21 Anticancer agents

21.1 Cancer: an introduction

21.1.1 Definitions

Cancer still remains one of the most feared diseases in the modern world. According to the World Health Organization, it affected one person in three and caused a quarter of all deaths in the developed world during the year 2000. After heart disease, it is the largest cause of death. Cancer cells are formed when normal cells lose the normal regulatory mechanisms that control growth and multiplication. They become 'rogue cells' and often lose the specialized characteristics that distinguish one type of cell from another (for example a liver cell from a blood cell). This is called a loss of **differentiation**. The term **neoplasm** means new growth and is a more accurate terminology for the disease. The terms **cancer** and **tumour**, however, are more commonly accepted and will be used throughout this chapter. (The word tumour actually means a local swelling.) If the cancer is localized it is said to be **benign**. If the cancer cells invade other parts of the body and set up secondary tumours—a process known as **metastasis**—the cancer is defined as **malignant**. It is malignant cancer that is life threatening. A major problem in treating cancer is the fact that it is not a single disease. There are more than 200 different cancers resulting from different cellular defects, and so a treatment that is effective in controlling one type of cancer may be ineffective on another.

21.1.2 Causes of cancer

Possibly as many as 30% of cancers are caused by smoking, while another 30% are diet related. Carcinogenic chemicals in smoke, food, and the environment may cause cancer by inducing gene mutations or interfering with normal cell differentiation. The birth of a cancer (**carcinogenesis**) can be initiated by a chemical—usually a **mutagen**—but other triggering events, such as exposure to further mutagens, are usually required before a cancer develops.

Viruses have been implicated in at least six human cancers and are the cause of about 15% of the world's cancer deaths. For example, the Epstein–Barr virus is the cause of Burkitt's lymphoma and nasopharyngeal carcinoma. Human papillomaviruses are sexually transmitted and can lead to cancer of the cervix. Hepatitis B may cause 80% of all liver cancers, and HIV can cause Kaposi's sarcoma and lymphoma. Viruses can bring about cancer in several ways. They may bring oncogenes (see below) into the cell and insert them into the genome. For example, Rous sarcoma virus carries a gene for an abnormal tyrosine kinase. Some viruses carry one or more promoters or enhancers. If these are integrated next to a cellular oncogene, the promoter stimulates its transcription leading to cancer. The bacterium *Helicobacter pylori* is responsible for many stomach ulcers (section 25.4) and is also implicated in stomach cancer.

The treatments used to combat cancer (radiotherapy and chemotherapy) can actually induce a different cancer in surviving patients. For example, 5% of patients cured of Hodgkin's disease developed acute leukaemia. Nevertheless, the risk of a second cancer is outweighed by the benefit of defeating the original one.

Some patients are prone to certain cancers for genetic reasons. Damaged genes can be passed from one generation to another, increasing the risk of cancer in subsequent generations (e.g. certain breast cancers).

21.1.3 Genetic faults leading to cancer: proto-oncogenes and oncogenes

21.1.3.1 Activation of proto-oncogenes

Proto-oncogenes are genes which normally code for proteins involved in the control of cell division and differentiation. If they are mutated, this disrupts the normal function and the cell can become cancerous. The proto-oncogene is then defined as an **oncogene**. The *ras* gene is one example. Normally, it codes for a protein called **Ras**

which is involved in the signalling pathway leading to cell division (section 5.4.2). In normal cells, this protein has a self-regulating ability and can 'switch itself off'. If the gene becomes mutated, an abnormal **Ras protein** is produced which loses this ability and is continually active, leading to continuous cell division. It has been shown that mutation of the *ras* gene is present in 20–30% of human cancers. Oncogenes may also be introduced to the cell by viruses.

21.1.3.2 Inactivation of tumour suppression genes (anti-oncogenes)

If DNA is damaged in a normal cell, there are cellular 'policemen' that can detect the damage and block DNA replication. This gives the cell time to repair the damaged DNA before the next cell division. If repair does not prove possible, the cell commits suicide (**apoptosis**). **Tumour suppression genes** are genes which code for proteins that are involved in these processes of checking, repair, and suicide. *TP53* is an important example of such a gene and codes for the protein of the same name (**p53 protein**). If the *TP53* gene is damaged, the repair mechanisms become less efficient, defects are carried forward from one cell generation to another, and, as the damage increases, the chances of the cell becoming cancerous increase.

21.1.3.3 The consequences of genetic defects

Genetic defects can lead to the following cellular defects, all of which are associated with cancer:

- abnormal signalling pathways;
- insensitivity to growth-inhibitory signals;
- abnormalities in cell cycle regulation;
- evasion of programmed cell death (apoptosis);
- limitless cell division (immortality);
- ability to develop new blood vessels (angiogenesis);
- tissue invasion and metastasis.

It is thought that most, if not all, of these conditions have to be met before a defective cell can spawn a life-threatening malignant growth. Thus, a single defect can be kept under control by a series of safeguards. This can explain why cancers may take many years to develop after exposure to a damaging mutagen, such as asbestos or coal dust. That first exposure may have caused mutations in some cells, but cellular chemistry has the control systems in place to cope and to keep the cells in check. However, a lifetime's exposure to other damaging mutagens, such as tobacco smoke, results in further genetic damage which overwhelm the safeguards one by one until the abnormal cell finally breaks free of its shackles and becomes cancerous.

The various hurdles and safeguards that a potential cancer cell has to overcome explains why cancers are relatively rare early on in life and are more common in later years. This also helps to explain why cancer is so difficult to treat once it does appear. As so many cellular safeguards have already been overcome, it is unlikely that tackling one specific cellular defect is going to be totally effective. As a result, traditional anticancer drugs have tended to be highly toxic agents and act against a variety of different cellular targets by different mechanisms. Unfortunately, because they are potent cellular poisons, they also affect normal cells and produce serious side effects. Such agents are said to be **cytotoxic** and dose levels have to be chosen which are high enough to affect the tumour but are bearable to the patient. In recent years, anticancer drugs have been developed which target specific abnormalities in a cancer cell, allowing them to be more selective and have less serious side effects. However, bearing in mind the number of defects in a cancer cell, it is unlikely that a single agent of this kind will be totally effective, and it is more likely that these new agents will be most effective when they are used in combination with other drugs having different mechanisms of action, or with surgery and radiotherapy.

We now look at the various defects that are common in cancer cells.

21.1.4 Abnormal signalling pathways

Whether a normal cell grows and divides depends on the various signals it receives from surrounding cells. The most important of these signals come from hormones called growth factors. These are extracellular chemical messengers which activate protein kinase receptors in the cell membrane (sections 4.8 and 5.4). The receptors concerned trigger a signal transduction pathway which eventually reaches the nucleus and instructs the transcription of the proteins and enzymes required for cell growth and division. Most, if not all, cancers suffer from some defect in this signalling process such that the cell is constantly instructed to multiply. The signalling process is complex, so there are various points at which it can go wrong.

Many cancer cells are capable of growing and dividing in the absence of external growth factors. They can do this by producing the growth factor themselves, then releasing it such that it stimulates its own receptors, often by autophosphorylation. Examples include **platelet-derived growth factor** (**PDGF**) and **transforming growth factor** α (**TGF-α**). Other cancer cells can produce abnormal receptors which are switched on constantly despite the lack of growth factors (e.g. **Erb-B2 receptors** in breast cancer cells). It is also possible for receptors to be overexpressed. This means that an oncogene is too active and

codes for excessive protein receptor. Once this is in the cell membrane, the cell becomes supersensitive to low levels of circulating growth factor.

There are many points where things could go wrong in the signal transduction pathways. For example, the **Ras protein** is a crucial feature in the signal transduction pathways leading to cell growth and division. Abnormal Ras protein is locked in the 'on' position and is constantly active despite the lack of an initial signal from a growth factor.

21.1.5 Insensitivity to growth-inhibitory signals

Several external hormones such as **transforming growth factor β** (**TGF-β**) counteract the effects of stimulatory growth factors, and signal the inhibition of cell growth and division. Insensitivity to these signals raises the risk of a cell becoming cancerous. This can arise from damage to the genes coding for the receptors for these inhibitory hormones—the tumour suppression genes.

21.1.6 Abnormalities in cell cycle regulation

A cycle of events takes place during cell growth and multiplication which involves four phases known as G_1, S, G_2, and M (Fig. 21.1). As part of this process, decisions have to be made by the cell whether to move from one stage to another, depending on the balance of those chemical signals promoting growth and those inhibiting it.

The G_1 phase (gap 1) is where a cell is actively growing in size and preparing to copy its DNA in response to various growth factors or internal signals. The next phase is the S phase (synthesis) where replication of DNA takes place. Once the cell's chromosomes are copied, there is another interval called the G_2 phase (gap 2) during which the cell readies itself for cell division. This gap, or interval, is crucial, as it gives the cell time to check the copied DNA and to repair any damaged copies. Finally, there is the M phase (mitosis) where cell division takes place to produce two daughter cells, each containing a full set of chromosomes. The daughter cells can then enter the cell cycle again (G_1). Alternatively, they may move into a dormant or resting state (G_0).

Within the cell cycle, there are various decision points which determine whether the cell should continue to the next phase. For example, there is a decision point called the **restriction point** (R) during the G_1 phase which frequently becomes abnormal in tumour cells. There are also various surveillance mechanisms known as checkpoints which assess the integrity of the process. For example, a delay will take place during the G_2 phase if DNA damage is detected. This gives sufficient time for damaged DNA to be repaired or for the cell to commit suicide (**apoptosis**). These checkpoints can also be defective in tumour cells.

Control of the cell cycle involves a variety of proteins called **cyclins** and enzymes called **cyclin-dependent kinases** (**CDKs**) (Fig. 21.2). There are at least 15 types of cyclin and nine types of CDK, and each has a role to play at different stages of the cell cycle. Examples are shown in Fig. 21.2. Binding of a cyclin with its associated kinase activates the enzyme and serves to move the cell from one phase of the cell cycle to another. For example, when a cell is in the G1 phase, a decision has to be made whether to move into the S phase and start copying DNA. This decision is taken depending on the balance of stimulatory versus inhibitory signals being received through signal transduction. If the balance is towards cell growth and division there is an increase in **cyclin D**. This binds to **CDK4** and **CDK6**. The resulting

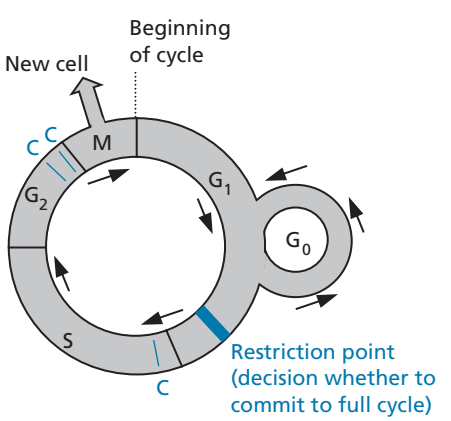


FIGURE 21.1 The cell cycle. G_1, gap 1—cell enlarges and makes new proteins; S, synthesis of DNA; G_2, gap 2—cell prepares to divide; M, mitosis—cell divides; G_0, resting stage—no growth; C, checkpoints.

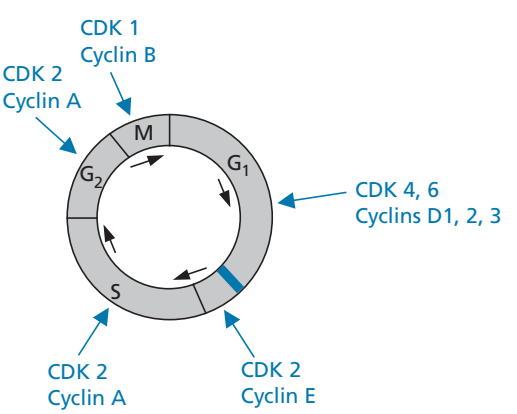


FIGURE 21.2 Control of the cell cycle by cyclins and cyclin-dependent kinases.

complexes phosphorylate a powerful growth-inhibitory molecule known as **pRB** which normally binds and inactivates a transcription factor. Phosphorylation alters pRB such that it can no longer bind to the transcription factor and the latter is free to bind to specific regions of DNA. This results in the transcription of specific genes which leads to the production of proteins capable of moving the cell towards the S phase (e.g. **cyclin E** and **thymidine kinase**). Once cyclin E has been produced, it combines with **CDK2** and this complex is responsible for progression from the G1 phase to the S phase. Other activated cyclin–CDK complexes are important in different phases of the cell cycle. For example, the **cyclin A–CDK2** complex is required for progression through the S phase and a **cyclin B–CDK1** complex is necessary for mitosis.

Restraining proteins are present which can modify the effect of cyclins (Fig. 21.3). These include **p15** and **p16** which block the activity of the cyclin D–CDK complex. Another is the inhibitory protein **p21** which is controlled by **p53**—an important protein that monitors the health of the cell and the integrity of DNA.

To sum up, progression through the cell cycle is regulated by sequential activation of cyclins and CDKs—a process which can be down-regulated by the CDK inhibitors. The whole process is normally tightly controlled, such that there is an accumulation of a relevant cyclin-CDK complex followed by rapid degradation of the complex once its task is complete.

Overactive cyclins or CDKs have been associated with several cancers. For example, breast cancer cells often produce excess cyclins D and E, and skin melanoma has lost the gene that codes for the inhibitory protein p16. Half of all human tumours lack a proper functioning p53 protein, which means that the level of the inhibitory protein p21 falls. In viral-related cervical cancers both the pRB and p53 proteins are often disabled.

Oncogenic alteration of cyclins, CDKs, **cyclin-dependent kinase inhibitors** (**CKIs**), and other components of the pRB pathway have been reported in 90% of human cancers, especially in the G1 phase. Thus, excessive production of cyclins or CDKs, or insufficient production of CKIs, can lead to a disruption of the normal regulation controls and result in cancer. Efforts have been made to identify how one can restore the control of the cancer cell cycle by targeting molecular abnormalities. These can include CDK inhibition, down-regulation of cyclins, up-regulation of CDK inhibitors, degradation of cyclins, or inhibition of tyrosine kinases that trigger the cell cycle activation in the first place.

21.1.7 Apoptosis and the p53 protein

There is a built-in cellular destruction process called **apoptosis**, which is the normal way in which the body protects itself against abnormal or faulty cells. Essentially, each cell monitors itself for a series of different chemical signals.

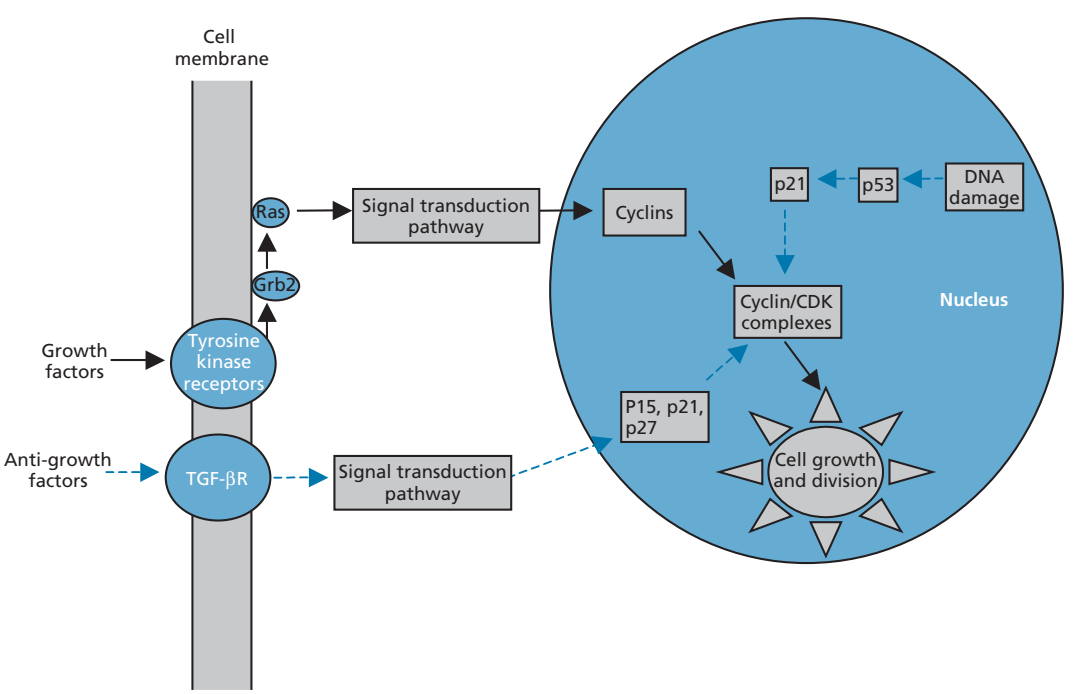


FIGURE 21.3 Cell signalling pathways. Black arrows indicate pathways that stimulate cell growth and division. Blue arrows indicate pathways that inhibit cell growth and division.

Should any of these be absent, a self-destruct mechanism is automatically initiated (Fig. 21.4). Apoptosis is also important in destroying cells that escape from their normal tissue environment. Cancer cells which metastasize have undergone genetic changes that allow them to avoid this process.

Two distinct pathways for apoptosis have been characterized.

- An **extrinsic route** where apoptosis results from external factors. This can take three forms. Firstly, there could be a sustained lack of growth factors or hormones. Secondly, there are proteins called **death activator proteins** which can bind to cell membrane proteins called **tumour necrosis factor receptors** (**TNF-R**). This triggers a signalling process initiating apoptosis. Finally, the immune system produces T-lymphocytes which circulate the body searching for damaged cells. Once found, the lymphocyte perforates the cell membrane of the damaged cell and injects an enzyme called **granzyme**, which initiates apoptosis.
- An **intrinsic pathway** can arise from factors such as DNA damage arising from exposure to chemicals, drugs, or oxidative stress. The cell has monitoring systems which can detect damage and lead to the increased production of the tumour suppressor protein **p53**. At sufficient levels, this protein will trigger apoptosis.

The various signals described above converge on the mitochondria which contain proteins capable of promoting apoptosis, in particular **cytochrome c**. Release of cytochrome c from mitochondria results in the assembly of a large oligomeric protein complex known as an **apoptosome**, which is made up of a scaffolding protein called **Apaf-1**. The apoptosome then recruits and activates an enzyme known as **procaspase 9**, which, in turn, activates **caspases**. Caspases are protease enzymes containing a cysteine residue in the active site which is important to the catalytic mechanism. Because they are proteases, they set about destroying the cell's proteins, which leads to destruction of the cell.

Considering the fatal effect caspases have on the cell, it is not surprising that there are various checks and balances to ensure that apoptosis does not occur too readily. A family of proteins regulate the process. Proteins such as **Bad** and **Bax** promote it, while others, such as **Bcl-2** and **Bcl-X**, suppress it. The relative levels of these proteins is dependent on the various monitoring procedures within the cell. For example, genetic damage leading to increased levels of p53 induces apoptosis by up-regulating the expression of Bax.

The survival of each cell in the body is, therefore, dependent on the balance of internal and external signals regulating cell growth, as well as the balance of regulatory chemicals promoting or inhibiting apoptosis. A defect in the complex systems leading to apoptosis could inhibit apoptosis, increasing the likelihood of carcinogenesis. For example, it has been found that the gene coding for p53 is the most frequently mutated gene

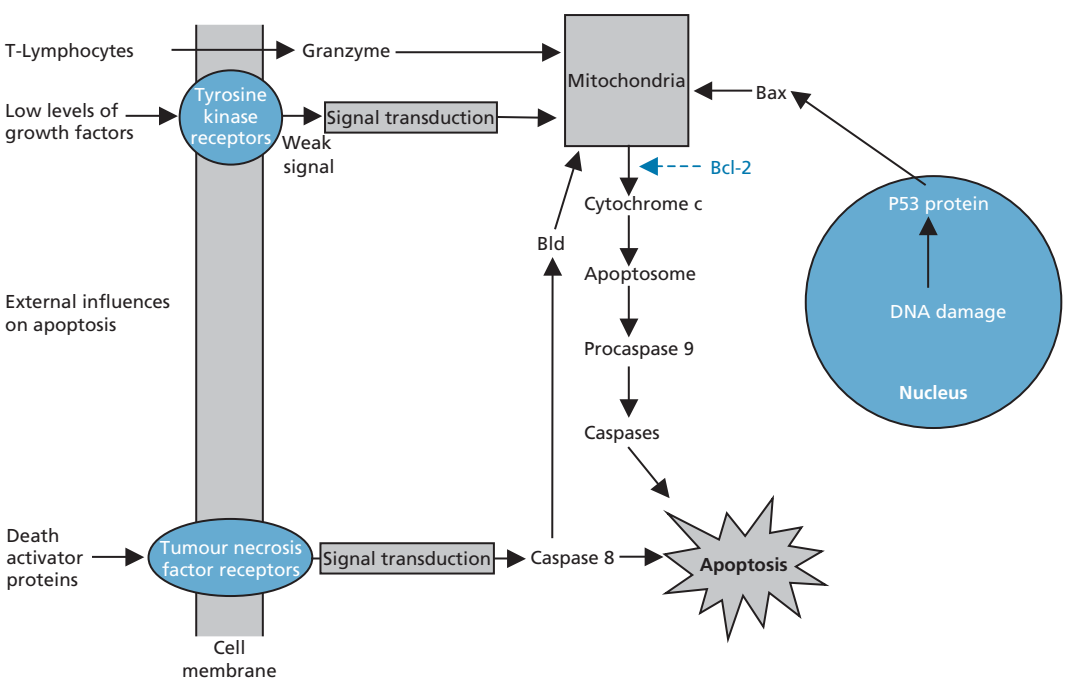


FIGURE 21.4 Signals leading to apoptosis.

in cancer (30–70%). Damage to this gene means a lack of the apoptosis-inducing p53 protein and an increased chance that the defective cell will survive to become cancerous. The genes coding for the apoptosis suppressors Bcl-2 and Bcl-X_L are also known to be overexpressed in several tumour types. Another genetic defect that has been found in many tumour cells is the overexpression of **HDM2**, a protein that binds to p53 and prevents it from functioning as a transcription factor (section 10.5).

Defects in the apoptosis mechanisms also have serious consequences for radiotherapy and many chemotherapeutic drugs, as both these procedures act by triggering apoptosis. For example, many traditional anticancer drugs damage DNA. This in itself may not be fatal to the cell, but the cell's monitoring system detects the damage and goes into self-destruct mode. If the mechanisms involved are defective then apoptosis does not occur, the drugs are not effective, and the cell becomes immortal.

21.1.8 Telomeres

Cancer cells are often described as becoming 'immortal'. This is because there is no apparent limit to the number of times they can divide. The lifetime of normal cells is predetermined by the possible number of times their DNA can be replicated (about 50–60 cell divisions).

Structures called **telomeres** play a key role in this immortalization process. Figure 21.5 shows the structure of a **chromatin**, which consists of a chromosome wrapped round a variety of proteins. The telomere consists of a polynucleotide region at the 3' end of a chromosome, and contains several thousand repeats of a short (six base-pair) sequence. The purpose of the telomere is to act as a 'splice' for the end of the chromosome and to stabilize and protect the DNA. After each replication process, about 50–100 base pairs are lost from the telomere because **DNA polymerase** is unable to completely replicate the 3' ends of chromosomal DNA. Eventually, the telomere becomes too short to be effective and the DNA becomes unstable, either unravelling or linking up with another DNA end to end. This proves fatal to the cell and apoptosis is triggered.

It is observed that in the early stages of cancer many cancer cells are also restricted to the number of times they can divide, but a cancer cell eventually develops which breaks free of this restriction and becomes immortal. These cells maintain the length of their telomere by expressing an enzyme called **telomerase**—a member of a group of enzymes called the **RNA-dependent DNA polymerases**. Telomerase has the ability to add hexanucleotide repeats on to the end of telomeric DNA and thus maintain its length. This is an important process during the development of an embryo when telomerase is responsible for creating the telomeres in the first place, but, after birth, the gene encoding the enzyme is suppressed. Immortal cells have found a way of removing that suppression such that the enzyme is expressed once more. The telomerase enzyme is expressed in over 85% of cancers.

