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 which 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.

Transport proteins can also be targeted as a means of transporting polar drugs across the cell membrane and into the cell (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 that 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).

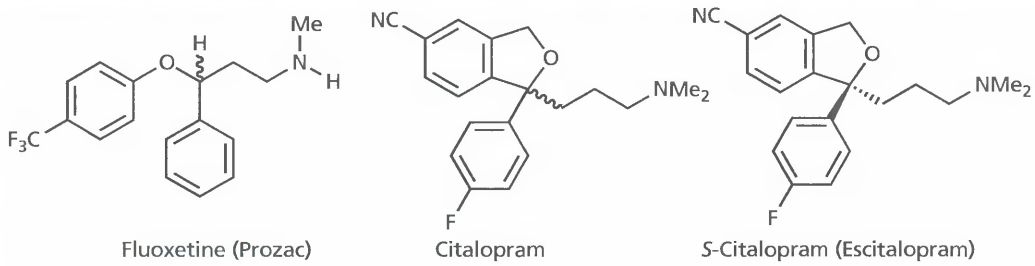
Capsid proteins are also important in the mechanism by which viruses infect host cells. The viral proteins interact with host cell proteins which are present in the cell membranes, and this triggers processes which allow the virus to enter the cell. Drugs which bind to viral proteins and inhibit this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

BOX 10.1 Antidepressant drugs acting on transport proteins

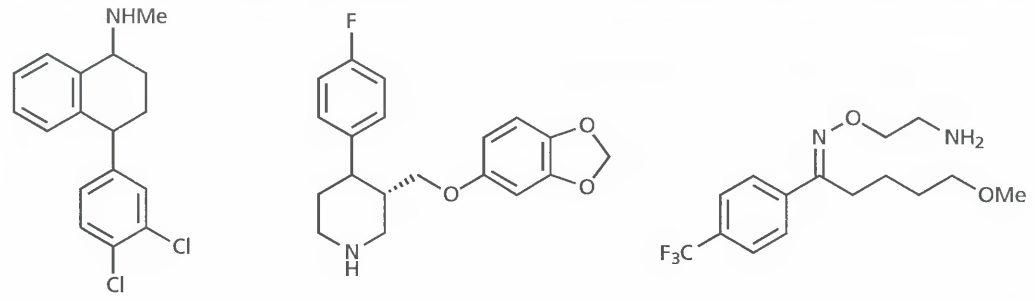
The antidepressant drugs **fluoxetine** (Prozac), **citalopram**, and **escitalopram** selectively block the transport protein responsible for the uptake of a neurotransmitter called **serotonin** from nerve synapses, and are called selective serotonin reuptake inhibitors (SSRIs). A lack of serotonin 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).



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

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

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



Sertraline Paroxetine Fluvoxamine

FIGURE 2 Further examples of SSRIs.

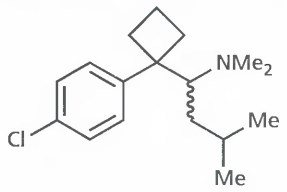


FIGURE 10.1 Sibutramine.

thus inhibit depolymerization. Either way, the balance between polymerization and depolymerization is disrupted leading 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

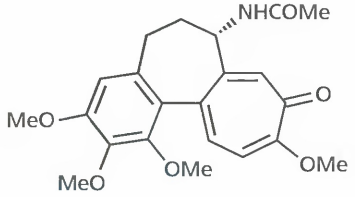


FIGURE 10.2 Colchicine.

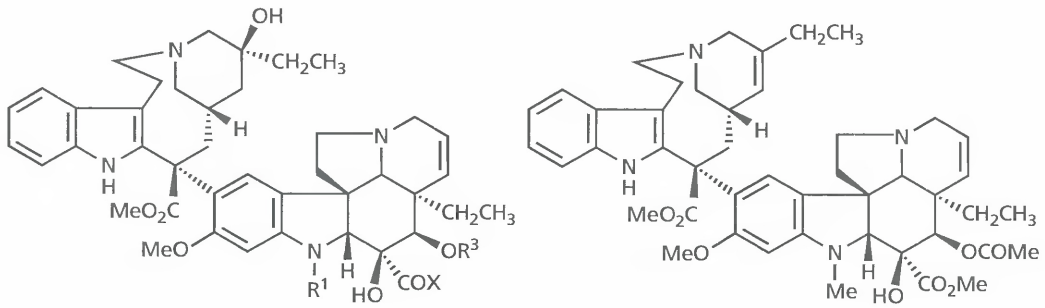
of neutrophils into joints. Unfortunately, colchicine has many side effects, and it is restricted, therefore, to the treatment of acute attacks of this disease.

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.

