binding by either method. This theory was used to explain the activity of partial agonists observed during the development of the anti-ulcer drug cimetidine (section 25.2.2.2). An alternative explanation is that a partial agonist has the ability to stabilize two different conformations of the

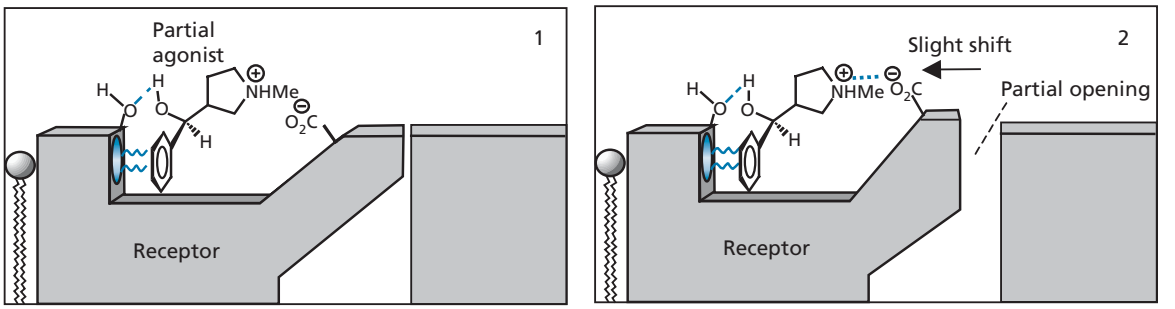



**FIGURE 8.15** Partial agonism.

receptor—one which is active and one which is not (Fig. 8.16).

- Receptors that bind the same chemical messenger are not all the same. The partial agonist may be capable of distinguishing between different receptor types or subtypes, acting as an agonist at one subtype, but as an antagonist at another subtype.

Examples of partial agonists in the opioid and antihistamine fields are discussed in Chapters 24 and 25 respectively.

## 8.5 Inverse agonists

Many antagonists that bind to a receptor binding site are, in fact, more properly defined as **inverse agonists**. An inverse agonist has the same effect as an antagonist in that it binds to a receptor, fails to activate it, and prevents the normal chemical messenger from binding. However, there is more to an inverse agonist than that. Some receptors (e.g. the **GABA***, **serotonin** and **dihydropyridine receptors**) are found to have an inherent activity, even in the absence of the chemical messenger. They are said to have **constitutional activity**. An inverse agonist is also capable of preventing this activity.

The discovery that some receptors have an inherent activity has important implications for receptor theory. It suggests that these receptors do not have a 'fixed' inactive conformation, but are continually changing shape such that there is an equilibrium between the active conformation and different inactive conformations. In that equilibrium, most of the receptor population is in an inactive conformation, but a small proportion of the receptors is in the active conformation. The action of agonists and antagonists is then explained by how that equilibrium is affected by binding preferences (Fig. 8.16).

If an agonist is introduced (Fig. 8.16, frame B), it binds preferentially to the active conformation and stabilizes it, shifting the equilibrium to the active conformation and leading to an increase in the biological activity associated with the receptor.

In contrast, it is proposed that an antagonist binds equally well to all receptor conformations (both active and inactive) (Fig. 8.16, frame C). In the absence of the natural ligand, the receptor's equilibrium is unaffected and there is no change in biological activity. The introduction of an agonist has no effect either, because all the receptor binding sites are already occupied by the antagonist. Antagonists such as these will have some structural similarity to the natural agonist.

An inverse agonist is proposed to have a binding preference for an inactive conformation. This stabilizes the inactive conformation and shifts the equilibrium away from the active conformation leading to a drop in inherent biological activity (Fig. 8.16, frame D). An inverse agonist need have no structural similarity to an agonist, as it could be binding to a different part of the receptor.

A partial agonist has a slight preference for the active conformation over any of the inactive conformations. The equilibrium is shifted to the active conformation but not to the same extent as with a full agonist, and so the increase in biological activity is less (Fig. 8.16, frame E). Moreover, the binding of the natural ligand may be suppressed.

## 8.6 Desensitization and sensitization

Desensitization can occur by a number of mechanisms. Some drugs bind relatively strongly to a receptor and switch it on, but then subsequently block the receptor after a certain period of time. Thus, they are acting as agonists, then antagonists. The mechanism of how this takes place is not clear, but it is believed that prolonged binding of the agonist to the receptor results in phosphorylation of hydroxyl or phenolic groups in the receptor. This causes the receptor to alter shape to an inactive conformation despite the binding site being occupied. In the

* GABA = γ-aminobutyric acid

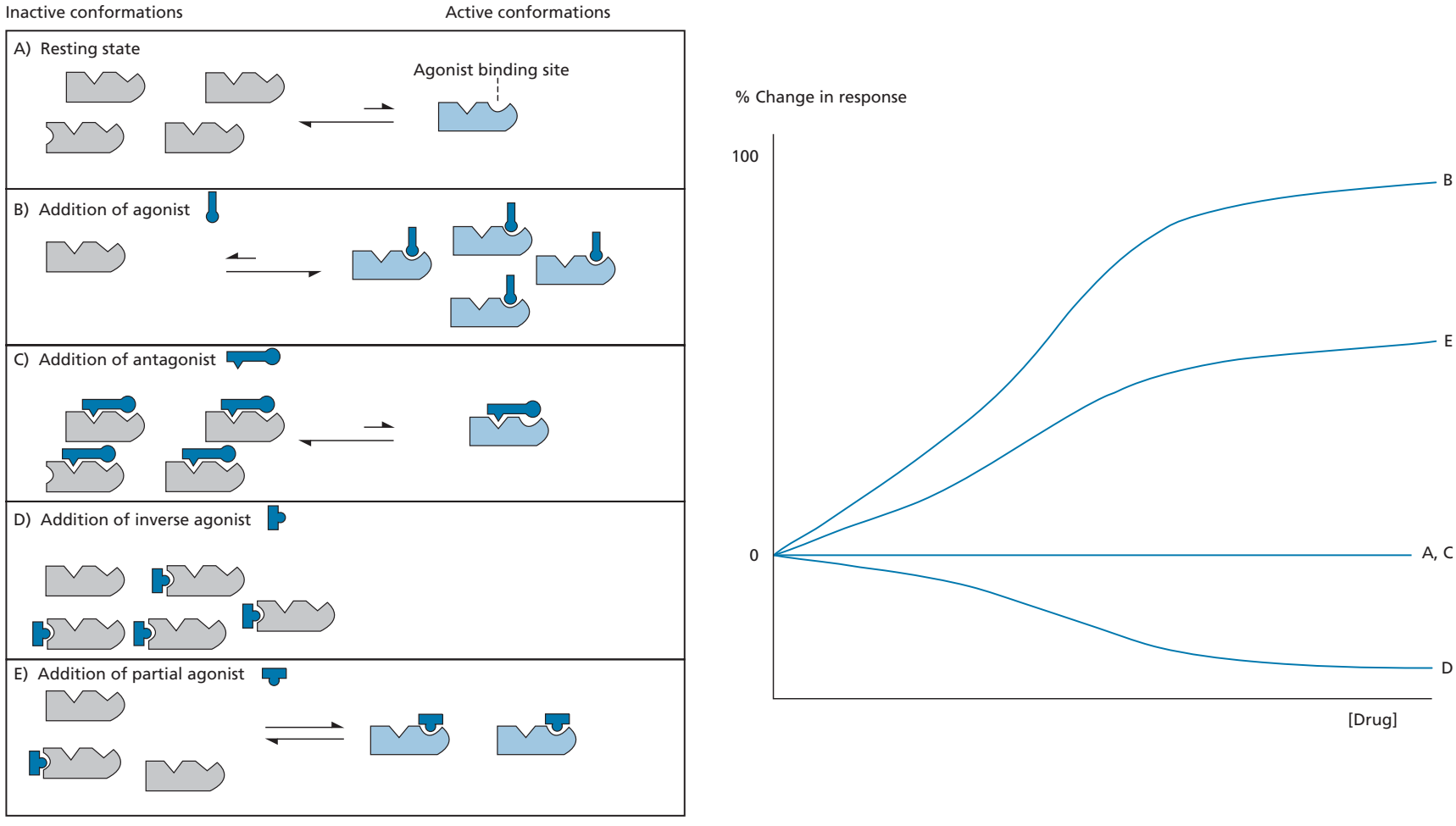



**FIGURE 8.16** Equilibria between active and inactive receptor conformations, and the effect of agonists, antagonists, inverse agonists, and partial agonists in the absence of the natural ligand.

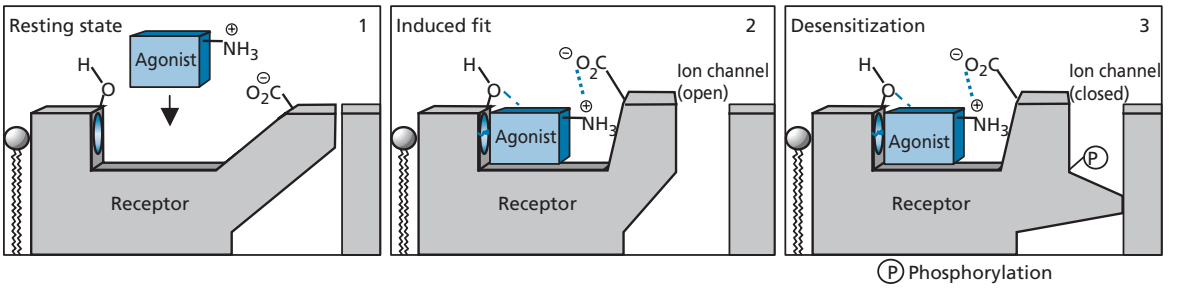



**FIGURE 8.17** Desensitization of a receptor following prolonged binding of an agonist.

case of an ion channel, this would mean that the channel is closed (Fig. 8.17). In the case of a G-protein-coupled receptor, the binding site for the G-protein is closed. This altered tertiary structure is then maintained as long as the binding site is occupied by the agonist. When the drug eventually leaves, the receptor is dephosphorylated and returns to its original resting shape.

On even longer exposure to a drug, the receptor/drug complex may be removed completely from the cell membrane by a process called **endocytosis**. Here, the relevant portion of the membrane is 'nipped out', absorbed into the cell, and metabolized. Receptor endocytosis may also occur after short exposures to a ligand, but, in this situation, the receptor is often recycled back to the cell membrane in a re-sensitization process.

Finally, prolonged activation of a receptor may result in the cell reducing its synthesis of the receptor protein. Consequently, it is generally true that the best agonists bind swiftly to the receptor, pass on their message, and then leave quickly.

Antagonists, in contrast, tend to be slow to add and slow to leave. Prolonged exposure of a target receptor to an antagonist may lead to the opposite of desensitization (i.e. **sensitization**). This is where the cell synthesizes more receptors to compensate for the receptors that are blocked. This is known to happen when some β-blockers are given over long periods (section 23.11.3).

## 8.7 Tolerance and dependence

As mentioned above, depriving a target receptor of its natural ligand by administering an antagonist may induce that cell to synthesize more receptors. By doing so, the cell gains a greater sensitivity for what little ligand is left. This process can explain the phenomena of tolerance and dependence (Fig. 8.18).

Tolerance is a situation where higher levels of a drug are required to get the same biological response. If a drug is acting to suppress the binding of a chemical messenger, then the cell may respond by increasing the number of receptors. This would require increasing the dose to regain the same level of antagonism.

If the drug is suddenly stopped, then all the receptors suddenly become available. There is now an excess of receptors, which makes the cell supersensitive to normal levels of messenger. This would be equivalent to receiving an overdose of an agonist. The resulting biological effects would explain the distressing withdrawal symptoms that result from the cessation of certain drugs. These withdrawal symptoms would continue until the number of receptors returned to their original level. During this period, the patient may be tempted to take the drug again in order to 'return to normal' and will have then acquired a dependence on the drug.

Tolerance and dependence also occur with agonists, such as the opioids. In this situation, increased doses are required because of receptor desensitization. Increased levels of agonist are required to activate the receptors that are still available.

## 8.8 Receptor types and subtypes

The receptors for a particular chemical messenger are not all identical. There are various types and subtypes of receptor, and it is found that these are not evenly distributed throughout the different organs and tissues of the body. This means that designing a drug which is selective for a particular type or subtype of receptor leads to selectivity of action against a particular organ in the body (see also sections 4.3 and 4.7.3)

Some examples of receptor types and subtypes are given in Table 8.1. The identification of many of these subtypes is relatively recent and the current emphasis in medicinal chemistry is to design drugs that are as selective as possible so that the drugs are tissue-selective and have fewer side effects. For example, there are five types of dopaminergic receptor. All clinically effective antipsychotic agents (e.g. **clozapine**, **olanzapine**, and **risperidone**; Fig. 8.19) antagonize the dopaminergic receptors D2 and D3. However, blockade of D2 receptors may lead to some of the side effects observed and a selective D3 antagonist may have better properties as an antipsychotic.

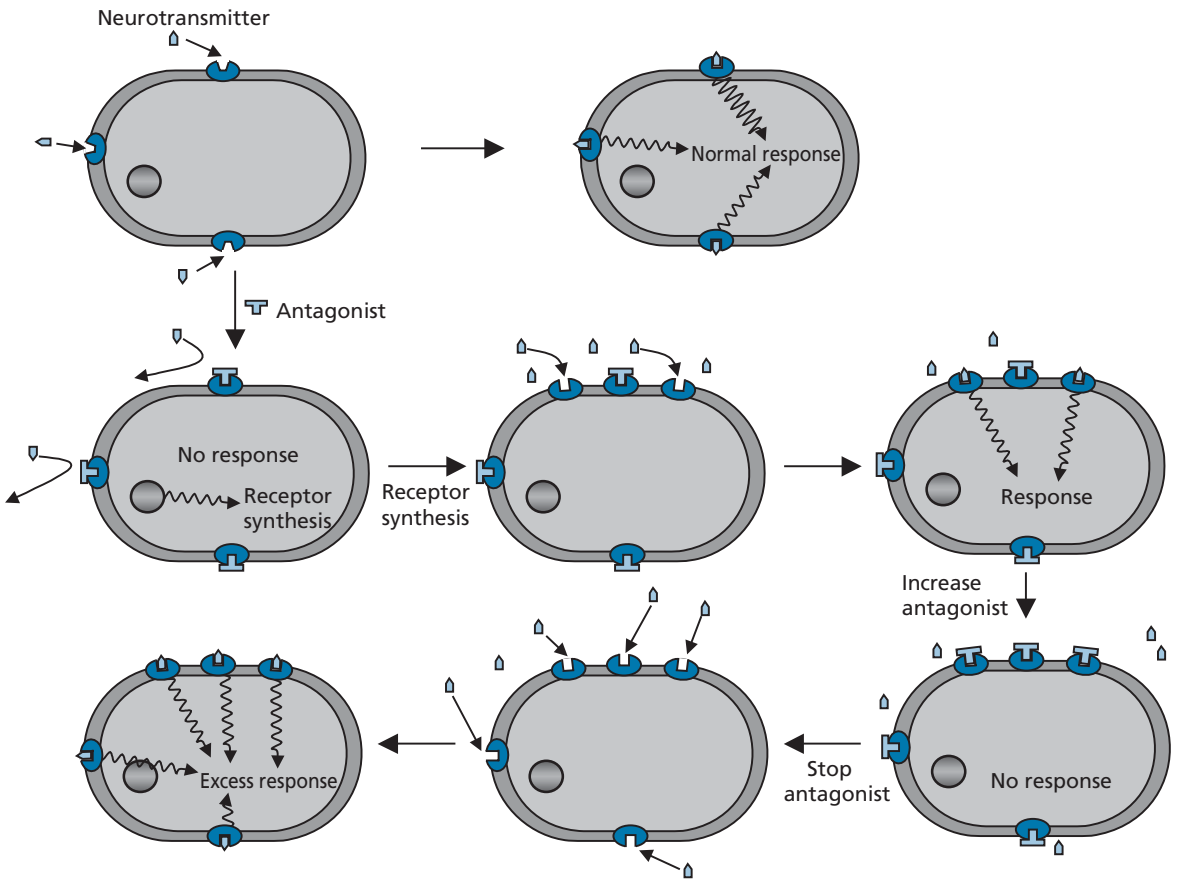
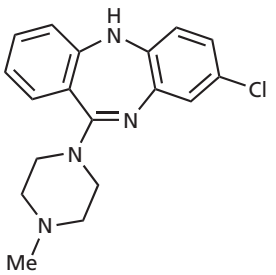


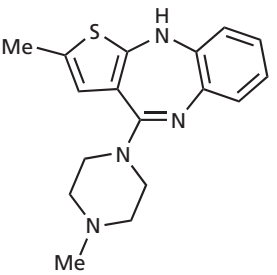

**FIGURE 8.18** Increasing cell sensitivity by the synthesis of more receptors.

**TABLE 8.1** Some examples of receptor types and subtypes

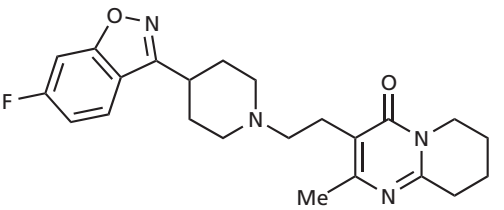
| Receptor | Type | Subtype | Examples of agonist therapies | Examples of antagonist therapies |
|---|---|---|---|---|
| Cholinergic (Chapter 22) | Nicotinic (N) Muscarinic (M) | Nicotinic (four subtypes) $M_1$–$M_5$ | Stimulation of gastrointestinal tract (GIT) motility ($M_1$) Glaucoma (M) | Neuromuscular blockers and muscle relaxants (N); Peptic ulcers ($M_1$); Motion sickness (M) |
| Adrenergic (adrenoceptors) (Chapter 23) | Alpha (α1, α2) Beta (β) | $\alpha_{1A}$ $\alpha_{1B}$ $\alpha_{1D}$ $\alpha_{2A}$–$\alpha_{2C}$ ($\beta_1$, $\beta_2$, $\beta_3$) | Anti-asthmatics ($\beta_2$) | β-blockers ($\beta_1$) |
| Dopamine | | $D_1$–$D_5$ | Parkinson's disease | Antidepressants ($D_2$/$D_3$) |
| Histamine (Chapter 25) | | $H_1$–$H_3$ | Vasodilation (limited use) | Treatment of allergies; Anti-emetics; sedation ($H_1$); Anti-ulcer agents ($H_2$) |
| Opioid and opioid-like (Chapter 24) | | μ, κ, δ, ORL1 | Analgesics | Antidote to morphine overdose |
| 5-Hydroxytryptamine (serotonin) | 5-$HT_1$ 5-$HT_7$ | 5-$HT_{1A}$, 5$HT_{1B}$, 5$HT_{1D-1F}$, 5$HT_{2A-2C}$, 5-$HT_{5A}$, 5-$HT_{5B}$ | Antimigraine (5-$HT_{1D}$) Stimulation of GIT motility (5-$HT_4$) | Anti-emetics (5-$HT_3$) |
| Estrogen (Section 21.4) | | | Contraception | Breast cancer (Tamoxifen) |




Clozapine




Olanzapine




Risperidone

**FIGURE 8.19** Antipsychotic agents.

Other examples of current research projects include:

- muscarinic ($M_2$) agonists for the treatment of heart irregularities;
- adrenergic ($\beta_3$) agonists for the treatment of obesity;
- *N*-methyl-D-aspartate (NMDA) antagonists for the treatment of stroke;
- cannabinoid ($CB_1$) antagonists for the treatment of memory loss.

**KEY POINTS**

- Agonists are compounds that mimic the natural ligand for the receptor.
- Antagonists are agents that bind to the receptor, but which do not activate it. They block binding of the natural ligand.
- Agonists may have a similar structure to the natural ligand.
- Antagonists bind differently from the natural ligand such that the receptor is not activated.
- Antagonists can bind to regions of the receptor that are not involved in binding the natural ligand. In general, antagonists tend to have more binding interactions than agonists and bind more strongly.
- Partial agonists induce a weaker effect than a full agonist.
- Inverse agonists act as antagonists, but also eliminate any resting activity associated with a receptor.
- Desensitization may occur when an agonist is bound to its receptor for a long period of time. Phosphorylation of the receptor results in a change of conformation.
- Sensitization can occur when an antagonist is bound to a receptor for a long period of time. The cell synthesizes more receptors to counter the antagonist effect.
- Tolerance is a situation where increased doses of a drug are required over time to achieve the same effect.
- Dependence is related to the body's ability to adapt to the presence of a drug. On stopping the drug, withdrawal symptoms occur as a result of abnormal levels of target receptor.
- There are several receptor types and subtypes, which vary in their distribution round the body. They also vary in their selectivity for agonists and antagonists.
- Pharmacodynamics is the study of how drugs interact with their targets to produce a pharmacological effect. Pharmacokinetics is the study of factors that affect the ability of drugs to reach their targets *in vivo*.

## 8.9 Affinity, efficacy, and potency

The **affinity** of a drug for a receptor is a measure of how strongly that drug binds to the receptor. **Efficacy** is a measure of the maximum biological effect that a drug can produce as a result of receptor binding. It is important to appreciate the distinction between affinity and efficacy. A compound with high affinity does not necessarily have high efficacy. For example, an antagonist can bind with high affinity but have no efficacy. The **potency** of a drug refers to the amount of drug required to achieve a defined biological effect—the smaller the dose required, the more potent the drug. It is possible for a drug to be potent (i.e. active in small doses) but have a low efficacy.