Several efforts have been made to design drugs which will inhibit telomerase, but, to date, none have reached the clinic.

21.1.9 Angiogenesis

As a tumour grows, its cancerous cells require a steady supply of amino acids, nucleic acid bases, carbohydrates, oxygen, and growth factors if they are to continue multiplying. This means that the tumour has to have a good blood supply. As a tumour grows in size, however, its cells become increasingly remote from the blood supply and become starved of these resources. Oxygen levels also fall resulting in a state of **hypoxia**. This is particularly true for

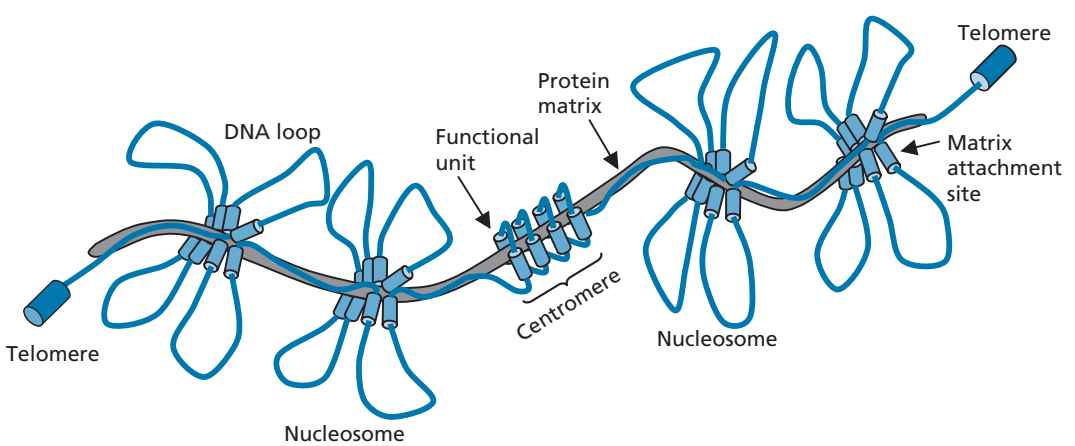


FIGURE 21.5 Chromatin, telomeres, and DNA.

the cells in the centre of the tumour. As a result of this, **hypoxia-inducible factors**, such as **HIF-1**, start to build up within the tumour cells, and these factors serve to up-regulate genes that promote survival in oxygen-starved environments. For example, growth factors are released from the cell, such as **vascular endothelial growth factor** (**VEGF**) and **fibroblast growth factor** (**FGF-2**), which interact with receptors on the endothelial cells of nearby blood vessels and stimulate these cells to divide, leading to the branching and extension of existing capillaries—a process known as **angiogenesis** (Fig. 21.6). Vascular growth factors are present in normal cells and are usually released when tissues have been damaged. The resulting angiogenesis helps in the repair of the injured tissues and is normally controlled by angiogenesis inhibitors, such as **angiostatin** and **thrombospondin**. Unfortunately, this balance is disturbed in tumour growth. As a result, tumours are able to receive the increased blood supply required for their survival. Moreover, the chances of cancer cells escaping from the primary source and metastasizing are increased, not only because of the increased availability of blood vessels, but also because the newly developing endothelial cells can release proteins, such as **interleukin-6**, that stimulate metastasis. The blood vessels arising from angiogenesis are abnormal in that they are disorganized in structure, dilated, and leaky. The cells also display molecules called **integrins** on their surface which are absent from mature vessels and which protect the new cells from apoptosis. Before angiogenesis can begin, the basement membrane round the blood vessels has to be broken down and this is carried out by enzymes known as **matrix metalloproteinases (MMPs)**. This then allows the endothelial cells to migrate towards the tumour. Dissolution of the matrix also allows angiogenesis factors to be released to encourage angiogenesis.

Inhibiting angiogenesis is a tactic which can help to tackle cancer, and drugs have been developed that inhibit angiogenesis and break down the abnormal blood vessels. Angiogenesis inhibitors are generally safer and less toxic than traditional chemotherapeutic agents, but are unlikely to be used on their own. Instead, they will probably be used alongside standard cancer treatments, such as surgery, chemotherapy, and radiation. Angiogenesis inhibitors appear to 'normalize' the abnormal blood vessels of tumours before they kill them. This normalization can help anticancer agents reach tumours more effectively. In the longer term it serves to stall tumour growth then shrink it by breaking up abnormal capillaries. As a result, the tumour becomes starved of nutrients and growth should decrease.

Some anticancer treatments take advantage of the leaky blood vessels which result from angiogenesis. Anticancer drugs can be encapsulated into liposomes, nanospheres, and other drug delivery systems which are too big to escape from normal blood vessels, but can escape through the walls of the leakier blood vessels supplying the tumour. As a result, the anticancer drug is concentrated at the tumour. As tumours do not generally develop an effective lymphatic system, the polymeric drug delivery systems tend to be trapped at the tumour site.

Even with angiogenesis, there are regions of a well-developed tumour which fail to receive an adequate blood supply. As a result, cells in the centre of the tumour are starved of oxygen and nutrients, and may well stop growing and become dormant. This can pose a serious problem, as most anticancer drugs act best on actively dividing cells. Anticancer therapy may well be successful in halting a cancer and eliminating most of it, but once the treatment is stopped, the dormant cells start multiplying and the tumour reappears. Worryingly, it has been observed that such cells are more likely to metastasize.

Another consequence of an insufficient blood supply and lack of oxygen (hypoxia) is that cells in the centre of

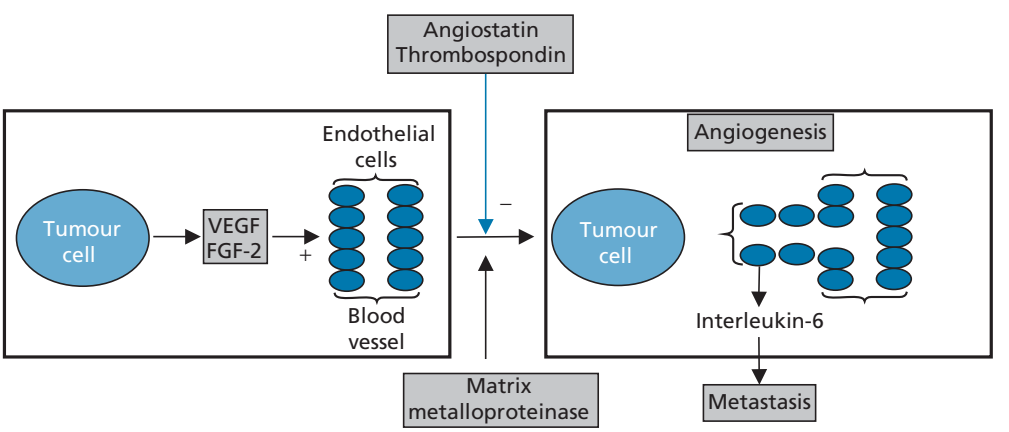


FIGURE 21.6 Angiogenesis.

the tumour are forced to revert to glycolysis in order to produce energy, which leads to a build-up of acidic by-products within the cell. The cells address this problem by exporting acidic protons into the extracellular space. As a result, the environment around tumours tends to be more acidic than in normal tissues. Several anticancer therapies have attempted to take advantage of this difference in acidities, for example, the selective localization of porphyrins in photodynamic therapy.

Angiogenesis inhibitors are relatively safe, so it may be possible to use these drugs as a prophylactic to prevent the appearance of cancer in susceptible individuals.

Several of the drugs described in the following sections inhibit angiogenesis. These include **combretastatin** (section 21.5.1), **VEGF receptor kinase inhibitors** (section 21.6.2.4), **matrix metalloproteinase inhibitors** (section 21.7.1), **TNP-470** (section 21.7.5), **thalidomide** (section 21.8.1), **endostatin** and **angiostatin** (section 21.8.3), and **bevacizumab** (Box 21.12).

21.1.10 Tissue invasion and metastasis

Not all cancers are life threatening. Benign tumours are growths which remain localized in a particular part of the body and can grow to the size of a football without a fatal result. Malignant cancers, however, are life threatening because the cells involved have the ability to break away from the primary tumour, invade a blood vessel or a lymphatic vessel, travel through the circulation, and set up tumours elsewhere in the body. In order to do this, these cells have to overcome a series of controls that are designed to keep cells in their place.

Cells have a molecular signature on their surface which identifies whether they are in the correct part of the body or not. These are cell adhesion molecules (e.g. **E-cadherin**) which ensure that cells adhere to cells of similar character and to an insoluble meshwork of protein filling the space between them—the extracellular matrix. This is particularly true of epithelial cells—the cell layers forming the outer surface of skin and the lining of the gut, lungs, and other organs.

Adhesion to the extracellular matrix is particularly important, as it is necessary if cells are to survive. Molecules called **integrins** are involved in the anchoring process. If a normal cell becomes detached, it stops growing and apoptosis is triggered. This prevents cells from one part of the body straying to other parts of the body. Moreover, normal cells can only survive if their adhesion molecules match the relevant extracellular matrix.

Cell adhesion molecules are missing in metastasized cancer cells, allowing them to break away from the primary tumour. Such cells also appear to be anchorage independent: they do not self-destruct once they have become free and can latch on to extracellular matrix in other parts of the body to set up secondary tumours. It is thought that oncogenes in these cells code for proteins which send false messages back to the nucleus implying that the cell is still attached.

It is noticeable that most cancers derive from epithelial cells. Once an epithelial cell has gained the ability to split away from its neighbours, it needs to gain access to the blood supply if it is to spread round the body. However, epithelial cells grow on a basement membrane—a thin layer of extracellular matrix which acts as a physical barrier to the movement of the cells. Cancer cells and white blood cells are the only cells capable of breaching this barrier. White blood cells need to do this in order to reach areas of infection, whereas cancer cells breach the barrier to spread the disease. Both types of cell contain the **matrix metalloproteinase** enzyme that hydrolyses the proteins composing the barrier. Once a cancer cell breaks through the basement barrier, it has to break down a similar barrier surrounding the blood vessel in order to enter the blood supply. It then spreads round the body carried by the blood supply until it finally adheres to the blood vessel and breaks out by the opposite process in order to reach new tissue. It is estimated that fewer than 1 in 10,000 such cells succeed in setting up a secondary tumour, but it only needs one such cell to do so, and once metastasis has occurred, the prospects of survival are slim. Circulating tumour cells usually get trapped in the first network of capillaries they meet and this is where they are most likely to set up secondary tumours. For most tissues, the focus for secondary tumours will be the lungs. In the case of cells originating from the intestines, it is the liver. Some cancer cells produce factors that cause platelets to initiate blood clotting around them such that they increase in size, become stickier, and stick to the blood vessel wall, allowing them to escape.

21.1.11 Treatment of cancer

There are three traditional approaches to the treatment of cancer—surgery, radiotherapy, and chemotherapy. This chapter is devoted to cancer chemotherapy, but it is important to appreciate that chemotherapy is normally used alongside surgery and radiotherapy. Moreover, it is often the case that combination therapy (the simultaneous use of various anticancer drugs with different mechanisms of action) is more effective than using a single drug. The advantages include increased efficiency of action, decreased toxicity, and evasion of drug resistance.

As cancer cells are derived from normal cells, identifying targets that are unique to cancer cells is not easy. As a result, most traditional anticancer drugs act against targets which are present in both types of cell. Therefore, the effectiveness and selectivity of such drugs is dependent on

them becoming more concentrated in cancer cells than normal cells. This often turns out to be the case, as cancer cells are generally growing faster than normal cells and so they accumulate nutrients, synthetic building blocks, and drugs more quickly. Unfortunately, not all cancer cells grow rapidly; cells in the centre of a tumour may be dormant and evade the effects of the drug. Conversely, there are normal cells in the body which grow rapidly, such as bone marrow cells. As a result, they too accumulate anticancer drugs, resulting in bone marrow toxicity—a common side effect of cancer chemotherapy which results in a weakening of the immune response and a decreased resistance to infection. Indeed, many cancer patients are prone to pathogens which would not normally be infectious. Such secondary infections can be difficult to treat and care has to be taken over which antibacterial drugs are used. For example, bacteriostatic antibacterial agents may not be effective as they rely on the normal functioning of the immune system. Other typical side effects of traditional anticancer drugs are impaired wound healing, loss of hair, damage to the epithelium of the gastrointestinal tract, depression of growth in children, sterility, teratogenicity, nausea, and kidney damage.

Most traditional anticancer drugs work by disrupting the function of DNA and are classed as cytotoxic. Some act on DNA directly; others (antimetabolites) act indirectly by inhibiting the enzymes involved in DNA synthesis. Having said that, cancer chemotherapy is now entering a new era which can be described as **molecular targeted therapeutics**—highly selective agents which target specific molecular targets that are abnormal or overexpressed in the cancer cell. Progress in this area has arisen from a better understanding of the cellular chemistry involved in particular cancer cells. The development of kinase inhibitors such as **imatinib (Glivec)** is a much-heralded illustration of this approach (section 21.6.2.2). The use of antibodies and gene therapy is another area of research which shows huge potential (section 21.9).

Knowledge of the cell cycle is important in chemotherapy. Some drugs are more effective during one part of the cell cycle than another. For example, drugs which affect microtubules are effective when cells are actively dividing (the M-phase), whereas drugs acting on DNA are more effective if the cells are in the S-phase. Some drugs are effective regardless of the phase; for example alkylating agents, such as **cisplatin.** For this reason, anticancer drugs are most effective against cancers which are proliferating rapidly as they are more likely to become susceptible when they reach the relevant part of the cell cycle. Conversely, slower growing cancers are less effectively treated.

A better understanding of the molecular mechanisms behind specific cancers is yielding better and more specific treatments, but the importance of detecting cancer early on cannot be overemphasized. Unfortunately, the physical symptoms of most tumours do not become apparent until they are well established. By that time, it may be too late. Therefore, it is preferable to detect actual, or potential, tumours before symptoms arise. **Personalized medicine** is an approach which is likely to become increasingly important (section 6.1.5). The genetic analysis of tumours in individual patients allows the early detection and identification of cancer, as well as identifying the best treatment to be used for a particular individual. This approach is already used in determining which patients will benefit from the anticancer agents **Herceptin** (Box 21.12) and imatinib. Genetic fingerprinting should also identify individuals at risk to particular cancers so that they can be screened regularly.

Although cancer is difficult to treat, there have been notable successes in treating rapidly growing cancers, such as Hodgkin's disease, Burkitt's lymphoma, testicular cancer, and several childhood malignancies. Early diagnosis also improves the chances of successful treatment in other cancers. At present, four cancers account for over half of all new cases (lung, breast, colon, and prostate).

Finally, one of the best ways of reducing cancers is to reduce the risk. Public education campaigns are important in highlighting the dangers of smoking, excessive drinking, and hazardous solvents, as well as promoting healthy diets and lifestyles. The benefits of eating high-fibre foods, fruit, and vegetables are clear. Indeed, there have been various research projects aimed at identifying the specific chemicals in these foods which are responsible for this protective property. For example, dithiolthiones are a group of chemicals in broccoli, cauliflower, and cabbage which appear to have protective properties, one of which involves the activation of enzymes in the liver to detoxify carcinogens. **Genistein** (Fig. 21.7) is a protective compound found in soy products used commonly in Asian diets. It is notable that Asian populations have a low incidence of breast, prostate, and colon cancers. **Epigallocatechin gallate**, an antioxidant present in green tea, is another potential protective agent. Synthetic drugs are also being investigated as possible cancer preventives (e.g. **finasteride**, **aspirin**, **ibuprofen**, and **difluoromethylornithine**).

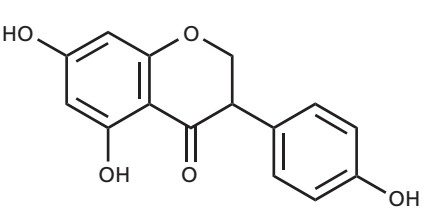


FIGURE 21.7 Genistein.

21.1.12 Resistance

Resistance to anticancer drugs is a serious problem. Resistance can be intrinsic or acquired.

- **Intrinsic resistance** means that the tumour shows little response to an anticancer agent from the very start. This can be a result of a variety of possible mechanisms, such as slow growth rate, poor uptake of the drug, or the biochemical/genetic properties of the cell. Tumour cells in the centre of the tumour may be in the resting state and be intrinsically resistant as a result. It has also been proposed that cancer stem cells might exist that are inherently resistant to current anticancer agents. Such stem cells could explain the re-emergence of certain tumours after successful initial treatment.
- When a tumour is initially susceptible to a drug, but becomes resistant, it is said to show **acquired resistance**. This is due to the presence of a mixture of drug-sensitive and drug-resistant cells within the tumour. The drug wipes out the drug-sensitive cells but this only serves to select out and enrich the drug-resistant cells. The survival of even one such cell can lead to failure of the treatment, as that one survivor can spawn a newer drug-resistant tumour. One might ask why a single tumour should contain drug-sensitive and drug-resistant cells as it is likely to have developed from a single cell in the first place. The reason is that cancer cells by their very nature are genetically unstable and so mutations are bound to have occurred during tumour growth, which will result in resistant cells.

There are several molecular mechanisms by which resistance can take place as a result of mutation. For example, resistance can be due to decreased uptake of drug by the cell or increased synthesis of the target against which the drug is directed. Some drugs need to be activated in the cell and the cancer cell may adapt such that these reactions no longer take place. Alternative metabolic pathways may be found to avoid the effects of antimetabolites. Drugs may be actively expelled from the cell in a process known as **efflux**. A cell membrane carrier protein called **P-glycoprotein** is particularly important in this last mechanism. This protein is a member of a group of energy-dependent transporters known as **ATP-binding cassette (ABC) transporters**. It normally expels toxins from normal cells, but mutations in cancer cells can result in an increased expression of the protein such that anticancer drugs are efficiently removed as soon as they enter the cell. Unfortunately, the P-glycoprotein can eject a wide diversity of molecules. As a result, cells with excess P-glycoprotein are resistant to a variety of different anticancer drugs, even if they have not been exposed to them before. This is known as **multidrug resistance** (**MDR**). For example, cells acquiring resistance to the **vinca alkaloids** are also resistant to **dactinomycin** (actinomycin D) and **anthracyclines**.

Efforts have been made to counter this form of resistance by developing drugs which compete for the P-glycoprotein or inhibit it. The calcium ion channel blocker **verapamil** effectively competes for the P-glycoprotein and allows the build up of an anticancer drug within the cancer cell. Unfortunately, verapamil cannot be used clinically because of its own inherent activity, but there is potential in this approach. **Ciclosporin A** and **quinine** have also been found to inhibit P-glycoprotein and have been investigated in clinical trials, as well as a range of newer agents (e.g. **laniquidar**, **oc144-093**, **zosuquidar, elacridar**, **biricodar**, and **tariquidar**). One of the difficulties faced in these studies is finding an agent that will block the transporter protein in cancer cells, but not in normal cells.

To conclude, regardless of the mechanism involved, it is likely that a drug-resistant cell may be present in a cancer. Therefore, it makes sense to use combinations of anticancer drugs with different targets to increase the chances of finding a weakness in every cell and not just those susceptible to a single drug.

KEY POINTS

- Cancer cells have defects in the normal regulatory controls governing cell growth and division. Such defects arise from mutations resulting in the activation of oncogenes and the inactivation of tumour suppression genes.
- Defects in signalling pathways are commonly found in cancer cells. The pathways stimulating cell growth and division are overactive as a result of the overproduction of a crucial protein in the pathway or the production of an abnormal protein. The proteins involved include growth factors, receptors, signal proteins, and kinases.
- The production of regulatory proteins which suppress cell growth and division is suppressed in many cancers.
- The cell cycle consists of four phases. Progression through the cell cycle is controlled by cyclins and cyclin-dependent kinases, moderated by restraining proteins. Defects in this system have been detected in 90% of cancers.
- Apoptosis is a destructive process leading to cell death. Cells have monitoring systems which check the general health of the cell and trigger the process of apoptosis if there are too many defects. Regulatory proteins have a moderating influence on apoptosis. Defects in apoptosis increase the chances of defective cells developing into cancerous cells and reduce the effectiveness of several drugs.
- Telomeres act as splices to stabilize the ends of DNA. Normally, they decrease in size at each replication until they are too short to be effective, resulting in cell death. Cancer cells activate the expression of an enzyme called telomerase to maintain the telomere and become immortal.

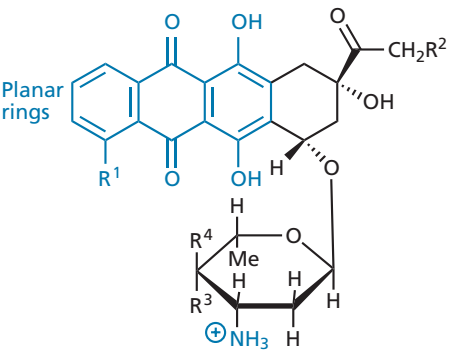
- Angiogenesis is the process by which tumours stimulate the growth of new blood vessels to provide the nutrients required for continued growth. Agents which inhibit angiogenesis are useful in anticancer therapy to inhibit tumour growth and to enhance the effectiveness of other drugs.
- Metastasis is the process by which cancer cells break free of the primary tumour, enter the blood supply and set up secondary tumours in other tissues. To do this, the regulatory controls which fix cells to a specific environment, and which destroy cells that become detached, are over-ruled.
- Surgery, radiotherapy, and chemotherapy are used to treat cancer. Chemotherapy usually involves combinations of drugs having different targets or mechanisms of action. Traditional anticancer drugs are generally cytotoxic; more modern drugs are selective in their action.
- Cancer cells can have intrinsic or acquired resistance to anticancer drugs. Resistance may be due to poor uptake of the drug, increased production of the target protein, mutations which prevent the drug binding to its target, alternative metabolic pathways, or efflux systems which expel drugs from the cell.

21.2 Drugs acting directly on nucleic acids

21.2.1 Intercalating agents

Intercalating drugs contain a planar aromatic or heteroaromatic ring system which can slip into the double helix of DNA and distort its structure. Once bound, the drug can inhibit the enzymes involved in the replication and transcription processes. Examples include **dactinomycin** and **doxorubicin**, described in section 9.1.

Doxorubicin (previously called **adriamycin**) (Fig. 21.8) belongs to a group of naturally occurring antibiotics called the **anthracyclines**, and was isolated from *Streptomyces peucetius* in 1967. It is very similar in structure to daunorubicin—differing only in one hydroxyl group. However, that has an important effect on activity and doxorubicin is one of the most effective anticancer agents ever discovered. The drug intercalates into DNA and is an example of a **topoisomerase II poison** as it stabilizes the complex formed between DNA and topoisomerase II—an enzyme that is crucial to the replication process (sections 9.1 and 6.1.3). It is thought that an excessive number of these stabilized DNA–enzyme complexes triggers apoptosis. Because these enzymes are active during cell growth and division, the topoisomerase-II poisons are most effective against rapidly proliferating cells. A second mechanism by which doxorubicin



Anthracyclines	R^1	R^2	R^3	R^4
Doxorubicin	OMe	OH	OH	H
Epirubicin	OMe	OH	H	OH
Daunorubicin	OMe	H	OH	H
Idarubicin	H	H	OH	H

FIGURE 21.8 The anthracyclines.

can prove harmful to DNA involves the hydroxyquinone moiety, which can chelate iron to form a doxorubicin–DNA–iron complex. Reactive oxygen species are then generated, leading to single-strand breaks in the DNA chain. This mechanism is considered less important than the interaction with topoisomerase II, but it has been implicated in the cardiotoxicity of doxorubicin. A third proposed mechanism involves intercalated doxorubicin inhibiting the **helicases** which unravel DNA into single DNA strands.