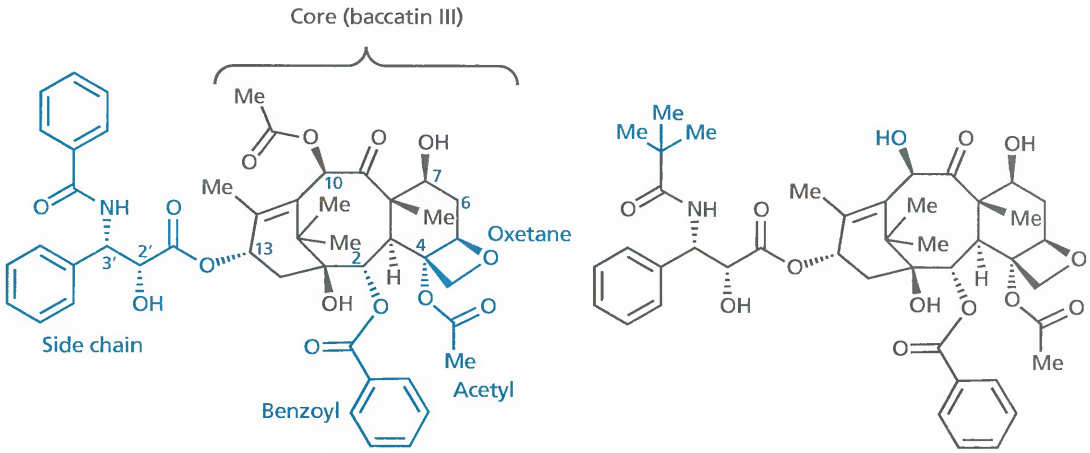
Tubulin is actually made up of two separate proteins and the taxoids are found to bind to the β-subunit of tubulin. In contrast to the drugs described in section 10.2.2.1, the binding of paclitaxel accelerates polymerization and stabilizes the resultant microtubules, which means that



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.



Paclitaxel

Docetaxel

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

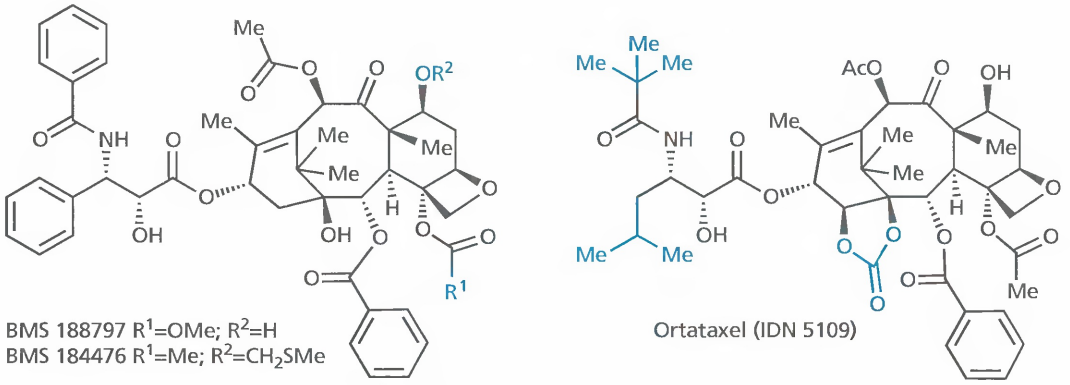


FIGURE 10.5 Analogues of paclitaxel.

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 which have recently been developed 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 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 as well as the uncoating process.
- Tubulin is a structural protein which is 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 molecular target for the antibacterial agent vancomycin is an interesting and rather unique example of a drug target in that the drug targets a biosynthetic building block. Essentially, vancomycin 'caps' the building block and prevents its incorporation into the growing bacterial cell wall. There is a small peptide chain on the building block which can bind to the drug by hydrogen bond interactions. Indeed, one could view the vancomycin molecule as providing a binding site for the building block—a kind of reversal of roles (see section 19.5.5.2).

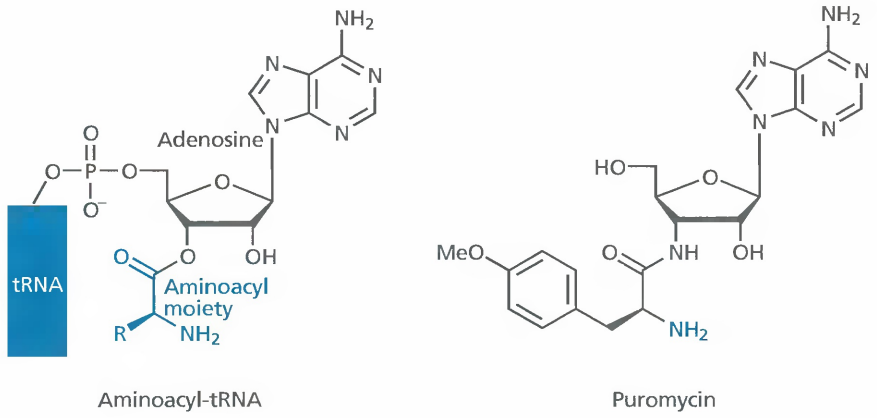


FIGURE 10.6 Comparison of puromycin and aminoacyl-tRNA.