Affinity can be measured using a process known as **radioligand labelling**. A known antagonist (or ligand) for the target receptor is labelled with radioactivity and is added to cells or tissue such that it can bind to the receptors present. Once an equilibrium has been reached, the unbound ligands are removed by washing, filtration, or centrifugation. The extent of binding can then be measured by detecting the amount of radioactivity present in the cells or tissue, and the amount of radioactivity that was removed. The equilibrium constant for bound versus unbound radioligand is defined as the **dissociation binding constant** ($K_d$).

$$L + R \rightleftharpoons \underset{\text{Receptor–lignad complex}}{LR} \qquad K_d = \frac{[L] \times [R]}{[LR]}$$

[L] and [LR] can be found by measuring the radioactivity of unbound ligand and bound ligand, respectively, after correction for any background radiation. However, it is not possible to measure [R], so we have to carry out

some mathematical manipulations to remove [R] from the equation.

The total number of receptors present must equal the number of receptors occupied by the ligand ([LR]) and those that are unoccupied ([R]), i.e.:

$$[R_{tot}] = [R] + [LR]$$

This means that the number of receptors unoccupied by a ligand is

$$[R] = [R_{tot}] - [LR]$$

Substituting this into the first equation and rearranging leads to the **Scatchard equation**, where both [LR] and [L] are measurable:

$$\frac{[\text{Bound ligand}]}{[\text{Free ligand}]} = \frac{[LR]}{[L]} = \frac{R_{tot} - [LR]}{K_d}$$

We are still faced with the problem that $K_d$ and $R_{tot}$ cannot be measured directly. However, these terms can be determined by drawing a graph based on a number of experiments where different concentrations of a known radioligand are used. [LR] and [L] are measured in each case and a **Scatchard plot** (Fig. 8.20) is drawn which compares the ratio [LR]/[L] versus [LR]. This gives a straight line; the point where it meets the x-axis represents the total number of receptors available ($R_{tot}$) (line A; Fig. 8.20). The slope is a measure of the radioligand's affinity for the receptor and allows $K_d$ to be determined.

We are now in the position to determine the affinity of a novel drug.

This is done by repeating the radioligand experiments in the presence of the unlabelled drug. The drug competes with the radioligand for the receptor's binding sites and is called a **displacer**. The stronger the affinity of the drug, the more effectively it will compete for binding sites and the less radioactivity will be measured for [LR]. This will result in a different line in the Scatchard plot.

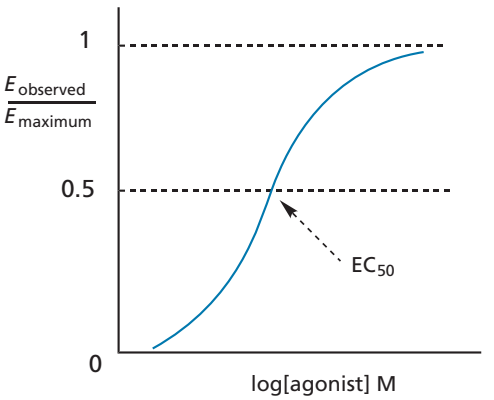



**FIGURE 8.21** Measurement of $EC_{50}$.

If the drug competes directly with the radiolabelled ligand for the same binding site on the receptor, then the slope is decreased but the intercept on the x-axis remains the same (line X in the graph). In other words, if the radioligand concentration is much greater than the drug it will bind to all the receptors available.

Agents that bind to the receptor at an allosteric binding site do not compete with the radioligand for the same binding site and so cannot be displaced by high levels of radioligand. However, by binding to an allosteric site they make the normal binding site unrecognizable to the radioligand and so there are fewer receptors available. This results in a line with an identical slope to line A, but crossing the x-axis at a different point, thus indicating a lower total number of available receptors (line Y).

The data from these displacement experiments can be used to plot a different graph which compares the percentage of the radioligand that is bound to a receptor versus the concentration of the drug (or displacer). This results in a sigmoidal curve termed the **displacement** or **inhibition curve**, which can be used to identify the **$IC_{50}$ value** for the drug (i.e. the concentration of compound that prevents 50% of the radioactive ligand being bound).

The **inhibitory** or **affinity constant ($K_i$)** for the drug is the same as the $[IC]_{50}$ value if non-competitive interactions are involved. For compounds that *are* in competition

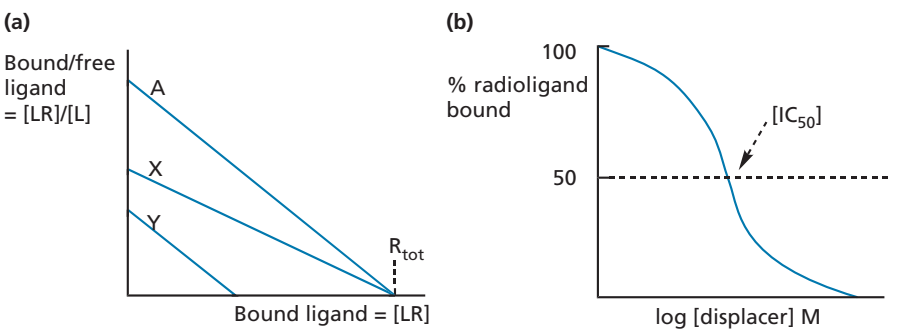



**FIGURE 8.20** (**a**) Scatchard plot (A = radioligand only, X = radioligand + competitive ligand, Y = radioligand + non-competitive ligand). (**b**) The displacement or inhibition curve.

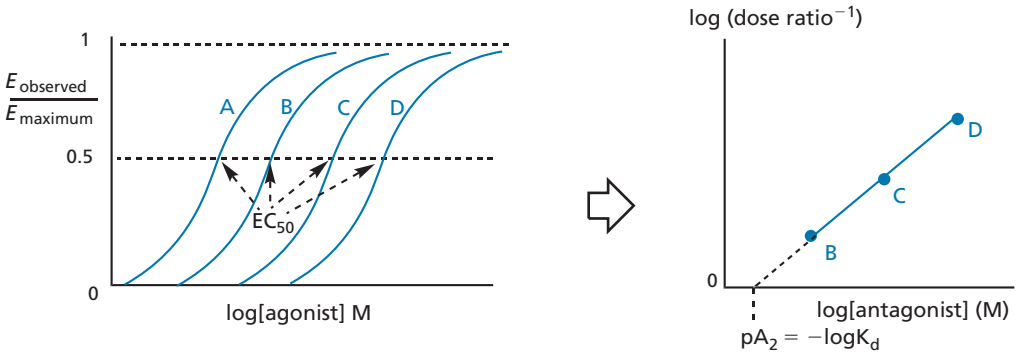



**FIGURE 8.22** Schild analysis (A = no antagonist present, B–D = increasing concentrations of antagonist present).

with the radioligand for the binding site, the inhibitory constant depends on the level of radioligand present and is defined as

$$K_i = \frac{IC_{50}}{1 + [L]_{tot} / K_d}$$

where $K_d$ is the dissociation constant for the radioactive ligand and $[L]_{tot}$ is the concentration of radioactive ligand used in the experiment.

**Efficacy** is determined by measuring the maximum possible effect resulting from receptor–ligand binding. Potency can be determined by measuring the concentration of drug required to produce 50% of the maximum possible effect (**$EC_{50}$**) (Fig. 8.21). The smaller the value of $EC_{50}$, the more potent the drug. In practice, **$pD_2$** is taken as the measure of potency where $pD_2 = -\log[EC]_{50}$.

A **Schild analysis** is used to determine the dissociation constant ($K_d$) of competitive antagonists (Fig. 8.22). An agonist is first used at different concentrations to activate the receptor and an observable effect is measured at each concentration. The experiment is then repeated several times in the presence of different concentrations of antagonist. Comparing the effect ratio ($E_{observed}/E_{maximum}$) versus the log of the agonist concentration (log[agonist]) produces a series of sigmoidal curves where the $EC_{50}$ of the agonist increases with increasing antagonist concentration. In other words, greater concentrations of agonist are required to compete with the antagonist. A **Schild plot** is then constructed, which compares the log of the reciprocal of the dose ratio with the log of the antagonist concentration. The **dose ratio** is the agonist concentration required to produce a specified level of effect when no antagonist is present compared with the agonist concentration required to produce the same level in the presence of antagonist. The line produced from these studies can be extended to the x-axis to find $pA_2$ ($= -\log K_d$), which represents the affinity of the competitive antagonist.

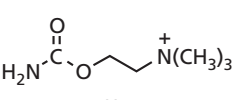
Schild plots can be used to determine whether different agonists show similar selectivity toward different types of receptor. The $pA_2$ values of a non-selective antagonist acting on a population of the different receptors types are determined in the presence of each of the agonists. If the $pA_2$ values are similar, it is indicative that the agonists show similar receptor selectivity.

**KEY POINTS**

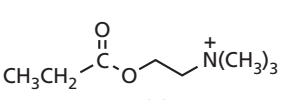
- Affinity is a measure of how strongly a drug binds to a receptor. Efficacy is a measure of the effect of that binding on the cell. Potency relates to how effective a drug is in producing a cellular effect.
- Affinity can be measured from Scatchard plots derived from radioligand displacement experiments.
- Efficacy is determined by the $EC_{50}$ value—the concentration of agent required to produce 50% of the maximum possible effect resulting from receptor activation.
- A Schild analysis is used to determine the dissociation constant of competitive antagonists.

## QUESTIONS

1. Structure I is an agonist which binds to the cholinergic receptor and mimics the action of the natural ligand acetylcholine. Structure II, however, shows no activity and does not bind to the receptor. Suggest why this might be the case.

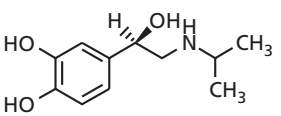



(I)

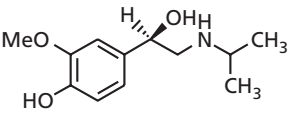



(II)

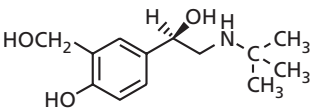
**2.** Isoprenaline undergoes metabolism to give the inactive metabolite shown. Suggest why this metabolite is inactive.



Isoprenaline



Inactive metabolite



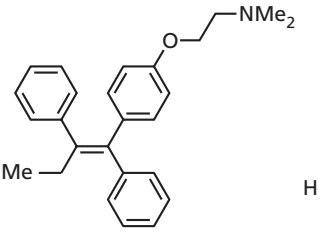
*R*-Salbutamol

**3.** Salbutamol is an anti-asthmatic agent that acts as an adrenergic agonist. Do you think it is likely to show any selectivity between the α- or β-adrenoceptors? Explain your answer.

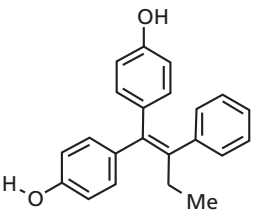
**4.** Propranolol is an adrenergic antagonist. Compare the structure of propranolol with noradrenaline and identify which features are similar in both molecules. Suggest why this molecule might act as an antagonist rather than an agonist, and whether it might show any selectivity between the different types of adrenergic receptor.

**5.** If you were asked to design drugs that acted as selective antagonists of the dopamine receptor, what structures might you consider synthesizing?

**6.** Tamoxifen acts as an antagonist for the estrogen receptor. Suggest how it might bind to the receptor in order to do this.



Tamoxifen



Tamoxifen metabolite

**7.** The tamoxifen metabolite shown in Question 6 acts as an estrogen agonist rather than an antagonist. Why?

**8.** The ability of the opioid antagonist naloxone to antagonize the opioid agonists normorphine, Met-enkephalin, and metkephamid was determined on tissue containing different types of opioid receptor. From the data provided, prepare Schild plots for the opioid antagonist naloxone in the presence of each of the opioid agonists and determine the $pA_2$ values in each case. Identify whether any of the opioid agonists show a similar selectivity for opioid receptors. ([I] is the concentration of naloxone present).

| | | | |
|---|---|---|---|
| Normorphine and naloxone | | | |
| [I] mol/l | $1 \times 10^{-6}$ | $1 \times 10^{-7}$ | $3.162 \times 10^{-8}$ |
| Dose ratio | 0.0018 | 0.0178 | 0.1122 |
| Metkephamid and naloxone | | | |
| [I] mol/l | $3.16 \times 10^{-7}$ | $1 \times 10^{-7}$ | $3.162 \times 10^{-8}$ |
| Dose ratio | 0.0562 | 0.1585 | 0.7943 |
| Met-enkephalin and naloxone | | | |
| [I] mol/l | $3.16 \times 10^{-7}$ | $1 \times 10^{-7}$ | $3.162 \times 10^{-8}$ |
| Dose ratio | 0.0398 | 0.2512 | 0.8913 |

## FURTHER READING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*Titles for general further reading are listed on p.763.*

# 9 Nucleic acids as drug targets

Although proteins are the target for the majority of clinically useful drugs, there are many important drugs which target nucleic acids, especially in the areas of antibacterial and anticancer therapy (see sections 19.7, 19.8, and 21.2). In this chapter, we concentrate on the mechanism of action of some of these drugs. Further information and clinical aspects are covered in Chapters 19 and 21. The structure and function of nucleic acids was discussed in Chapter 6.

We shall first consider the drugs that interact with DNA. In general, we can group these under the following categories:

- intercalating agents;
- topoisomerase poisons (non-intercalating);
- alkylating agents;
- chain cutters;
- chain terminators.

## 9.1 Intercalating drugs acting on DNA

Intercalating drugs are compounds that contain planar or heteroaromatic features which slip between the base-pair layers of the DNA double helix. Some of these drugs prefer to approach the helix via the major groove; others prefer access via the minor groove. Once they are inserted between the nucleic acid base pairs, the aromatic/heteroaromatic rings are held there by van der Waals interactions with the base pairs above and below. Several intercalating drugs also contain ionized groups which can interact with the charged phosphate groups of the DNA backbone, thus strengthening the interaction. Once the structures have become intercalated, a variety of other processes may take place which prevent replication and transcription, leading, finally, to cell death. The following are examples of drugs that are capable of intercalating DNA.

**Proflavine** (Fig. 9.1) is an example of a group of antibacterial compounds called the **aminoacridines**, which were used during World Wars I and II to treat deep surface wounds. They proved highly effective in preventing infection and reduced the number of fatalities resulting from wound infections. Proflavine is completely ionized at pH 7 and interacts directly with bacterial DNA. The flat tricyclic ring intercalates between the DNA base pairs and interacts with them by van der Waals forces, while the aminium cations form ionic bonds with the negatively charged phosphate groups on the sugar phosphate

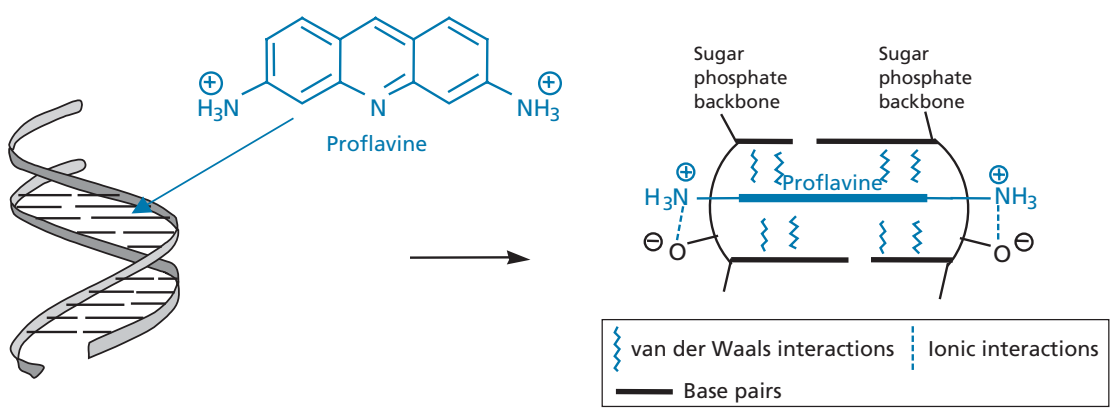



**FIGURE 9.1** The intercalation of proflavine with DNA.

backbone. Once inserted, proflavine deforms the DNA double helix and prevents the normal functions of replication and transcription.

**Dactinomycin** (Fig. 9.2) (previously called actinomycin D) is a naturally occurring antibiotic that was first isolated from *Streptomyces parvullis* in 1953, and was shown to be an effective anticancer agent in children. It contains two cyclic pentapeptides, but the important feature is a flat, tricyclic, heteroaromatic structure which slides into the double helix via the minor groove. It appears to favour interactions with guanine–cytosine base pairs and, in particular, between two adjacent guanine bases on alternate strands of the helix. The molecule is further held in position by hydrogen bond interactions between the nucleic acid bases of DNA and the cyclic pentapeptides positioned on the outside of the helix. The 2-amino group of guanine plays a particularly important role in this interaction. The resulting bound complex is very stable and prevents the unwinding of the double helix. This, in turn, prevents DNA-dependent RNA polymerase from catalysing the synthesis of messenger RNA (mRNA) and thus prevents transcription.

**Doxorubicin** (Fig. 9.2) is one of the most effective anticancer drugs ever discovered, and belongs to a group of naturally occurring antibiotics called the **anthracyclines**. It was first isolated from *Streptomyces peucetius* in 1967 and contains a tetracyclic system where three of the rings are planar. The drug approaches DNA via the major groove of the double helix and intercalates using the planar tricyclic system. The charged amino group attached to the sugar is also important, as it forms an ionic bond with the negatively charged phosphate groups of the DNA backbone. This is supported by the fact that structures lacking the aminosugar have poor activity. Intercalation prevents the normal action of an enzyme called **topoisomerase II**—an enzyme that is crucial to replication and mitosis. The mechanism by which this enzyme works is described in section 6.1.3 and includes the formation of a DNA–enzyme complex where the enzyme is covalently linked to the DNA. When doxorubicin is intercalated into DNA it stabilizes this DNA–enzyme complex and stalls the process. Agents such as doxorubicin are referred to as topoisomerase II poisons rather than inhibitors, as they do not prevent the enzyme functioning directly. Other mechanisms of action for doxorubicin and its analogues have also been proposed (see section 21.2.1).

**Bleomycins** (Fig. 9.3) are complex natural products that were isolated from *Streptomyces verticillus* in 1962 and are some of the few anticancer drugs not to cause bone marrow depression. Their structure includes a bithiazole ring system which intercalates with DNA. Once the structure has become intercalated, the nitrogen atoms of the primary amines, pyrimidine ring, and imidazole ring chelate a ferrous ion which then interacts with oxygen and is oxidized to a ferric ion, leading to the generation of superoxide or hydroxyl radicals. These highly reactive species abstract hydrogen atoms from DNA, which results in the DNA strands being cut—particularly between purine and pyrimidine nucleotides. Bleomycin also appears to prevent the enzyme **DNA ligase** from repairing the damage caused.

## 9.2 Topoisomerase poisons: non-intercalating

The following structures are classed as poisons rather than inhibitors because they stabilize the normally transient cleavable complex that is formed between DNA and

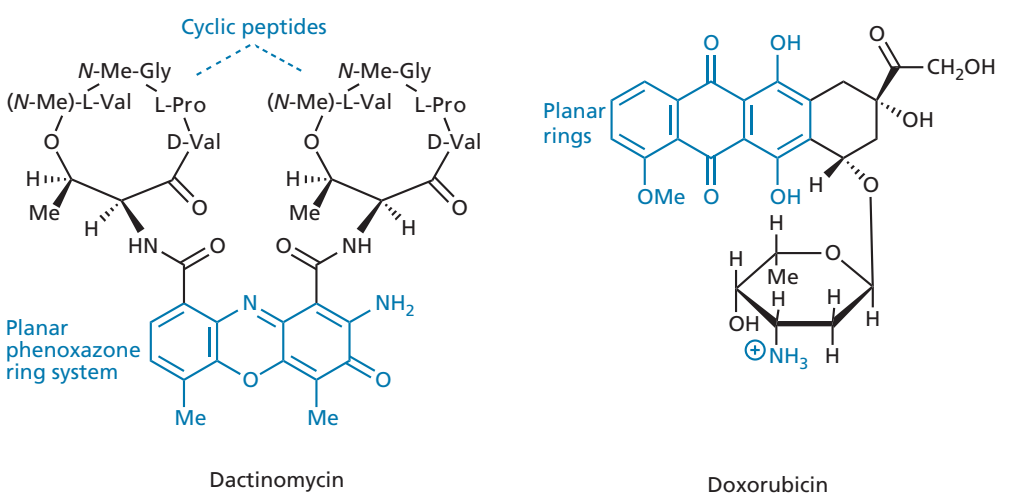



**FIGURE 9.2** Dactinomycin and doxorubicin.

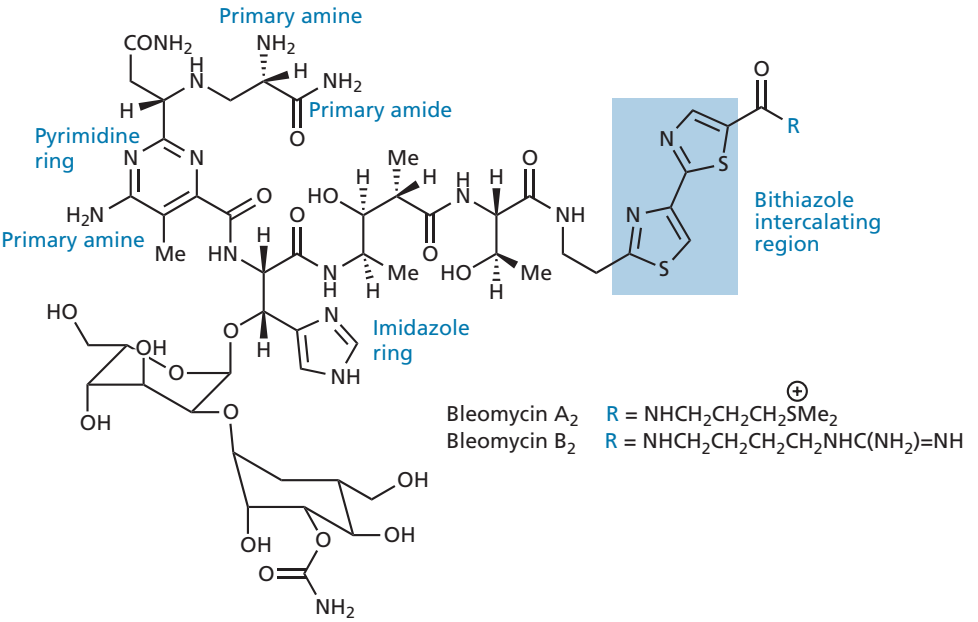



**FIGURE 9.3** Bleomycins.

topoisomerase enzymes, thus inhibiting the rejoining of the DNA strand or strands (section 6.1.3). We have already mentioned topoisomerase poisons in section 9.1, where we discussed the anthracyclines. In this section, we look at topoisomerase poisons which do not intercalate into the DNA structure. However, as DNA is part of the target complex, we can view these poisons as targeting DNA, as well as the topoisomerase enzyme.