A variety of other anthracyclines are used in cancer chemotherapy, mainly **daunorubicin** (also called **cerubidine**, **daunomycin**, or **rubidomycin**), and the second-generation anthracyclines **epirubicin** and **idarubicin** (**idamycin**) (Fig. 21.8). Idarubicin lacks the methoxy group at R^1, so it is more polar and has an altered metabolism which prolongs its half-life. A third generation of anthracyclines is being studied where the aminium ion on the sugar ring is replaced with an azido group or triazole ring. Such compounds do not appear to be susceptible to the efflux mechanism which leads to drug resistance (Box 21.1) and also show decreased general toxicity.

Mitoxantrone (Fig. 21.9) is a simplified, synthetic analogue of the anthracyclines where the tetracyclic ring system has been 'pruned' back to the planar tricyclic system required for intercalation. There are two identical substituent chains present which make the molecule symmetrical and easier to synthesize. The sugar ring is lacking because it is thought to be responsible for cardiotoxic side effects. However, the amino substituent that is normally present on the sugar is still present within the substituent chains. Structure–activity relationship (SAR) studies on mitoxantrone identify a pharmacophore involving one of the phenol groups, a

BOX 21.1 Clinical aspects of intercalating agents

Most of the anthracyclines are orally inactive and have to be administered by intravenous injection. Another drawback is that they have cardiotoxic side effects which can be irreversible and lead to heart failure, while multi-drug resistance can develop owing to amplification of the gene coding for the P-glycoprotein (section 21.1.12). This results in increased efflux of the drug from the tumour cell. Many of the anthracyclines cannot be used alongside radiotherapy owing to enhanced toxic effects, but are widely used otherwise. **Doxorubicin** is used to treat a broad spectrum of solid tumours, as well as acute leukaemias, lymphomas, and childhood tumours. Liposomes (section 11.10) can be useful as carriers to deliver doxorubicin to target tumours and this approach is associated with less cardiac toxicity. **Daunorubicin** is indicated for acute leukaemias. **Epirubicin** is considered effective against breast cancer. **Idarubicin** is used in the treatment of haematological malignancies and can be given orally. Both epirubicin and idarubicin are second-generation anthracyclines with less cardiac toxicity than doxorubicin or daunorubicin.

Mitoxantrone is used for the treatment of certain leukaemias and lymphomas, and for advanced breast cancer. It does not have the same level of cardiotoxicity associated with the anthracyclines. **Amsacrine** is given intravenously and is used occasionally for the treatment of acute myeloid leukaemia.

Dactinomycin is given mainly intravenously to treat paediatric solid tumours, including Wilm's tumour and Ewing's tumour. It has similar side effects to doxorubicin, but lacks similar cardiac toxicity.

Bleomycin is a mixture of bleomycin A_2 and bleomycin B_2, and is used intravenously or intramuscularly in combination therapies for the treatment of certain types of skin cancer, testicular carcinoma, and lymphomas. Unlike most anticancer agents, it produces very little bone marrow depression, but it is quite toxic—particularly to the skin and mucous membranes. The drug is normally inactivated by an enzyme which hydrolyses a primary amide to a carboxylic acid, but this enzyme is present in only very small quantities in the skin. As a result, the active drug can accumulate here to toxic levels.

carbonyl group, and the amino group in the side chain. Because the molecule is symmetrical, there are two such pharmacophores, but activity remains much the same for analogues containing only one. It was also demonstrated that the amino group linking the side chain to the tricyclic ring system was important to activity. Mitoxantrone intercalates DNA preferentially at guanine–cytosine base pairs such that the side chains lie in the minor groove of DNA, and it is thought to interact with topoisomerase II in a similar fashion to doxorubicin. Other mechanisms of action have been proposed, including inhibition of microtubule assembly and inhibition of **protein kinase C**.

Amsacrine (Fig. 21.9) contains an acridine tricyclic system capable of intercalating into DNA. It also stabilizes topoisomerase-cleavable complexes.

Another important group of intercalating, anticancer agents are the **bleomycins**, which are large, water-soluble glycoproteins derived from *Streptomyces verticillus*. Once they have intercalated with DNA, they are responsible for the production of free radical species that cause oxidative cleavage of DNA strands (section 9.1).

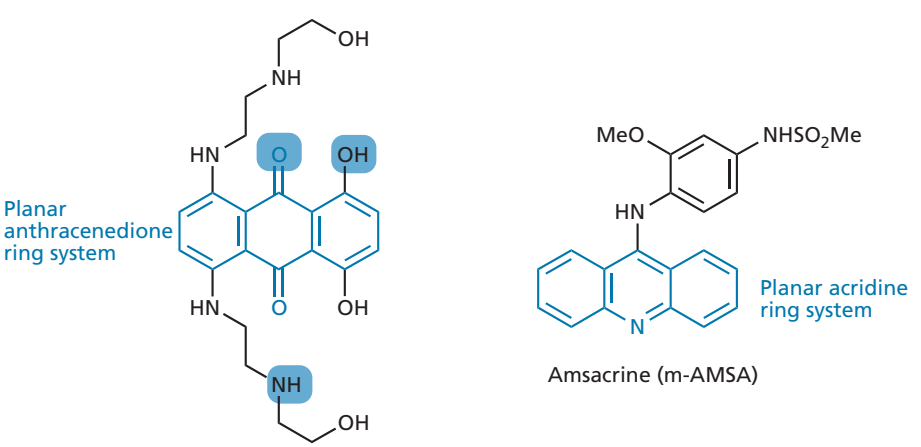


FIGURE 21.9 Mitoxantrone (pharmacophoric groups highlighted in boxes) and amsacrine.

21.2.2 Non-intercalating agents which inhibit the action of topoisomerase enzymes on DNA

21.2.2.1 Podophyllotoxins

Etoposide and **teniposide** (Fig. 9.4) are potent anticancer agents which stabilize the covalent intermediate formed between DNA and topoisomerase II, and are also thought to produce strand breakage by free radical production (section 9.2). The drugs show selectivity for cancer cells, despite the fact that topoisomerase II is present in both cancer cells and normal cells. This is thought to be a result of elevated enzyme levels or enzyme activity in the cancer cells. It has also been found that teniposide is more readily taken up by cells than etoposide and has a greater cytotoxic effect. This is thought to be because teniposide is less polar and can cross cell membranes more easily.

Etoposide suffers from poor water solubility but this can be improved by using a phosphate ester prodrug. A range of etoposide analogues has been synthesized in an effort to find agents which have better aqueous solubility, improved activity against drug-resistant cancer cells, and less susceptibility to metabolic inactivation.

21.2.2.2 Camptothecins

Camptothecin (Fig. 21.10) is a naturally occurring cytotoxic alkaloid which was extracted from a Chinese bush (*Camptotheca acuminata*) in 1966. It targets the complex between DNA and **topoisomerase I** (section 9.2). This leads to DNA cleavage and cell death if DNA synthesis is in progress, but it has been observed that these agents are also toxic to cancer cells which are not synthesizing new DNA. This is due to an alternative mechanism of action—possibly the induction of destructive enzymes such as serine proteases and endonucleases.

The camptothecins show selectivity for cancer cells over normal cells when the cancer cells in question show higher levels of topoisomerase I than normal cells. Topoisomerase I can also be more active in certain cancer cells, which may also account for the anti-tumour selectivity observed.

The lactone group is important for activity, but at blood pH it is in equilibrium with the less active ring-opened carboxylate structure. Introducing substituents into the A and B rings can alter the relative binding affinities of these structures to serum albumin such that the level of the lactone present is altered favourably. Unfortunately, camptothecin itself shows poor aqueous solubility and has unacceptable toxic side effects.

Irinotecan and **topotecan** (Fig. 21.10) are clinically useful, semi-synthetic analogues of camptothecin. They retain the important lactone group and were designed to have aqueous solubility by adding suitable polar functional groups such as alcohols and amines. Irinotecan is a urethane prodrug that is converted to the active phenol (**SN-38**) by carboxylesterases, predominantly in the liver.

21.2.3 Alkylating and metallating agents

Alkylating agents are highly electrophilic compounds that react with nucleophilic groups on DNA to form strong covalent bonds (section 9.3). Drugs with two alkylating groups can cause cross-linking that disrupts replication or transcription. Unfortunately, alkylating agents can also alkylate nucleophilic groups on proteins, which means they have poor selectivity. Nevertheless, alkylating drugs have been useful in the treatment of cancer. Tumour cells often divide more rapidly than normal cells and so disruption of DNA function affects these cells more drastically than normal cells. However, it should be noted that these drugs can be mutagenic and carcinogenic in their own right. This results from the damage that they wreak on DNA in normal, healthy cells. The simple alkylating agents that are used commonly in organic synthesis (e.g. iodomethane and dimethylsulfate) are considered carcinogenic because they have the capability to alkylate DNA.

21.2.3.1 Nitrogen mustards

Chlormethine (Fig 21.11) was the first alkylating agent to be used medicinally and was introduced in 1942. The

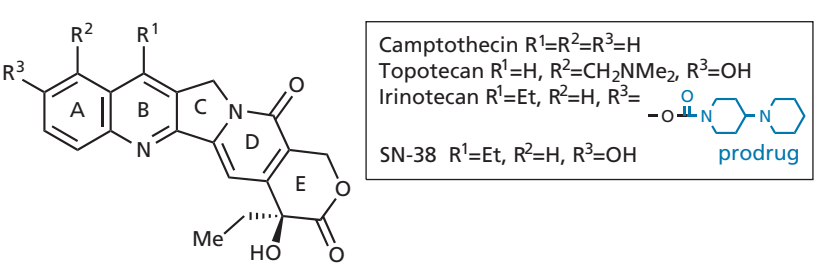


FIGURE 21.10 Camptothecins.

BOX 21.2 Clinical aspects of non-intercalating agents inhibiting the action of topoisomerase enzymes on DNA

Etoposide and **teniposide** are used clinically for a variety of conditions, such as testicular cancer and small cell lung cancer. Resistance can arise as a result of overexpression of the P-glycoprotein involved in the efflux mechanism, or to mutations in the topoisomerase enzyme which weaken interactions with the drug.

Topotecan is an intravenous drug that is used in the treatment of advanced ovarian cancer when previous treatments have failed. **Irinotecan** is given intravenously and is a prodrug used in combination therapy with **fluorouracil** and **folinic acid** (**leucovorin**) for the treatment of advanced colorectal cancer. It has a potential role in treating a variety of other cancers. Unfortunately, the carboxylesterases required to activate the structure are not very efficient and only 2–5% of an injected dose is actually converted. Gene therapy and ADEPT strategies are being explored to try to improve this process (section 21.9). Resistance to these drugs arises from mutations to the topoisomerase I enzyme.

Severe diarrhoea can be a major side effect of irinotecan which limits its use. This arises from activation of the drug in the intestines by gut flora, resulting in it killing intestinal cells. Research is being carried out to try and find an enzyme inhibitor that will inhibit the activation process.

mechanism involves the cross-linking of guanine groups on DNA, as described in section 9.3.1. Chlormethine is highly reactive and can react with water, blood, and tissues. It is too reactive to survive the oral route and has to be administered intravenously.

The side reactions mentioned above can be reduced by lowering the reactivity of the alkylating agent. For example, replacing the *N*-methyl group with an *N*-aryl group (I in Fig. 21.11) has such an effect. The lone pair of the nitrogen interacts with the π system of the ring and is less

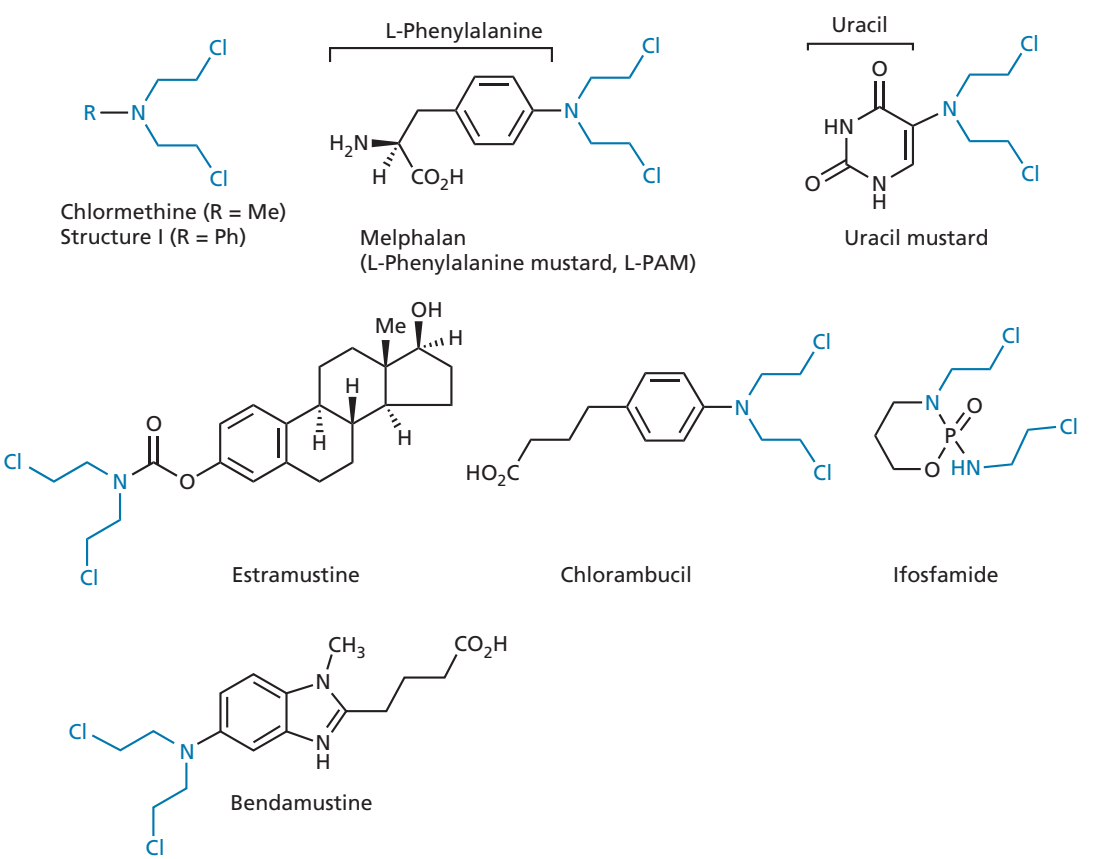


FIGURE 21.11 Mustard-like alkylating agents.

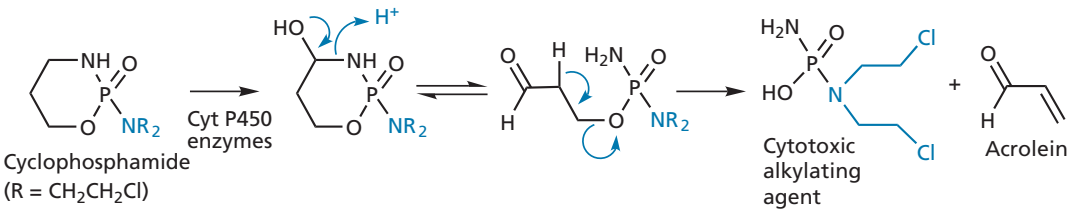


FIGURE 21.12 Phosphoramide mustard from cyclophosphamide.

available to displace the chloride ion. As a result, the intermediate aziridinium ion (see Fig. 9.9) is less easily formed and only strong nucleophiles, such as guanine, will react with it. The alkylating agent **melphalan** (Fig. 21.11) takes advantage of this property and has the added advantage of having a moiety which mimics the amino acid phenylalanine. As a result, the drug is more likely to be recognized as an amino acid and get taken into cells by transport proteins. The increased stability also means that the drug can be given orally. Phenylalanine is a biosynthetic precursor for melanin and it was hoped that this would help to target the drug to skin melanomas. Unfortunately, such targeting has not been particularly significant.

A similar approach has been to attach a nucleic acid building block to the alkylating group. For example, **uracil mustard** (Fig. 21.11) contains the nucleic acid base uracil and shows a certain amount of selectivity for tumour cells over normal cells. Because tumour cells generally divide faster than normal cells, nucleic acid synthesis is faster and so tumour cells need more of the nucleic acid building blocks. The tumour cells scavenge more than their fair share of the building blocks and accumulate the cytotoxic drug more effectively. Unfortunately, this approach has not achieved the high levels of selectivity desired for effective eradication of all relevant tumour cells.

Other examples of alkylating agents include **chlorambucil** and **estramustine** (Fig. 21.11). In the latter drug, the alkylating group is linked to the hormone **estradiol**. As estradiol normally crosses cell membranes into cells, it carries the alkylating agent with it. The link to the steroid is through a urethane functional group which lowers the nitrogen's nucleophilicity. **Bendamustine** is a more recently approved agent.

Cyclophosphamide (Fig. 21.12) is the most commonly used alkylating agent in cancer chemotherapy. It is a non-toxic prodrug which is converted in the body to the active drug (Fig. 21.12). Metabolism takes place in the liver where cytochrome P450 enzymes oxidize the ring. Ring-opening then takes place and a non-enzymatic hydrolysis splits acrolein from the molecule to generate the cytotoxic alkylating agent. The nucleophilicity of the nitrogen is tempered by it being part of a phosphoramide group, and so the active agent is more selective for stronger nucleophiles, such as guanine.

Cyclophosphamide itself is relatively non-toxic and can be taken orally without causing damage to the gut wall. It was also hoped that the high level of **phosphoramidase** enzyme present in some tumour cells would lead to a greater concentration of alkylating agent in these cells and result in some selectivity of action. Unfortunately, the acrolein released can sometimes prove toxic to the kidneys and the bladder. One possible explanation is that acrolein alkylates cysteine residues in cell proteins. Certainly, toxicity can be reduced by co-administrating sulphydryl donors such as ***N*-acetylcysteine** or **sodium-2-mercaptoethane sulfonate (mesna)** ($HSCH_2CH_2SO_3^-$) which interact with the acrolein. **Ifosfamide** (Fig. 21.11) is a related drug with a similar mechanism and similar problems. **TH-302** (Fig. 21.13) is structurally related to ifosfamide and acts as a hypoxia-activated prodrug. In other words, it is activated in environments where there

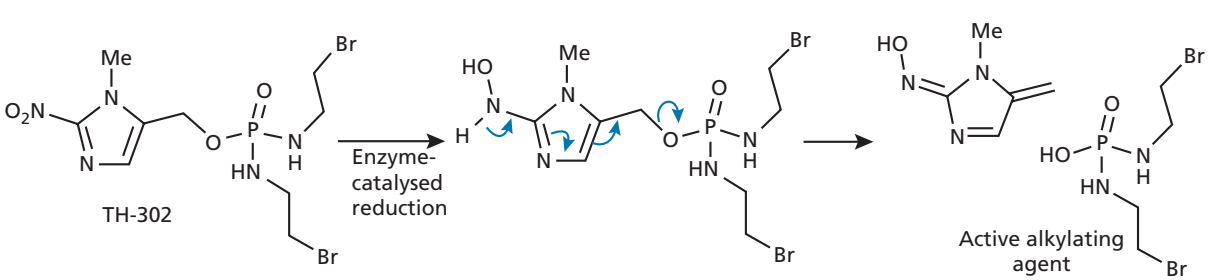


FIGURE 21.13 Activation of the prodrug TH-32 under hypoxic conditions.

are low oxygen concentrations, such as the centre of solid tumours. Under such conditions, the prodrug undergoes reduction and degradation to the active alkylating agent. TH-302 is currently undergoing phase III clinical trials.

21.2.3.2 Cisplatin and cisplatin analogues: metallating agents

Cisplatin (Fig. 21.14) is one of the most frequently used anticancer drugs. The structure is activated within cells and produces intrastrand cross-linking (section 9.3.4). **Carboplatin** (Fig. 21.14) is a derivative of cisplatin with reduced side effects.

A range of other platinum drugs (Fig. 21.14) (such as the first orally active compound **JM216)** have been developed in an attempt to overcome tumour resistance (Box 21.3). Most of these compounds are still undergoing clinical trials, but **oxaliplatin** was approved in 1999 and is effective against tumours that have gained resistance to cisplatin and carboplatin. This lack of cross-resistance is due to the presence of the diaminocyclohexane ring. The ring is bad for water solubility, but this can be counteracted by introducing an oxalato ligand as the leaving group.

A lot of research is being carried out to try and tackle problems such as side effects and resistance. These include designing prodrugs of metallating agents that will only be activated at target tumour cells. For example, cancer cells have an oxidizing environment and prodrugs are being designed which are activated by hydrogen peroxide. Another approach has been to link the metallating agent to a molecule which will target an overexpressed target in the tumour cells; for example linking the agent to a steroid such that it targets tumour cells which overexpress a steroid receptor.

21.2.3.3 CC 1065 analogues

CC 1065 (Fig. 21.15) is a naturally occurring anticancer agent which binds to the minor groove of DNA then alkylates an adenine base. It is 1000 times more active *in vitro* than doxorubicin and cisplatin. **Adozelesin** is a simplified synthetic analogue and is being considered for use in antibody–drug conjugates (section 21.9.2).

21.2.3.4 Other alkylating agents

There are several other anticancer agents that act as alkylating agents, including **dacarbazine**, **procarbazine**, **lomustine**, **carmustine**, **temozolomide**, **busulfan**, and **mitomycin C**. The mechanisms of by which these agents work are described in section 9.3.

21.2.4 Chain cutters

Calicheamicin γ^1 is an anti-tumour agent that was isolated from a bacterium. It binds to DNA and is responsible for generating radical species which lead to the cutting of the DNA chain (section 9.4). It is an extremely potent agent and is one of the structures being studied in the design of antibody–drug conjugates (section 21.9.2 and Box 21.13).

21.2.5 Antisense therapy

The biopharmaceutical company Genta has developed an antisense drug (section 9.7.2) called **oblimersen** which consists of 18 deoxynucleotides linked by a phosphorothioate backbone (section 14.10). It binds to the initiation codon of the m-RNA molecule carrying the genetic instructions for **Bcl-2**. Bcl-2 is a protein which suppresses cell death (apoptosis) and so suppressing its synthesis will increase the chances of apoptosis taking place when chemotherapy or radiotherapy is being employed. This is currently being tested in phase III clinical trials in combination with the anticancer drugs **docetaxel** and **irenotecan**.

Phosphorothioate oligonucleotides are also being investigated that will target the genetic instructions for **Raf** and **PKCγ**—two proteins that are involved in signal transduction pathways.

KEY POINTS

- Intercalating drugs contain planar aromatic or heteroaromatic ring systems which can slide between the base pairs of the DNA double helix.
- 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.

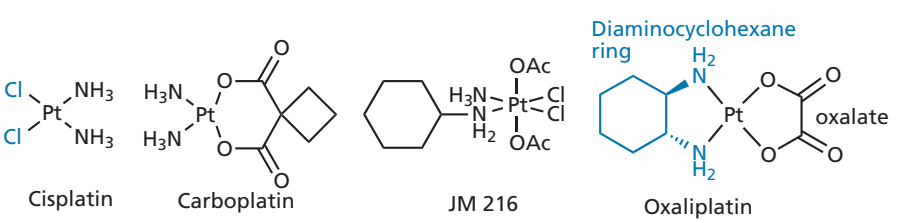


FIGURE 21.14 Platinum-based anticancer drugs.

BOX 21.3 Clinical aspects of alkylating and metallating agents

Chlormethine has been used for the treatment of Hodgkin's lymphoma as part of a multidrug regime. The related structure **melphalan** is currently used in the treatment of multiple myeloma, as well as advanced ovarian and breast cancers. **Uracil mustard** has been used successfully in the treatment of chronic lymphatic leukaemia. **Estramustine** can be given orally and is used predominantly for the treatment of prostate cancer. **Chlorambucil** is an orally active drug used primarily in the treatment of chronic lymphocytic leukaemia and Hodgkin's disease. **Bendamustine** was approved in 2008 for the treatment of chronic lymphatic leukaemia and lymphomas. Resistance to alkylating agents can arise through reaction with cellular thiols and decreased cellular uptake.