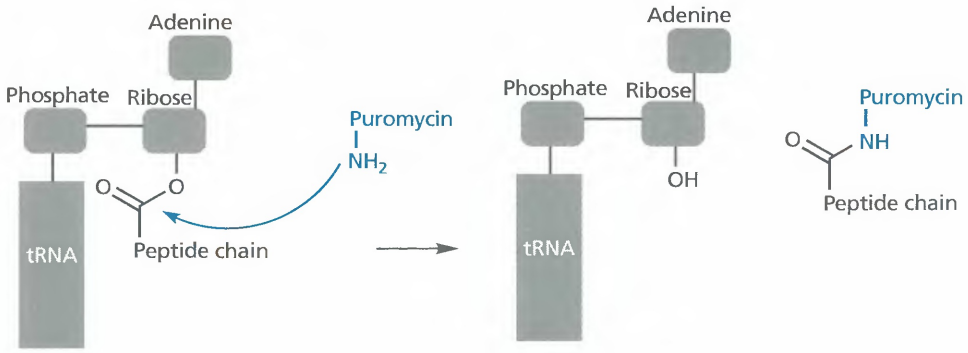


FIGURE 10.7 Transfer of peptide chain to puromycin.

10.4 Biosynthetic processes as drug targets: chain terminators

In section 9.5, we looked at antiviral drugs which 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 since it mimics the aminoacyl terminus of an aminoacyl-tRNA molecule (Fig. 10.6). Aminoacyl-tRNA is the molecule which 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 in doing so prevents 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 process. 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).

One way of inhibiting protein–protein interactions is to use antibodies (section 10.7.2), and these agents have been 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 which is used as an immunosuppressant in kidney transplants

while **abciximab** is an antibody fragment which inhibits blood clotting following angioplasty procedures aimed at unblocking coronary arteries. Successful though antibodies may be, they are limited in application to extracellular proteins, and so 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

BOX 10.2 Targeting transcription factor–coactivator interactions

The transcription of a gene is initiated by a protein complex that is formed between a transcription factor and a coactivator protein (Box 8.2). A drug that inhibits the interactions between these proteins would prevent formation of the complex, prevent transcription, and be potentially 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 coactivator 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 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.

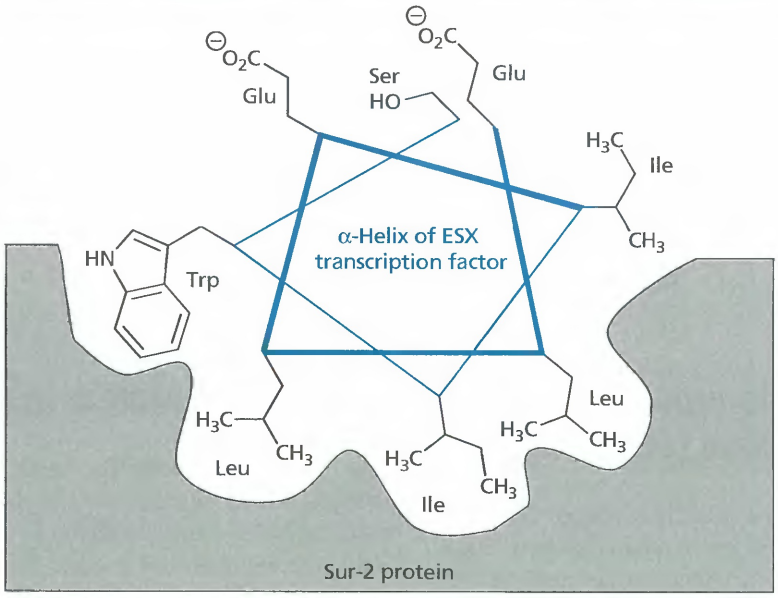


FIGURE 1 Interaction of an α-helix of the ESX transcription factor with the protein Sur-2.

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. It may also bind to a hydrophobic pocket in the coactivator 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 since 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.

(Continued)

BOX 10.2 Targeting transcription factor–coactivator interactions *(Continued)*

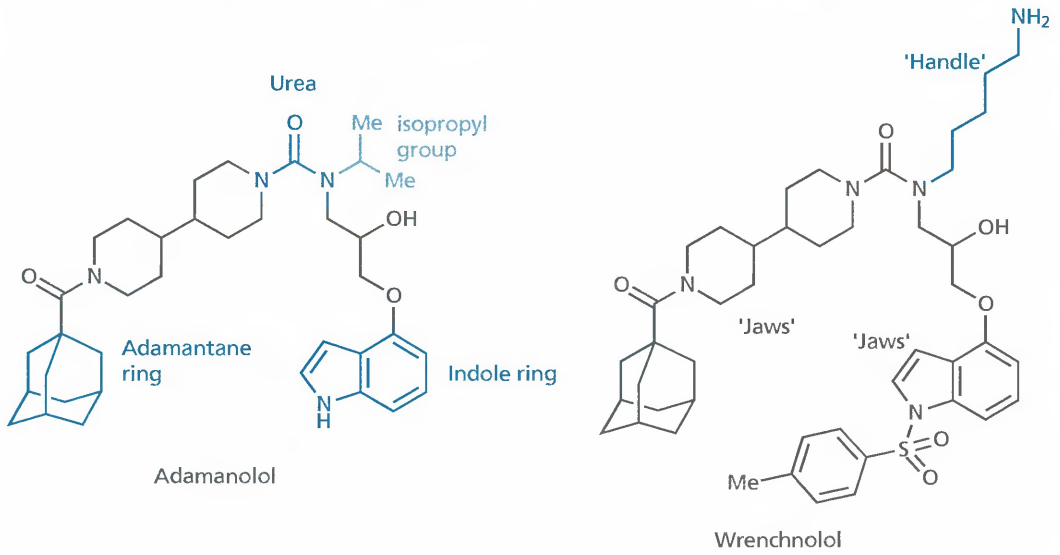


FIGURE 2 Structures of adamanolol and wrenchnolol.

protein, and protein–protein interactions involve large surface areas of the proteins involved. 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 involves 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 8 residues of the hormone interacting with 9 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 since 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, 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 for the same purpose as daclizumab above. It prevents 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) that is found in fibrinogen and 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!