The anticancer agents **etoposide** and **teniposide** (Fig. 9.4) belong to a group of compounds called the **podophyllotoxins**, and are semi-synthetic derivatives of **epipodophyllotoxin**—an isomer of a naturally occurring agent called **podophyllotoxin**. Both agents act as topoisomerase poisons. DNA strand breakage is also thought to occur by a free radical process involving oxidation of the 4′-phenolic group and the production of a semiquinone free radical. Evidence supporting this comes from the fact that the 4′-methoxy structures are inactive. The presence of the glucoside sugar moiety also increases the ability to induce breaks.

**Camptothecin** (Fig. 9.5) is a natural product that was extracted from a Chinese bush (*Camptotheca acuminata*) in 1966. It stabilizes the cleavable complex formed between DNA and the enzyme **topoisomerase I** (section 6.1.3). As a result, single-strand breaks accumulate in the DNA. These can be repaired if the drug departs, but if replication is taking place when the drug–enzyme–DNA complex is present, an irreversible double-strand break

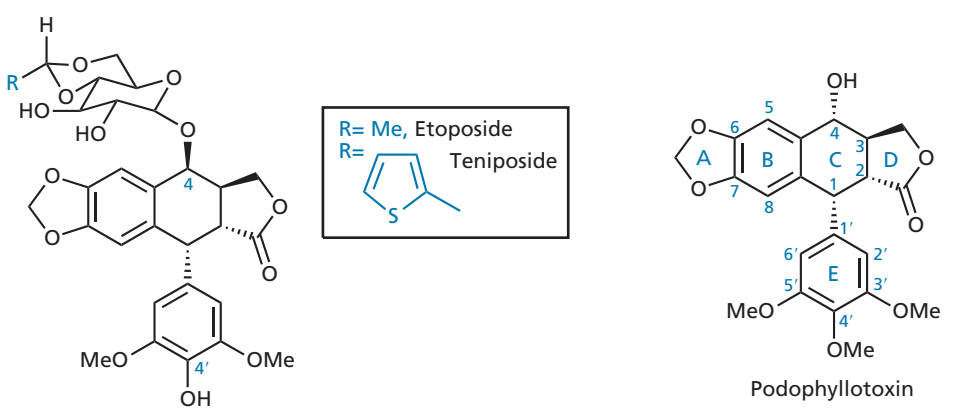



**FIGURE 9.4** Podophyllotoxins.

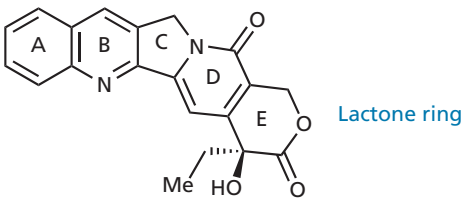



**FIGURE 9.5** Camptothecin.

takes place, which leads to cell death. Semi-synthetic analogues of camptothecin have been developed as clinically useful anticancer agents (section 21.2.2.2).

The antibacterial **quinolones** and **fluoroquinolones** (section 19.8.1) are synthetic agents that inhibit the replication and transcription of bacterial DNA by stabilizing the complex formed between DNA and bacterial topoisomerases. Inhibition arises by the formation of a ternary complex involving the drug, the enzyme, and bound DNA (Fig. 9.6). The binding site for the fluoroquinolones only appears once the enzyme has 'nicked' the DNA strands, and the strands are ready to be crossed over. At that point, four fluoroquinolone molecules are bound in a stacking arrangement such that their aromatic rings are coplanar. The carbonyl and carboxylate groups of the fluoroquinolones interact with DNA by hydrogen bonding, while the fluoro-substituent at position 6, the substituent at C-7, and the carboxylate ion are involved in binding interactions with the enzyme.

**KEY POINTS**

- Intercalating drugs contain planar aromatic or heteroaromatic ring systems which can slide between the base pairs of the DNA double helix.
- The anthracyclines are intercalating drugs that act as topoisomerase II poisons, stabilizing the cleavage complex formed between the enzyme and DNA.
- Bleomycins are intercalating drugs which form complexes with ferrous ions. These complexes generate reactive oxygen species that cleave the strands of DNA.
- Etoposide and teniposide are non-intercalating drugs that act as topoisomerase II poisons.
- Camptothecin is a non-intercalating drug that acts as a topoisomerase I poison. It stabilizes an enzyme–DNA complex where a single strand of DNA has been cleaved.

## 9.3 Alkylating and metallating agents

Alkylating agents are highly electrophilic compounds that react with nucleophiles to form strong covalent bonds. There are several nucleophilic groups present on the nucleic acid bases of DNA which can react with electrophiles—in particular the *N*-7 of guanine (Fig. 9.7).

Drugs with two alkylating groups can react with a nucleic acid base on each chain of DNA to cross-link the strands such that replication or transcription is disrupted. Alternatively, the drug could link two nucleophilic groups on the same chain such that the drug is attached like a limpet to the side of the DNA helix. That portion of DNA then becomes masked from the enzymes required to catalyse DNA replication and transcription.

Miscoding due to alkylated guanine units is also possible. The guanine base usually exists as the keto tautomer,

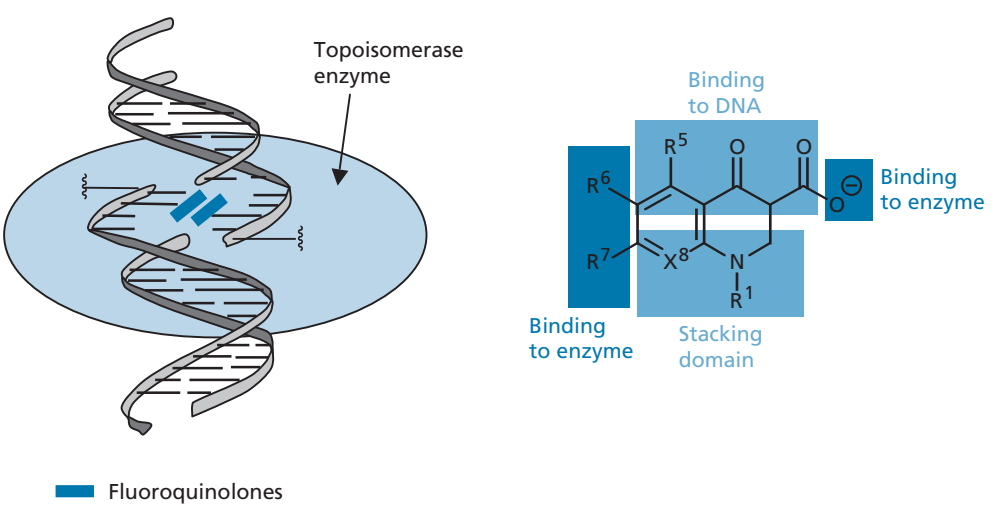



**FIGURE 9.6** Complex formed between DNA, the topoisomerase enzyme and fluoroquinolones; $R^6$ = F for fluoroquinolones.

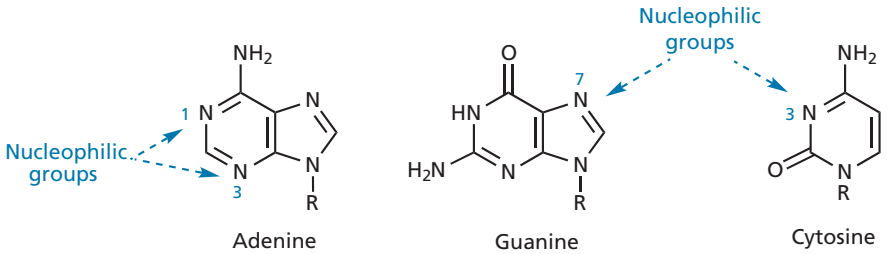



**FIGURE 9.7** Nucleophilic groups on adenine, guanine, and cytosine.

allowing it to base-pair with cytosine. Once alkylated, however, guanine prefers the enol tautomer and is more likely to base pair with thymine (Fig. 9.8). Such miscoding leads ultimately to an alteration in the amino acid sequence of proteins, which, in turn, can lead to disruption of protein structure and function.

Unfortunately, alkylating agents can alkylate nucleophilic groups on proteins, as well as DNA, which means they have poor selectivity and have toxic side effects. They can even lead to cancer in their own right. Nevertheless, alkylating drugs are still useful in the treatment of cancer (section 21.2.3). Examples of how some of these drugs alkylate DNA are now given (see also Case study 4 for an example of an anti-parasitic drug that alkylates DNA).

### 9.3.1 Nitrogen mustards

The **nitrogen mustards** get their name because they are related to the sulphur-containing mustard gases used during World War I. In 1942, the nitrogen mustard compound **chlormethine** (Fig. 9.9) was the first alkylating agent to be used medicinally, although full details were not revealed until after the war owing to the secrecy surrounding all nitrogen mustards. The nitrogen atom is able to displace a chloride ion intramolecularly to form the highly electrophilic aziridinium ion. This is an example of a **neighbouring group effect**, also called **anchimeric assistance**. Alkylation of DNA can then take place. As the process can be repeated, cross-linking between chains or within the one chain will occur. Monoalkylation of DNA guanine units is also possible if the second alkyl halide reacts with water, but cross-linking is the major way in which these drugs inhibit replication and act as anticancer agents.

Analogues of chlormethine have been designed to improve selectivity and to reduce side effects (section 21.2.3.1). Other agents, such as **cyclophosphamide**, have been designed as prodrugs and are converted into the alkylating drug once they have been absorbed into the blood supply (section 21.2.3.1).

### 9.3.2 Nitrosoureas

The anticancer agents **lomustine** and **carmustine** (Fig. 9.10) were discovered in the 1960s, and are chloroethylnitrosoureas which decompose spontaneously in the body to form two active compounds—an alkylating agent and a carbamoylating agent (Fig. 9.11). The organic isocyanate that is formed carbamoylates lysine residues in proteins and may inactivate DNA repair enzymes. The alkylating agent reacts initially with a guanine moiety on one strand of DNA, then with a guanine or cytosine unit on the other strand to produce interstrand cross-linking (Figs. 9.11 and 9.12). **Streptozotocin** (Fig. 9.10) is a naturally occurring nitrosourea isolated from *Streptomyces achromogenes*.

### 9.3.3 Busulfan

Busulfan (Fig. 9.13) was synthesized in 1950 as part of a systematic search for novel alkylating agents. It is an anticancer agent which causes interstrand cross-linking between guanine units. The sulphonate groups are good

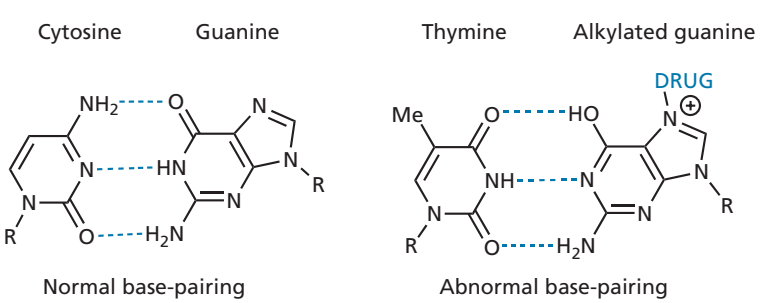



**FIGURE 9.8** Normal and abnormal base-pairing of guanine.

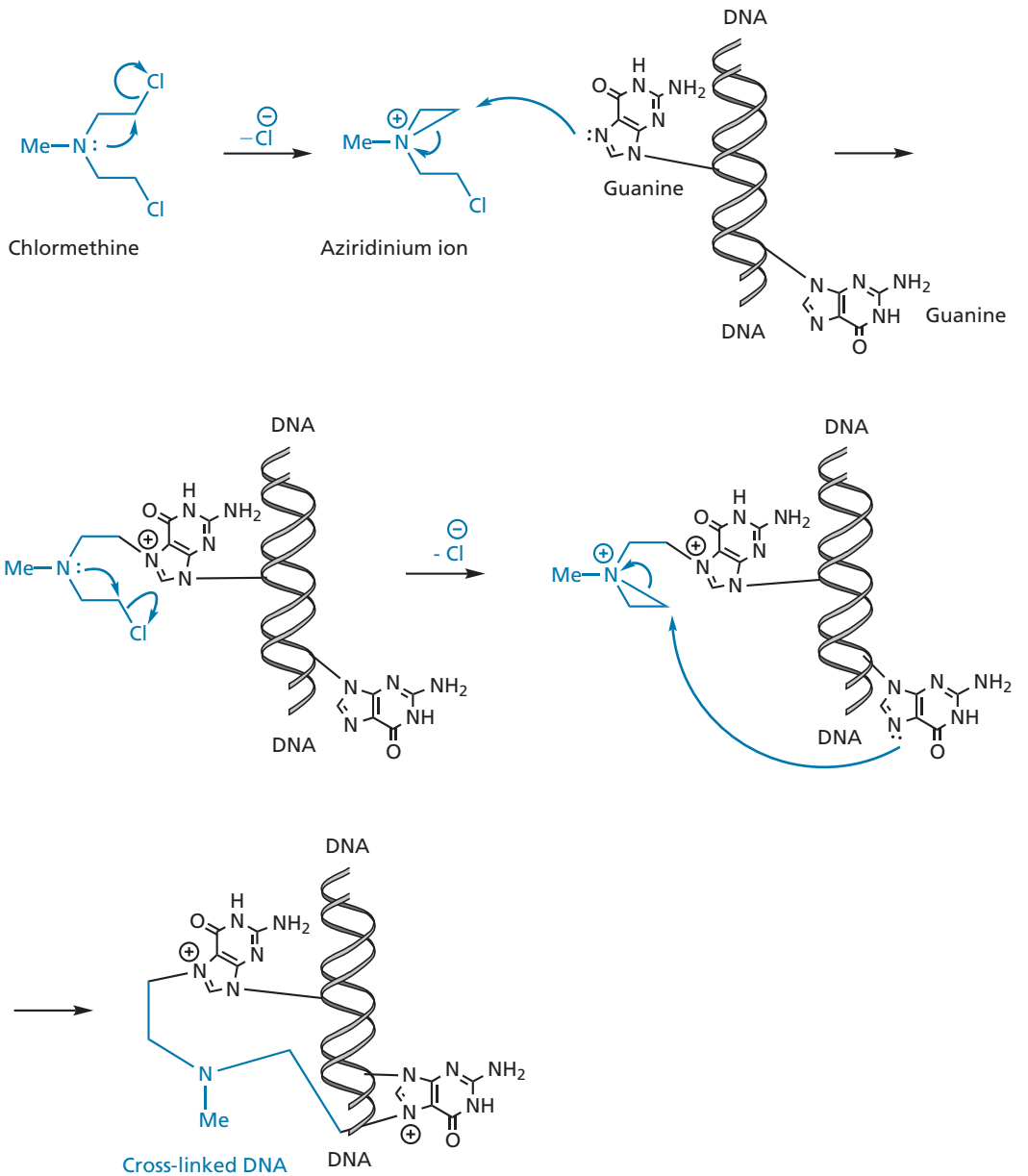



**FIGURE 9.9** Alkylation of DNA by chlormethine.

leaving groups and play a similar role to the chlorines in the nitrogen mustards. However, the mechanism involves a direct $S_N2$ nucleophilic substitution of the sulphonate groups and does not involve any intermediates such as the aziridinium ion.

## 9.3.4 Cisplatin

Cisplatin (Fig. 9.14) is one of the most frequently used anticancer drugs in medicine. Its discovery was fortuitous in the extreme, arising from research carried out in the 1960s to investigate the effects of an electric current on bacterial growth. During these experiments, it was discovered that bacterial cell division was inhibited. Further research led to the discovery that an electrolysis product from the platinum electrodes was responsible for the inhibition and the agent was eventually identified as cis-diammonia dichloroplatinum (II), now known as cisplatin.

The structure consists of a central platinum atom, covalently linked to two chloro substituents, while the two ammonia molecules act as ligands. The overall structure is neutral and unreactive. Once cisplatin enters cells, however, it enters an environment which has a low concentration of chloride ions. This leads to aquation where the chloro substituents of cisplatin are

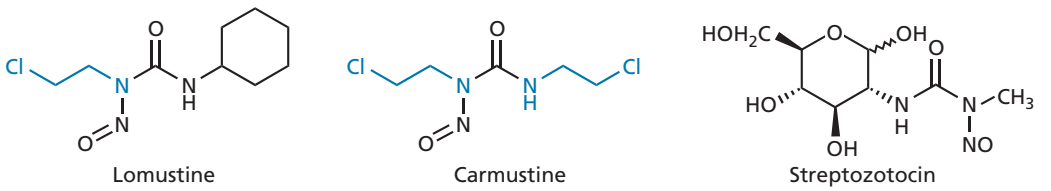



**FIGURE 9.10** Nitrosourea alkylating agents.

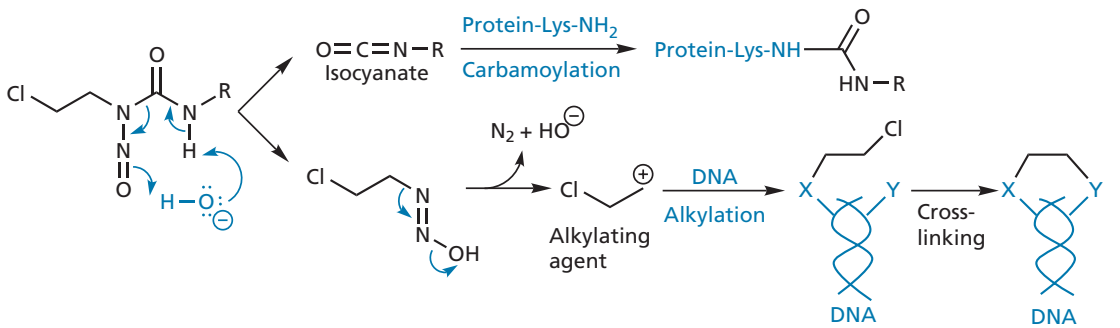



**FIGURE 9.11** Mechanisms of action for nitrosoureas.

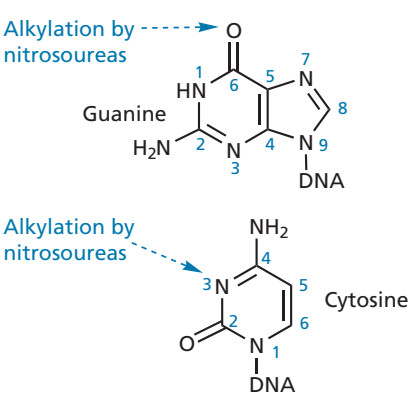



**FIGURE 9.12** Alkylation sites on guanine and cytosine for nitrosoureas.

displaced by neutral water ligands to give reactive, positively charged species which act as metallating agents. These bind strongly to DNA in regions containing adjacent guanine units, forming covalent Pt-DNA links within the same strand (intrastrand cross-linking). It is likely that this takes place to the N-7 and O-6 positions of adjacent guanine molecules. The hydrogen bonds that are normally involved in base-pairing guanine to cytosine are disrupted by the cross-links, leading to localized unwinding of the DNA helix and inhibition of transcription. Derivatives of cisplatin have been developed with reduced side effects (section 21.2.3.2).

## 9.3.5 Dacarbazine and procarbazine

Dacarbazine and procarbazine (Fig. 9.15) are prodrugs which generate a methyldiazonium ion as the alkylating agent (Fig. 9.16). The antitumour properties of procarbazine were discovered in the 1960s following the **screening** of several hundred compounds that had been prepared as potential antidepressants.