Cyclophosphamide is given orally or intravenously, and is widely used for the treatment of leukaemias, lymphomas, soft tissue sarcoma, and solid tumours. Haemorrhagic cystitis is a rare, but serious, side effect which results in inflammation, oedema, bleeding, ulceration, and cell death. This is caused by the metabolite acrolein and can be countered by increased fluid intake or by administering **mesna**. The related drug **ifosfamide** is given intravenously along with mesna.

Lomustine and **carmustine** are lipid-soluble and can cross the blood–brain barrier. As a result, they have been used in the treatment of brain tumours and meningeal leukaemia. Lomustine can be given orally, but carmustine is given intravenously because it is rapidly metabolized. Carmustine implants have also been approved. **Streptozotocin** has been used for the treatment of pancreatic islet cell carcinoma. There is a specific uptake of the drug into the pancreas where it carbamoylates proteins.

Busulfan is given orally in the treatment of chronic myeloid leukaemia and may increase the life expectancy of patients by about a year. It is also administered alongside cyclophosphamide prior to stem cell transplantation. It acts selectively on the bone marrow and has little effect on lymphoid tissue or the gastrointestinal tract. However, excessive use may lead to irreversible damage to the bone marrow. Resistance to busulfan is related to the rapid removal and repair of the DNA cross-links.

Cisplatin is a very useful antitumour agent which is used alone or in combination with other drugs for the intravenous treatment of lung, cervical, bladder, head, neck, testicular, and ovarian tumours. It is also used in various combination therapies to treat other forms of cancer. Unfortunately, cisplatin is associated with very severe nausea and vomiting, but the administration of the 5-HT_3 receptor antagonist **ondansetron** (Box 12.2) is effective in combating this problem. **Carboplatin** is now preferred over cisplatin for the intravenous treatment of advanced ovarian tumours, and is also used to treat lung cancers. It is better tolerated than cisplatin and has less severe side effects. **Oxaliplatin** was approved in 1999 for the treatment of colorectal cancer and shows a better safety profile than cisplatin or carboplatin. It is used in combination with **fluorouracil** and **folinic acid**.

Tumour resistance to cisplatin and similar agents has been attributed to a number of factors. Cisplatin requires a transporter protein in order to enter the cell and resistance can occur if there are low levels of the transport protein. The activated species arising from cisplatin (section 9.3.4) reacts easily with cellular thiols, such as glutathione, and resistance can occur if these thiols are present in high concentration. The agent is 'mopped up' before it has a chance to react with DNA. Finally, resistance may arise because of increased efflux of the drug from the cell.

Dacarbazine is used clinically in combination therapies for the treatment of melanoma and soft tissue sarcomas. **Procarbazine** is most often used for the treatment of Hodgkin's disease and is given orally. **Temozolomide** is used for the treatment of certain types of brain tumour and is administered orally in capsules at least one hour before a meal.

Mitomycin C is used intravenously for the treatment of upper gastrointestinal and breast cancers. It can also be used to treat superficial bladder cancers. It has many side effects and is one of the most toxic anticancer drugs in clinical use. Prolonged use can lead to permanent bone marrow damage.

- Nitrogen mustards react with guanine groups on DNA to produce cross-linking. The reactivity of the agents can be lowered by attaching electron-withdrawing groups to the nitrogen to increase selectivity against DNA over proteins. Incorporation of important biosynthetic building blocks aids the uptake into rapidly dividing cells.
- Cisplatin and its analogues are metallating agents which cause intrastrand cross-linking. They are commonly used for the treatment of testicular and ovarian cancers.
- CC-1065 analogues are highly potent alkylating agents which are being considered for use in antibody–drug conjugates.
- Calicheamicin is a natural product which reacts with nucleophiles to produce a diradical species. Reaction with DNA ultimately leads to cutting of the DNA chains.
- Antisense molecules have been designed to inhibit the mRNA molecules that code for the proteins which suppress apoptosis.

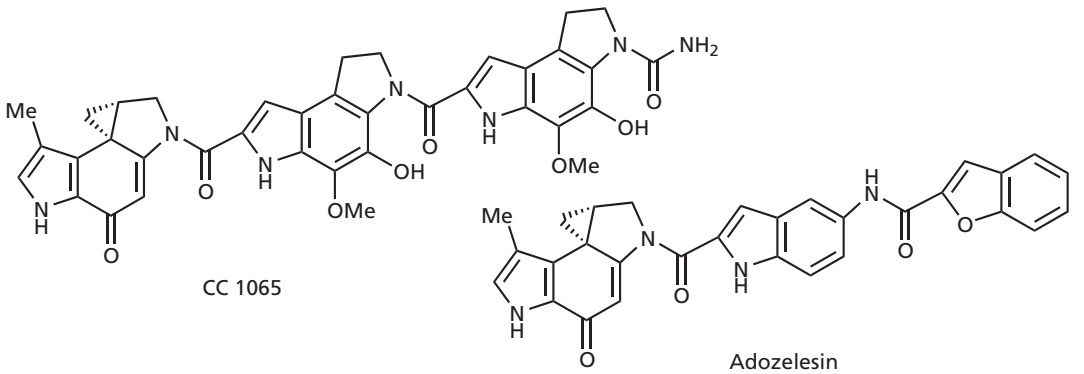


FIGURE 21.15 CC 1065 and adozelesin.

21.3 Drugs acting on enzymes: antimetabolites

The drugs described in section 21.2 interact directly with DNA to inhibit its various functions. Another method of disrupting DNA function is to inhibit the enzymes involved in the synthesis of DNA or its nucleotide building blocks. The inhibitors involved are described as **antimetabolites**. The action of antimetabolites leads to the inhibition of DNA function or the synthesis of abnormal DNA, which may trigger the processes leading to apoptosis.

21.3.1 Dihydrofolate reductase inhibitors

Dihydrofolate reductase (**DHFR**) is an enzyme which is crucial in maintaining levels of the enzyme cofactor **tetrahydrofolate** (**FH_4**) (Figs. 21.16 and 21.17).

Without this cofactor, the synthesis of the DNA building block (dTMP) would grind to a halt, which, in turn, would slow down DNA synthesis and cell division. The enzyme catalyses the reduction of the vitamin **folic acid** to FH_4 in two steps via **dihydrofolate** (**FH_2**). Once formed, FH_4 picks up a single carbon unit to form **N^5, N^{10}-methylene FH_4**, which then acts as a source of one-carbon units for various biosynthetic pathways, including the methylation of deoxyuridine monophosphate (dUMP) to form deoxythymidine monophosphate (dTMP). N^5, N^{10}-methylene FH_4 is converted back to FH_2 in the process and dihydrofolate reductase is vital in restoring the N^5, N^{10}-methylene FH_4 for further reaction.

Methotrexate (Fig. 21.18) is one of the most widely used antimetabolites in cancer chemotherapy. It is very similar in structure to the natural folates, differing only in additional amino and methyl groups. It has a stronger binding affinity for the enzyme owing to an additional hydrogen bond or ionic bond which is not present when FH_2 binds. As a result, methotrexate prevents the binding of FH_2 and its conversion to N^5,N^{10}- methylene FH_4. Depletion of the cofactor has its greatest effect on the enzyme **thymidylate synthase**, resulting in the lowered synthesis of dTMP.

Methotrexate tends to accumulate in cells as a result of **polyglutamylation**. This is an enzyme-catalysed process which involves the addition of glutamate groups to the glutamate moiety already present in the molecule. This also happens to natural folates, and the reaction serves to increase the charge and size of the folates such that they

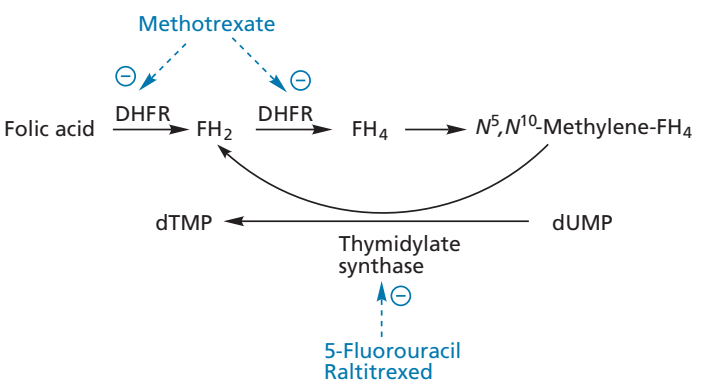


FIGURE 21.16 Reactions catalysed by dihydrofolate reductase and thymidylate synthase.

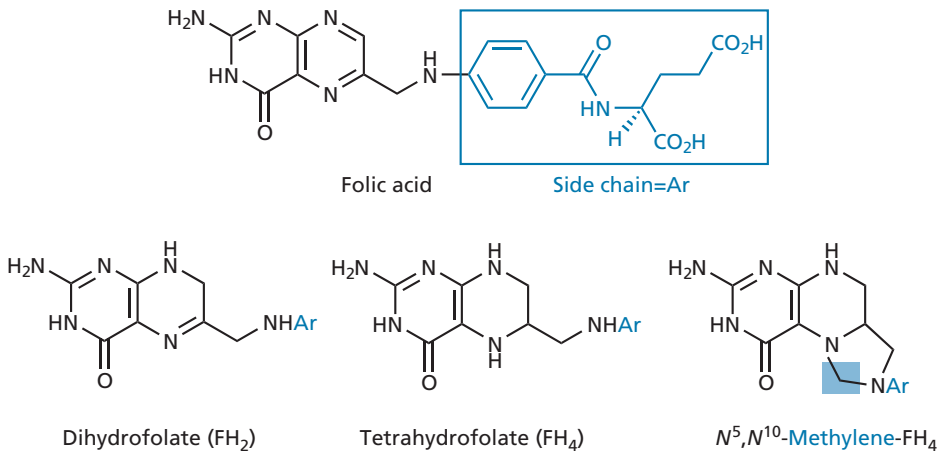


FIGURE 21.17 Structures of folic acid and related cofactors.

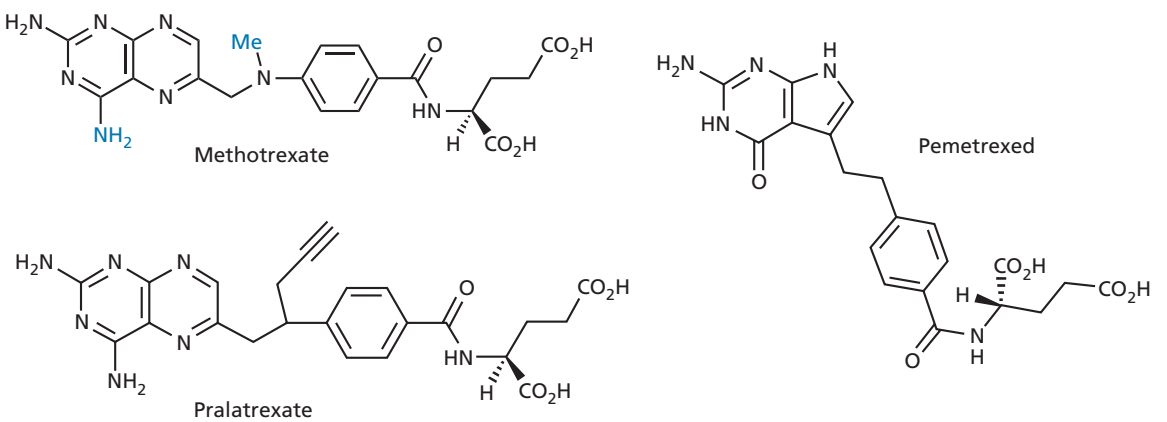


FIGURE 21.18 Methotrexate, pemetrexed, and pralatrexate.

are trapped within the cell. **Pemetrexed** and **Pralatrexate** are related drugs that were approved in 2004 and 2009 respectively.

21.3.2 Inhibitors of thymidylate synthase

Methotrexate has an indirect effect on thymidylate synthase by lowering the amount of N^5, N^{10}-methylene FH_4 cofactor required. **5-Fluorouracil** (Fig. 21.19) is an anticancer drug which inhibits this enzyme directly.

It does so by acting as a prodrug for a **suicide substrate** (section 7.5). 5-Fluorouracil is converted in the body to the fluorinated analogue of 2′-deoxyuridylic acid monophosphate (FdUMP) (Fig. 21.19), which then combines with the enzyme and the cofactor (Fig. 21.20). Up until this point, nothing unusual has happened and the reaction mechanism has been proceeding normally. The

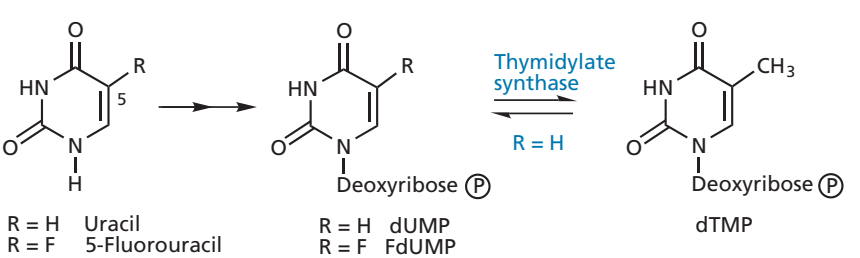


FIGURE 21.19 Biosynthesis of dTMP. Ⓟ = phosphate.

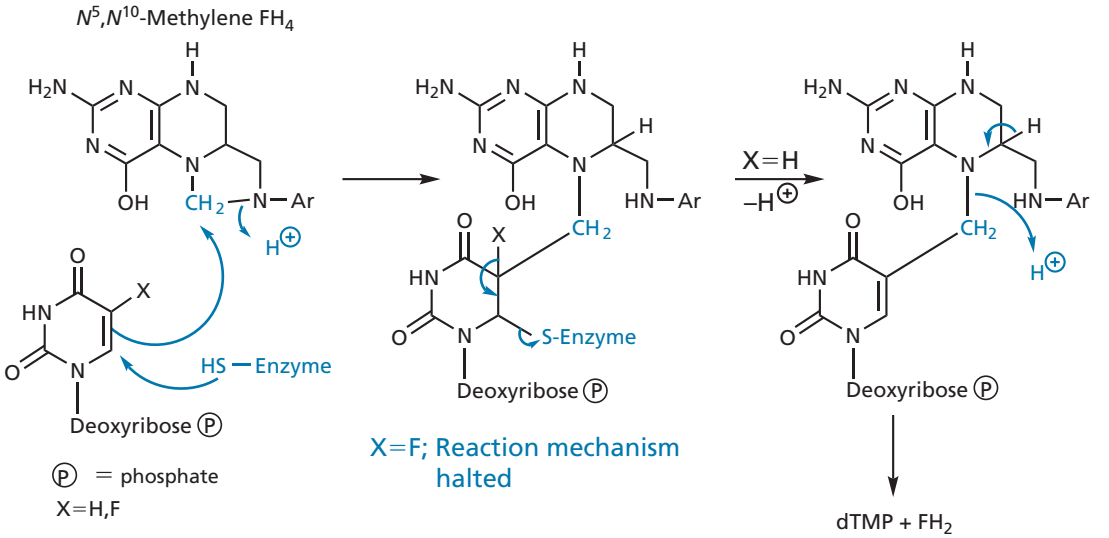


FIGURE 21.20 Use of 5-fluorouracil as a prodrug for a suicide substrate.

tetrahydrofolate has formed a covalent bond to the uracil skeleton via the methylene unit which is usually transferred to uracil, but things start to go wrong. At this stage, a proton is usually lost from position 5 of uracil (X=H). However, 5-fluorouracil has a fluorine atom at that position instead of hydrogen (X=F). Further reaction is impossible, as it would require fluorine to leave as a positive ion. Fluorine is too electronegative for this to occur because it prefers to ionize as the fluoride ion (F^-). As a result, the fluorouracil skeleton remains covalently and irreversibly bound to the active site. The synthesis of thymidine is now terminated, which, in turn, stops the synthesis of DNA. Consequently, replication and cell division are blocked. **Capecitabine** (Box 21.4) is a prodrug for 5-fluorouracil.

5-Fluorouracil binds to the same region of the active site as uracil. Inhibitors which bind to the cofactor binding region have also been developed. **Raltitrexed** (Fig. 21.21) is the first of a new generation of highly specific folate-based thymidylate synthase inhibitors. Another agent under study is **ZD 9331**, which is not a substrate for **folylpolyglutamate synthetase** (FPGS), and can overcome cell resistance where cells have decreased FPGS expression.

BOX 21.4 Clinical aspects of antimetabolites

Methotrexate can be administered orally and by various other methods. It is used to treat a wide variety of cancers, either alone or in combination with other drugs. Examples include childhood acute lymphoblastic leukaemia, non-Hodgkin's lymphoma, and a number of solid tumours. Resistance to methotrexate can arise from enhanced expression of dihydrofolate reductase (DHFR) or diminished uptake of methotrexate by the **reduced folate carrier** (RFC)—a membrane transport protein responsible for the cellular uptake of both folates and antifolates. **Pemetrexed** is approved for the treatment of pleural mesothelioma and non-small cell lung cancer. **Pralatrexate** is approved for certain blood tumours.

5-Fluorouracil is usually given intravenously as oral absorption is unpredictable. It is commonly used alongside **folinic acid** (**leucovorin**) to treat colorectal cancer and is also used for the treatment of various solid tumours, including breast cancer and gastrointestinal tract cancers. Used topically, it is a particularly useful drug for the treatment of skin cancer because it shows a high level of selectivity for cancer cells over normal skin cells. Unfortunately, it has neurotoxic and cardiotoxic side effects. Resistance can occur if the cell produces excess quantities of dUMP to compete with the drug for the active site. **Capecitabine** (Fig. 1) is taken orally and is metabolized to fluorouracil. It can be used as a monotherapy for metastatic colorectal cancer instead of fluorouracil plus folinic acid. It is also licensed for the first-line treatment of advanced gastric cancer in combination with a platinum agent. The drug can also be useful in the treatment of advanced colon cancer or metastatic breast cancer. A curious side effect in some patients is the elimination of fingerprints as a result of mild inflammation.

Nelarabine was given accelerated approval by the US Food and Drug Administration (FDA) in 2005 for the treatment of T-cell acute lymphoblastic leukaemia and T-cell

(*Continued*)

BOX 21.4 Clinical aspects of antimetabolites (*Continued*)

lymphoblastic lymphoma. It acts as a water-soluble prodrug and is demethylated by adenosine deaminase to the less water soluble **ara-G**, which is then phosphorylated by kinases to the active trinucleotide. This is incorporated into DNA, resulting in the triggering of apoptosis.

Raltitrexed is an injectable cytotoxic drug which is used in the treatment of advanced colorectal cancer. Acquired resistance includes impaired cellular uptake, decreased polyglutamation by folylpolyglutamate synthetase (FPGS), or increased thymidylate synthase expression.

Hydroxycarbamide is administered orally for the treatment of busulfan-resistant chronic granulocytic leukaemia and has been used in combination therapy for the treatment of head, neck, and cervical cancers. Resistance can arise owing to increased expression of the enzyme.

Pentostatin is a specialist anticancer drug which is given intravenously for the treatment of hairy cell leukaemia.

Cytarabine is used intravenously, subcutaneously, or intrathecally for the treatment of a wide variety of leukaemias.

Gemcitabine has fewer side effects, and is used intravenously to treat pancreatic cancer and non-small-cell lung cancer. It is also administered alongside **cisplatin** for the treatment of advanced bladder cancer or with **paclitaxel** for breast cancer. **Fludarabine** is administered orally or intravenously for the treatment of chronic lymphatic leukaemia, and is available as the 5' monophosphate prodrug (**Fludara**) to improve solubility.

6-Mercaptopurine and **6-tioguanine** are used primarily for the treatment of acute leukaemias, and are more effective in children than in adults.

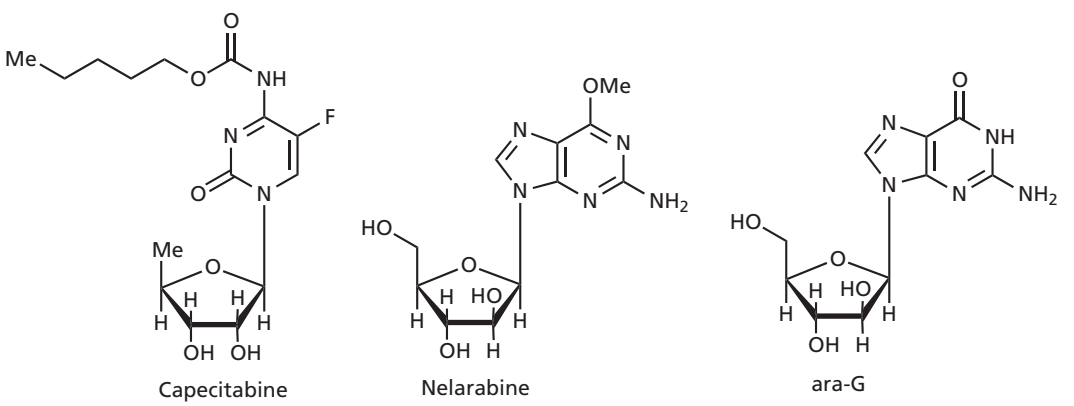


FIGURE 1 Antimetabolites.

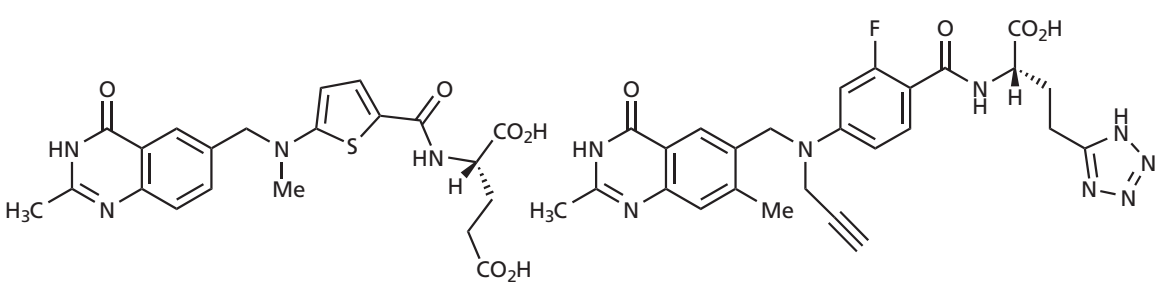


FIGURE 21.21 Raltitrexed and ZD 9331.

21.3.3 Inhibitors of ribonucleotide reductase

Ribonucleotide reductase is responsible for the conversion of ribonucleotide diphosphates to deoxyribonucleotide diphosphates (Fig. 21.22). The enzyme contains an iron cofactor which is crucial to the reaction mechanism. This involves the iron reacting with a tyrosine residue to generate and stabilize a tyrosine free radical, which then abstracts a proton from the substrate and initiates the

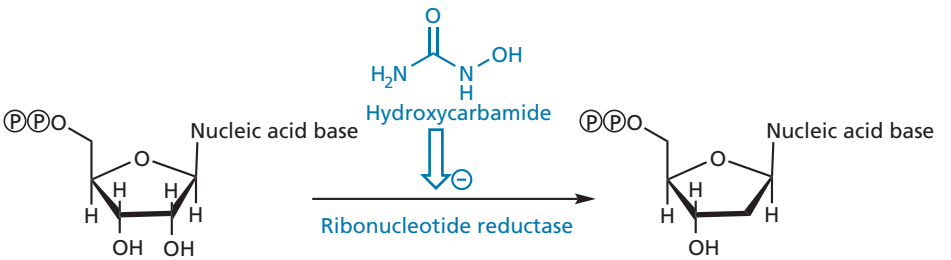


FIGURE 21.22 Reaction catalysed by ribonucleotide reductase (Ⓟ=phosphate).