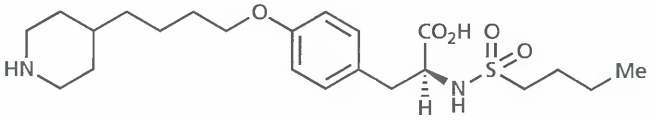


FIGURE 10.8 Tirofibin.

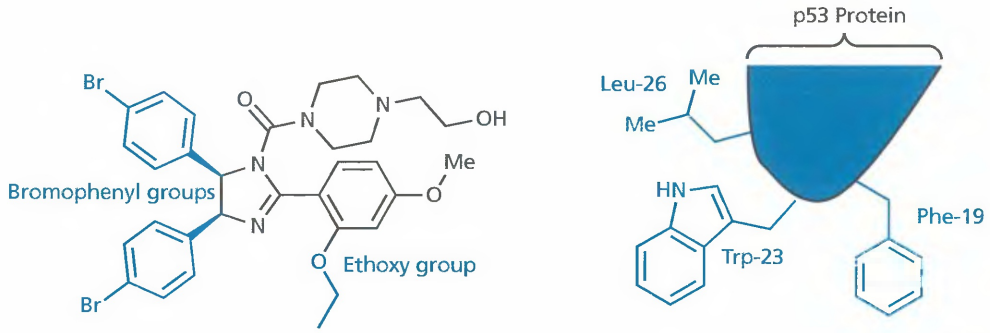


FIGURE 10.9 Nutlin-2 mimicking the three amino acid 'finger' residues of p53.

An important example of a protein–protein interaction involves the proteins p53 and MDM2 (or HDM2[1]). The former protein is produced in cells that are damaged or 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, since it suppresses the growth of defective cells such as tumour cells. MDM2 is a protein which 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 which 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 the interaction of three amino acid residues present on p53 (Leu-26, Trp-23, Phe-19). One can imagine these three amino acid residues acting as three fingers which insert into three 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 substituents 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 since 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. 10.10 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 which 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

[1] Note: MDM2 is produced in mice and is used for research. HDM2 is the human version of MDM2.

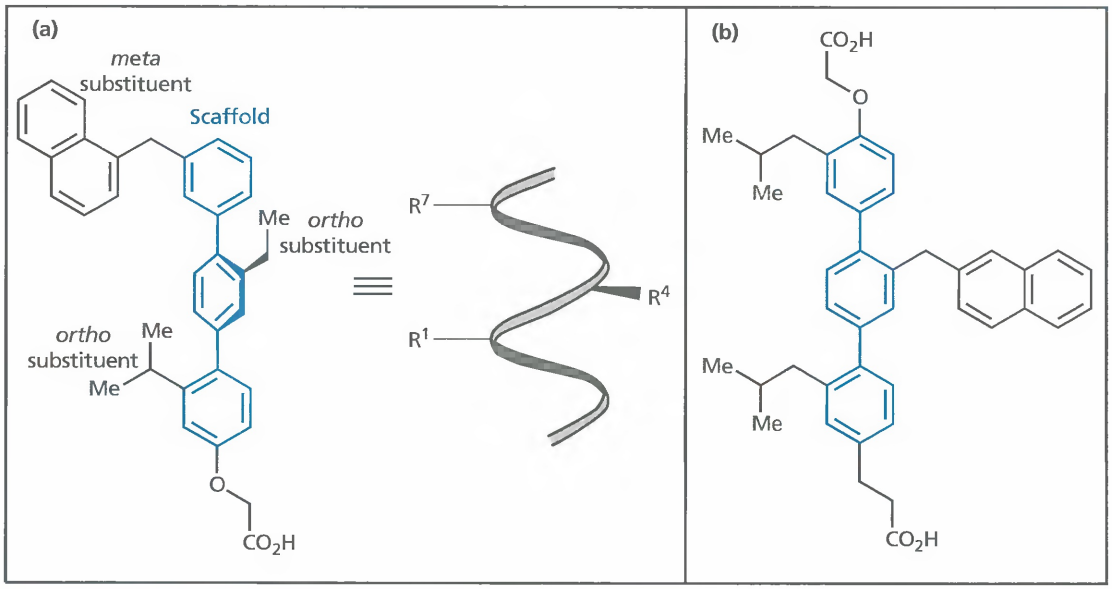


FIGURE 10.10 (a) Terphenyl-based structure mimicking an α-helix. (b) Terphenyl structure that binds to the protein BCl-x_L.

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).

Drugs which 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 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 synthesised 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 to the formation of new blood vessels (sections 21.1.9 and 21.6.2.4), and 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 11.9).