Dacarbazine is activated by *N*-demethylation in the liver—a reaction catalysed by cytochrome P450 enzymes

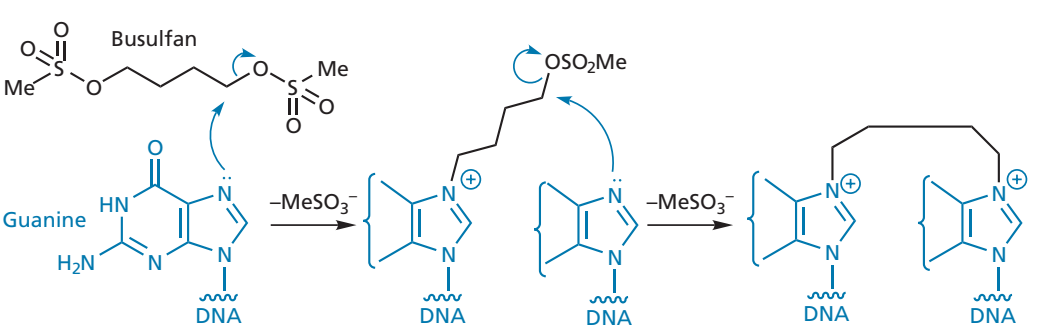



**FIGURE 9.13** Cross-linking mechanism involving busulfan.

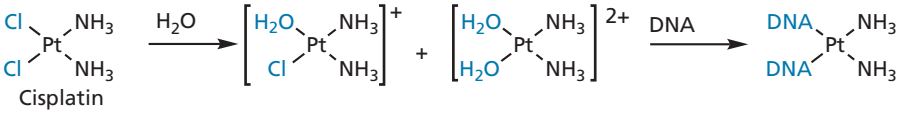



**FIGURE 9.14** Activation of cisplatin and intrastrand cross-linking of DNA.

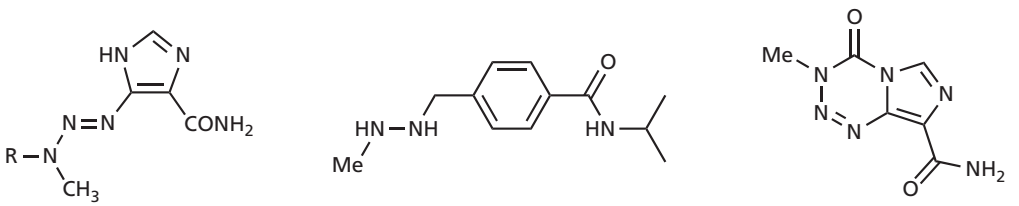



**FIGURE 9.15** Dacarbazine, procarbazine, and temozolomide.

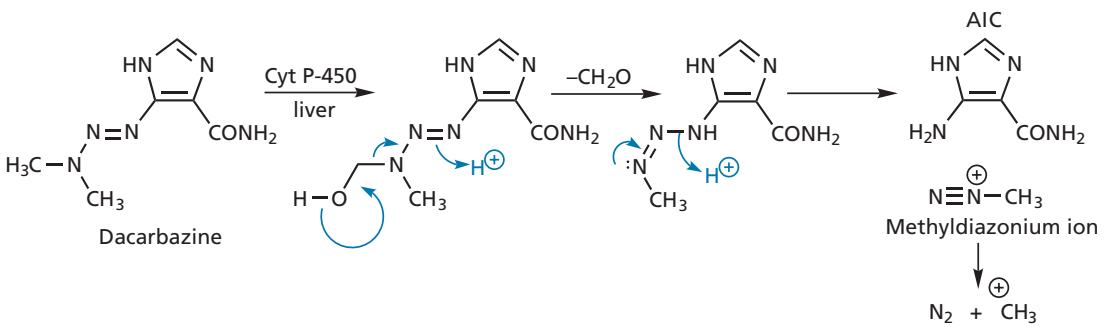



**FIGURE 9.16** Mechanism of action of dacarbazine.

(section 11.5.2) (Fig. 9.16). Formaldehyde is then lost to form a product which spontaneously degrades to form 5-aminoimidazole-4-carboxamide (AIC) and the methyldiazonium ion. Reaction of this ion with RNA or DNA results in methylation, mainly at the 7-position of guanine. DNA fragmentation can also occur. AIC has no cytotoxic effect and is present naturally as an intermediate in purine synthesis. **Temozolomide** (Fig. 9.15) also acts as a prodrug and is hydrolysed in the body to form MTIC (5-(3-methyltriazen-1-yl)imidazole-4-carboxamide), which decomposes in a similar fashion to form the methyldiazonium ion. The O6 oxygen atom of guanine groups is particularly methylated by this agent.

## 9.3.6 Mitomycin C

Mitomycin C (Fig. 9.17) was discovered in the 1950s and is a naturally occurring compound obtained from the microorganism *Streptomyces caespitosus*. It is one of the most toxic anticancer drugs in clinical use and acts as a prodrug, being converted to an alkylating agent within the body. The process by which this takes place is initiated by an enzyme-catalysed reduction of the quinone ring system to a hydroquinone. Loss of methanol and opening of the three-membered aziridine ring then takes place to generate the alkylating agent. Guanine residues on different DNA strands are then alkylated, leading to interstrand cross-linking, and the inhibition of DNA replication and cell division. As a reduction step is involved in the mechanism, it has been proposed that this drug should be more effective against tumours in an oxygen-starved (hypoxic) environment, such as the centre of solid tumour masses.

### KEY POINTS

- Alkylating agents contain electrophilic groups that react with nucleophilic centres on DNA. If two electrophilic groups are present, interstrand and/or intrastrand cross-linking of the DNA is possible.
- Nitrogen mustards react with guanine groups on DNA to produce cross-linking.
- Nitrosoureas have a dual mechanism of action whereby they alkylate DNA and carbamoylate proteins.
- Cisplatin is an alkylating agent which causes intrastrand cross-linking.

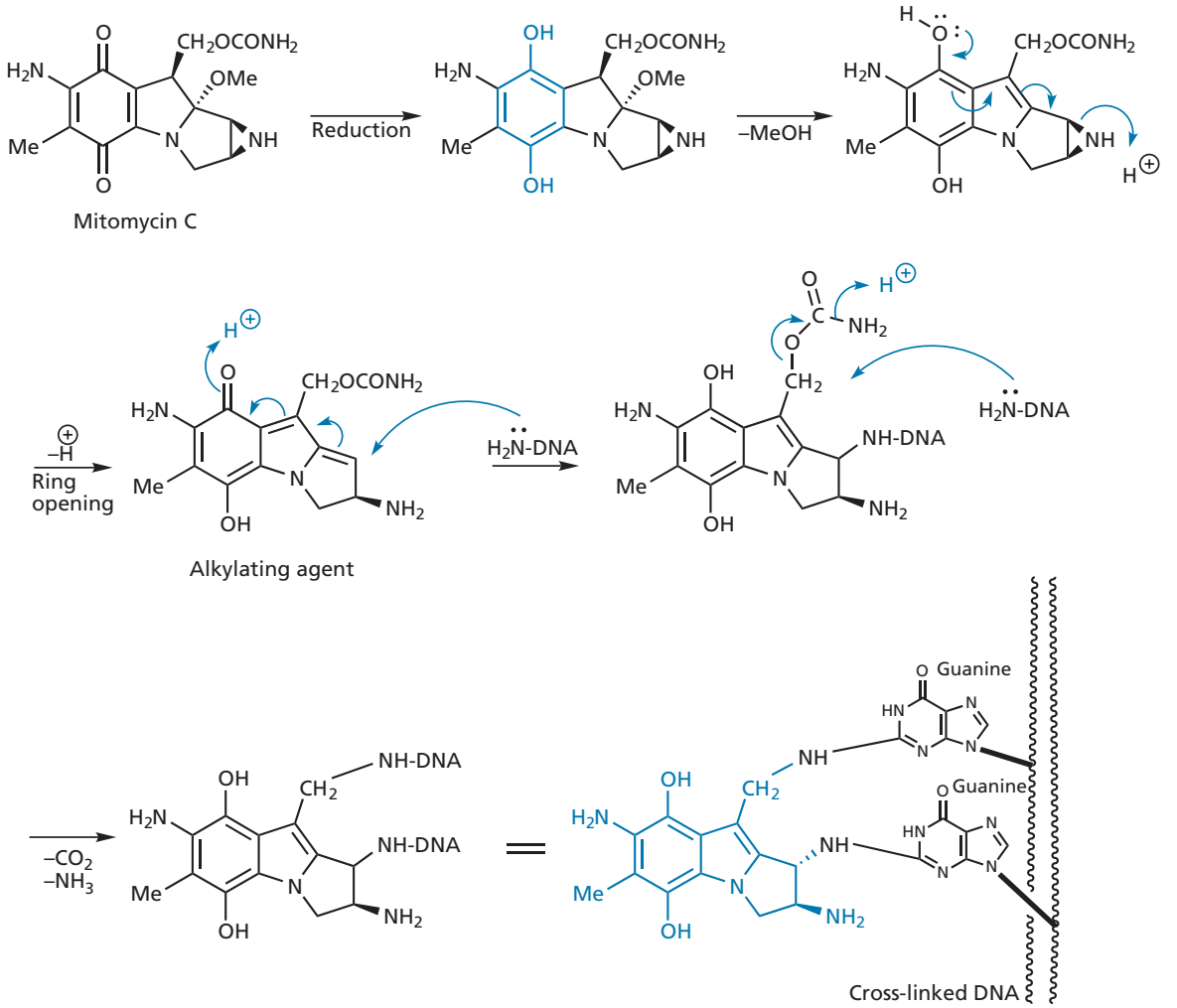



**FIGURE 9.17** DNA cross-linking by mitomycin C.

- Dacarbazine and procarbazine are prodrugs that are activated by enzymes to produce a methyldiazonium ion which acts as an alkylating agent.
- Mitomycin C is a natural product that is converted to an alkylating agent by enzymatic reduction. Interstrand cross-linking takes place between guanine groups.

## 9.4 Chain cutters

'Chain cutters' cut the strands of DNA and prevent the enzyme **DNA ligase** from repairing the damage. They appear to act by creating radicals on the DNA structure. These radicals react with oxygen to form peroxy species and the DNA chain fragments. The bleomycins (section 9.1) and the podophyllotoxins (section 9.2) are examples of drugs that can act in this way, as are the **nitroimidazoles** and **nitrofurantoin**, which target bacterial DNA and are used as antibacterial agents (section 19.8.4). Another example is the antitumour agent **calicheamicin** $\gamma^1$ (Fig. 9.18), which was isolated from a bacterium. This compound binds to the minor groove of DNA and cuts the DNA chain by the mechanism shown in Fig. 9.19. The driving force behind the reaction mechanism is the formation of an aromatic ring from the unusual enediyne system. The reaction starts with a nucleophile attacking the trisulphide group. The thiol which is freed then undergoes an intramolecular Michael addition with a reactive α, β-unsaturated ketone. The resulting intermediate then cycloaromatizes (a reaction known as the **Bergman cyclization**) to produce an aromatic diradical species which snatches two hydrogens from DNA. As a result, the DNA becomes a diradical. Reaction with oxygen then leads to chain cutting.

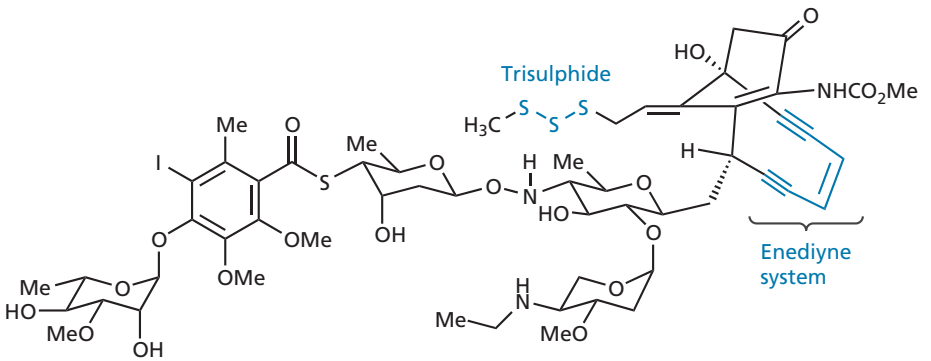



**FIGURE 9.18** Calicheamicin $\gamma^{I}$.

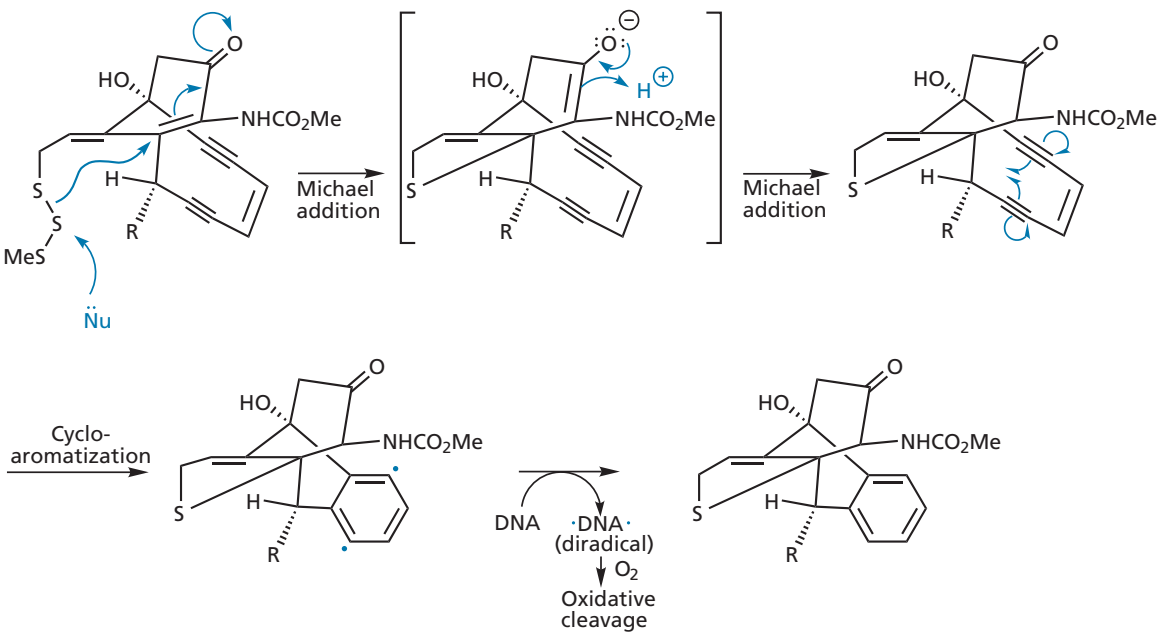



**FIGURE 9.19** Mechanism of action of calicheamicin $\gamma^{I}$.

## 9.5 Chain terminators

Chain terminators are drugs that act as 'false substrates' and are incorporated into the growing DNA chain during replication. Once they have been added, the chain can no longer be extended and chain growth is terminated. The drugs which act in this way are 'mistaken' for the nucleotide triphosphates that are the authentic building blocks for DNA synthesis. The mechanism by which these nucleotides are added to the end of the growing DNA chain is shown in Fig. 9.20 and involves the loss of a diphosphate group—a process catalysed by the enzyme **DNA polymerase**. Before each building block is linked to the chain, it has to be 'recognized' by the complementary nucleic acid base on the template chain. This involves base-pairing between a nucleic acid base on the template and the nucleic acid base on the nucleotide.

Chain terminators, therefore, have to satisfy three conditions. Firstly, they have to be recognized by the DNA template by interacting with a nucleic acid base on the template strand. Secondly, they should have a triphosphate group such they can undergo the same enzyme-catalysed reaction mechanism as the normal building blocks. Thirdly, their structure must make it impossible for any further building blocks to be added.

Aciclovir (Fig. 9.21) is an important antiviral drug that was discovered in the 1970s, and acts as a chain terminator, satisfying all three requirements. Firstly, it contains a guanine base which means that it can base pair to cytosine moieties on the template chain. Secondly, although it does not contain a triphosphate group, this is added to the

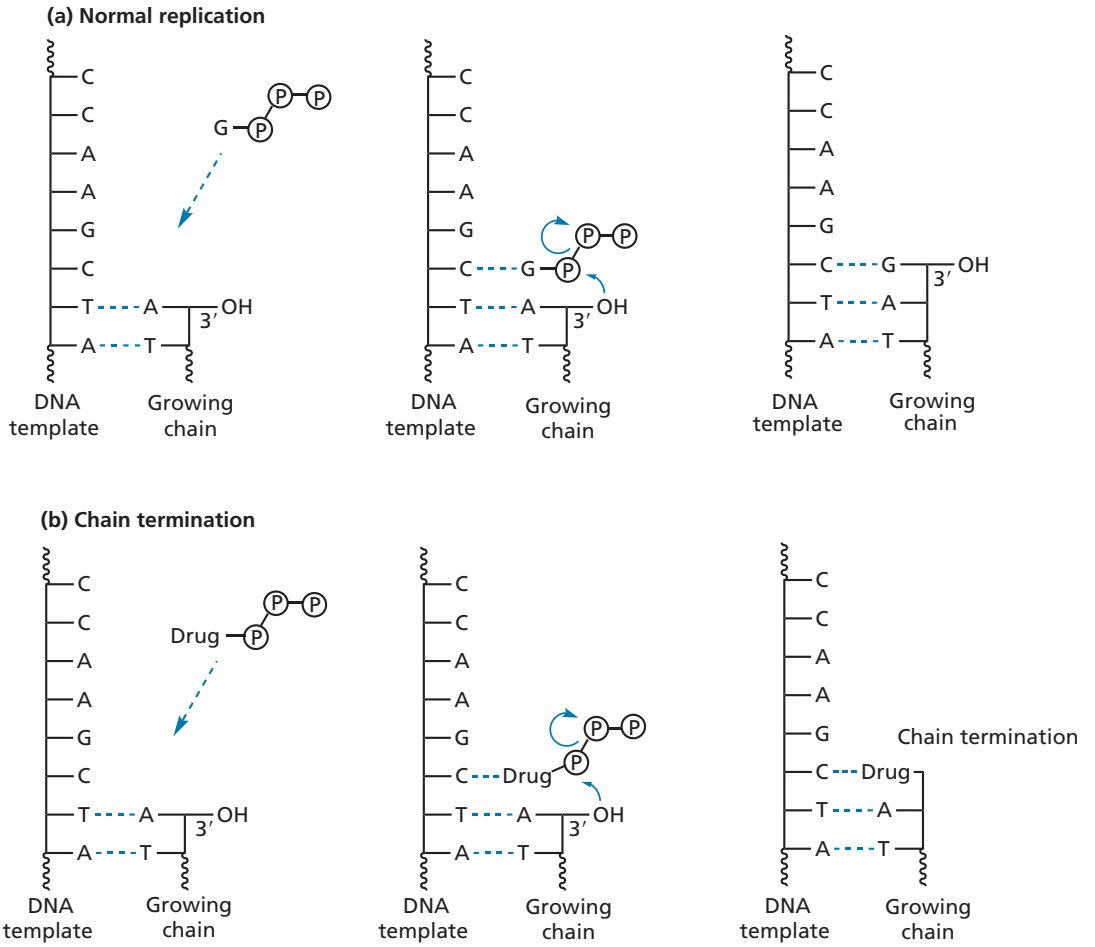



**FIGURE 9.20** (**a**) The normal replication mechanism. (**b**) A drug acting as a chain terminator. (Ⓟ = Phosphate)

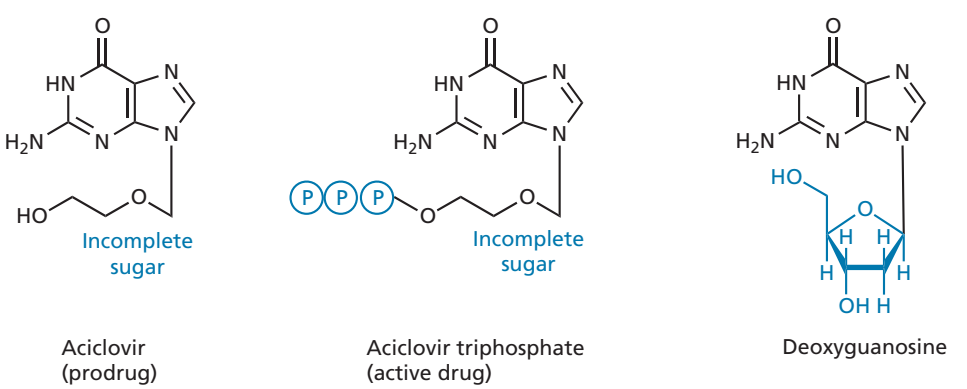



**FIGURE 9.21** Structure of aciclovir, aciclovir triphosphate, and deoxyguanosine. (Ⓟ = Phosphate)

molecule in virally infected cells. Thirdly, the sugar unit is incomplete and lacks the required OH group normally present at position 3′—compare the structure of deoxyguanosine in Fig 9.21. Therefore, the nucleic acid chain cannot be extended any further. Several other structures acting in a similar fashion are used in antiviral therapies and are described in sections 20.6.1 and 20.7.3.1.