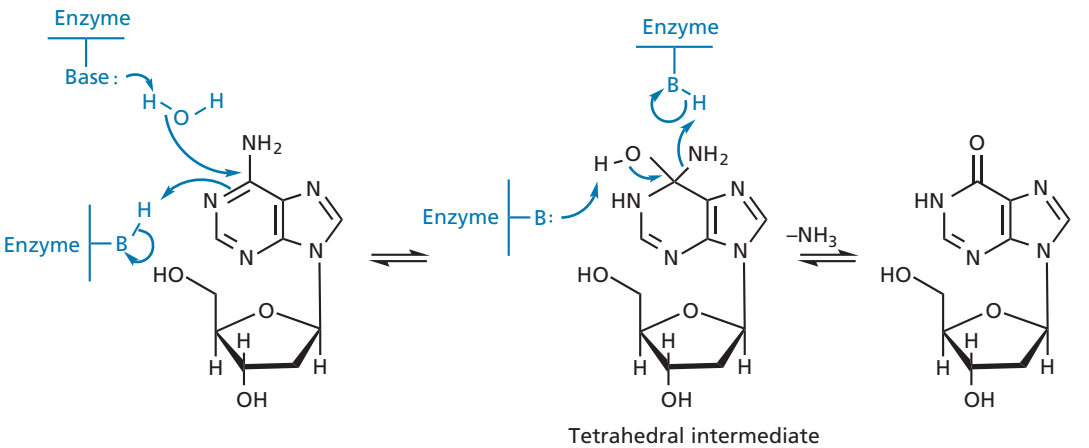


FIGURE 21.23 Mechanism of adenosine deaminase (B = base).

reaction mechanism. **Hydroxycarbamide** (Fig. 21.22) is a clinically useful agent which inhibits the enzyme by destabilizing the iron centre.

21.3.4 Inhibitors of adenosine deaminase

Ribonucleotide reductase is inhibited directly by hydroxycarbamide, but it can also be inhibited indirectly by increasing the level of natural allosteric inhibitors such as dATP (allosteric inhibitors are described in section 3.6). The enzyme **adenosine deaminase** catalyses the deamination of adenosine to inosine (Fig. 21.23) and it is found that inhibition of the enzyme leads to a build-up of dATP in the cell, which, in turn, inhibits ribonucleotide reductase.

The anti-leukaemia drug **pentostatin** (Fig. 21.24) is a natural product isolated from *Streptomyces antibioticus*, and is a powerful inhibitor of adenosine deaminase (K_i = 2.5 pM). It acts as a transition-state inhibitor, mimicking the proposed tetrahedral nature of the transition state, which is believed to be similar to the tetrahedral intermediate in Fig. 21.23.

21.3.5 Inhibitors of DNA polymerases

DNA polymerases catalyse the synthesis of DNA using the four deoxyribonucleotide building blocks dATP, dGTP, dCTP, and dTTP (Chapter 6). The anticancer drug **cytarabine** (Fig. 21.25) is an analogue of 2′ deoxycytidine and acts as a prodrug. It is phosphorylated in cells to the corresponding triphosphate (**ara-CTP**) which acts as a competitive inhibitor. In addition, ara-CTP can act as a substrate for DNA polymerases and become incorporated into the growing DNA chain. This can lead to chain termination or prevent replication of the modified DNA. All of these effects result in the inhibition of DNA synthesis and repair. **Gemcitabine** is an analogue of cytarabine with fewer side effects. The purine analogue **fludarabine** is also metabolized to a triphosphate and has the same mechanism of action as cytarabine. It, too, inhibits transcription and can be incorporated into RNA.

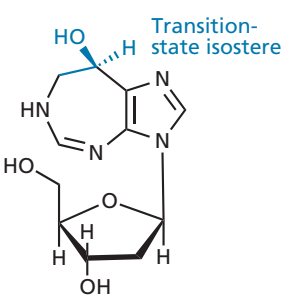


FIGURE 21.24 Pentostatin.

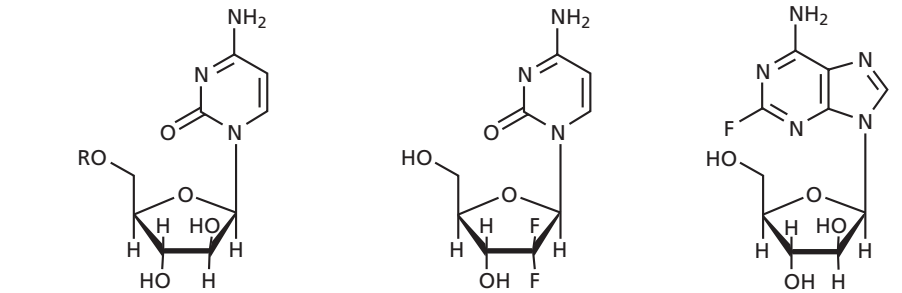


FIGURE 21.25 Inhibitors of DNA polymerase.

21.3.6 Purine antagonists

The thiopurines **6-mercaptopurine** and **6-tioguanine** (Fig. 21.26) are prodrugs which are converted to their corresponding nucleoside monophosphates by cellular enzymes. The monophosphates then inhibit purine synthesis at a number of points. They are also incorporated into RNA and DNA, leading to complex effects which end in cell death. Both agents are converted to a common product (**thio-GMP**) which is subsequently converted to **thio-GTP** and **thio-dGTP**, before incorporation into RNA and DNA respectively.

21.3.7 Inhibitors of poly ADP ribose polymerase

A number of research groups are investigating **poly ADP ribose polymerase (PARP) inhibitors** as potential anticancer agents, although none have reached the market to date. The enzyme repairs single strand breaks in DNA and so its inhibition can eventually result in double strand breaks in DNA and cell death. Some tumour cells are likely to be more susceptible to PARP inhibitors than normal cells.

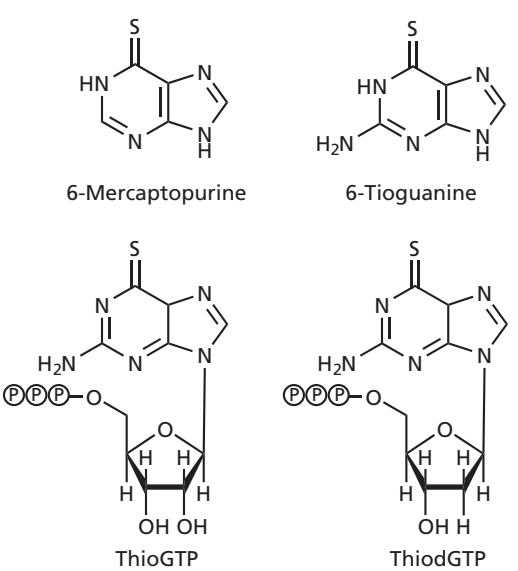


FIGURE 21.26 Purine antagonists, (P) represents a phosphate group.

KEY POINTS

- Antimetabolites are agents which inhibit the enzymes involved in the synthesis of DNA or its building blocks.
- Thymidylate synthase catalyses the synthesis of dTMP from dUMP. The cofactor required for this reaction is regenerated by the enzyme dihydrofolate reductase. Inhibition of either enzyme is useful in anticancer therapy.
- Ribonucleotide reductase catalyses the conversion of ribonucleotide diphosphates to deoxyribonucleotide diphosphates. It can be inhibited directly by drugs or indirectly by inhibiting adenosine deaminase. In the latter case, a build-up of dATP results in allosteric inhibition.
- Various nucleosides and purines act as prodrugs and are converted in the cell to agents that inhibit DNA polymerases. The active agents also act as substrates and are incorporated into growing DNA leading to chain termination or the inhibition of replication.

21.4 Hormone-based therapies

Hormone-based therapies are used for cancers which are hormone dependent. If the cancer cell requires a specific hormone, then a hormone can be administered which has an opposing effect. Alternatively, hormone antagonists can be used to block the action of the required hormone. Steroid hormones combine with intracellular receptors to form complexes that act as **nuclear transcription factors**. In other words, they control whether transcription takes place or not (see also Box 8.2 and section 4.9).

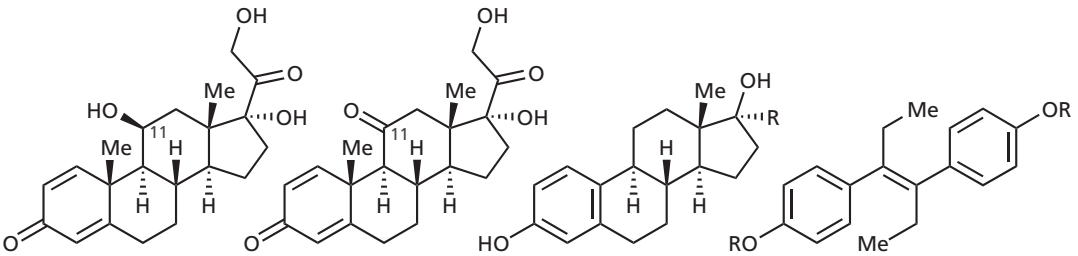


FIGURE 21.27 Glucocorticoids and estrogens.

21.4.1 Glucocorticoids, estrogens, progestins, and androgens

There are various types of hormones that are used in anticancer therapy, such as the **glucocorticoids prednisolone** and **prednisone** (Fig. 21.27). Prednisone acts as a prodrug and is converted enzymatically to prednisolone in the body.

Estrogens inhibit the production of **luteinizing hormone** (**LH**) and, by doing so, decrease the synthesis of **testosterone**. The most commonly used agents are **ethinylestradiol** (a derivative of **estradiol**) and **diethylstilbestrol** (a non-steroidal estrogen) (Fig. 21.27). **Fosfestrol** is the diphosphate prodrug of diethylstilbestrol.

Progestins used as anticancer agents include **medroxyprogesterone acetate** and **megestrol acetate** (Fig. 21.28). **Androgens** are thought to suppress production of LH, resulting in a decrease in estrogen synthesis. The most commonly used agents are **fluoxymesterone** and **testosterone propionate** (Fig. 21.28). The latter is a prodrug which is converted to **dihydrotestosterone**.

21.4.2 Luteinizing hormone-releasing hormone agonists

Luteinizing hormone-releasing hormone (**LHRH**) (also called **gonadotropin-releasing hormone**) is a decapeptide hormone which binds to receptors on anterior pituitary cells and stimulates the release of LH. On long-term exposure to LHRH, the receptor becomes desensitized leading to a drop in LH levels. Since LH stimulates the synthesis of testosterone, this results in lowered testosterone levels. The two agents most commonly used are **leuprolide** and **goserelin** (Fig. 21.29), which are both decapeptide analogues of LHRH designed to be more resistant to peptidase degradation. This normally takes place next to glycine at

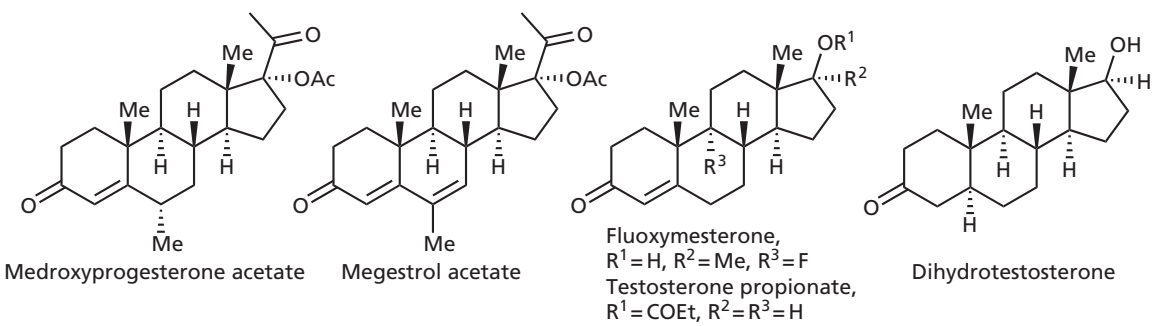
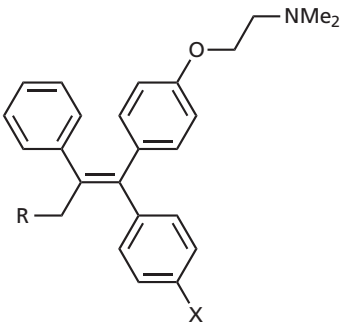


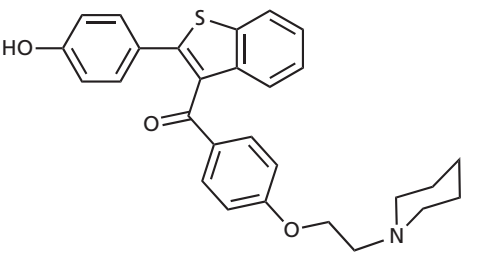
FIGURE 21.28 Progestins and androgens.

1 2 3 4 5 6 7 8 9 10	
pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2	LHRH
pyroGlu-His-Trp-Ser-Tyr-(D-Leu)-Leu-Arg-Pro-ethylamide	Leuprolide
pyroGlu-His-Trp-Ser-Tyr-(D-(t-Bu)Ser)-Leu-Arg-Pro-Azgly-NH_2	Goserelin

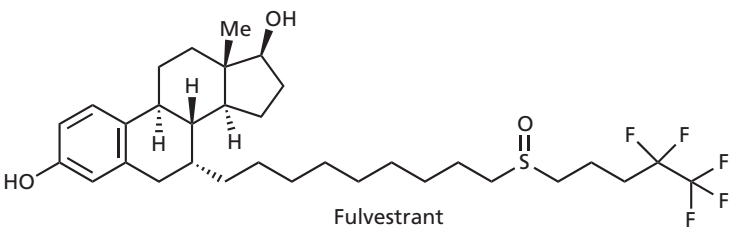
FIGURE 21.29 Luteinizing hormone-releasing hormone (LHRH) agonists.



Tamoxifen; X=H, R=Me
4-Hydroxytamoxifen; X=OH, R=Me
Toremifene ; X=H, R=CH_2Cl



Raloxifene



Fulvestrant

FIGURE 21.30 Anti-estrogens.

position 6 and replacing this amino acid with an unnatural D-amino acid makes this region unrecognizable to the enzyme. Substitution of the glycine residue at position 10 with a suitable group also increases receptor affinity.

21.4.3 Anti-estrogens

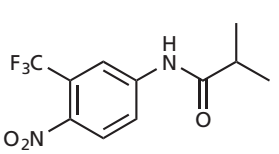
Tamoxifen and **raloxifene** (Fig. 21.30) are synthetic agents which antagonize estrogen receptors and prevent estradiol from binding. The mechanism by which these agents work has been studied extensively and is described in Box 8.2. More recent **anti-estrogens** include **toremifene** and **fulvestrant** (approved in 2002).

21.4.4 Anti-androgens

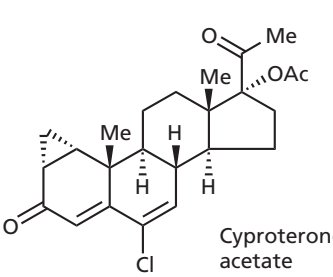
Flutamide and **cyproterone acetate** (Fig. 21.31) are used to treat prostate cancer and work by blocking the action of androgens at their receptors. Until recently, prostate cancer was treated with a combined therapy of a LHRH agonist and an **anti-androgen**. A different approach which has recently proved successful is to inhibit a metabolic enzyme called **17α-hydroxylase-17(20)-lyase**. This is a cytochrome P450 enzyme which is involved in the biosynthesis of androgens from cholesterol and so its inhibition results in lowered androgen levels. **Abiraterone** (Fig. 21.31) is a potent and selective inhibitor of this enzyme, and was approved in 2011 for the treatment of prostate cancer. The pyridine ring plays a key role in its action by interacting with the iron of haem in the enzyme's active site.

21.4.5 Aromatase inhibitors

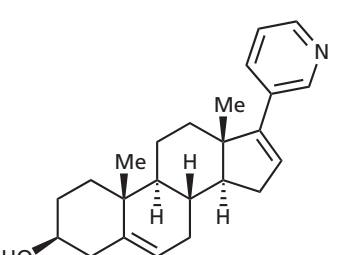
Aromatase inhibitors tend to be used as second-line drugs for the treatment of estrogen-dependent breast cancers that prove resistant to tamoxifen. **Aromatase** is



Flutamide

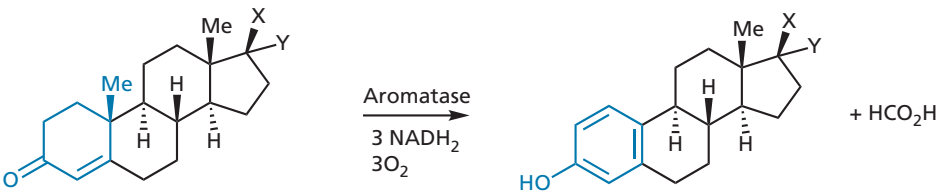


Cyproterone acetate



Abiraterone

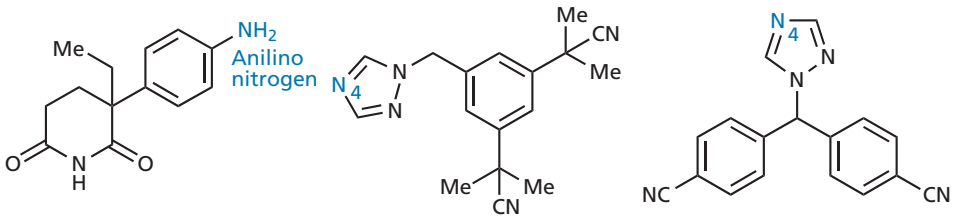
FIGURE 21.31 Anti-androgens.



Androstenedione, X,Y = ketone
Testosterone, X = OH, Y = H

Estrone, X,Y = ketone
Estradiol, X = OH, Y = H

FIGURE 21.32 Reaction catalysed by aromatase.



Aminoglutethimide

Anastrozole

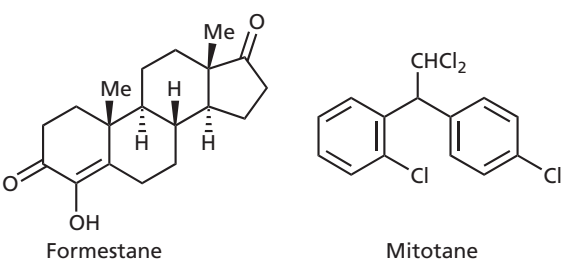
Letrozole

FIGURE 21.33 Reversible, competitive inhibitors of aromatase.

a membrane-bound enzyme complex consisting of two proteins: one is a cytochrome P450 enzyme containing haem (CYP19) and the other is a reductase enzyme using NADPH as cofactor. Aromatase catalyses the last stage in the biosynthesis of estrogens from androgens where an aromatic ring is formed (Fig. 21.32). The cytochrome enzyme contains haem, which serves to bind the steroid substrate and oxygen then catalyse the oxidation. Since the enzyme catalyses the last step of this synthesis, it has been seen as an important target for the design of anti-estrogenic drugs. Two types of inhibitor are used clinically—reversible, competitive inhibitors and irreversible inhibitors acting as suicide substrates.

Aminoglutethimide (Fig. 21.33) is an early example of a reversible, competitive inhibitor, but has disadvantages in that it binds to various cytochrome P450 enzymes and inhibits a range of steroid hydroxylations. This results in undesirable side effects. Drug design based on aminoglutethimide as the lead compound resulted in more selective inhibitors, such as **anastrozole** and **letrozole**, which are used to treat breast cancer. The *N*-4 nitrogen of the triazole ring interacts with the haem iron of aromatase and prevents binding of the steroid substrate. The anilino nitrogen of aminoglutethimide serves the same purpose.

Formestane (Fig. 21.34) acts as a suicide substrate that permanently inactivates aromatase and is more selective in its action than aminoglutethimide.



Formestane

Mitotane

FIGURE 21.34 Formestane and mitotane.

KEY POINTS

- Hormone-based therapy is used against cancers which are hormone dependent. Hormones can be administered which counteract the offending hormone. Alternatively, antihormonal compounds are administered to prevent the offending hormone from binding to its receptor.
- Glucocorticoids, estrogens, progestins, androgens, and LHRH are used in hormone-based therapy.
- Agents which act as receptor antagonists are used to block estrogens and androgens.
- Enzyme inhibitors are used to block the synthesis of hormones. An important target is the enzyme aromatase which catalyses the last step leading to estrogens.

21.5 Drugs acting on structural proteins

Tubulin is a structural protein which is crucial to cell division (section 2.7.1). The protein acts as a building block for microtubules which are polymerized and depolymerized during cell division. Drugs can block this process

BOX 21.5 Clinical aspects of hormone-based therapies

Prednisolone is used widely for the oral treatment of leukaemias and lymphomas. **Ethinylestradiol** is the most potent estrogen available and is used to treat prostate cancer. **Diethylstilbestrol** is rarely used to treat prostate cancer because of side effects, but is occasionally used for breast cancer. **Fosfestrol** is the diphosphate prodrug of diethylstilbestrol and has been used for the treatment of hormone-resistant metastatic prostate cancer. It is only activated in target cells, where it can reach higher concentrations than using diethylstilbestrol itself.

Progestins are used primarily to treat advanced endometrial carcinoma that cannot be treated by surgery or radiation. They have also been used as a second-line drug for the treatment of kidney cancers and metastatic breast cancer, but their use in tackling these diseases is now declining. The most commonly used agents are **medroxyprogesterone acetate** and **megestrol acetate**, which can both be administered orally.

Androgens such as **fluoxymesterone** and **testosterone propionate** are sometimes used to treat metastatic breast cancer. Unfortunately, they have a masculizing effect and so they are only used in a minority of cases.

LHRH agonists are used to treat advanced prostate and breast cancers. The two agents most commonly used are **leuprolide** and **goserelin**. Both agents are administered as their acetates. Leuprolide acetate can be administered daily. Alternatively, it can be inserted into microspheres and administered once-monthly, whereupon the drug is released slowly over several weeks. Goserelin acetate can be provided as a slow release implant where the drug is contained within a biodegradable cylindrical polymer rod. This can be implanted into subcutaneous fat every 28 days.

Tamoxifen, **toremifine**, and **fulvestrant** are used for the treatment of hormone-dependent breast cancer. The role of **raloxifene** as an anticancer agent is unclear as yet and so it is currently used only for the treatment and prevention of postmenopausal osteoporosis.

Flutamide and **cyproterone acetate** are used in the treatment of prostate cancer.

Anastrozole and **letrozole** are used to treat breast cancer, but are only effective in postmenopausal women.

Mitotane (Fig. 21.34) interferes with the synthesis of adrenocortical steroids and is used in the treatment of advanced or inoperable adrenocortical tumours. As it inhibits the activity of the adrenal cortex, corticosteroid replacement therapy is required during its use.

Octreotide is an analogue of somatostatin and is used to treat hormone-secreting tumours of the gastrointestinal tract.

by either binding to tubulin to prevent polymerization or binding to the microtubules to prevent depolymerization.

Agents which prevent polymerization do not prevent depolymerization, and so this eventually leads to dissolution of the microtubules and destruction of the mitotic spindle required for cell division.

21.5.1 Agents which inhibit tubulin polymerization

Vincristine, **vinblastine**, **vindesine**, and **vinorelbine** are alkaloids (Fig. 10.3) derived from the Madagascar periwinkle plant (*Catharanthus roseus*, formerly known as *Vinca rosea*), and can bind to tubulin to prevent polymerization. These are discussed in section 10.2.2.

Phyllanthoside (Fig. 21.35) is another natural product that is thought to bind to tubulin and prevent polymerization. It was obtained from the roots of a Costa Rican tree in the early 1970s and entered clinical trials. A variety of other naturally occurring agents have been extracted from marine sources and shown to inhibit microtubule formation. For example, **spongistatin 1** (Fig. 21.35) was extracted from a marine sponge in the Maldives and shows potential as an anticancer agent.

Analogues of the naturally occurring compound **podophyllotoxin** (Fig. 9.4) have already been mentioned in section 21.2.2.1 for their effect on topoisomerase II. Curiously, podophyllotoxin itself has a completely different mechanism of action where it forms a complex with tubulin and prevents the synthesis of microtubules.