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 a drug target

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 cell membrane. Another agent which is 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 which disrupts multiple functions of the bacterial cell membrane (section 19.6.4).

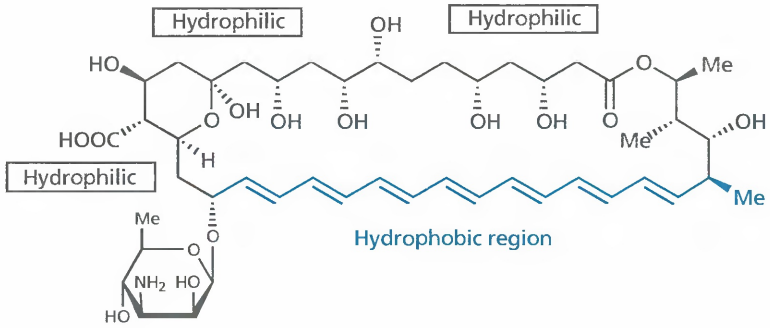


FIGURE 10.11 Amphotericin B.

10.6.1 'Tunnelling molecules'

The antifungal agent **amphotericin B** (Fig. 10.11) (used topically against athletes foot and systemically against life-threatening fungal diseases) interacts with the lipids 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 as such 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 are to the exterior and 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. 10.12). The compound is a natural product derived from a microorganism (*Streptomyces nodosus*).

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 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, then cause the segments of

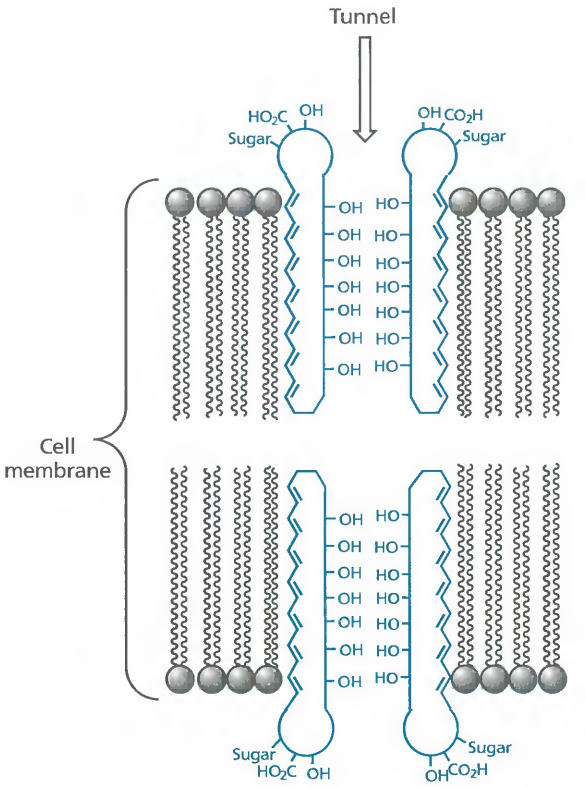


FIGURE 10.12 Amphotericin-formed channel through the cell membrane.

lipid membrane to bend back on itself 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 that 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 lead to cell death. The cyclic peptides concerned are designed

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.

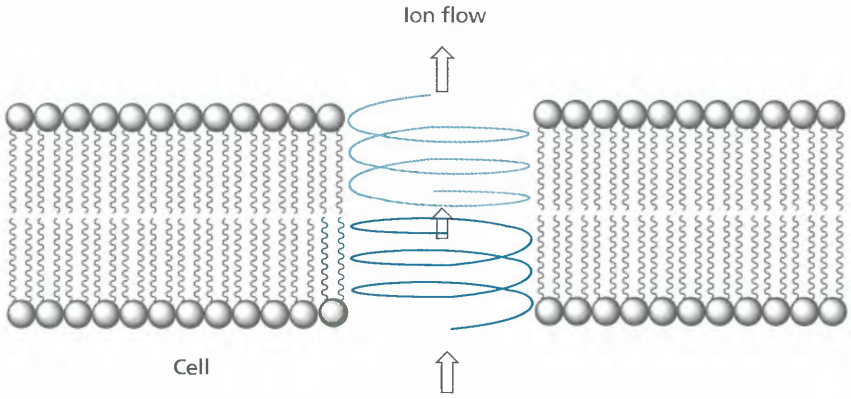


FIGURE 10.14 Gramicidin helices aligned end-to-end to traverse the cell membrane.