## 9.6 Control of gene transcription

Various research groups are looking into the design of synthetic molecules that can bind to DNA by recognizing nucleic acid base pairs, and, by doing so, control gene transcription. It has been found that 'hairpin' polyamide

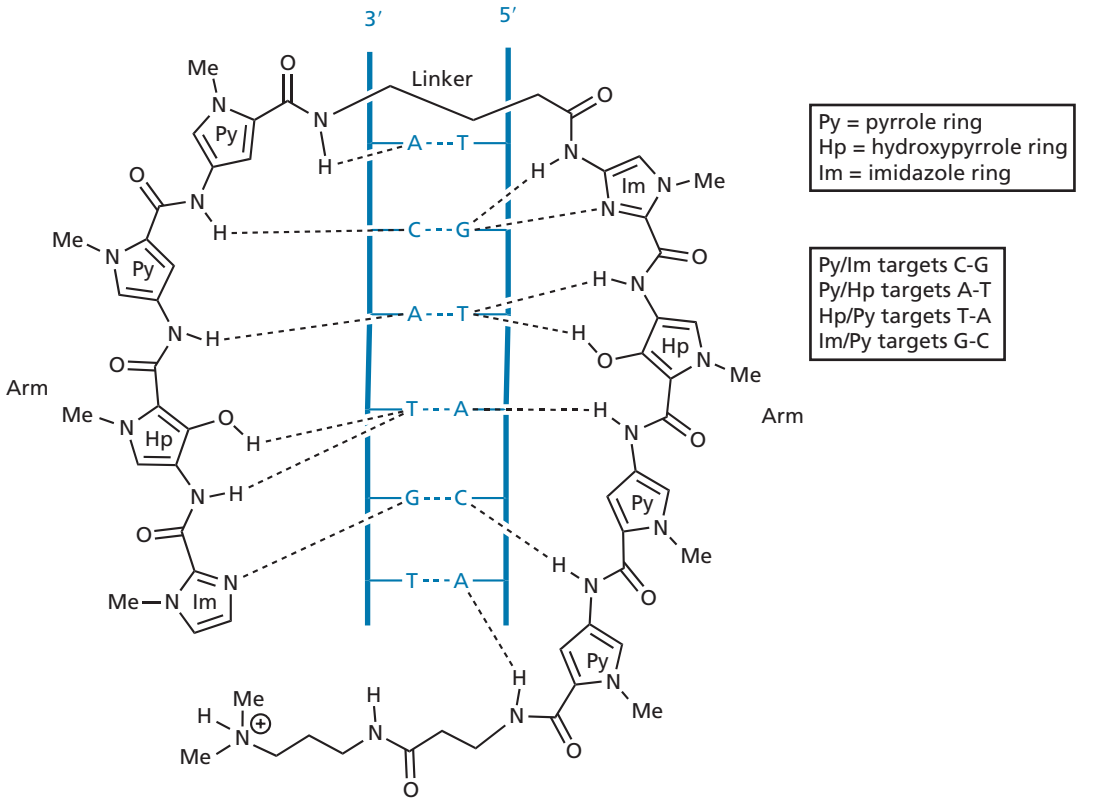



**FIGURE 9.22** Synthetic polyamides capable of recognizing and binding to a particular sequence of nucleic acid base pairs.

structures containing heterocyclic rings have this capacity and bind in the minor groove of DNA (Fig. 9.22). The molecule is made up of two arms connected by means of a linker unit. The molecule attaches itself to DNA like a clamp with each arm binding to one of the DNA strands. The binding interactions are through hydrogen bonding to the base pairs of DNA, and involve both the heterocyclic rings and the amide bonds. Polyamides containing eight heterocyclic rings bind with an affinity and specificity that is comparable to naturally occurring DNA-binding proteins. Experiments have shown that it is feasible for these drugs to cross the cell membrane and to inhibit transcription by binding to the regulatory element of a gene—in other words where a transcription factor would normally bind. Binding at this specific region is achieved by designing the drug to recognize the base pair sequences in that region. This is possible by using particular patterns of pyrrole, hydroxypyrrole, and imidazole rings on each arm of the molecule. Binding to the regulatory element of the gene is crucial, as polyamides that bind to the coding region of the gene do not appear to prevent transcription. Presumably, they are displaced during the transcription process. However, it may be possible to attach an alkylating agent to the molecule such that a covalent bond is formed and the gene is 'knocked out'.

It may also be possible to design polyamides that activate the transcription process, rather than switch it off. Initial work has involved linking the polyamide to a peptide. The polyamide acts as the binding unit for DNA, while the attached peptide acts as the activating unit for transcription. It will be interesting to see whether any of these approaches leads to a clinically useful drug.

## 9.7 Agents that act on RNA

### 9.7.1 Agents that bind to ribosomes

A large number of clinically important antibacterial agents prevent protein synthesis in bacterial cells by binding to ribosomes and inhibiting the translation process. These are described in section 19.7.

### 9.7.2 Antisense therapy

A great deal of research has been carried out into the possibility of using **oligonucleotides** to block the coded messages carried by mRNA. This is an approach known as **antisense therapy** and has great potential. The rationale is as follows (Fig. 9.23). Assuming that the primary

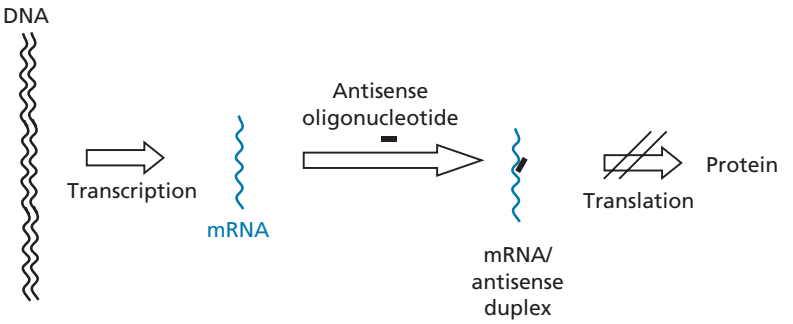



**FIGURE 9.23** The principles of antisense therapy.

sequence of a mRNA molecule is known, an oligonucleotide can be synthesized containing nucleic acid bases that are complementary to a specific stretch of the mRNA molecule. As the oligonucleotide has a complementary base sequence, it is called an **antisense oligonucleotide**. When mixed with mRNA, the antisense oligonucleotide recognizes its complementary section in mRNA, interacts with it, and forms a duplex structure such that the bases pair up by hydrogen bonding. This section now acts as a barrier to the translation process and blocks protein synthesis.

There are several advantages to this approach. First of all, it can be highly specific. Statistically, an oligonucleotide of 17 nucleotides should be specific for a single mRNA molecule and block the synthesis of a single protein. The number of possible oligonucleotides containing 17 nucleotides is $4^{17}$, assuming four different nucleic acid bases. Therefore, the chances of the same segment being present in two different mRNA molecules is remote. Secondly, because one mRNA leads to several copies of the same protein, inhibiting mRNA should be more efficient than inhibiting the resulting protein. Both these factors should allow the antisense drug to be used in low doses and result in fewer side effects than conventional protein inhibition.

However, there are several difficulties involved in designing suitable antisense drugs. mRNA is a large molecule with a secondary and tertiary structure. Care has to be taken to choose a section that is exposed. There are also problems relating to the poor absorption of nucleotides and their susceptibility to metabolism.

Nevertheless, antisense oligonucleotides are potential antiviral and anticancer agents, as they should be capable of preventing the biosynthesis of 'rogue' proteins and have fewer side effects than currently used drugs. Design strategies aimed at solving many of the pharmacokinetic problems of oligonucleotides are described in section 14.10. The first antisense oligonucleotide to be approved for the market was the antiviral agent **fomivirsen** (**Vitravene**) in 1998 (section 20.6.3).

Antisense oligonucleotides are also being considered for the treatment of genetic diseases such as **muscular dystrophy** and **β-thalassaemia**. Abnormal mRNA is sometimes produced as a result of a faulty splicing mechanism (section 6.2.3). Designing an antisense molecule which binds to the faulty splice might disguise that site and prevent the wrong splicing mechanism taking place.

A surprising discovery in recent years is the finding that short segments of double-stranded RNA (21–23 nucleotides) can prevent translation by both inhibiting and degrading mRNA. Further research has revealed a natural process by which translation is regulated within the cell in the following manner.

In the nucleus, an endonuclease enzyme excises segments of base-paired RNA from normal RNA. These segments exit the nucleus into the cytoplasm and are further cleaved by an endonuclease enzyme called **Dicer** to produce short segments of double-stranded molecules called **micro-RNAs** (**miRNA**), which are typically 21 nucleotides in length (Fig. 9.24).

Each miRNA is recognized and bound by a complex of enzymes called **RNA induced silencing complex (RISC)**, which catalyses the unravelling of the strands to produce single-stranded segments of RNA called small interfering or **small inhibitory RNAs** (**siRNA**) (Fig. 9.25). One of the strands is discarded while the other remains bound to the protein and base-pairs with any mRNA molecule that contains a complementary sequence of nucleic acid bases. This brings mRNA and RISC together, and the enzyme complex then cleaves the mRNA.

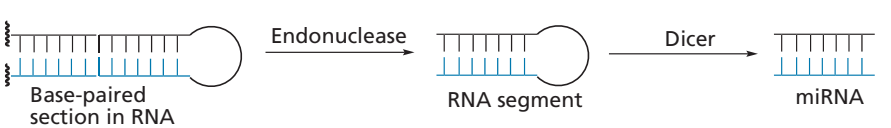



**FIGURE 9.24** Cleavage of RNA to produce micro-RNAs (miRNA).

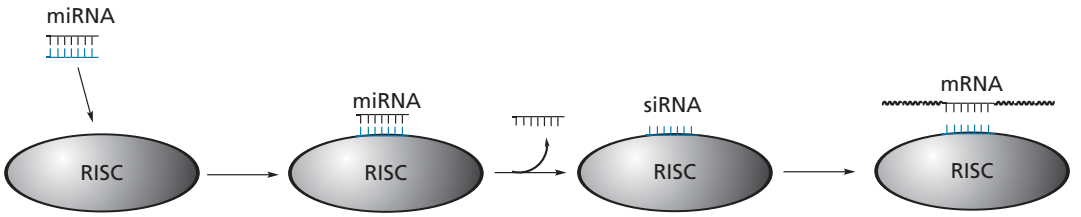



**FIGURE 9.25** Interaction of micro-RNA (miRNA) with RNA induced silencing complex (RISC).

An alternative process can take place where miRNA is bound to a protein complex called **miRNP** (**micro-RNA-protein**). This protein also unwinds miRNA and discards one of the strands. Base-pairing of the bound siRNA with relevant mRNA then takes place. The mRNA is not cleaved, but the mRNA is 'locked up' and so translation is suppressed.

Both of these processes are important to the normal development of the cell and to the development of tumours, but work is now in progress to design drugs that will take advantage of these mechanisms. For example, siRNAs have been shown to regulate HIV-1 expression in cultured cells and have the potential to be used in gene therapy for the treatment of AIDS. One of the advantages of these mechanisms over conventional antisense therapy is a greater efficiency in suppressing translation.

One siRNA molecule can be responsible for the cleavage of several mRNA molecules through the RISC pathway.

However, there are many difficulties still to be overcome. If siRNAs are to be effective as drugs, they will have to be metabolically stable (section 14.10). There are also difficulties in ensuring that they:

- reach their target cells;
- are taken up into the target cell.

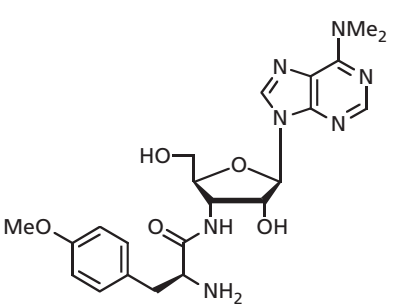
One method that is being tried to solve these problems is to encapsulate the siRNA into small stable nucleic acid–lipid particles that remain stable in the bloodstream and are then taken up by target cells. For example, experiments have shown that it is possible to deliver siRNA molecules to liver cells by this method. If siRNA molecules could be designed to 'knock out' the mRNA that codes for low-density lipoproteins (LDPs), this could be an effective way of lowering cholesterol levels. LDPs play an important role in transporting cholesterol round the body (see Case study 1).

**KEY POINTS**

- Calicheamicin is a natural product that reacts with nucleophiles to produce a diradical species. Reaction with DNA ultimately leads to cutting of the DNA chains.
- Aciclovir and related antiviral agents act as prodrugs that are converted to incomplete or unnatural nucleotides which act as DNA chain terminators.
- Synthetic agents are being designed which can bind to the regulatory elements of DNA in order to control gene transcription.
- Antisense therapy involves the use of oligonucleotides that are complementary to small sections of mRNA. They form a duplex with mRNA and prevent translation.
- Small inhibitory RNA molecules can inhibit protein synthesis by binding to mRNA and either blocking translation or cleaving mRNA.

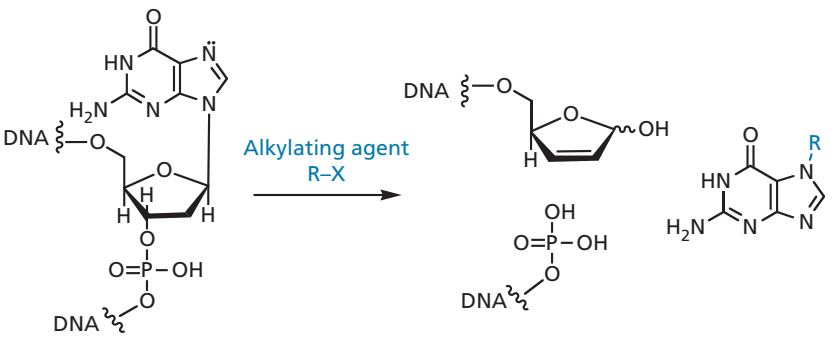
## QUESTIONS

1. Puromycin is an antibiotic that inhibits the translation of proteins. When inhibition is taking place, partially constructed proteins are found to be present in the cytoplasm and are covalently linked to the drug. Suggest a mechanism by which this drug causes inhibition.

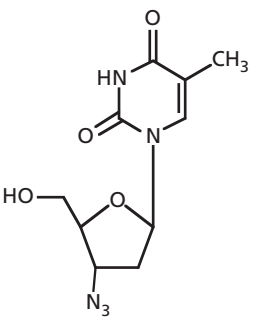



Puromycin

**2.** Alkylating agents have been observed to cause breaks in the DNA chain as shown below. Suggest a mechanism.




**3.** The following structure is an important antiviral agent. Suggest what mode of action it may have and the mechanism by which it works.



**4.** Propose a mechanism showing how the anticancer drug temozolomide acts as a prodrug for MTIC.

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# 10 Miscellaneous drug targets

In Chapters 7–9 we looked at the most common drug targets in medicinal chemistry (i.e. enzymes, receptors, and nucleic acids). In this chapter, we shall look at other important drug targets to illustrate the variety of ways in which drugs can act.

## 10.1 Transport proteins as drug targets

Transport proteins were described in section 2.7.2. They have a binding site which 'recognizes' and binds a specific guest molecule, but it is sometimes possible to fool a transport protein into accepting a drug that resembles the usual guest. If that drug remains strongly bound to the transport protein, it will prevent the protein from carrying out its normal role. Some important drugs operate in this way. For example, **cocaine** and the **tricyclic antidepressants** bind to transport proteins, and prevent neurotransmitters, such as **noradrenaline** or **dopamine**, from re-entering nerve cells (section 23.12.4). This results in an increased level of the neurotransmitter at nerve synapses and has the same effect as adding drugs that mimic the neurotransmitter. Other antidepressant drugs act on the transport proteins for serotonin (Box 10.1). Drugs which inhibit the reuptake of neurotransmitters may affect more than one type of neurotransmitter. For example, several antidepressant drugs inhibit more than one type of transport protein (section 23.12.4). Another example is the antiobesity drug **sibutramine** (Fig. 10.1), which acts centrally to inhibit the reuptake of serotonin, noradrenaline, and, to a lesser extent, dopamine. It is thought that the increase in serotonin levels dulls the appetite. Sibutramine was introduced in 1997 and is chemically related to the amphetamines. However, it was withdrawn in 2010 as a result of side effects.

Transport proteins can also be targeted as a means of transporting polar drugs across the cell membrane and into the cell (see Case study 1, and sections 14.6.1.3 and 23.12.4).

## 10.2 Structural proteins as drug targets

In general, there are not many drugs which target structural proteins. However, some antiviral drugs have been designed to act against viral structural proteins, and there are established anticancer agents which target the structural protein, tubulin.

### 10.2.1 Viral structural proteins as drug targets

Viruses consist of a nucleic acid encapsulated within a protein coat called a **capsid**. If a virus is to multiply within a host cell, this protein coat has to be dismantled in order to release the nucleic acid into the cell. Drugs have been designed which bind to the structural proteins that make up the capsid and which prevent the uncoating process. The drugs concerned show potential as antiviral agents against the cold virus (section 20.9).

Capsid proteins are also important in the mechanism by which viruses infect host cells. The viral proteins interact with host cell proteins, which are present in the cell membranes. This triggers processes which allow the virus to enter the cell. Drugs that bind to viral proteins and inhibit this protein–protein interaction can, therefore, act as antiviral agents. **Enfuvirtide** was approved in March 2003 and is an example of an antiviral agent that works in this way (section 20.7.5).

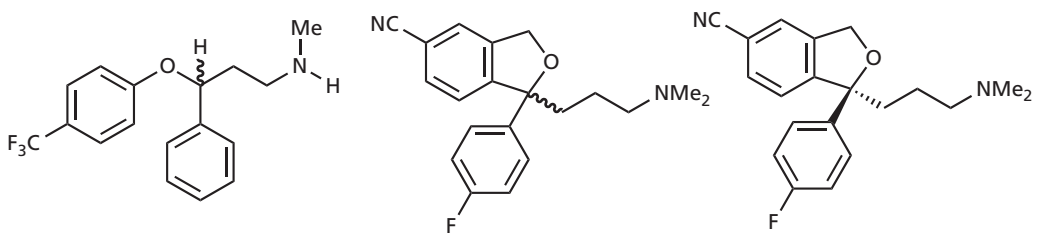
### 10.2.2 Tubulin as a drug target

In section 2.7.1 we described the role of the structural protein tubulin in cell division—a process which involves

## BOX 10.1 Antidepressant drugs acting on transport proteins

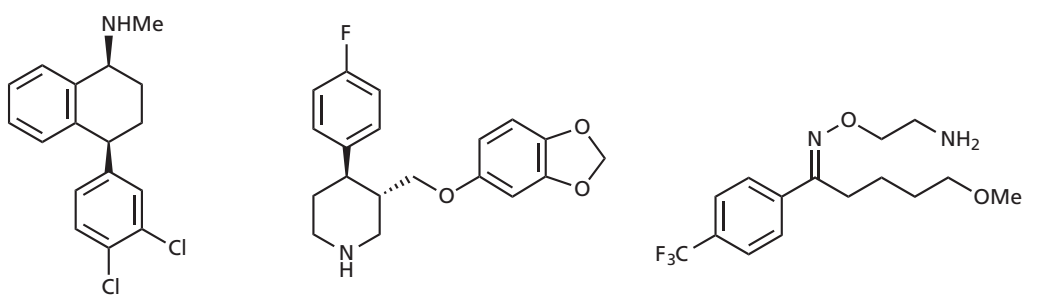
The antidepressant drugs **fluoxetine** (Prozac), **citalopram**, and **escitalopram** (Fig. 1) selectively block the transport protein responsible for the uptake of a neurotransmitter called **serotonin** from nerve synapses, and are called **selective serotonin reuptake inhibitors** (SSRIs) (see also Case study 7). A lack of serotonin in the brain has been linked with depression and by blocking its uptake, the serotonin that *is* released has a longer duration of action. Fluoxetine and citalopram are chiral molecules which are marketed as racemates. The *S*-enantiomer of citalopram is more active than the *R*-enantiomer and is now marketed as escitalopram. Replacing a racemic drug with a more effective enantiomer is known as **chiral switching** (section 15.2.1).

Other examples of clinically important SSRIs include sertraline, paroxetine, and fluvoxamine (Fig. 2).




Fluoxetine (Prozac) Citalopram *S*-Citalopram (Escitalopram)

**FIGURE 1** Antidepressant drugs acting to block the uptake of serotonin.




Sertraline Paroxetine Fluvoxamine

**FIGURE 2** Further examples of SSRIs.

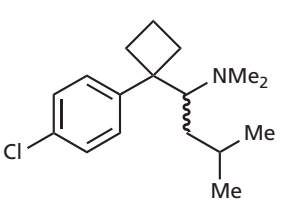



**FIGURE 10.1** Sibutramine.

the polymerization and depolymerization of microtubules using tubulin proteins as building blocks. A variety of drugs interfere with this process by either binding to tubulin and inhibiting the polymerization process, or binding to the microtubules to stabilize them and thus inhibit depolymerization. Either way, the balance between polymerization and depolymerization is disrupted, which leads to a toxic effect and the inability of the cell to divide. Drugs that target tubulin have been found to be useful anticancer and anti-inflammatory agents, and some of the most important are described below.

### 10.2.2.1 Agents that inhibit tubulin polymerization

**Colchicine** (Fig. 10.2) is an example of a drug that binds to tubulin and prevents its polymerization. It can be used in the treatment of gout by reducing the mobility

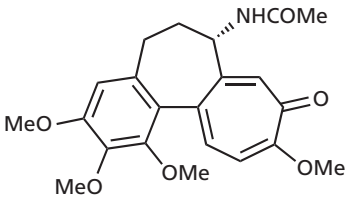



**FIGURE 10.2** Colchicine.

of neutrophils into joints. Unfortunately, colchicine has many side effects and it is restricted, therefore, to the treatment of acute attacks of this disease.

The **Vinca alkaloids vincristine**, **vinblastine**, **vindesine**, and **vinorelbine** (Fig. 10.3) bind to tubulin to prevent polymerization and are useful anticancer agents. A range of other natural products have also been found to prevent the polymerization of microtubules and are currently being studied as potential anticancer agents (section 21.5.1).