Podophyllotoxins belong to a group of compounds called **lignans** and have been isolated from plant sources, such as the American mandrake or May apple (*Podophyllum peltatum*), and from the Himalayan plant *Podophyllum emodi*. Extracts of these plants have been used for over 1000 years to treat a variety of diseases, including cancers. For example, it has been recorded that the roots of the wild chervil (*Anthriscus sylvestris*) were used as a treatment for cancer, and it has been shown that these roots contain **deoxypodophyllotoxin**. The crude extract from the above plants is known as **podophyllum** and was shown in 1942 to be effective in the treatment of venereal warts. Podophyllotoxin was eventually isolated from this extract and was used as an anticancer agent for a while. However, its use had to be restricted because of severe side effects. A structural similarity has been noted between podophyllotoxin and **colchicine**—another compound which interacts with tubulin (section 10.2.2).

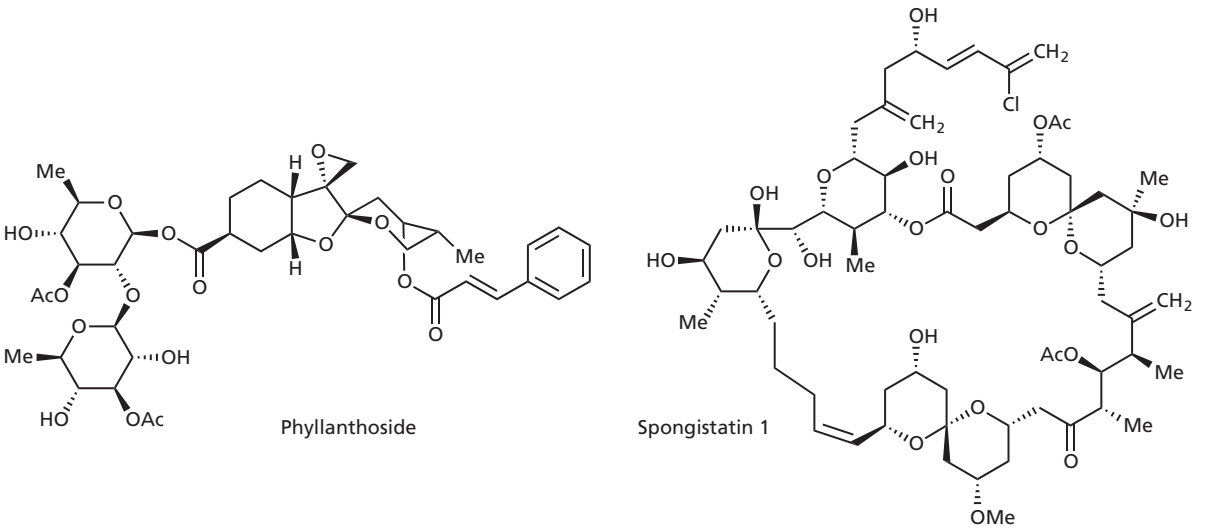


FIGURE 21.35 Natural products inhibiting microtubule formation.

It is interesting to note that the activity of **epipodophyllotoxin** (section 9.2) against tubulin polymerization is an order of magnitude lower than podophyllotoxin and when bulky sugar molecules are present (as in **etoposide**—section 21.2.2.1) activity is removed altogether. This implies that the sugar moieties in etoposide form a bad steric interaction with tubulin which prevents binding.

Cryptophycins (Fig. 21.36) have been isolated from blue–green algae and shown to have an anticancer mechanism which involves the inhibition of microtubule formation. They also inhibit the mechanisms by which microtubules and mitotic spindles function. **Cryptophycin 52** is being considered for clinical trials.

Maytansine 1 (Fig. 21.36) belongs to a group of natural products called the **maytansinoids** which were extracted from an Ethiopian shrub. It has some similarities in structure to the cryptophycins and also inhibits tubulin polymerization, having an activity 1000 times greater than vincristine. Clinical trials had to be abandoned owing to its toxic effects and poor therapeutic window, but it is now being considered as a suitable drug for antibody–drug conjugates (section 21.9.2).

Combretastatins (Fig. 21.37) are natural products derived from the African bush willow (*Combretum caffrum*), a plant which was used by the Zulus as a medicine and as a charm to ward off enemies. **Combretastatin A-4** is the most active structure in this family and has reached clinical trials as its more water-soluble phosphate prodrug. It shares many of the structural features of other tubulin-binding drugs, such as colchicine and podophyllotoxin, and binds to tubulin at the same binding region as colchicine. The relative orientation of the two aromatic rings is important and so the *cis*-geometry of the double

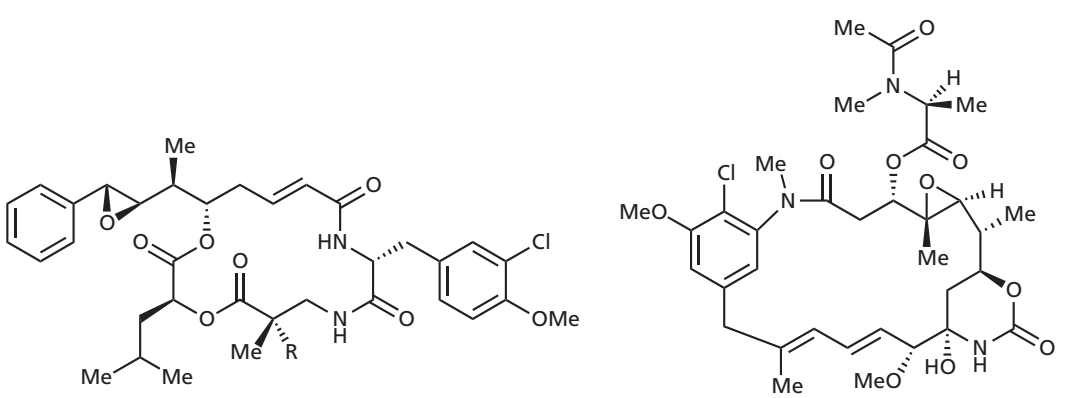
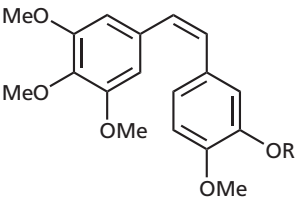


FIGURE 21.36 Cryptophycins and maytansine 1.



Combretastatin A-4; R = H
Combretastatin A-4 prodrug; R = phosphate

FIGURE 21.37 Combretastatins.

bond is crucial to activity. The drug has been shown to selectively inhibit the blood supply to tumours and prevent angiogenesis.

21.5.2 Agents which inhibit tubulin depolymerization

The **taxoids** are an important group of compounds which inhibit tubulin depolymerization and are discussed in section 10.2.2. The best known example is **paclitaxel** (**Taxol**). Semi-synthetic taxoids are currently being investigated to find compounds with better oral bioavailability, improved pharmacological properties, and activity against drug-resistant cancers containing the P-glycoprotein efflux pump.

Since the discovery of paclitaxel, various other natural products have been found to have a similar mechanism of action, and are currently being studied as potential anticancer agents (Fig. 21.38). These include bacterial metabolites called **epothilones** and marine natural products, such as **eleutherobin** isolated from coral. These compounds show several advantages over paclitaxel. Firstly, the epothilones do not appear to be substrates for the P-glycoprotein efflux system, and are potentially effective against drug-resistant cancer cells. Secondly, the epothilones have better aqueous solubility than paclitaxel, which may allow the development of better formulations.

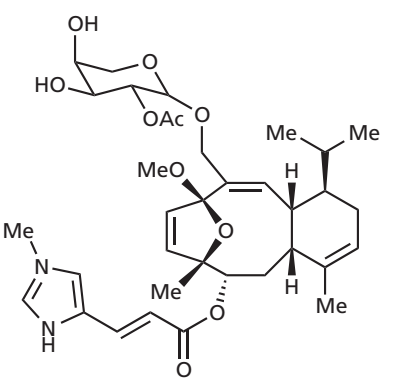
A drawback with the epothilones is their metabolic lability, which results from the cleavage of the lactone ring by esterases. They have also been shown to be highly toxic in animal studies. Therefore, research is being carried out to find analogues with improved properties. This has led to **BMS 247550** (Fig. 21.38), which has entered clinical trials. The lactone in this structure has been replaced by a more stable amide group which stabilizes the molecule to metabolism and also reduces its toxic side effects. Thus, the amide acts as a bioisostere for the lactone group.

These novel agents bind to the same region of tubulin as paclitaxel. A three-dimensional pharmacophore has been developed which encompasses the different structures and which is being used as the basis for the design of hybrid molecules that may lead to a third generation of taxoids.

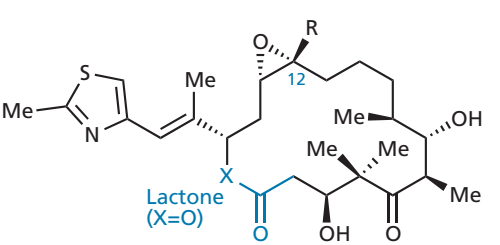
Sarcodictyins (Fig. 21.39) are simplified analogues of eleutherobin and are also active against drug-resistant cancers. Structure–activity relationship (SAR) studies of these compounds have demonstrated the importance of the coloured groups in Fig. 21.39. **Eribulin** is a simplified synthetic analogue of a marine sponge natural product called **halichondrin B**. It was approved in 2010 and is now available in Europe, the USA, and Japan.

KEY POINTS

- Agents which inhibit the polymerization or depolymerization of microtubules are important anticancer agents.
- The vinca alkaloids, podophyllotoxin, the combretastatins, and a variety of other natural products bind to tubulin and inhibit the polymerization process.



Eleutherobin



Epothilone A: R=H, X=O
Epothilone B: R=Me, X=O
BMS 247550: R=Me, X=NH

FIGURE 21.38 Recently discovered natural products which inhibit microtubule depolymerization.

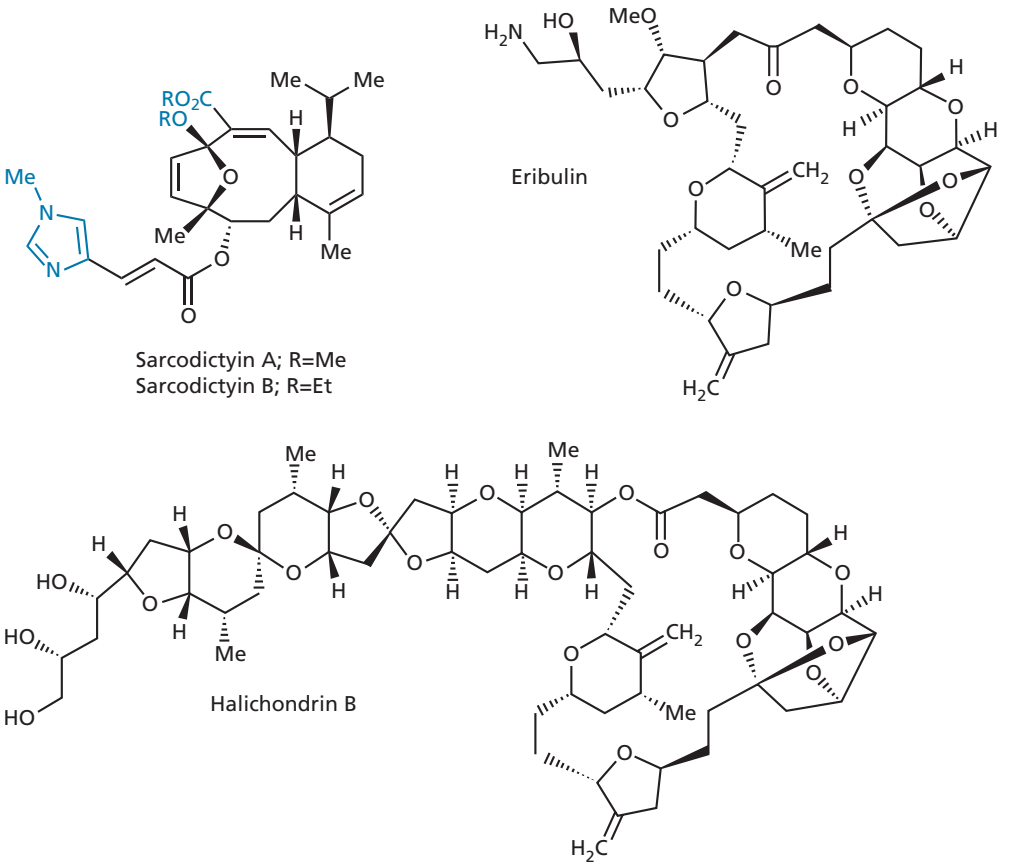


FIGURE 21.39 Sarcodictyins, eribulin, and halichondrin B.

BOX 21.6 Clinical aspects of drugs acting on structural proteins

The Vinca alkaloids **vincristine**, **vinblastine**, and **vindesine** are used intravenously to treat a variety of cancers, including leukaemias, lymphomas, and some solid tumours, such as breast and lung cancer.

Vinorelbine is used widely for the intravenous treatment of advanced breast cancer and non-small-cell lung carcinomas. Neurotoxicity occurs with all the Vinca alkaloids and may limit their use in some patients. Resistance can arise from overexpression of the P-glycoprotein involved in transporting drugs out of the cell.

Podophyllotoxin is the agent of choice for the treatment of genital warts, but it must be handled with care because of its toxicity. Preparations are available which contain the pure compound or the plant extract (**podophyllum**)

Paclitaxel shows outstanding therapeutic activity against solid tumours and was approved for clinical use in 1992 for the treatment of breast and ovarian cancers. **Docetaxel** was approved for the treatment of advanced breast cancer in 1996. Both drugs are in clinical trials for the treatment of a variety of other cancers. They both halt the cell division cycle mainly at the G2/M stage. Apoptosis then takes place. A problem with the use of taxoids is the fact that they cannot be taken orally and they also have various undesirable side effects. Moreover, therapy often leads to the development of multidrug resistance. This involves several mechanisms, including tubulin mutation which results in weaker binding interactions, and overexpression of the P-glycoprotein transport protein which leads to faster efflux from the cell. Another problem with paclitaxel is its poor solubility, which makes formulation difficult. Indeed, some patients cannot tolerate the solvents required. Research is being carried out to design solvent-free drug delivery methods, such as nanoparticles consisting of albumin-bound paclitaxel.

Eribulin binds to the ends of microtubules to prevent depolymerization, which triggers apoptosis of cancer cells. It is approved for the treatment of inoperable and recurrent breast cancers.

- Paclitaxel and its derivatives bind to tubulin and accelerate polymerization by stabilizing the resulting microtubules. Newer analogues are being investigated which show better oral bioavailability, improved pharmacological properties, and activity against drug-resistant cancers.
- A variety of natural products have been discovered which have a similar mechanism of action to paclitaxel.

21.6 Inhibitors of signalling pathways

Most traditional anticancer drugs are cytotoxic both to cancer and normal cells, and any selectivity relies on a greater concentration of the agents within cancer cells. Nowadays, cancer chemotherapy is on the verge of a revolution. Advances in genetics and molecular biology have led to an ever-increasing understanding of the molecular processes behind specific cancers and the identification of a variety of molecular targets which are either unique to a cancer cell or are overexpressed compared with normal cells. The design of agents that will act on these targets promises the development of more selective anticancer agents with less toxic side effects. Understanding the defects in a cell's signalling pathways and identifying suitable targets have already resulted in clinically useful drugs. Suitable targets include the receptors for growth hormones, and the various signal proteins and kinases in the signal transduction pathways. The following sections illustrate some of the most promising lines of research, but it should be appreciated that there is a vast amount of research being carried out in this area and it is not possible to give a comprehensive coverage of it all.

21.6.1 Inhibition of farnesyl transferase and the Ras protein

It has been observed that an abnormal form of the signalling protein **Ras** (sections 5.4.1 and 5.4.2) is present in 30% of human cancers, and is particularly prevalent in colonic and pancreatic cancers. Abnormal Ras derives from a mutation of the *ras* gene to form a *ras* oncogene. Ras proteins are an inherent component of the cellular signalling pathways which control cell growth and multiplication. They are small G-proteins which bind GDP when they are in the resting state, and GTP when they are in the active state. Binding to GTP is temporary, as the protein can auto-catalyse its hydrolysis back to GDP and return to the resting state. Mutant Ras proteins persistently bind GTP, however, and fail to hydrolyse it, such that they are constantly active. As Ras is an integral part of the signalling pathways that control cell growth and division, it is believed that this contributes to the development of cancer. Therefore, finding methods of 'neutralizing' Ras could be useful in combating cancer.

One of these approaches centres around a zinc metalloenzyme called **farnesyl transferase** (**FT**). This enzyme is responsible for attaching a 15-carbon farnesyl group to the Ras protein when it is in the cytoplasm of the cell. The farnesyl group is hydrophobic and acts as a hook and an anchor to hold the Ras protein to the inner surface of the cell membrane. This is necessary if the Ras protein is to interact with other elements of the signal transduction process. Inhibitors of the FT enzyme have been shown *in vitro* to reverse malignancy in cancer cells containing the *ras* oncogene, without affecting normal cells.

The enzyme mechanism (Fig. 21.40) involves the binding of **farnesyl diphosphate** (**FPP**) to the active

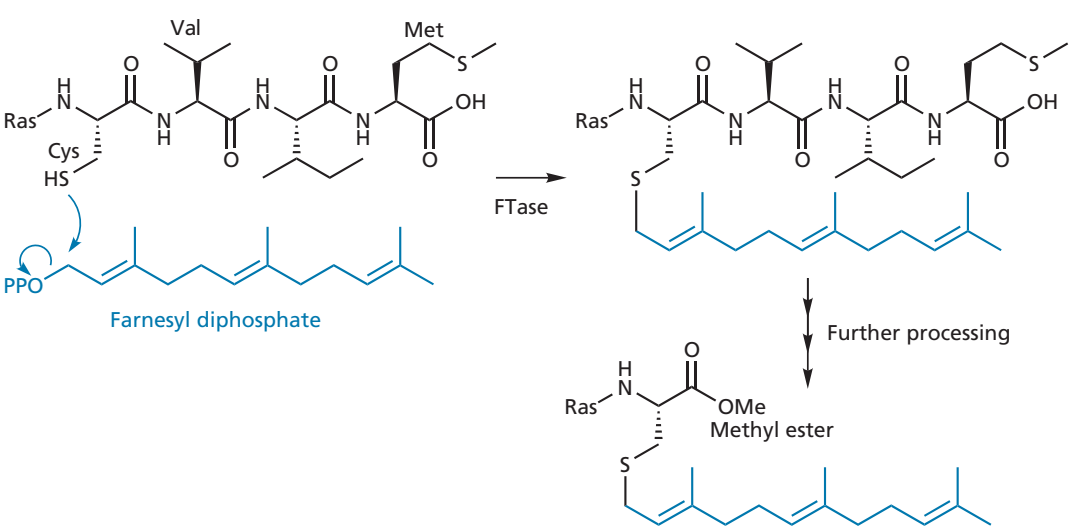


FIGURE 21.40 Mechanism of farnesyl transferase.

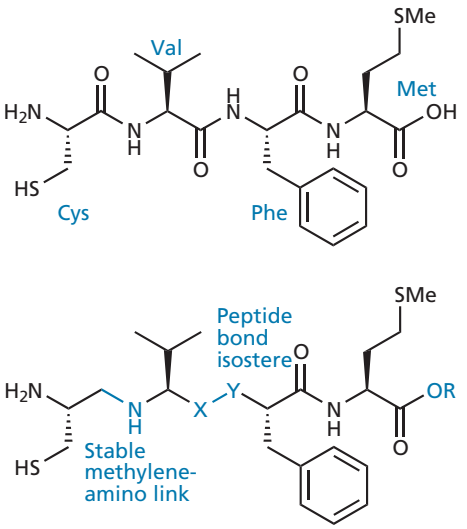


FIGURE 21.41 Design of farnesyl transferase inhibitors.

site, followed by the Ras substrate. This order of binding is important, as FPP is actually involved in binding the Ras protein. Farnesylation can then take place where a cysteine residue on the Ras protein displaces a pyrophosphate leaving group from FPP. Magnesium and iron ions are present in the active site as cofactors. The former is involved in complexing the negatively charged pyrophosphate group to make it a good leaving group, while the latter interacts with the thiol group of cysteine, enhancing its nucleophilicity. Following farnesylation, the terminal tripeptide of Ras is cleaved and the resulting carboxylic acid is methylated to a methyl ester. Methylation is important or the charged carboxylic acid would hinder the binding of the farnesyl chain to the cell membrane.

Inhibitors have been developed which mimic the terminal tetrapeptide moiety of the Ras protein. This region, which is common to different types of Ras protein, is known as the **CaaX peptide** (C stands for cysteine, a is valine, isoleucine, or leucine, and X is methionine, glutamine, or serine). Studies on a variety of tetrapeptides were carried out to see what effect modification of these positions would have. These studies showed that placing an aromatic amino acid such as phenylalanine next to X transformed the tetrapeptide from a substrate into an inhibitor (Fig. 21.41). This then served as a lead compound for further development which involved the replacement of two of the peptide links with stable isosteric groups, and masking of the carboxylic acid. We shall now look more closely at why these modifications were carried out.

As the target enzyme is within the cell, inhibitors have to cross cell membranes, show metabolic stability, and have suitable pharmacokinetic properties. However, the lead compound is a tetrapeptide and suffers from various disadvantages.

- Firstly, it has a polar carboxylic acid group which is bad for absorption. This problem can be overcome by masking the acid group as an ester prodrug such that the molecule can cross cell membranes more easily. Once inside the cell, the ester can be hydrolysed to give the required carboxylic acid.
- Secondly, the lead compound has peptide bonds that are susceptible to metabolism, particularly by **aminopeptidases**. Replacing two of these bonds with stable methyleneamino groups avoided this problem but introduced a different one. The resulting amines are more nucleophilic than the original peptide groups, and one of these was able to carry out an intramolecular cyclization with the terminal ester to form an inactive diketopiperazine structure (Fig. 21.42). This could be avoided by replacing the offending amine with an ether.

These features are seen in some of the most notable inhibitors studied so far, mainly **L 739750**, **FTI 276**, and their respective ester prodrugs (Fig. 21.43). These have shown promising *in vivo* results on cancers in transgenic mice, without obvious toxicity. Both structures contain the important thiol group as a ligand for the zinc ion cofactor, the stable methyleneamino moiety, and the aromatic substituent which is beneficial to inhibitory activity. The isostere for the middle peptide link is a methyleneoxy group in L 739750 and an aromatic ring in FTI 276. The methylthio substituent has been replaced by a sulfone group in L 739750, as this was found to increase activity.

The terminal amino group in both drugs is important for binding interactions. It is ionized and forms an ionic bond with an ionized phosphate group of FPP. The carboxylic acid group is also important for binding.

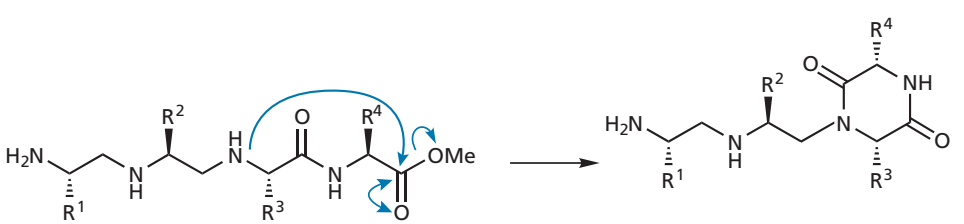


FIGURE 21.42 Diketopiperazine formation.