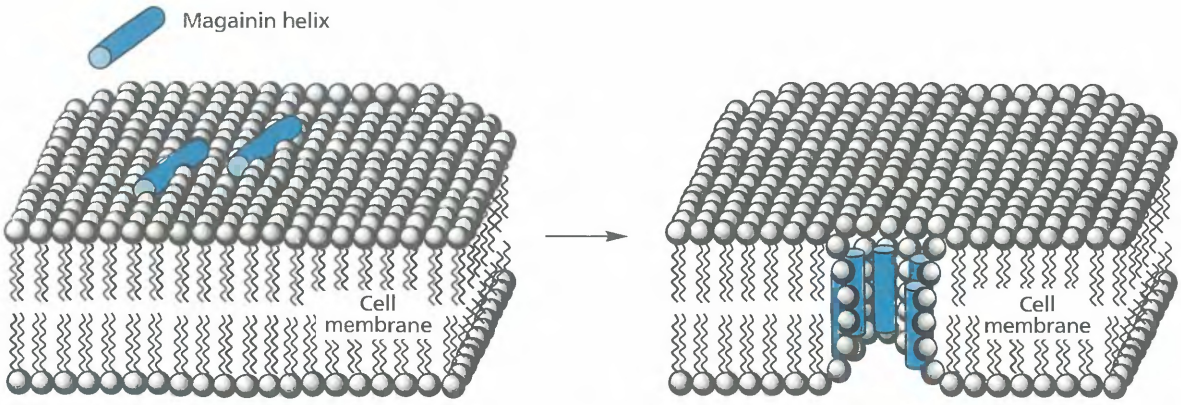


FIGURE 10.15 The wormhole or toroidal model for magainin antibiotic action.

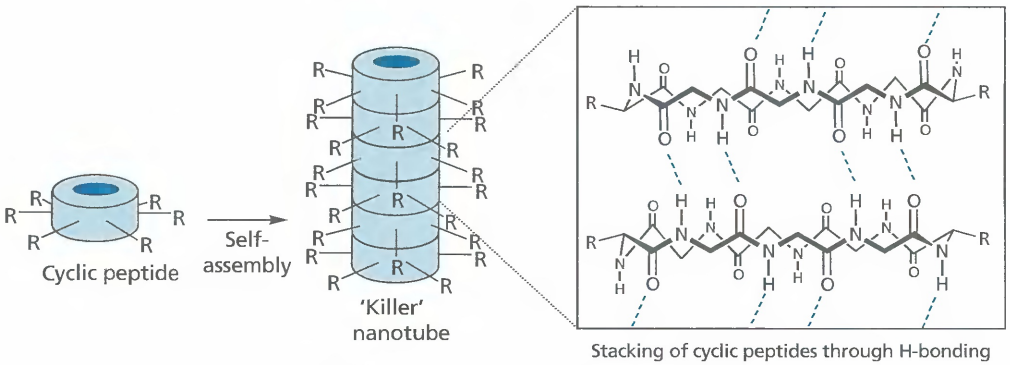


FIGURE 10.16 Self-assembly of 'killer nanotubes'.

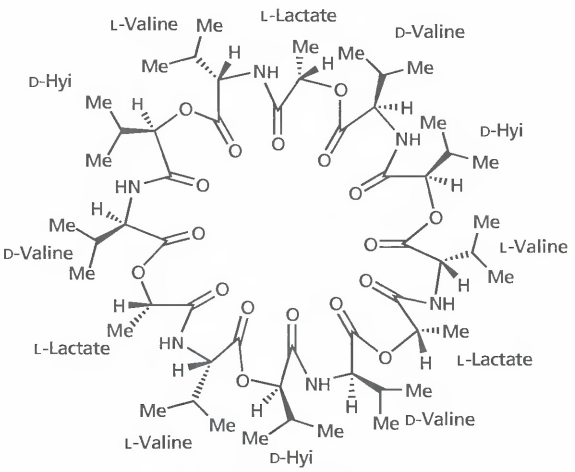
to have 6–8 alternating D and L amino acids, such that the amide groups are perpendicular to the plane of the cyclic structure, with the residues pointing outwards in the same plane. This means that the residues do not interfere with the stacking process and the amide groups in each cyclic peptide form 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, since the latter tend to have a negative charge on their surface. *In vivo* studies have also been carried out successfully on mice.

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. Since 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).



D-Hyi = D-Hydroxyisovaleric acid

FIGURE 10.17 Valinomycin.

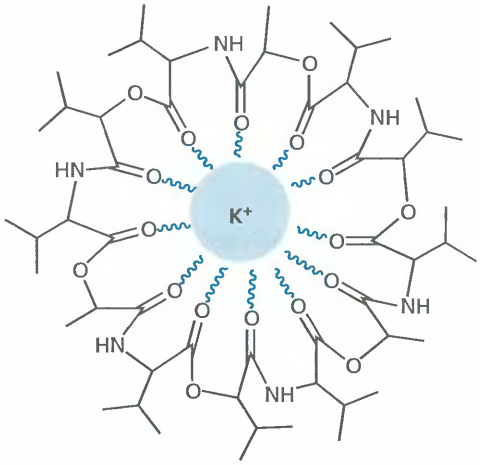


FIGURE 10.18 Potassium ion in the hydrophilic centre of valinomycin.

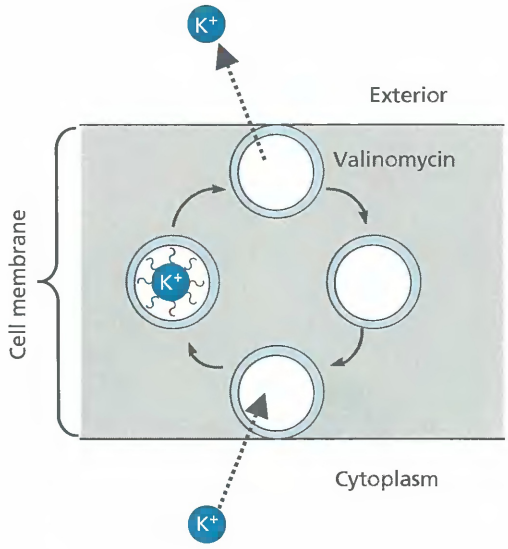


FIGURE 10.19 Valinomycin disrupts the ionic equilibrium of a cell.