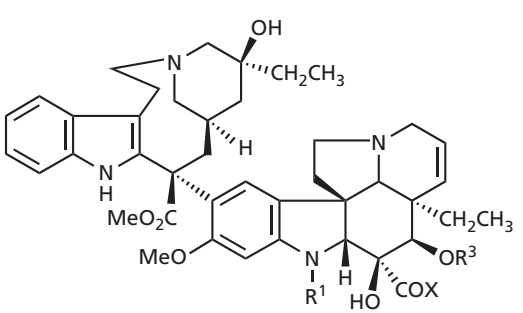
### 10.2.2.2 Agents that inhibit tubulin depolymerization

**Paclitaxel (Taxol)** and the semi-synthetic analogue **docetaxel** (Fig. 10.4) are important anticancer agents that inhibit tubulin depolymerization (section 21.5.2). Paclitaxel itself was isolated from the bark of yew trees (*Taxus* spp.) and identified in 1971 following a screening programme for new anticancer agents carried out by the US National Cancer Institute. Obtaining sufficient paclitaxel was initially a problem, as the bark from two yew trees was required to supply sufficient paclitaxel for one patient! A full synthesis of paclitaxel was achieved in 1994, but was impractical for large-scale production because it involved 30 steps and gave a low overall yield. Fortunately, it has been possible to carry out a semi-synthetic synthesis (section 15.3.4) using a related natural product which can be harvested from the yew needles without damaging the tree. The semi-synthetic route involves docetaxel as an intermediate. The term **taxoids** is used generally for paclitaxel and its derivatives.

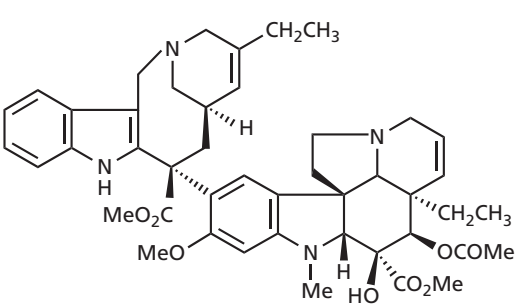
Tubulin is actually made up of two separate proteins and the taxoids are found to bind to the β-subunit of tubulin. In contrast to the drugs described in section 10.2.2.1, the binding of paclitaxel accelerates polymerization and stabilizes the resultant microtubules, which means that depolymerization is inhibited. As a result, the cell division cycle is halted.

The benzoyl and acetyl substituents, at positions 2 and 4, respectively, play an important role in this binding interaction, as do the side chain and the oxetane ring. These groups dominate the 'lower' or 'southern' half of the molecule (as the structure is normally presented), and so the variations that are possible in this region are restricted when making analogues. In contrast, it is possible to carry out more variations in the 'northern' half of the molecule. This can affect the *in vivo* efficacy of the molecule, allowing modification of aqueous solubility and pharmacokinetic properties. **BMS 188797** and **BMS 184476** (Fig. 10.5) are two taxoids that have been developed recently and have reached clinical trials.

More substantial variations have resulted in a second generation of taxoids, where potency has been increased by 2–3 orders of magnitude. For example, it was possible to replace the aromatic rings of paclitaxel with other hydrophobic groups. Having a suitable acyl group at position 10 has also been found to increase activity against drug-resistant strains of cancers. Such compounds have the ability not only to bind to tubulin, but to inhibit the P-glycoprotein efflux pump. This is a protein which is present in the cell membrane of cancer cells and




Vinblastine ($R^1$=Me; X=OMe; $R^3$= COMe)
Vincristine ($R^1$=CHO; X=OMe; $R^3$= COMe)
Vindesine ($R^1$=Me; X=$NH_2$; $R^3$= H)




Vinorelbine

**FIGURE 10.3** The vinca alkaloids.

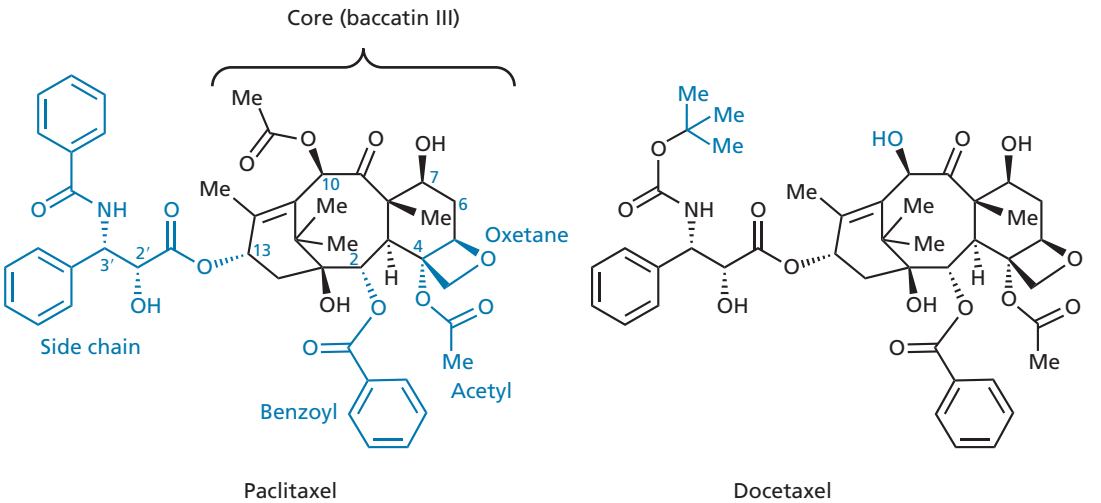



**FIGURE 10.4** Paclitaxel (Taxol), with important binding groups in colour, and docetaxel (Taxotere).

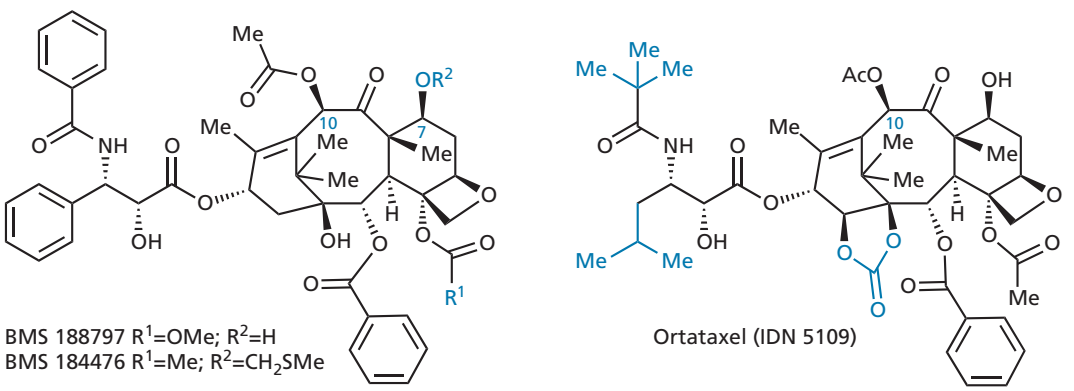



**FIGURE 10.5** Analogues of paclitaxel.

can pump drugs out of the cell before they get the chance to work effectively. Further work has demonstrated that acylating the 7-hydroxy group with hydrophobic groups is also effective in blocking efflux.

Finally, the addition of a methyl substituent at C-2′ has been found to increase activity by inhibiting rotation of the C-2′–C-3′ bond. The first orally active taxoid structure **ortataxel** (Fig. 10.5) has now been developed and has entered clinical trials.

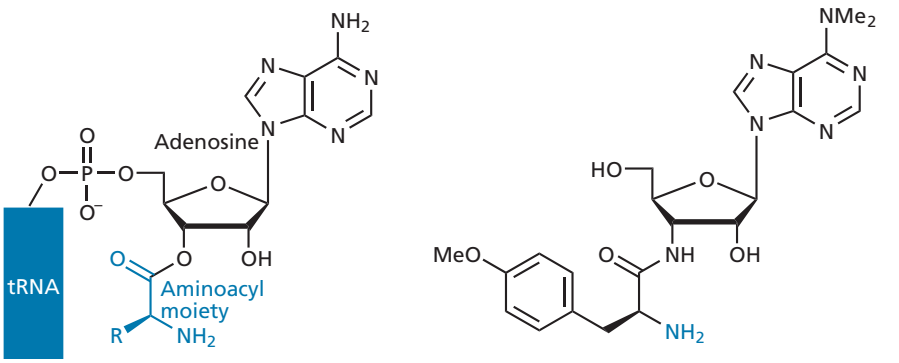
Since the discovery of paclitaxel a variety of other natural products have been found to have a similar mechanism of action and are currently being studied as potential anticancer agents (section 21.5.2).

**KEY POINTS**

- Transport proteins transport polar molecules across the hydrophobic cell membrane. Drugs can be designed to take advantage of this transport system in order to gain access to cells or to block the transport protein.
- Drugs that target viral structural proteins can prevent viruses entering host cells. They can also inhibit the uncoating process.
- Tubulin is a structural protein crucial to cell division and cell mobility, and which is the target for several anticancer drugs.
- The vinca alkaloids bind to tubulin and inhibit the polymerization process.
- Paclitaxel and its derivatives bind to tubulin and accelerate polymerization by stabilizing the resulting microtubules.

## 10.3 Biosynthetic building blocks as drug targets

The target for the antibacterial agent **vancomycin** is rather unique in that it is a biosynthetic building block. Essentially, vancomycin ‘caps’ the building block and




Aminoacyl-tRNA Puromycin

**FIGURE 10.6** Comparison of puromycin and aminoacyl-tRNA.

prevents its incorporation into the growing bacterial cell wall. There is a small peptide chain on the building block which can bind to vancomycin by hydrogen bond interactions. Indeed, vancomycin acts like a receptor by providing a binding site for the building block (see section 19.5.5.2).

## 10.4 Biosynthetic processes as drug targets: chain terminators

In section 9.5 we looked at antiviral drugs that act as chain terminators for the synthesis of new DNA. Puromycin is an antibiotic which can be viewed in the same light, except that it terminates the growth of protein chains during translation. It is able to carry out this role because it mimics the terminus of an aminoacyl-tRNA molecule (Fig. 10.6). Aminoacyl-tRNA is the molecule that brings an amino acid to the ribosome such that it can be added to the growing protein chain (section 6.2.2).

Because puromycin resembles the aminoacyl and adenosine moieties of aminoacyl-tRNA, it is able to enter the A site of the ribosome and prevent aminoacyl-tRNA molecules from binding. It has the amino group required for the transfer reaction and so the peptide chain is transferred from tRNA in the P binding site to puromycin in the A binding site. Puromycin departs the ribosome carrying a stunted protein along with it (Fig. 10.7).

## 10.5 Protein–protein interactions

Many important cellular processes involve the association of two or more proteins (section 2.7.4) and so several research teams are trying to develop drugs that might interfere with this association. Such drugs could be useful in a variety of medicinal fields. For example, a drug that prevents protein–protein interactions as part of a signal transduction process (Chapter 5) could inhibit cell growth and cell division, and hence be a useful anticancer agent. An agent which prevents the formation of transcription factor complexes could prevent the transcription of specific genes (Box 10.2). There is also evidence that the abnormal protein structures observed in Alzheimer's disease result from protein–protein interactions (section 22.15).

One way of inhibiting protein–protein interactions is to use antibodies (section 10.7.2). These agents have been

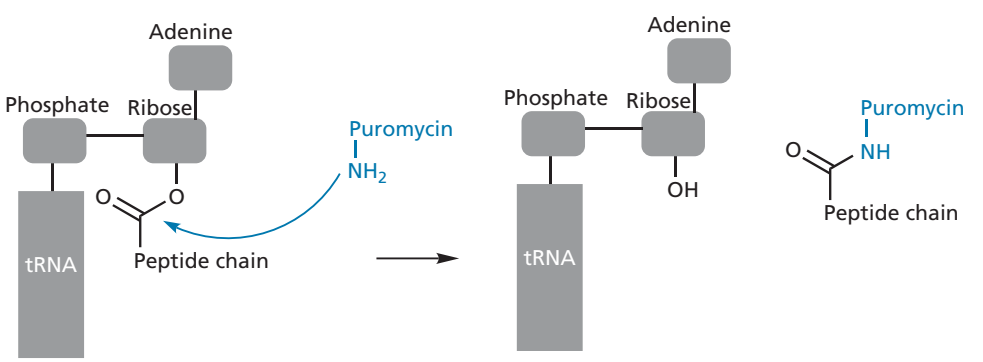



**FIGURE 10.7** Transfer of peptide chain to puromycin.

particularly successful in preventing protein–protein interactions for a family of extracellular proteins called **integrins**. The integrins are adhesive proteins which are important to processes such as blood clotting, inflammation, cell protection, and the immune response. Indeed, **daclizumab** is an antibody used as an **immunosuppressant** in kidney transplants, while **abciximab** is an antibody fragment that inhibits blood clotting following **angioplasty** procedures aimed at unblocking coronary arteries. Successful though antibodies may be, they are

## BOX 10.2 Targeting transcription factors: co-activator interactions

The transcription of a gene is initiated by a protein complex that is formed between a transcription factor and a co-activator protein (Box 8.2). A drug that inhibits the interactions between these proteins would prevent formation of the complex, prevent transcription, and, potentially, be useful in treating some cancers The crucial interactions between two proteins can often involve a relatively short α-helical segment. For example, the interaction between the ESX transcription factor and its co-activator protein Sur-2 involves an eight amino acid α-helix present on the transcription factor (Fig. 1). One of these eight amino acids is a tryptophan residue (Trp), which plays a particularly important binding role, and so one research group screened a number of chemical libraries for compounds containing indole rings that could mimic this residue. This led to the discovery of a lead compound called **adamanolol** (Fig. 2), which was found to inhibit the interaction between the proteins.

Structure–activity studies showed that:

- the indole ring system was essential and mimics the tryptophan residue;
- the adamantane ring is important and is thought to mimic a cluster of isoleucine and leucine residues that are on the α-helix, and it may also bind to a hydrophobic pocket in the co-activator protein;
- the isopropyl group can be replaced with bulky substituents. These substituents enforce a configuration around the urea linker where the molecule forms a helix-like shape with the adamantane and indole rings in close proximity.

From these results, a more active, water-soluble agent called **wrenchnolol** was designed—so named as it resembles the shape of a wrench. The molecule has two hydrophobic 'jaws' and a polar 'handle'. The non-polar components are clustered on one face of the molecule with the polar handle angled away, resulting in an amphiphilic molecule that mimics the amphiphilic α-helix of the transcription factor. The hydrophobic jaws make contact with the Sur-2 protein and mimic the amino acid residues of tryptophan, leucine, and isoleucine.

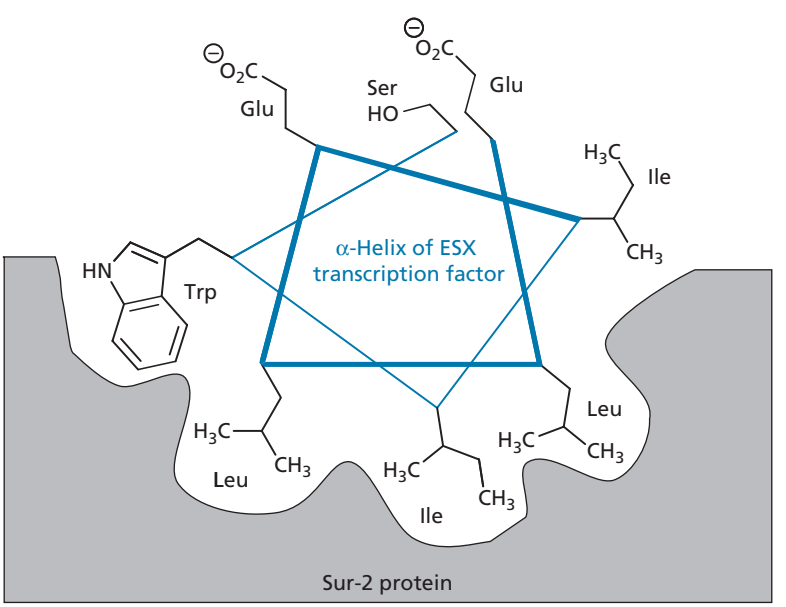



**FIGURE 1** Interaction of an α-helix of the ESX transcription factor with the protein Sur-2.

*(Continued)*

**BOX 10.2** Targeting transcription factors: co-activator interactions (*Continued*)

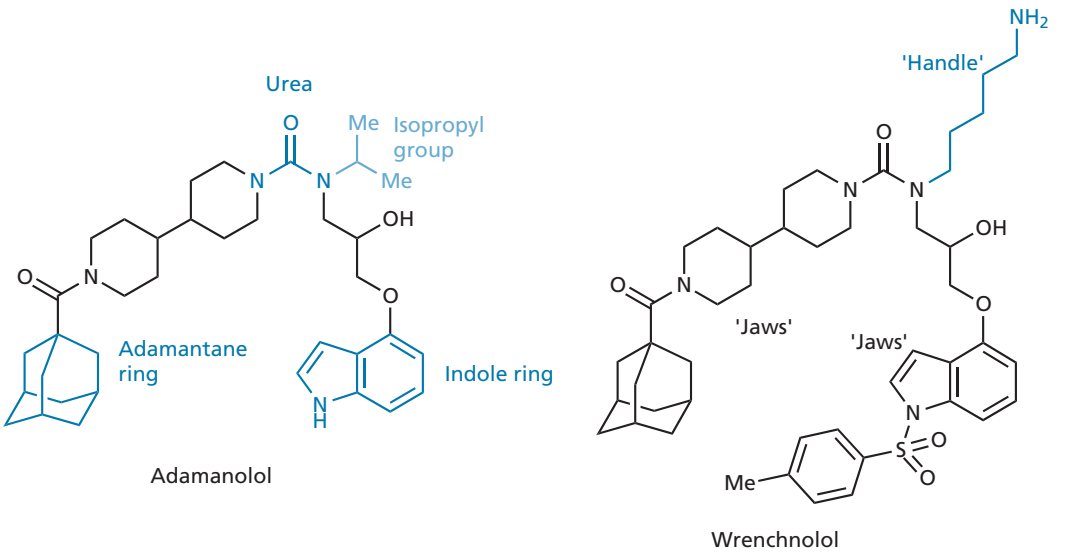



**FIGURE 2** Structures of adamanolol and wrenchnolol.

limited in application to extracellular proteins. Therefore, it would be advantageous to design drug-sized molecules which could have the same action on protein targets, both extracellularly and intracellularly.

Finding a drug to do this might seem a tall order. Drugs, after all, are small molecules in comparison to a protein, and protein–protein interactions involve large surface areas of the associated proteins. The idea of binding a drug to a protein surface in order to ward off another protein seems a bit optimistic. It might be equated with landing a spacecraft on the moon and expecting it to ward off meteorites. Fortunately, it has been found that the interactions between proteins often involve a small number of particularly important interactions involving relatively small areas. For example, the binding of human growth factor with its receptor certainly involves large surface areas of both proteins, where 31 amino acid residues of the human growth factor protein interact with 33 residues of the receptor. However, 85% of the binding energy is associated with eight residues of the hormone interacting with nine residues of the receptor. Therefore, it is conceivable that a drug could be designed to bind to some of these crucial residues and hinder the association of these proteins.

However, there are other potential problems to consider. The protein surfaces involved in protein–protein interactions are often relatively flat and do not contain the kind of binding sites that we are used to with enzymes and receptors. Therefore, identifying a particular feature on a protein surface that could be 'recognized' by a drug might be difficult. A final problem is that drugs which inhibit protein–protein interactions are likely to be larger than the average-sized drug. This might pose problems as the drugs must pass through cell membranes in order to reach intracellular targets and do so in sufficient quantity to be effective.

Despite these problems, there is active research in finding drugs that can inhibit protein–protein interactions. Such drugs are known as **protein–protein binding inhibitors** (PPBIs). PPBIs have potential as anticancer agents (section 21.8.4), antiviral agents (section 20.7.5), analgesics, and anti-inflammatory agents, and could also be useful in the treatment of autoimmune diseases and osteoporosis. It is worth pointing out that there are already drugs on the market which interfere with protein–protein interactions, mainly those that interact with tubulin (section 10.2.2). Drugs have also been found that bind to various integrins to prevent their interaction with other proteins. One example is the clinical agent **tirofiban** (Fig. 10.8), which is used as an anticoagulant by preventing protein–protein binding between an integrin and the blood-clotting agent fibrinogen. It is thought that the drug mimics a tripeptide sequence (Arg–Gly–Asp) in fibrinogen that plays an important role in the binding process between the two proteins. When the drug binds to integrin, it prevents this interaction taking place and so one could view the drug as an ultra-simplified analogue of the fibrinogen protein!