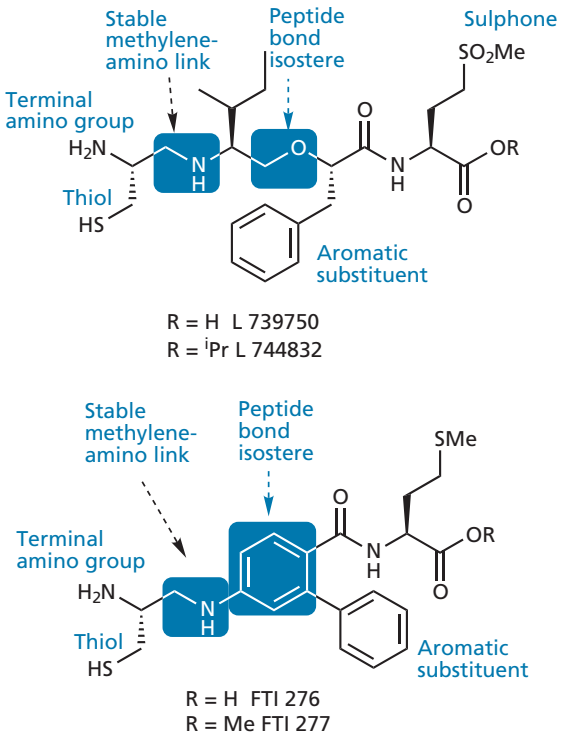


FIGURE 21.43 FTase inhibitors.

Although the thiol group is important as a ligand for zinc, there are problems related to its potential toxicity and its susceptibility to oxidation by metabolic enzymes. The AstraZeneca compound **AZD-3409** (Fig. 21.44) has the thiol and carboxylic acid groups masked in a double prodrug strategy to try to alleviate this problem. A pyrrolidine ring is also present and introduces conformational rigidity to this portion of the molecule. The drug is a potent inhibitor of FTase ($K_i < 1$ nM) and it also inhibits a related prenylating[1] enzyme called **geranylgeranyltransferase** (**GGTase**) (K_i = 8 nM). This enzyme can also catalyse prenylations, but uses a 20-carbon structure called **geranylgeranyl diphosphate** as the prenylating agent. Normally, GGTase prenylates proteins having the CaaX motif where X = Leu, but it is possible that it may prenylate proteins which are normally prenylated by FT if the latter enzyme is inhibited. Such a reaction would allow these proteins to bind to cell membranes and still be functional, thus bypassing the inhibition of FT. Therefore, an agent capable of inhibiting both enzymes may be beneficial.

Non-peptide inhibitors have also been designed and are undergoing clinical trials (see also Box 21.7). Structure I (Fig. 21.45) has an imidazole ring acting as the zinc ligand rather than a thiol group. This is to avoid the undesirable side effects of the latter group. The imidazole ring has previously been shown to act as a zinc ligand in other structures.

Lonafarnib (Fig. 21.45) was developed from a lead compound that was discovered by screening compound libraries, and is 10,000 times more active than the original structure. Remarkably, it has no ligand for the zinc cofactor and so structure-based drug design was carried out to introduce a suitable group at the correct position, resulting in **Sch 226374** (Fig. 21.46). The imidazole ring acts as the zinc ligand while an aromatic ring acts as a steric shield to protect it from metabolism.

Although farnesyl transferase inhibitors (FTIs) show promise as anticancer agents, it is questionable whether their observed activity is caused solely by their action in inhibiting the farnesylation of the Ras protein. For example, there are three human Ras proteins (H-Ras, N-Ras, and K-Ras). FTIs inhibit the farnesylation of all three but can only inhibit the *cellular* functions of H-Ras, as the other two Ras proteins can be prenylated by GGTase and become linked to the cell membrane. Nevertheless, anticancer effects are still observed in cancers where it is the K-Ras protein that is being expressed. As farnesyl transferase can accept a variety of different protein substrates other than Ras, it is possible that inhibition affects other cellular processes to produce the observed anticancer activity. These proteins could include several nuclear proteins, such as centromere-associated proteins and protein phosphatases. The former are associated with chromosome alignment and the mitotic checkpoint. Inhibition of these proteins could prevent the cell entering the mitosis phase.

As a final point, it has been observed that **statins** inhibit tumour cell growth and this has been ascribed to an effect on Ras farnesylation. These structures inhibit the HMGR enzyme (Case study 1) involved in the biosynthetic pathway to both steroids and isoprenoids. Consequently, statins can influence Ras protein farnesylation by lowering the level of farnesyl diphosphate. Other structures are

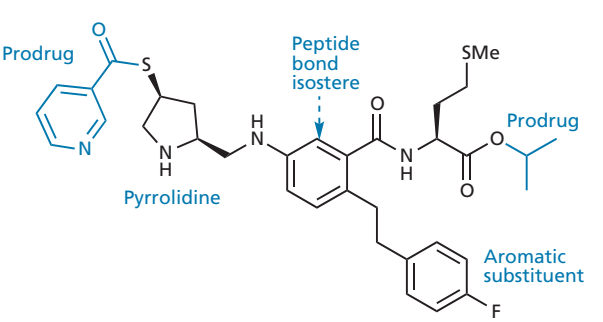
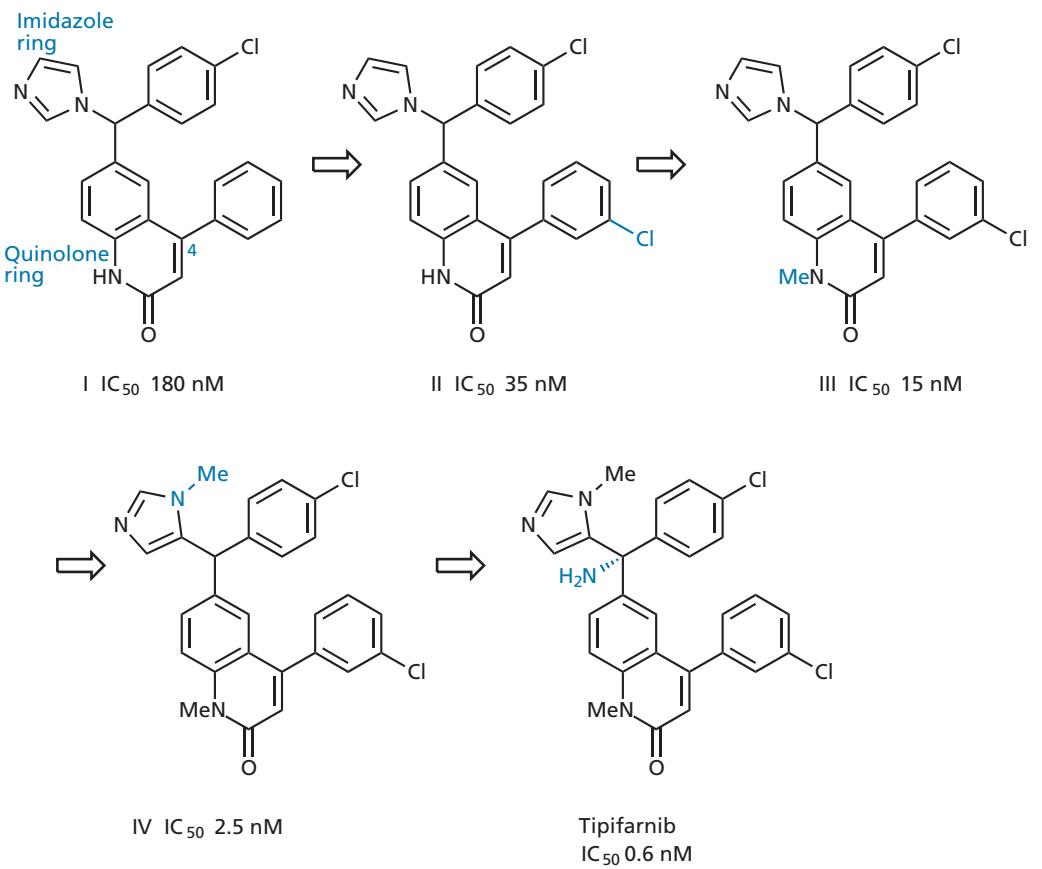


FIGURE 21.44 AZD-3409.

[1] Prenylation is the term used to describe the formation of a covalent bond between a molecule such as a protein, and a prenyl moiety such as a farnesyl or geranylgeranyl group.

BOX 21.7 Development of a non-peptide farnesyl transferase inhibitor

The screening of compound libraries produced the lead compound (I) for **tipifarnib**—an agent undergoing clinical trials as a farnesyl transferase inhibitor. SAR studies were carried out on structure (I) and established the importance of both aromatic rings to activity. Alterations were then carried out to improve activity, namely the introduction of a *meta*-chloro substituent (structure II), *N*-methylation of the quinolone (structure III), altering the position of the nitrogens on the imidazole ring (structure IV), and, finally, the introduction of a primary amino group. The imidazole ring acts as a ligand for the zinc cofactor in the enzyme's active site.



Development of tipifarnib.

being investigated that can block Ras cell signalling by interacting with Ras itself or targets involved in the signalling pathway, for example **rasfonin** (section 12.4.1.2).

21.6.2 Protein kinase inhibitors

Protein kinases are enzymes which phosphorylate specific amino acids in protein substrates. It is estimated that there may be over 500 different types of protein kinase and a vast amount of research is currently being undertaken on potential inhibitors of these enzymes. Many are enzymes within the cytoplasm of the cell (sections 5.2 and 5.3), while others (protein kinase receptors) traverse the cell membrane and play a dual role as receptor and enzyme (sections 4.8 and 5.4). The latter structures have an extracellular binding site to receive an external molecular messenger, and an intracellular kinase active site which is activated when the messenger binds to the receptor's binding site. The chemical messengers involved are a wide variety of growth hormones

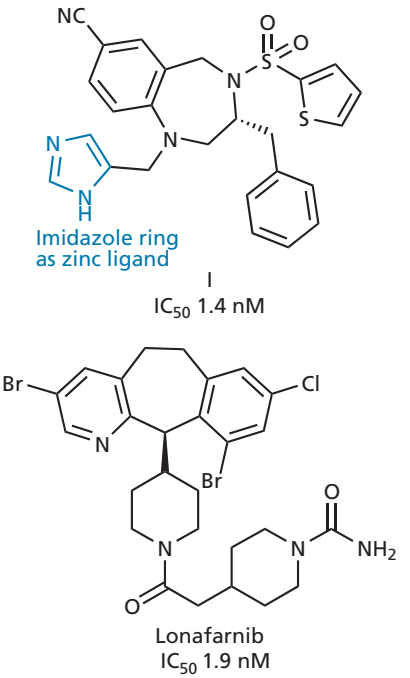
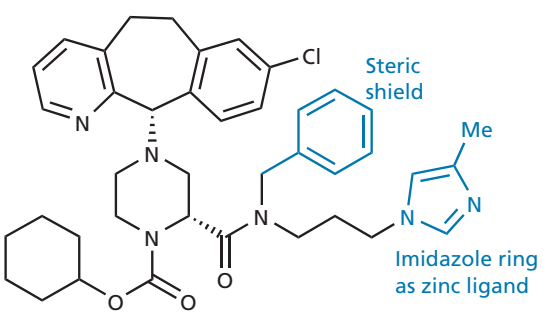


FIGURE 21.45 Non-peptide-like inhibitors of FTase undergoing clinical trials.

and growth factors which trigger the start of a signalling cascade that involves the various cytoplasmic protein kinases. This process ultimately controls transcription of specific genes in DNA leading to cell growth and cell division. In many cancers, it has been observed that there is an excess of a particular growth hormone or growth factor, or an excessive quantity of a particular protein kinase or protein kinase receptor. As these structures are intimately involved in the signal transduction processes which drive cell growth and cell division, it is reasonable to assume that protein kinase inhibitors will be useful anticancer agents.



Sch 226374; IC_{50} 0.36 nM

FIGURE 21.46 Development of a lonafarnib analogue.

Protein kinases can be divided into two main categories—the tyrosine kinases and the serine–threonine kinases. More recently, histidine kinases have been discovered which phosphorylate the nitrogen of a histidine residue. The tyrosine kinases phosphorylate the phenol group of tyrosine residues, whereas the serine–threonine kinases phosphorylate the alcohol group of serine and threonine residues (section 5.2.2). All the kinases use the cofactor **adenosine triphosphate** (ATP) as the phosphorylating agent and so there is a region within the active site that binds ATP and a neighbouring region that binds the substrate. In theory, it should be possible to design inhibitors that bind to one or other of these regions, but, so far, the best results have been achieved with inhibitors capable of binding to the cofactor binding region. Considering the fact that there are so many kinases and that they all use ATP as the phosphorylating agent, it was originally thought that achieving selectivity between kinases would be a major problem. This has not turned out to be the case. Crystal structures of protein kinases containing bound ATP reveal that ATP fits quite loosely to the active site and that there are areas which remain unoccupied. There are also significant differences between kinases with respect to the amino acids present in these unoccupied areas. As a result, it is quite possible to design selective inhibitors.

A knowledge of how ATP is bound to the kinase active site has helped enormously in the design of potent and selective agents. For example, the binding interactions of ATP with the kinase active site of the **epidermal growth factor receptor** (**EGF-R**) are shown in Fig. 21.47 and are representative of all the kinase active sites. The purine base is buried deep in the active site and makes two important hydrogen bonding interactions with the protein backbone in a region of the protein known as the **hinge region**, so called because it connects two distinct lobes of the enzyme. The heterocyclic ring also forms van der Waals interactions with the amino acids round about it. The ribose sugar is bound into a **ribose binding pocket** and the triphosphate chain lies along a cleft leading to the surface of the enzyme. The ionized triphosphate interacts with two metal ions and with several amino acids through hydrogen bonding. There are also various areas of unoccupied space, one of which is particularly important and consists of a hydrophobic pocket opposite the ribose binding pocket (**hydrophobic pocket I**). At the entrance to this pocket, there is an important amino acid residue which is called the **gatekeeper residue**. In some kinases, the gatekeeper residue is large and blocks access to the pocket, whereas in other kinases the gatekeeper residue is small, allowing drugs to be designed that will access and interact with the pocket. The hydrophobic pocket is also lined by different amino acids depending on the kinases involved, which opens

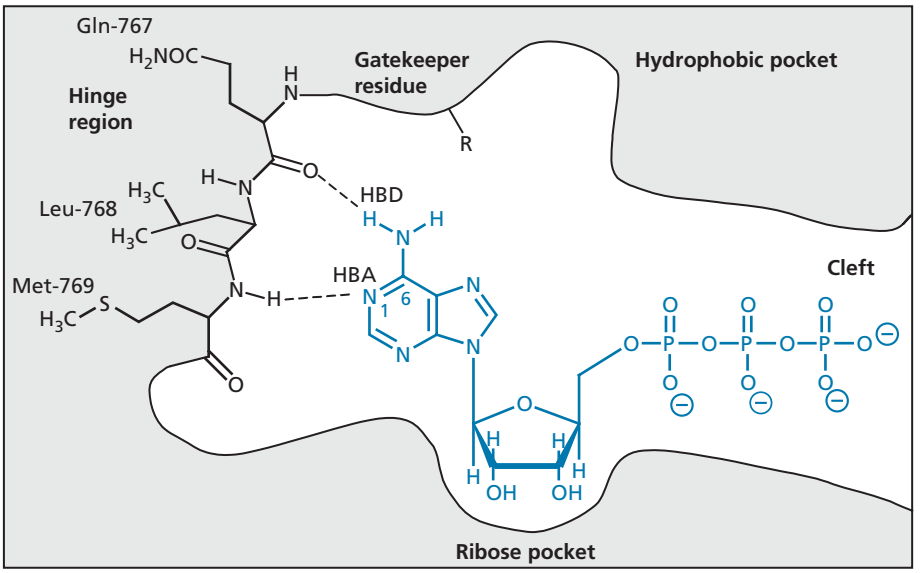


FIGURE 21.47 Binding of ATP to the kinase active site of the epidermal growth factor receptor.

up the possibility of designing drugs that can distinguish between the hydrophobic pocket of one kinase active site from the hydrophobic pocket of another.

Kinases exist in an active conformation, as well as one or more inactive conformations. The switch by which a kinase changes from an inactive conformation to the active conformation is controlled by an **activation loop**. Activation usually occurs by phosphorylation of residues on this loop, which causes the loop to move position. This has a marked effect on the position of a conserved triad of amino acids (Asp, Phe, and Gly) near the start of the activation loop, whereby Asp and Phe are orientated towards the binding site (**DFG-in**). In inactive forms of the enzyme, an extra hydrophobic region (**hydrophobic pocket II**) is exposed that is not exposed in the active form, and which is close to hydrophobic pocket I. This provides the potential for designing novel kinase inhibitors capable of binding and stabilizing an inactive conformation.

Kinase inhibitors are classed as **type I** or **type II inhibitors**. In both cases they bind to the active site and prevent the binding of the cofactor and substrate. Type I inhibitors normally bind to the active conformation of the enzyme, whereas type II inhibitors bind to an inactive conformation. Protein kinase inhibitors, such as **gefitinib**, **erlotinib**, **SU11248**, and **seliciclib** are type I inhibitors, while agents such as **imatinib**, **nilotinib**, **sorafenib**, and **vatalanib** are type II inhibitors. **Sunitinib** and **dasatinib** are able to bind to both active and inactive forms of the same kinase enzyme and could be defined as type I or type II. The story is further complicated by the fact that some inhibitors act as a type I inhibitor at one kinase target and as a type II inhibitor at another.

It has been observed that there is a significant variation of amino acids in hydrophobic region II between different kinases, suggesting that type II inhibitors have the potential to be more selective. However, as the amino acids in the additional hydrophobic region are less conserved, there is a greater possibility of drug resistance caused by random mutations. These could result in a viable kinase that would fail to bind the inhibitor. There is also the problem that type II inhibitors tend to be larger molecules, which may limit their ability to cross cell membranes.

To date, all clinically important inhibitors have binding interactions that mimic the adenine interactions of the cofactor ATP, namely two or three hydrogen bonds to the hinge region, plus van der Waals interactions with surrounding amino acids. Selectivity is obtained by designing interactions with regions of the active site not occupied by ATP, such as hydrophobic pocket I, or with the gatekeeper residue. In the case of type II inhibitors, additional van der Waals interactions are possible with the extra hydrophobic region II, as well as hydrogen bonds to two conserved amino acids (Glu and Asp) in that same region. The aspartate residue is part of the conserved triad mentioned above.

There are also investigations into **type III inhibitors** which bind purely to regions unoccupied by ATP, such as hydrophobic pockets I and II. Such agents have been classed as allosteric inhibitors.

21.6.2.1 Kinase inhibitors of the epidermal growth factor receptor (EGF-R)

EGF-R is a membrane-bound tyrosine kinase receptor that has an extracellular binding site for epidermal growth

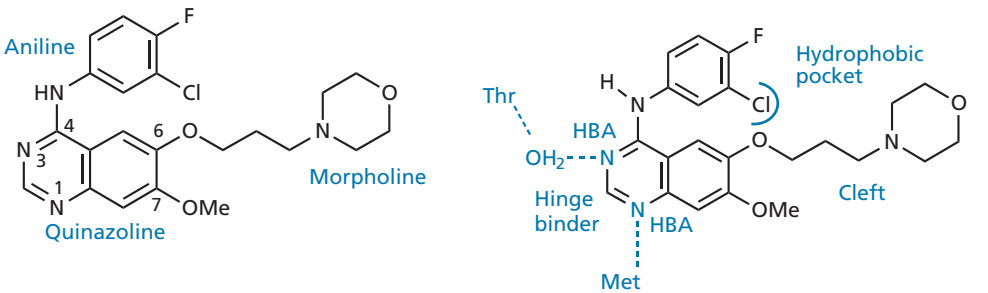


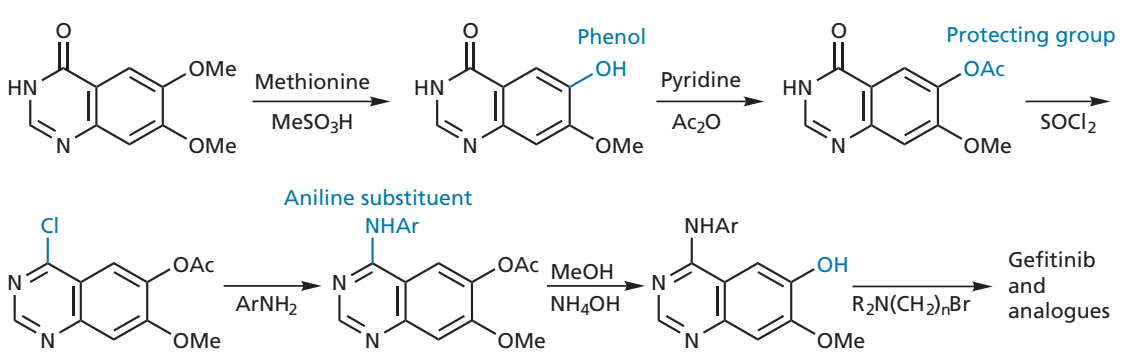
FIGURE 21.48 Structure and binding interactions of gefitinib (Iressa).

factor (EGF) and an intracellular kinase active site (sections 4.8.2 and 4.8.3). Several agents have been studied as EGF-R kinase inhibitors and the first of these to reach the clinic was **gefitinib** (**Iressa**) (Fig. 21.48 and Box 21.8).

Gefitinib was developed by Astra Zeneca and belongs to a group of structures known as the 4-anilinoquinazolines. It was developed from a potent inhibitor (I in Fig. 21.49) which had various important features previously identified by SAR studies, namely a secondary amine, electron-donating substituents at positions 6 and 7, and a small lipophilic substituent on the aromatic ring. The structure had useful *in vitro* activity, but its *in vivo* activity was hampered by the fact that it was metabolized rapidly by cytochrome P450 enzymes to give two metabolites. Oxidation of the aromatic methyl group resulted in metabolite II and oxidation of the aromatic *para*-position resulted in metabolite III. Both these types of positions are well known to be vulnerable to oxidative metabolism (section 11.5). Therefore, it was decided to modify the structure such that both metabolic routes were blocked. In structure IV in Fig. 21.49, the methyl group was replaced by a chloro substituent. This can be viewed as a bioisostere for the methyl group as it is of similar size and lipophilicity, but it has the advantage that it is resistant to oxidation. A fluoro substituent was chosen to block oxidation of the aromatic *para*-position. Fluorine is essentially the same size as hydrogen and so there is little risk of any adverse steric effects arising from its introduction. Although the

BOX 21.8 General synthesis of gefitinib and related analogues

A general synthesis for gefitinib and its analogues starts from a quinazolinone starting material which acts as the central scaffold for the molecule. The synthesis is then a case of introducing the two important substituents. Selective demethylation reveals a phenol which is then protected by an acetate group to prevent it reacting with subsequent reagents. Chlorination is now carried out on the carbonyl group and the resulting chloro substituent is substituted by an aniline to introduce the first important substituent. Deprotection of the phenol group and reaction with an alkyl halide introduces the second important substituent.



Synthesis of gefitinib (Iressa) and related analogues.

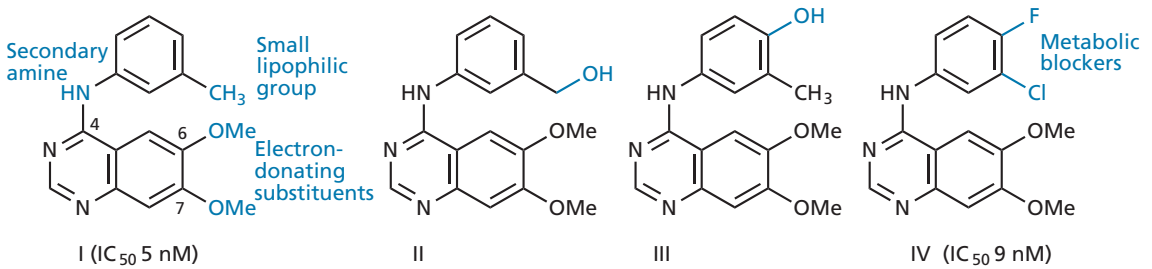


FIGURE 21.49 Design of a metabolically stable analogue of structure (I).

resulting compound was less active *in vitro* as an enzyme inhibitor, it showed better *in vivo* activity since it proved resistant to metabolism. Further modifications were then carried out to optimize the pharmacokinetic properties of the drug. A variety of alkoxy substituents at the 6-position were tried, culminating in the discovery of gefitinib. This contains a morpholine ring, which is often introduced to enhance water solubility. Because the morpholine ring includes a basic nitrogen, it is possible to protonate it and form water-soluble salts of the drug (e.g. hydrochloride or succinate salts). Note that the addition of a water-soluble 'handle' is a common feature in many kinase inhibitors. The group plays no role in target binding and it is important that it is positioned in such a way that it is in a solvent-exposed region of the drug when the latter is bound to the target binding site. In other words, the group should protrude from the binding site and be exposed to the surrounding aqueous environment. This avoids the energy penalty that would be required if the surrounding solvation coat had to be stripped away from such a polar group (see section 1.3.6). The acidity or basicity of this group also plays an important role in plasma–protein binding, which affects the distribution and metabolism of these inhibitors.