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.

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.

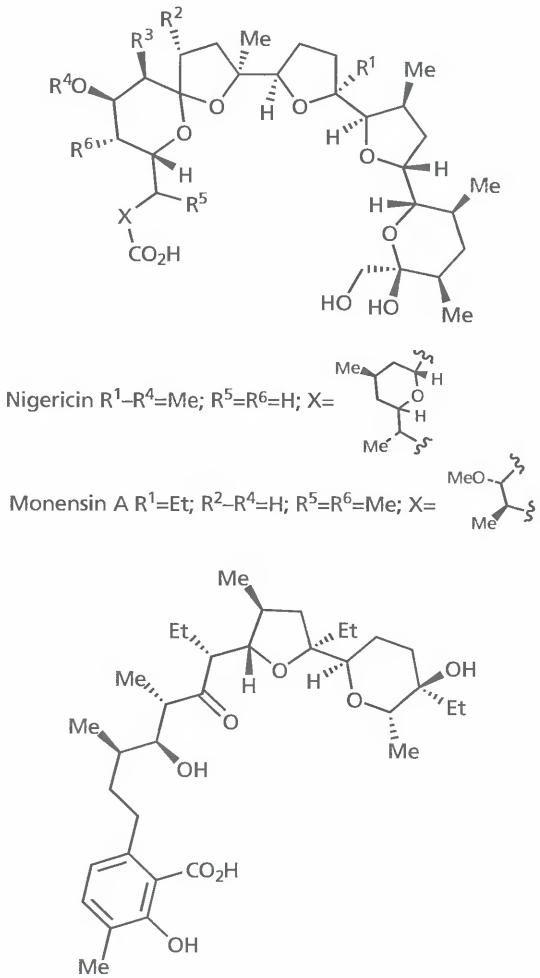


FIGURE 10.20 Ionophores used in veterinary medicine.

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.

10.7 Carbohydrates as drug targets

10.7.1 Glycomics

The term **glycomics** is used to describe the study of carbohydrates as drug targets or as drugs themselves. 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).

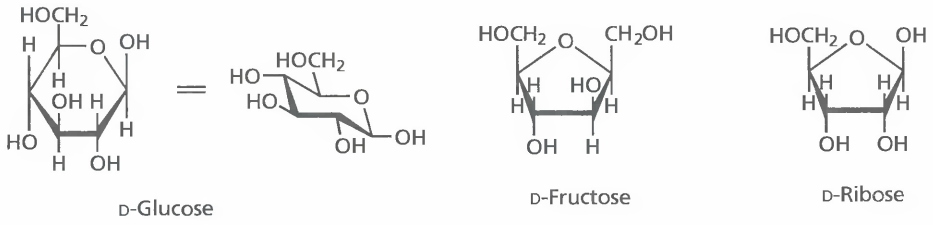


FIGURE 10.21 Examples of monosaccharides.

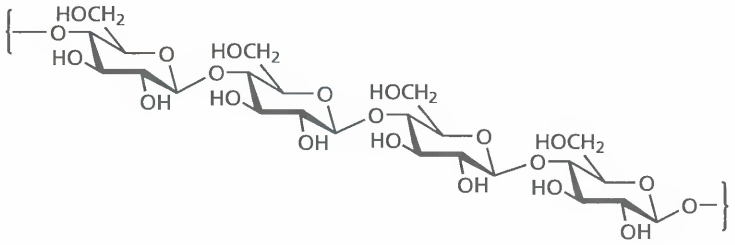


FIGURE 10.22 Cellulose, where glucosyl units are linked β-1,4.

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** (Box 10.3). 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.

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 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 the molecular tag as foreign and produces **antibodies** which bind to it and trigger an immune response aimed at destroying the invader.

Antibodies are Y shaped molecules which 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 drugs may also be required to allow transplants to be accepted. Another problem can arise when proteins are being used as drugs since 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

BOX 10.3 Glycosphingolipids

Glycosphingolipids are glycoconjugates that are thought to be important in the regulation of cell growth, and consequently have a direct bearing on diseases such as cancer. They are also responsible for labelling red blood cells and hence identifying which blood group one belongs to (A, B, AB, or O). The glycosphingolipids are made up of three components—a carbohydrate structure, which can be highly variable and complex, a structure called **sphingosine** that consists of a 2-amino-1,3-diol unit linked to a long chain hydrocarbon, and a fatty acid (e.g. **stearic acid**). The portion of the molecule consisting of the sphingosine and the fatty acid is called a **ceramide** (Fig. 1).