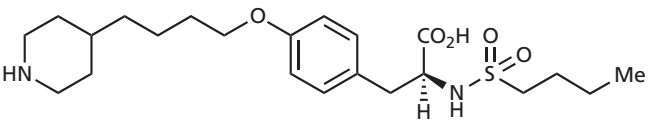



**FIGURE 10.8** Tirofiban.

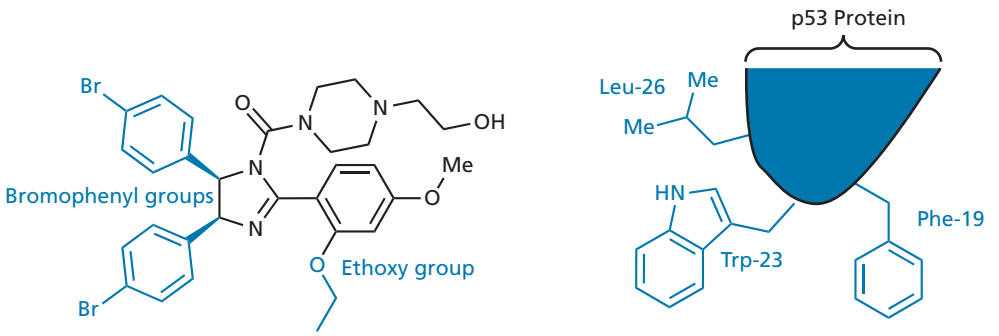



**FIGURE 10.9** Nutlin-2 mimicking the three amino acid 'finger' residues of p53.

An important example of a protein–protein interaction involves the proteins p53 and MDM2 (or HDM2*). The former protein is produced in cells that are damaged or are under stress, and serves to restrict cell growth or even induce cell death (section 21.1.7). This activity is important to the health and survival of an organism because it suppresses the growth of defective cells, such as tumour cells. MDM2 is a protein that down-regulates the activity of p53 by binding or interacting with it. In some tumour cells, a genetic defect results in excess levels of MDM2, which means that p53 can no longer function, allowing tumour cells to multiply. Therefore, drugs that prevent this interaction could be useful anticancer drugs. **Nutlin-2** (Fig. 10.9) is an example of a series of structurally related compounds which are capable of preventing this protein–protein interaction. It binds to a region of MDM2 that is normally involved in the protein–protein interaction with p53 and mimics three amino acid residues present on p53 (Leu-26, Trp-23, Phe-19). These three amino acid residues normally fit like three fingers into complementary pockets on the MDM2 surface. The ethoxy group and the two bromophenyl groups of nutlin-2 mimic these three fingers.*

One easy way of designing a PPBI is to identify a peptide that will mimic a crucial peptide binding region for one of the proteins. This peptide would then be recognized by the complementary protein and bind with it, thus preventing protein–protein binding. However, peptides have many disadvantages as drugs (section 14.9), and non-peptide drugs are preferable. To that end, medicinal chemists have attempted to design peptide mimics. In order to achieve that goal, molecules need to be designed with substituents that will mimic the side chains of amino acids. The substituents also need to be attached to a stable molecular scaffold in such a way that they are positioned in the same relative positions as amino acid residues in common protein features, i.e. α-helices, β-sheets, β-turns, and loops. A lot of work has been carried out designing drugs to mimic β-turns, but, more recently, researchers have been turning their attention to structures that mimic α-helices—an extremely important area as α-helices play crucial roles in many protein–protein interactions.

An example of this research involves **terphenyl structures** (Fig. 10.10). The three aromatic rings that are directly linked together in these compounds are not coplanar. Instead, they are at different angles with respect to each other and mimic the twist of the α-helix. These rings act as the scaffold onto which different substituents can be placed to mimic amino acid side chains. The *meta*-substituent and the two *ortho*-substituents shown in Fig. 10.10a mimic the side chains of amino acids which would be at the first, fourth, and seventh positions of an α-helix. This structure has been shown to act as an antagonist for the protein **calmodulin**, but by varying the nature of the substituents one can obtain structures that are recognized by different proteins. For example, the terphenyl structure shown in Fig. 10.10b binds to a protein called BCl-$x_L$. This protein plays an important role in apoptosis—the process by which cells are destroyed (section 21.1.7). Another terphenyl structure bearing three aliphatic residues has been shown to bind to a viral protein that is crucial to the process by which HIV enters a host cell, and so the terphenyl structure can inhibit that process (section 20.7.5).

* MDM2 is produced in mice and is used for research. HDM2 is the human version of MDM2.

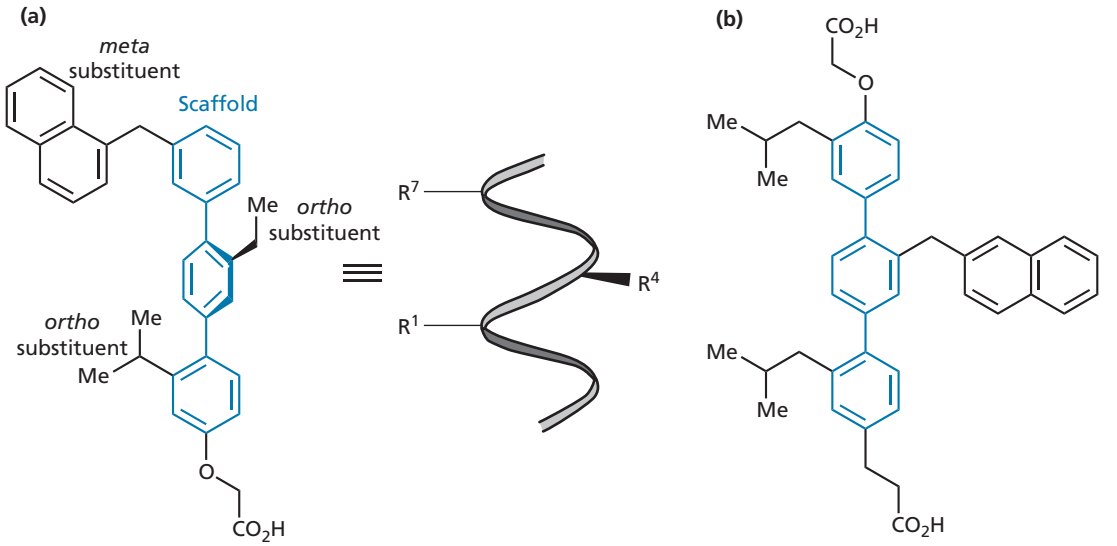



**FIGURE 10.10** (**a**) Terphenyl-based structure mimicking an α-helix. (**b**) Terphenyl structure that binds to the protein BCl-$x_L$.

Drugs that mimic β-sheets are also being investigated. Such drugs have potential as antiviral agents in the treatment of AIDS. One of the important viral proteins in the life cycle of HIV is a protease enzyme which is made up of two identical proteins interacting with each other by means of an antiparallel β-sheet (section 20.7.4.1). A drug which could mimic this feature might prevent dimerization of the protein and prevent it from functioning. Other antiviral drugs are being designed to target a variety of other protein–protein interactions involving HIV, especially those involved in the process of cell entry (section 20.7.5).

A different approach to inhibiting protein–protein interactions is to use an oligonucleotide. Oligonucleotide–protein interactions are common in the biological world and it has been shown that it is possible to obtain oligonucleotides that bind to specific protein targets with a high degree of selectivity. Such oligonucleotides are called **aptamers** (derived from the Latin *aptus,* to fit, and the Greek *meros,* part or region). A procedure called SELEX has been developed that allows researchers to find an aptamer that will bind to virtually any protein target. A library of oligonucleotides is synthesized using mixed combinatorial synthesis (Chapter 16). Each oligonucleotide is 20–40 nucleotides in length and the library contains in the order of $10^{15}$ potential aptamers. The library is tested against a particular protein target and aptamers that bind to the target are selected and amplified through cloning. Further cycles of selection and amplification can then be carried out to find the aptamer with the greatest selectivity and binding strength. This approach has been successful in generating a clinically useful aptamer called **pegaptanib**, which binds to a hormone called **vascular endothelial growth factor** (VEGF) and prevents it from binding to its receptor (VEGF-R). Activation of this receptor is important in the formation of new blood vessels (sections 21.1.9 and Box 21.11). Pegaptanib was approved in 2004 for the treatment of an eye disease where there is an overproduction of blood vessels. The aptamer is linked to polyethylene glycol (PEG) to improve the **half-life** of the agent (section 11.10).

The antibody **bevacizumab** works in a similar manner by binding to VEGF and is used as an anticancer agent (sections 21.1.9 and Box 21.12).

## 10.6 Lipids as drug targets

The number of drugs that interact with lipids is relatively small and, in general, they all act in the same way—by disrupting the lipid structure of cell membranes. For example, it has been proposed that general anaesthetics work by interacting with the lipids of cell membranes to alter the structure and conducting properties of the membranes. Another agent thought to disrupt cell membrane structure is the anticancer agent **cephalostatin I**, which is thought to span the phospholipid bilayer (section 21.8.2). Finally, **daptomycin** is an antibiotic that disrupts multiple functions of the bacterial cell membrane (section 19.6.4).

### 10.6.1 'Tunnelling molecules'

The antifungal agent **amphotericin B** (Fig. 10.11) (used topically against athlete's foot and systemically against

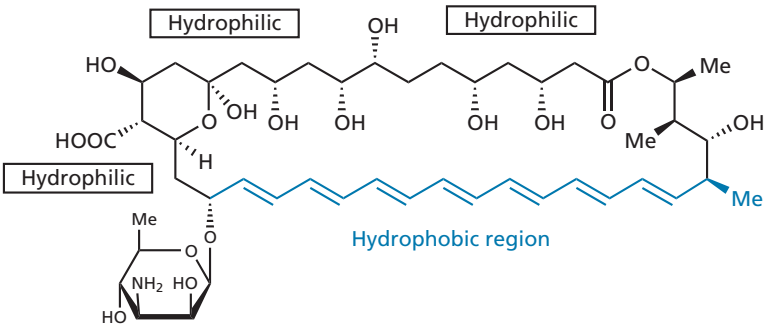



**FIGURE 10.11** Amphotericin B.

life-threatening fungal diseases) interacts with the lipids and sterols of fungal cell membranes to build 'tunnels' through the membrane. Once in place, the contents of the cell are drained away and the cell is killed.

Amphotericin B is a fascinating molecule in that one half of the structure is made up of double bonds and is hydrophobic, whereas the other half contains a series of hydroxyl groups and is hydrophilic. It is a molecule of extremes and is ideally suited to act on the cell membrane in the way that it does. Several amphotericin molecules cluster together such that the alkene chains face outwards to interact favourably with the hydrophobic centre of the cell membrane. The tunnel resulting from this cluster is lined with the hydroxyl groups and so it is hydrophilic, allowing the polar contents of the cell to drain away (Fig. 10.12a). The compound is a natural

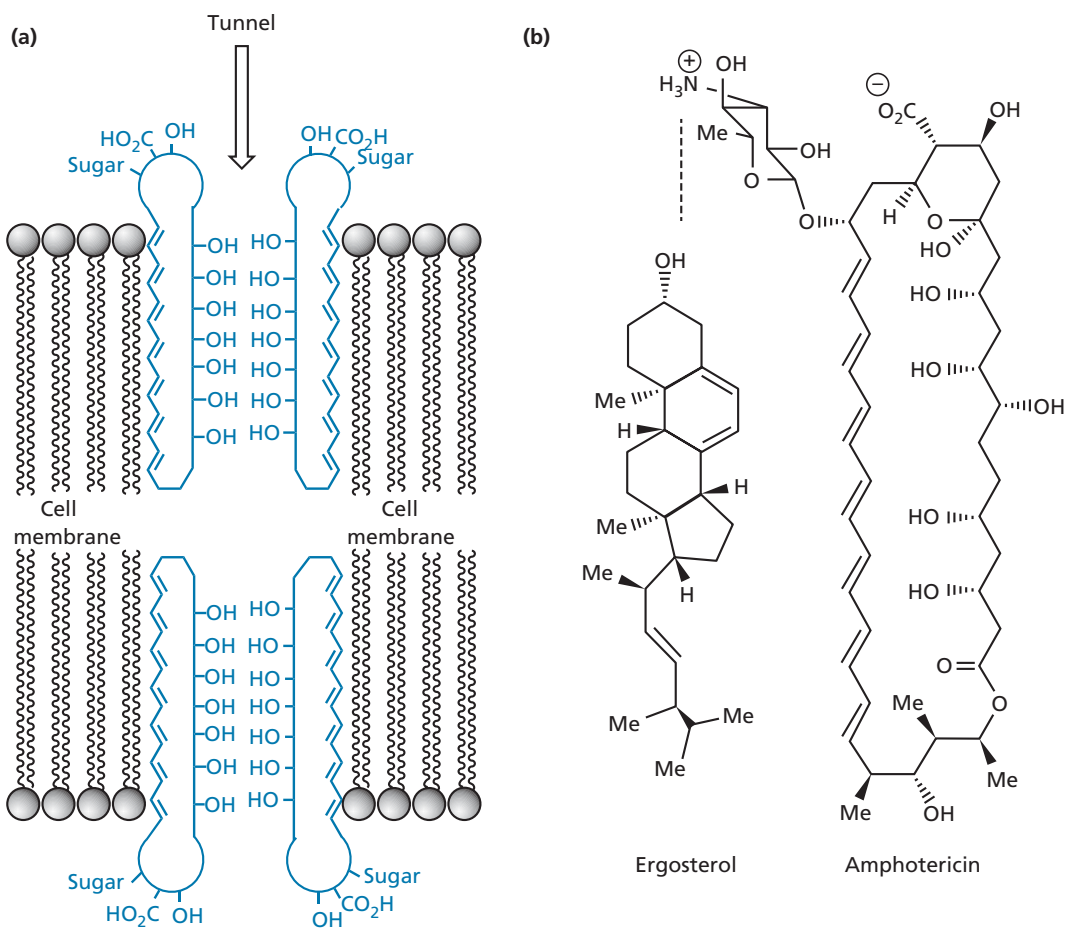



**FIGURE 10.12** (**a**) Ion channel pore through the cell membrane formed by amphotericin (ergosterol not shown). (**b**) Interaction between amphotericin and ergosterol in the ion pore channel.

Val-Gly-Ala-Leu-Ala-Val-Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp-NH-$CH_2$-$CH_2$-OH

**FIGURE 10.13** Gramicidin A.

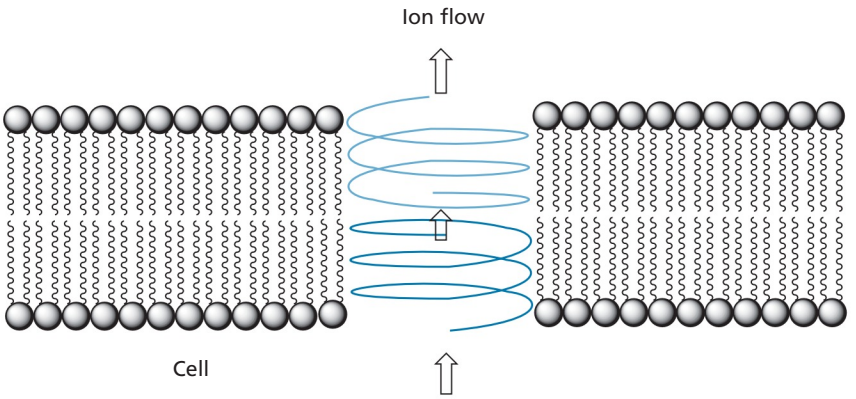



**FIGURE 10.14** Gramicidin helices aligned end-to-end to traverse the cell membrane.

product derived from a microorganism (*Streptomyces nodosus*). Recently, it has been established that each molecule of amphotericin forms a hydrogen bonding interaction with a molecule of **ergosterol** in order to create the ion pore channel. Therefore, the ion pore is actually made up of both amphotericin and ergosterol. Ergosterol is the fungal equivalent of cholesterol and is an important constituent of the fungal cell membrane. The crucial interaction involves the charged aminium group on the carbohydrate ring of amphotericin (Fig. 10.12b).

The antibiotic **gramicidin A** (Fig. 10.13) is a peptide containing 15 amino acids which is thought to coil into a helix such that the outside of the helix is hydrophobic and interacts with the membrane lipids, while the inside of the helix contains hydrophilic groups, thus allowing the passage of ions. Therefore, gramicidin A could also be viewed as an escape tunnel through the cell membrane. In fact, one molecule of gramicidin would not be long enough to traverse the membrane and it has been proposed that two gramicidin helices align themselves end-to-end in order to achieve the length required (Fig. 10.14).

**Magainins** (section 12.4.1.4) are 23-residue polypeptide antibiotics which form helical structures that also disrupt the permeability of cell membranes. However, the helices are thought to associate only with the head-groups of the cell membrane and then cause segments of the lipid membrane to bend back on themselves to form a toroidal structure or wormhole (Fig. 10.15). The magainin helices remain associated with the head-groups of the cell membrane to stabilize the pores that are formed.

Work is currently in progress to design cyclic peptides which will self-assemble in the cell membranes of bacteria to form tubules. These tubules have been labelled as 'killer nanotubes' (Fig. 10.16). Once formed, the nanotubes would allow molecules to leach out from the cell and cause cell death. The cyclic peptides concerned are

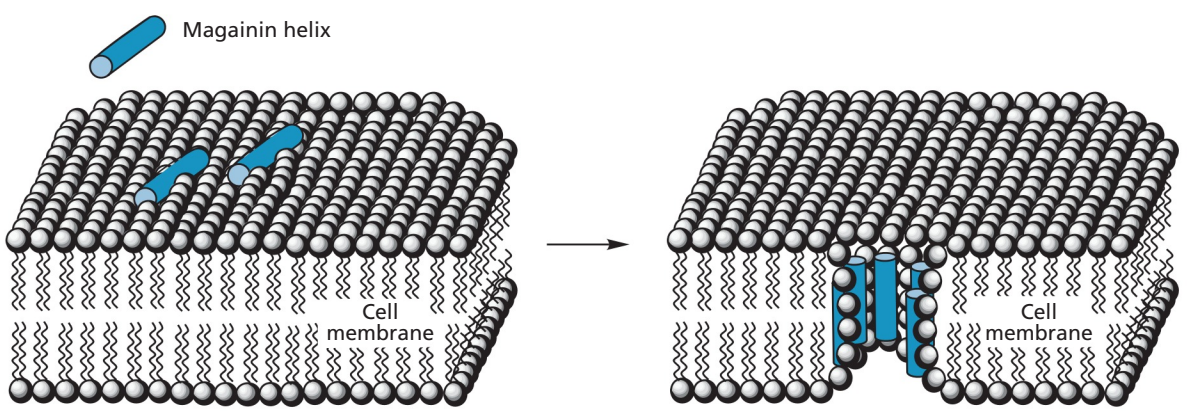



**FIGURE 10.15** The wormhole or toroidal model for magainin antibiotic action.

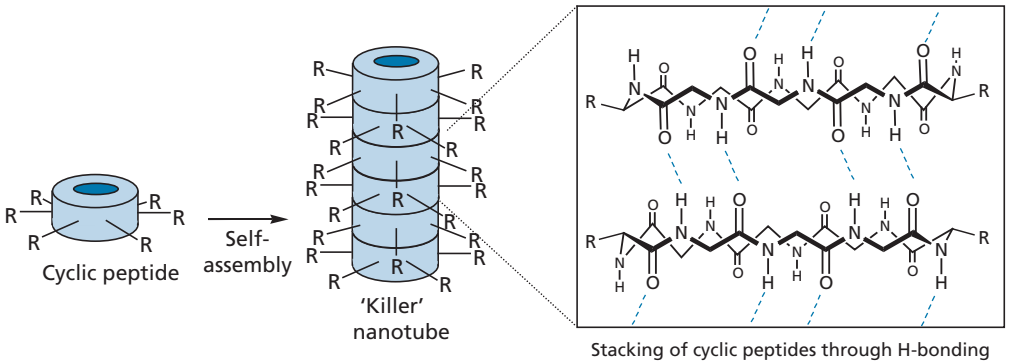



**FIGURE 10.16** Self assembly of 'killer nanotubes'.

designed to have 6–8 alternating D and L amino acids such that the amide groups are perpendicular to the plane of the cyclic structure with the side chains pointing outwards in the same plane. This means that the side chains do not interfere with the stacking process while the amide groups in each cyclic peptide form hydrogen bonds to the cyclic peptides above and below it, thus promoting the stacking process. Modifying the types of residues present has been successful in introducing selectivity *in vitro* for bacterial cells versus red blood cells. For example, the inclusion of a basic amino acid, such as lysine, is useful for selectivity. Lysine has a primary amino group which can become protonated and gain a positive charge. This encourages the structures to target bacterial membranes because the latter tend to have a negative charge on their surface. *In vivo* studies have also been carried out successfully on mice.

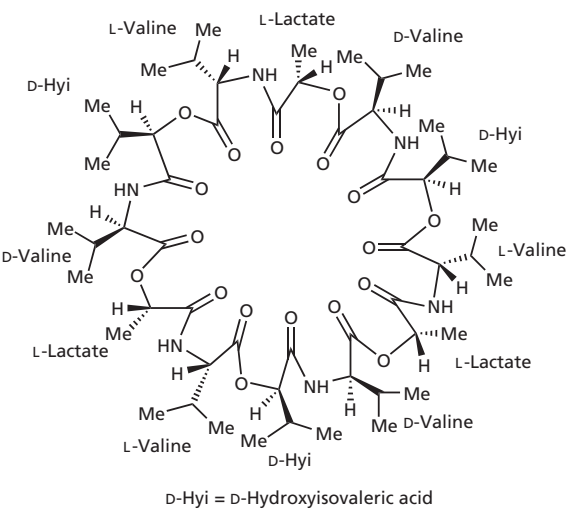



**FIGURE 10.17** Valinomycin.

## 10.6.2 Ion carriers

**Valinomycin** (Fig. 10.17) is a cyclic structure obtained from *Streptomyces* fermentation. It contains three molecules of L-valine, three molecules of D-valine, three molecules of L-lactic acid, and three molecules of D-hydroxyisovalerate. These four components are linked in an ordered fashion such that there is an alternating sequence of ester and amide linking bonds around the cyclic structure. This is achieved by the presence of a lactic or hydroxyisovaleric acid unit between each of the six valine units. Further ordering can be observed by noting that the L and D portions of valine alternate around the cycle, as do the lactate and hydroxyisovalerate units.

Valinomycin acts as an ion carrier and could be looked upon as an inverted detergent. As it is cyclic, it forms a doughnut-type structure where the polar carbonyl oxygens of the ester and amide groups face inwards, while the hydrophobic side chains of the valine and hydroxyisovalerate units point outwards. This is clearly favoured because the hydrophobic side chains can interact via van der Waals interactions with the fatty lipid interior of the cell membrane, while the polar hydrophilic groups are clustered together in the centre of the doughnut to produce a hydrophilic environment. This hydrophilic centre is large enough to accommodate an ion and it is found that a 'naked' potassium ion (i.e. one with no surrounding water molecules) fits the space and is complexed by the amide carboxyl groups (Fig. 10.18).

Valinomycin can, therefore, collect a potassium ion from the inner surface of the membrane, carry it across the membrane and deposit it outside the cell, thus disrupting the ionic equilibrium of the cell (Fig. 10.19). Normally, cells contain a high concentration of potassium ions and a low concentration of sodium ions. The fatty cell membrane prevents passage of ions between the cell and its environment, and ions can only pass through the cell membrane aided by specialized and controlled ion transport systems. Valinomycin introduces an uncontrolled ion transport system which proves fatal.

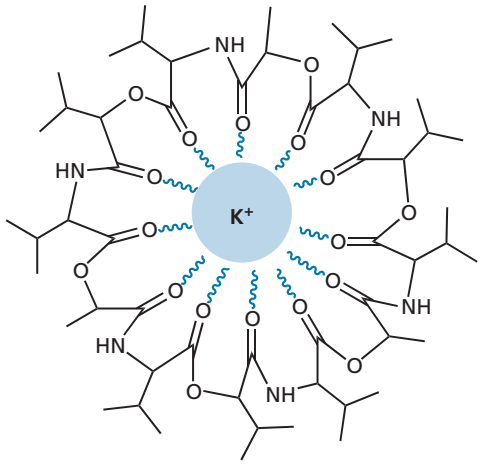



**FIGURE 10.18** Potassium ion in the hydrophilic centre of valinomycin.

Valinomycin is specific for potassium ions over sodium ions and one might be tempted to think that sodium ions would be too small to be properly complexed. The real reason is that sodium ions do not lose their surrounding water molecules very easily and would have to be transported as the hydrated ion. As such, they are too big for the central cavity of valinomycin.

The **ionophores nigericin**, **monensin A**, and **lasalocid A** (Fig. 10.20) function in much the same way as valinomycin and are used in veterinary medicine to control the levels of bacteria in the rumen of cattle and the intestines of poultry.

The polypeptide antibiotic **polymyxin B** (section 19.6.2) acts like valinomycin, but it causes the leakage of small molecules (e.g. nucleosides) from the cell, rather than ions.

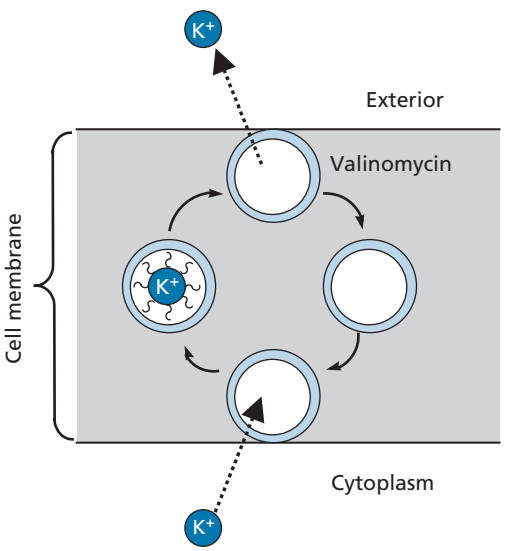



**FIGURE 10.19** Valinomycin disrupts the ionic equilibrium of a cell.

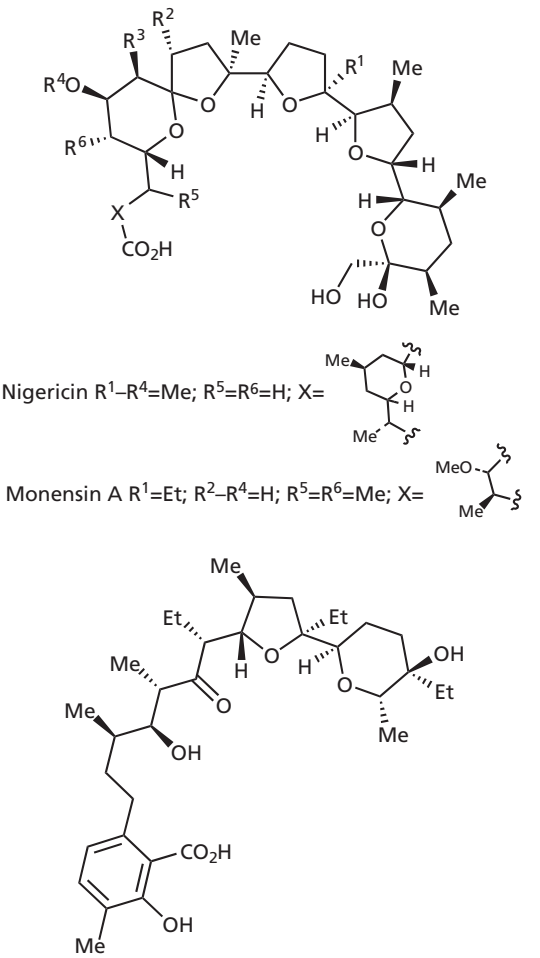



**FIGURE 10.20** Ionophores used in veterinary medicine.

## 10.6.3 Tethers and anchors

Several drugs contain hydrophobic groups that are designed to anchor the drug to the membranes of cells and organelles. These drugs are not targeting the membrane itself, but are tethered such that they interact more easily with molecular targets that are also tethered to the membrane (see sections 14.4.4 and 19.5.5.2).

**KEY POINTS**

- 'Tunnelling' molecules and ion carriers act on the plasma membrane and result in the uncontrolled movement of ions across the cell membrane leading to cell death.

- Cyclic peptides are being designed which will self-assemble to form nanotubes in the cell membranes of bacteria.
- Tethering drugs to a membrane is a useful method of targeting them against structures that are attached to membranes.

## 10.7 Carbohydrates as drug targets

### 10.7.1 Glycomics

The term **glycomics** is used to describe the study of carbohydrates, either as drugs or as drug targets. Carbohydrates are polyhydroxy structures, many of which have the general formula $C_nH_{2n}O_n$. Examples of some simple carbohydrate structures include **glucose, fructose**, and **ribose** (Fig. 10.21). These are called **monosaccharides** because they can be viewed as the monomers required to make more complex polymeric carbohydrates. For example, glucose monomers are linked together to form the natural polymers **glycogen, cellulose** (Fig. 10.22), or **starch**.

Until relatively recently, carbohydrates were not considered useful drug targets. The main roles for carbohydrates in the cell were seen as energy storage (e.g. glycogen) or structural (e.g. starch and cellulose). It is now known that carbohydrates have important roles to play in various cellular processes, such as cell recognition, cell regulation, and cell growth. Various disease states are associated with these cellular processes. For example, bacteria and viruses have to recognize host cells before they can infect them and so the carbohydrate molecules involved in cell recognition are crucial to that process (sections 20.3, 20.7.1, and 20.8.1). Designing drugs to bind to these carbohydrates may well block the ability of bacteria and viruses to invade host cells. Alternatively, vaccines or drugs may be developed based on the structure of these important carbohydrates (section 20.8.3).

It has also been observed that autoimmune diseases and cancers are associated with changes in the structure of cell surface carbohydrates (section 21.1.10). Understanding how carbohydrates are involved in cell recognition and cell regulation may well allow the design of novel drugs to treat these diseases (section 21.9).

Many of the important cell recognition roles played by carbohydrates are not acted out by pure carbohydrates, but by carbohydrates linked to proteins (**glycoproteins** or **proteoglycans**) or lipids (**glycolipids**). Such molecules are called **glycoconjugates**. Usually, the lipid or protein portion of the molecule is embedded within the cell membrane with the carbohydrate portion hanging free on the outside, like the streamer of a kite. This allows the carbohydrate portion to serve the role of a molecular tag that labels and identifies the cell. The tag may also play the role of a receptor, binding other molecules or cells.

For additional material see Web article 3: **glycosphingolipids**.

There is actually good sense in having a carbohydrate as a molecular tag rather than a peptide or a nucleic acid, because more structural variations are possible for carbohydrates than for other types of structure. For example, two molecules of alanine can only form one possible dipeptide, as there is only one way in which they can be linked (Fig. 10.23). However, because of the different hydroxyl groups on a carbohydrate, there are

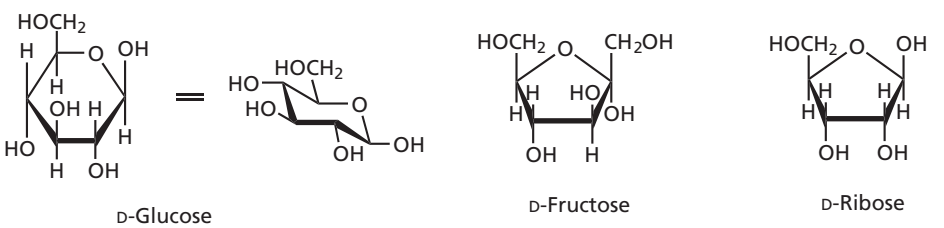



**FIGURE 10.21** Examples of monosaccharides.

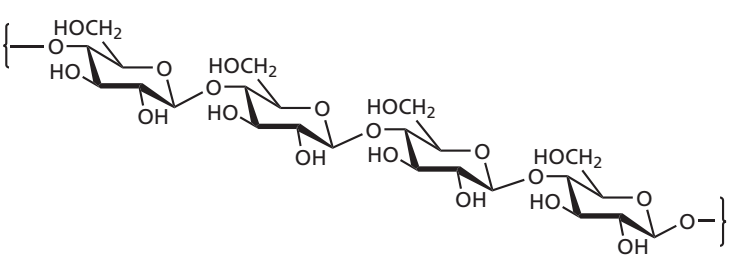


**FIGURE 10.22** Cellulose, where glucosyl units are linked β-1,4.

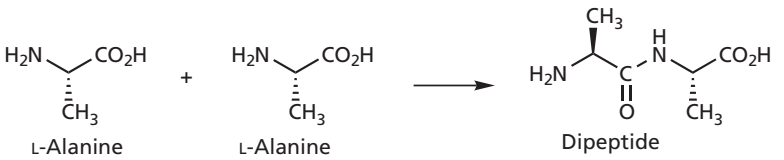



**FIGURE 10.23** Dipeptide formed from linking two L-alanines.

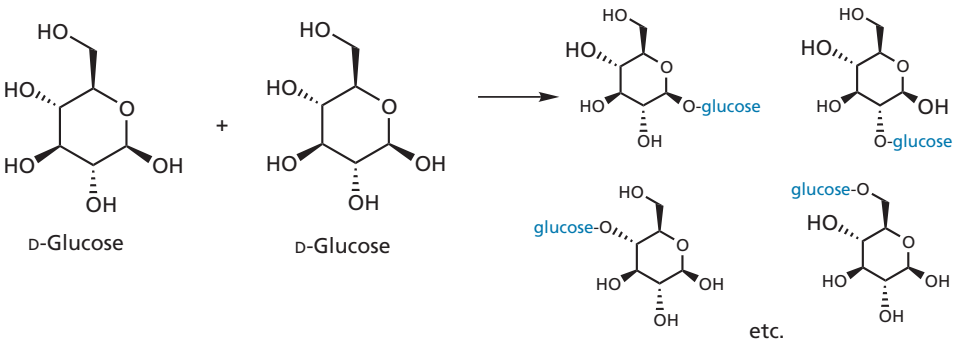



**FIGURE 10.24** Variety of carbohydrate structures formed from two glucose molecules.

11 possible disaccharides that can be formed from two glucose molecules (Fig. 10.24). This allows nature to create an almost infinite number of molecular tags based on different numbers and types of sugar units. Indeed, it has been calculated that 15 million possible structures can be derived from combining just four carbohydrate monomers.

## 10.7.2 Antigens and antibodies

The molecular tags that act as cell recognition molecules commonly act as **antigens** if that cell is introduced into a different individual. In other words, they identify that cell as being foreign. For example, bacteria have their own cell recognition molecules which are different from our own. When we suffer a bacterial infection, the immune system recognizes foreign molecular tags and produces **antibodies** which bind to them and trigger an immune response aimed at destroying the invader.

Antibodies are Y-shaped molecules that are made up of two heavy and two light peptide chains (Fig. 10.25). At the *N*-terminals of these chains there is a highly variable region of amino acids which differs from antibody to antibody. It is this region which recognizes particular antigens. Once an antigen is recognized, the antibody binds to it and recruits the body's immune response to destroy the foreign cell (Fig. 10.26). All cells (including our own) have antigens on their outer surface. They act as a molecular signature for different cells, allowing the body to distinguish between its own cells and 'foreigners'. Fortunately, the body does not normally produce antibodies against its own cells and so we are safe from attack. However, antibodies will be produced against cells from other individuals, and this poses a problem when it comes to organ transplants and blood transfusions. Therefore, it is important to get as close a match as possible between donor and recipient. Immunosuppressant

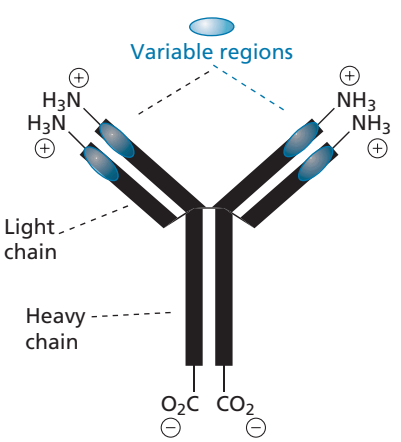



**FIGURE 10.25** Structure of an antibody.

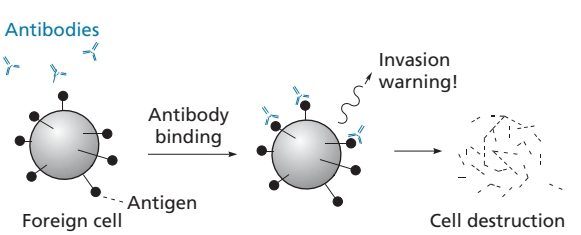



**FIGURE 10.26** Role of antibodies in cell destruction.

## **BOX 10.3** Cyclodextrins as drug scavengers

**Sugammadex** (Fig. 1) is a cyclodextrin which has been designed to scavenge the steroidal neuromuscular blocking agent **rocuronium** in order to reduce its lifetime in the blood supply. This, in turn, results in faster recovery times for patients who have undergone surgery.

Sugammadex consists of eight identical carbohydrate molecules. The faces of the carbohydrate rings form the interior of the macrocycle creating a relatively hydrophobic environment, while the hydroxyl and carboxylate groups interact with water. This makes the cyclodextrin water soluble.

The dimensions of the cyclodextrin cavity are such that the steroid is neatly encapsulated inside the cyclodextrin ring. The cavity diameter of sugammadex is 7.5–8.3Å which matches the molecular width of rocuronium (about 7.5Å). The carboxylate groups help to lock the steroid into the cyclodextrin by forming ionic interactions with the quaternary ammonium ion of the drug (Fig. 2).

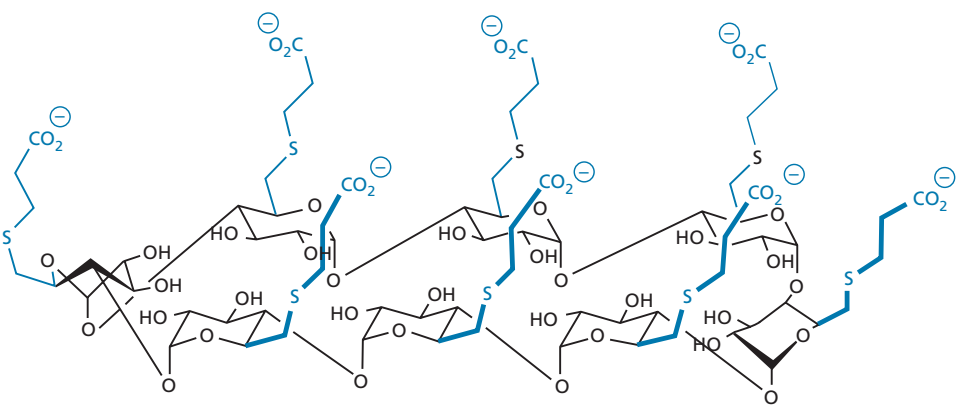


**FIGURE 1** Structure of sugammadex.

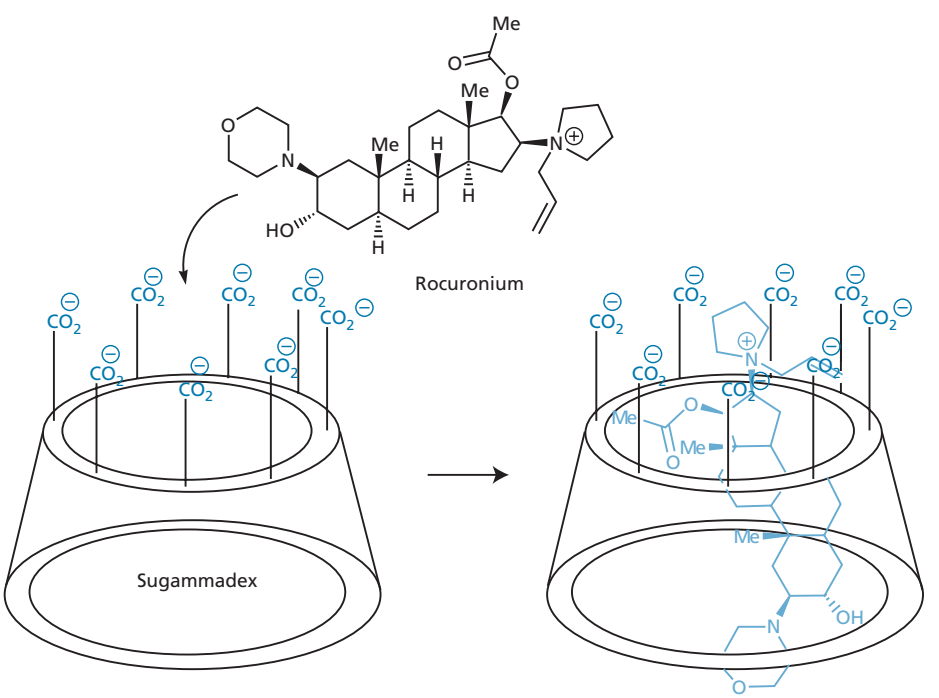



**FIGURE 2** Scavenging of rocuronium by sugammadex.

drugs may also be required to allow transplants to be accepted. Another problem can arise when proteins are being used as drugs, as these are large enough to stimulate the immune response.

There has been a lot of progress in using antibodies in the treatment of cancer by producing antibodies which will target antigens that are overexpressed on the surface of cancer cells. They can either be used by themselves to mark cancer cells out for destruction or as a means of delivering anticancer drugs to cancer cells. This is covered in more detail in sections 14.8.3 and 21.9. Antibodies have also been used in the treatment of autoimmune and inflammatory diseases (section 14.8.3).

### 10.7.3 Cyclodextrins

Cyclodextrins are macrocyclic structures made up of carbohydrate building blocks. As the interior of cyclodextrins is relatively hydrophobic and can accommodate drug-sized molecules, cyclodextrins have been extensively studied as a means of drug delivery for hydrophobic drugs. Moreover, a novel application has recently been approved for a cyclodextrin called **sugammadex**, which acts as a 'scavenger' for the neuromuscular agent **rocuronium** (Box 10.3).

For additional material see Web article 4: inside the 'doughnut': the versatile chemistry of cyclodextrins.

**KEY POINTS**

- Carrier proteins transport essential polar molecules across the hydrophobic cell membrane. Drugs can be designed to take advantage of this transport system in order to gain access to cells or to block the carrier protein.
- Tubulin is a structural protein which is crucial to cell division and cell mobility, and which is the target for several anticancer and anti-inflammatory drugs.
- Viral capsid proteins are promising targets for new antiviral agents.
- Drugs are being designed to inhibit protein–protein interactions. The drugs concerned mimic features of protein secondary structure, such as α-helices.
- General anaesthetics target the phospholipid bilayer of cell membranes.
- Several antifungal and antibacterial agents act on the cell membrane of cells. Some agents form tunnels through the cell membrane, while others act as ion carriers. In both situations, an uncontrolled passage of ions or small molecules takes place across the cell membrane, leading to cell death.
- Carbohydrates are of increasing importance as drugs or drug targets in developing new therapies for infection, cancer, and autoimmune disease.
- Carbohydrates are more challenging to synthesize than peptides, but offer a greater variety of potential novel structures.
- Antibodies are proteins that are important to the body's immune response and which can identify foreign cells or macromolecules, marking them out for destruction. They have been used therapeutically and can also be used to carry drugs to specific targets.

## QUESTIONS

1. The carboxylate groups in sugammadex play an important binding role in locking rocuronium into the central cavity of the cyclodextrin, but they also have an important role in allowing the drug access to the cavity. Suggest possible reasons for this.
2. The carboxylate groups in sugammadex are linked to the carbohydrate rings by a four-atom linker chain. Suggest whether a shorter or longer chain would make any difference, and whether there are any advantages in having the linker chain used.

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