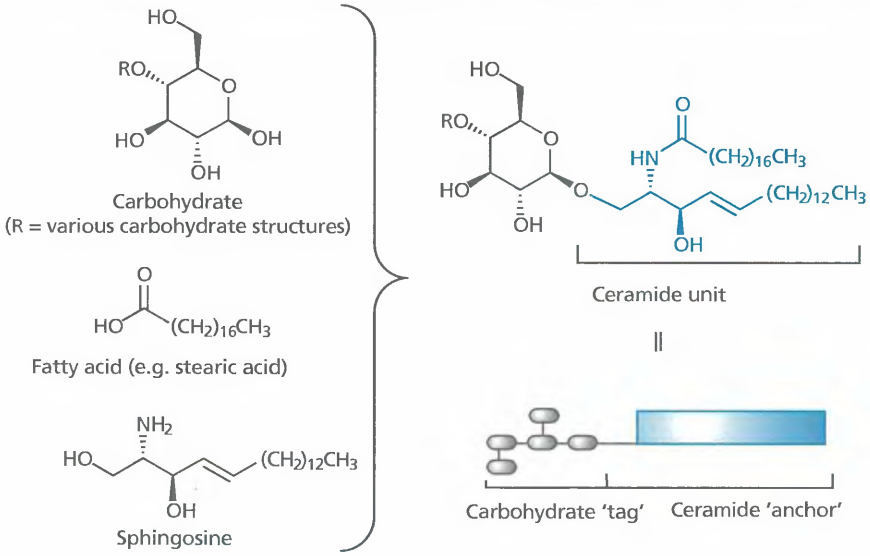


FIGURE 1 Structure of glycosphingolipids.

The ceramide portion of the molecule is hydrophobic and is embedded within the cell membrane, thus acting as an anchor for the highly polar carbohydrate section. This portion lies outside the cell membrane and acts as the molecular tag for the cell (Fig. 2).

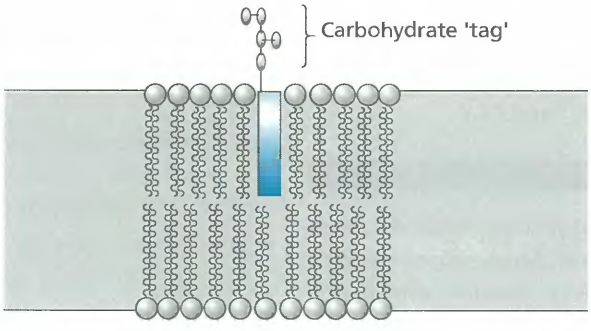


FIGURE 2 Glycosphingolipids as molecular 'tags'.

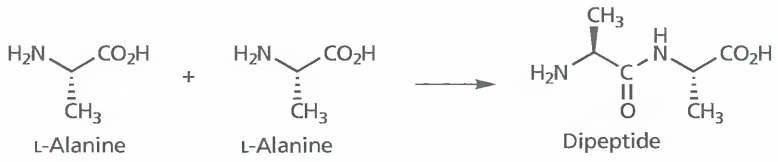


FIGURE 10.23 Dipeptide formed from linking two L-alanines.

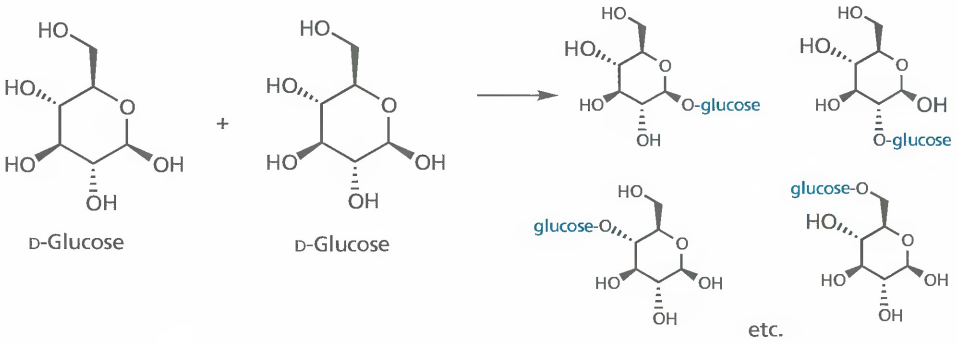


FIGURE 10.24 Variety of carbohydrate structures formed from two glucose molecules.

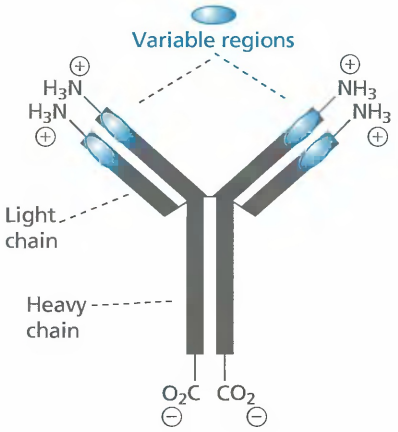


FIGURE 10.25 Structure of an antibody.

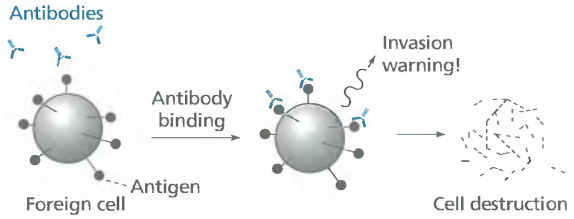


FIGURE 10.26 Role of antibodies in cell destruction.

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.

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 as 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 which 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.

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