Other EGF-R kinase inhibitors include **PKI-166**, which is undergoing clinical trials, and **erlotinib** (Fig. 21.50) which is now approved. PKI-166 is a pyrrolopyrimidine structure which binds differently from the quinazoline structures above. Here, the important hydrogen bonding interactions to the adenine binding region involve an N on the pyrimidine ring and an NH on the pyrrole ring. Erlotinib binds like gefitinib, and the acetylene group fits into the hydrophobic pocket guarded by the gatekeeper residue threonine.

The binding interactions of ATP, quinazolines, and pyrrolopyrimidines illustrate an important point. All three classes of compound contain a pyrimidine ring with an NH substituent at position 4. Having seen how this group binds for ATP (Fig. 21.47) it would be tempting to assume that it binds in the same manner for the other structures containing it. The fact that it does not illustrates the importance of analysing crystal structures of enzyme–inhibitor complexes and not making assumptions. The binding site for ATP is quite spacious, so it is perfectly feasible for molecules to bind in different modes. Indeed, it is possible for different molecules within the same structural class to bind in different modes depending on the substituents that are present.

Lapatinib (Fig. 21.51) has the same quinazoline 'core' as erlotinib and gefitinib and was approved in March 2007. Unlike its older cousins, lapatinib binds to an inactive form of the kinase which exposes a hydrophobic pocket that is not exposed in the active form. The fluorobenzyloxy substituent forms extra interactions with this pocket and results in potent activity for an additional kinase called **ErbB2** (**HER-2**). Thus, lapatinib is a **dual-action inhibitor** that can be used for cancers which overexpress both EGFR and ErbB2. The chain containing the amine and the sulphonyl group increases aqueous solubility and is located in a region

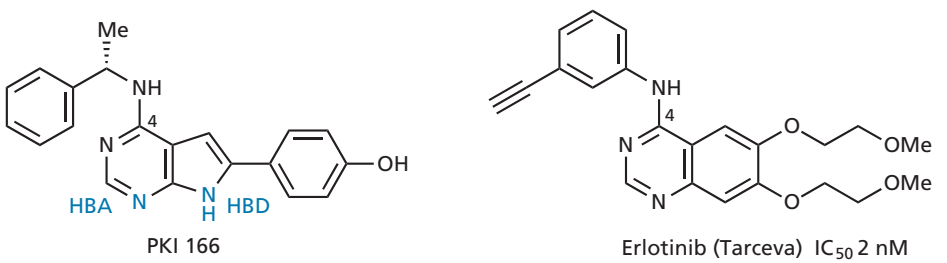


FIGURE 21.50 Inhibitors of the epidermal growth factor receptor kinase.

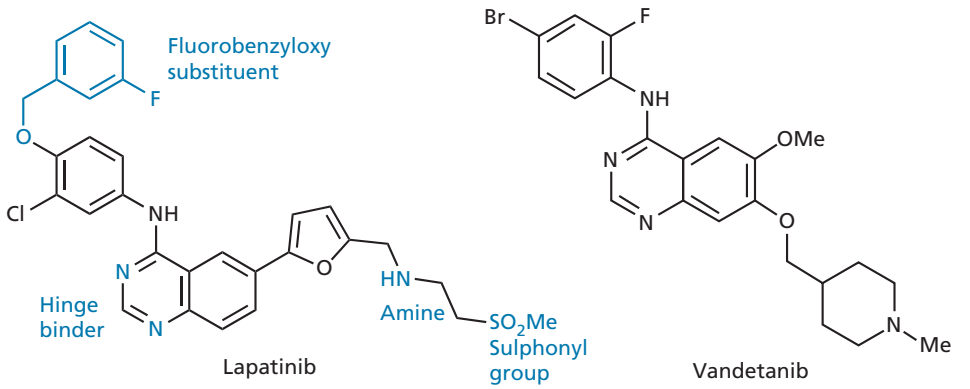


FIGURE 21.51 Lapatinib and vandetanib (ZD6474).

of the active site that is exposed to solvent. **Vandetanib** was approved in 2011 and is another dual-action inhibitor of EGF-R and Erb2. In addition, it inhibits the kinase activity of the **vascular endothelial growth factor receptor** (VEGF-R), which is associated with angiogenesis. Kinase inhibitors of VEGF-R disrupt the angiogenesis process and starve tumours of nutrients. Because it blocks both the EGFR and VEGFR signal transduction pathways, vandetanib is an example of an **extended-spectrum agent**.

21.6.2.2 Kinase inhibitors of Abelson tyrosine kinase, c-Kit, PDFG-R, and SRC

As the first protein kinase inhibitor to reach the market, **imatinib** (**Glivec** or **Gleevec**; Fig. 21.52 and Box 21.9) represents a milestone in anticancer therapy. It was also the first drug designed to target a molecular structure which is unique to a cancer cell. It acts as a selective inhibitor for a hybrid tyrosine kinase called **Bcr-Abl**, which is active in certain tumour cells. The tyrosine

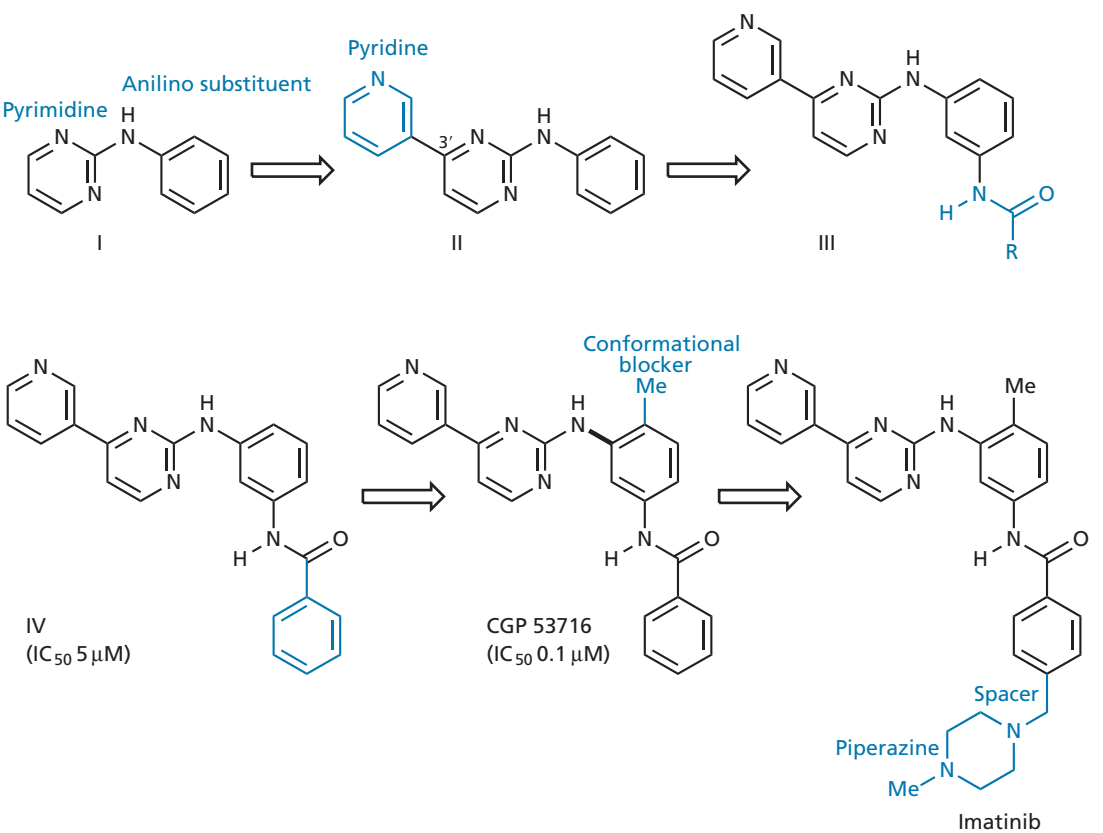
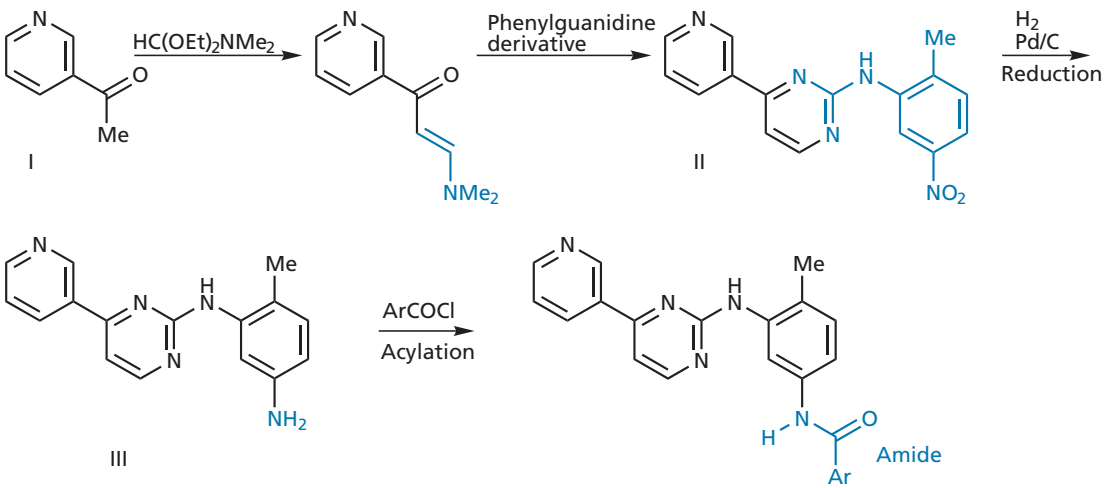


FIGURE 21.52 Development of imatinib.

BOX 21.9 General synthesis of imatinib and analogues

The synthesis of imatinib and its analogues involves the pyridine structure (I) as starting material. The central pyrimidine ring is then constructed in two stages to give structure (II). The remaining two steps involve reduction of an aromatic nitro group to an amine and acylation to give the final product. The route described allows the synthesis of a large variety of amides from intermediate (III).



General synthesis of imatinib and analogues.

kinase active site resides on the Abl portion of the hybrid protein.

The lead compound (I in Fig. 21.52) used for the development of imatinib was a phenylaminopyrimidine structure identified by random screening of large compound libraries. The original aim of this search was to find inhibitors of a different protein kinase known as protein kinase C (PKC)—a serine–threonine kinase. Strong inhibition of PKC was achieved by adding a pyridyl substituent at the 3′-position of the pyrimidine (II). Adding an amide group to the aromatic ring then led to structures which also showed inhibitory activity against tyrosine kinases. For example, structure IV inhibited serine–threonine protein kinases, such as PKC-α, and was also a relatively weak inhibitor of tyrosine kinases. A series of chemically related structures was then synthesized to test SAR against a variety of protein kinases and to optimize activity against tyrosine kinases. Introduction of an *ortho* methyl group as a conformational blocker (section 13.3.10) resulted in CGP 53716 which had enhanced activity against tyrosine kinases and no activity against serine–threonine kinases, demonstrating that the molecule had been forced to adopt a conformation which suited binding to tyrosine kinases but not to serine–threonine kinases. The conformational blocker hinders rotation of the Ar-N bond shown in bold (Fig. 21.52) such that the pyridine and pyrimidine rings are positioned away from the conformational blocker. Further modifications were then carried out to maximize activity and selectivity with the addition of a piperazine ring. This ring is also important for aqueous solubility as it contains a basic nitrogen which allows the formation of water-soluble salts. A one-carbon spacer was introduced between the aromatic ring and the piperazine ring, as aniline moieties are known to have mutagenic properties.

The X-ray crystal structure of imatinib bound to an inactive conformation of Abl kinase has been determined. This demonstrates the importance of the amide group within imatinib which serves as an **anchoring group** (Fig. 21.53). The amide forms hydrogen bonds to conserved glutamate and aspartate residues. These interactions orientate the molecule allowing either half of the structure to access hydrophobic pockets which determine target selectivity. There is a hydrogen bonding interaction between an amino group in imatinib and the 'gatekeeper' threonine residue in the active site. The importance of this interaction is emphasized by the loss of activity observed when the amino group is alkylated. The pyridine and pyrimidine rings are located within one of the hydrophobic regions,

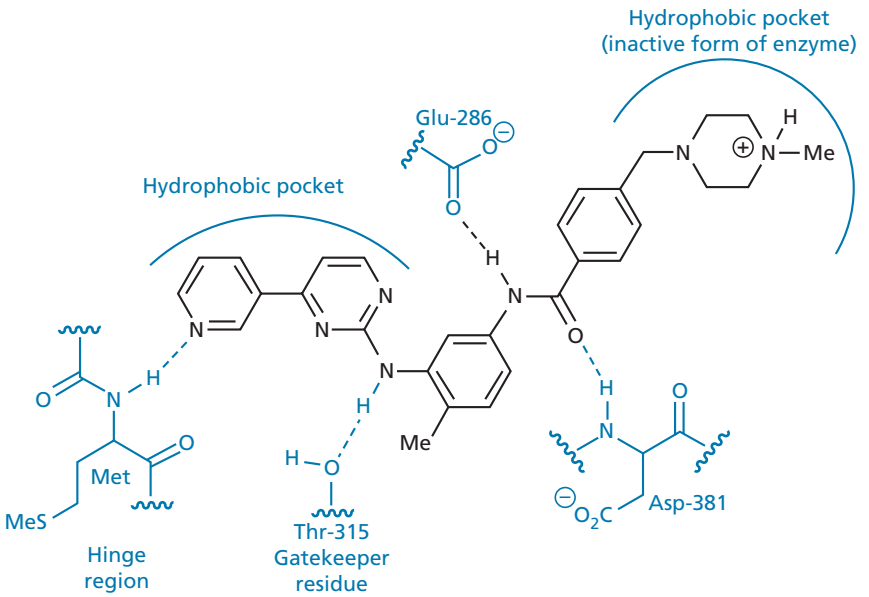


FIGURE 21.53 Binding interactions of imatinib in the active site of Abl kinase.

and the piperazine ring is in the other. Separate modelling studies suggest that the piperazinyl group forms an ionic interaction with a glutamate residue. This residue is conserved in three protein kinases (Abl, c-Kit, and PDGF-R) and imatinib is an inhibitor of all three. In contrast, the glutamate residue is absent from the tyrosine kinases (EGFR and c-SRC) and these kinases are not inhibited by imatinib. Therefore, this ionic interaction is likely to be important to the selectivity of the agent. Selectivity is also favoured by the *ortho* methyl group that was introduced as a conformational blocker. The methyl group is able to bind to a hydrophobic pocket that would not be accessible if a larger gatekeeper residue was present.

The fact that imatinib is not totally selective and inhibits a number of different kinases led to the concern that it would have serious side effects. Fortunately, this is not the case. It appears that normal cells are able to survive inhibition of these kinases, whereas the survival of cancer cells containing Bcr-Abl relies crucially on that protein. Therefore, reliance of a cancer cell on an abnormally functioning protein sensitizes it to agents which target that protein.

Acquired resistance to imatinib has been observed owing to mutations in the Abl kinase domain that prevent the drug from binding. Specifically, a mutation that alters the gatekeeper threonine residue to isoleucine has

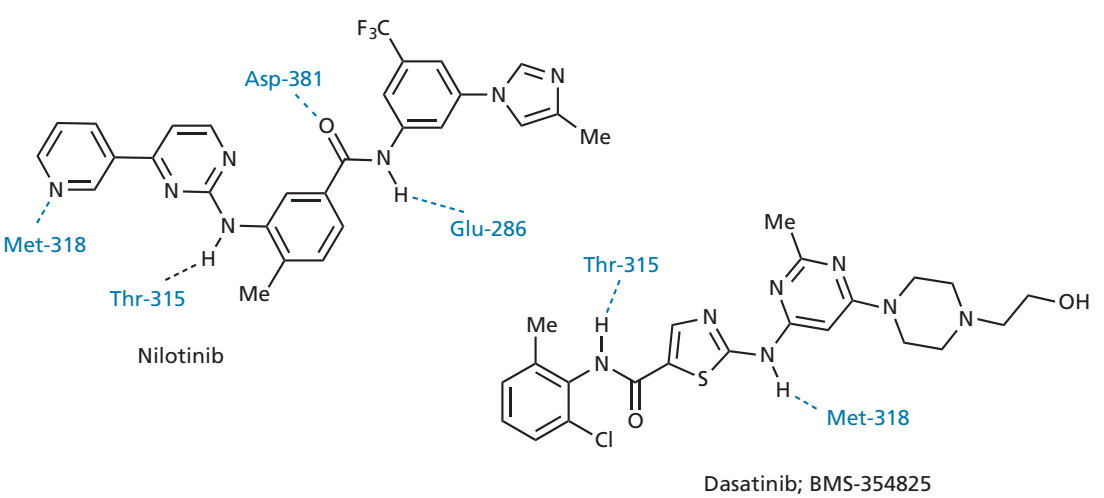


FIGURE 21.54 Nilotinib and dasatinib.

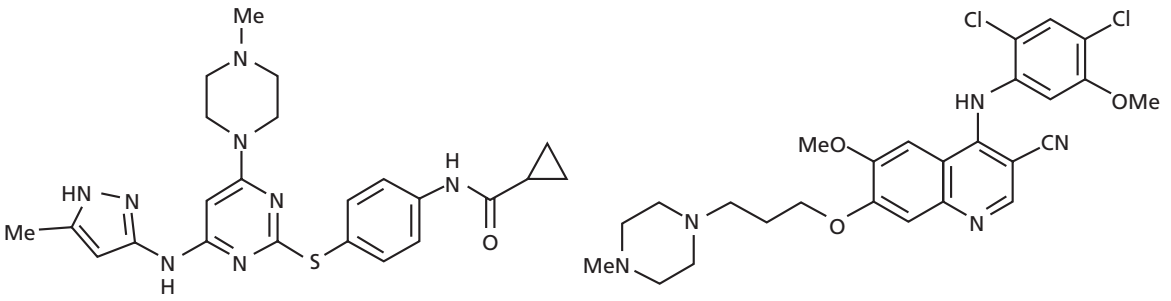


FIGURE 21.55 Tozasertib and bosutinib.

been observed at position 315 (the T315I mutation). Imatinib forms an important hydrogen bond to Thr-315 which is not possible with an isoleucine residue. Other point mutations have also been observed. Alternative signalling pathways may be adopted in some resistant cells and an increased expression of the target receptor may occur in others.

Nilotinib and **dasatinib** (Fig. 21.54) represent a second generation of Bcr-Abl inhibitors that are active against most imatinib-resistant tumours, but not against the T315I mutant; the interaction with Thr315 is crucial for both structures. The *N*-methylpiperazine ring in imatinib has been replaced by an imidazole ring in nilotinib, increasing affinity for Bcr-Abl 20–30 fold, while retaining activity for c-Kit and PDGF-R.

Dasatinib binds with greater affinity than either nilotinib or imatinib, and can bind to both active and inactive forms of the enzyme.

There are several projects aiming to design kinase inhibitors that are less likely to fall prey to the problems of drug resistance, such as structures that do not rely on the interaction with Thr315. Such structures should prove effective against the T315I mutant, especially if they take advantage of any unique features in the mutant binding site. **Tozasertib** is one such compound currently undergoing clinical trials.

Another way of tackling the problem of resistance is to design drugs which have a dual target. For example, dasatinib inhibits the kinase active sites of Abl and another kinase enzyme called **Src**; the latter plays a crucial role in cell movement and proliferation. **Bosutinib** (Fig. 21.55) is another structure currently in clinical trials.

Another strategy is to develop kinase inhibitors which bind to Bcr-Abl at different parts of the active site from the ATP binding region. For example, allosteric inhibitors are being studied that stabilize the inactive form of the protein by binding to an autoregulatory binding cleft which is distant from the active site. **GNF-2** (Fig. 21.56) is one such compound undergoing clinical trials which shows extremely good selectivity and has the potential to be the first anticancer drug to truly target leukaemia. Also under study are agents such as **ON012380** (Fig. 21.56), which binds to the substrate binding region rather than the ATP binding region. Many researchers feel that such inhibitors could be more selective and safer to use.

Finally, combination therapies that use drugs capable of targeting different regions of the same protein kinase may be therapeutically important in the future and help to combat resistance against any one drug.

Molecular modelling exercise 21.1.

21.6.2.3 Inhibitors of cyclin-dependent kinases (CDKs)

CDKs are involved in the control of the cell cycle (section 21.1.6), but are overexpressed or overactive in many

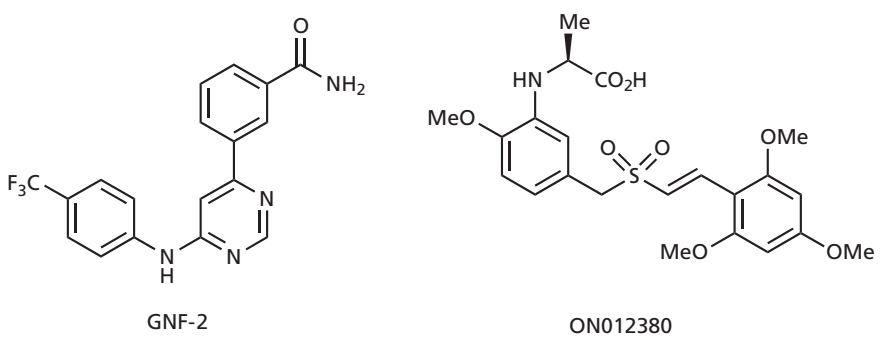


FIGURE 21.56 Structures of GNF-2 and ON012380.

cancer cells. As these enzymes are inactive in normal resting cells, drugs that target them should have fewer, and less toxic, side effects than conventional cytotoxic drugs. CDKs are serine–threonine kinases which are activated by **cyclins** and inhibited by **cyclin-dependent kinase inhibitors** (**CKIs**). There are at least nine CDKs and they are typically small proteins of about 300 amino acids. At present, a variety of inhibitors have been identified which compete with ATP for the kinase active site. **Flavopiridol** (Fig. 21.57) is one such structure which is undergoing clinical trials and looks promising as part of a combination therapy. It is a semi-synthetic flavone derived from **rohitukine**—a natural product extracted from an Indian plant. One possible problem with flavopiridol is its lack of selectivity between different CDKs and so analogues which do show selectivity may prove beneficial. As far as the binding interactions are concerned, flavopiridol binds to the same region of the active site as ATP. The benzopyran ring lies in the adenine binding region such that the ketone acts as a hydrogen bond acceptor and the OH acts as a hydrogen bond donor. The piperidine ring lies in the region normally occupied by the first phosphate moiety of ATP, where it makes several hydrogen bonding interactions with water and nearby amino acid residues. The chlorophenyl group lies over the ribose binding pocket.

Flavopiridol is also thought to inhibit the expression of **cyclins D1** and **D3**. It has been found to have an anti-angiogenic effect and can induce apoptosis.

Another CDK inhibitor which has entered clinical trials is **7-hydroxystaurosporin** (Fig. 21.57). This is a derivative of a natural compound called **staurosporine**, which is a non-selective inhibitor of protein kinases. Staurosporine has been an extremely important lead compound for a variety of projects aimed at developing selective kinase inhibitors. The 7-hydroxy derivative shows greater selectivity than staurosporine itself but still inhibits a variety of kinases including CDKs.

Roscovitine (**seliciclib**) (Fig. 21.57) shows selectivity for CDK2 and competes with ATP for the binding site. It induces apoptosis and is currently undergoing clinical trials.

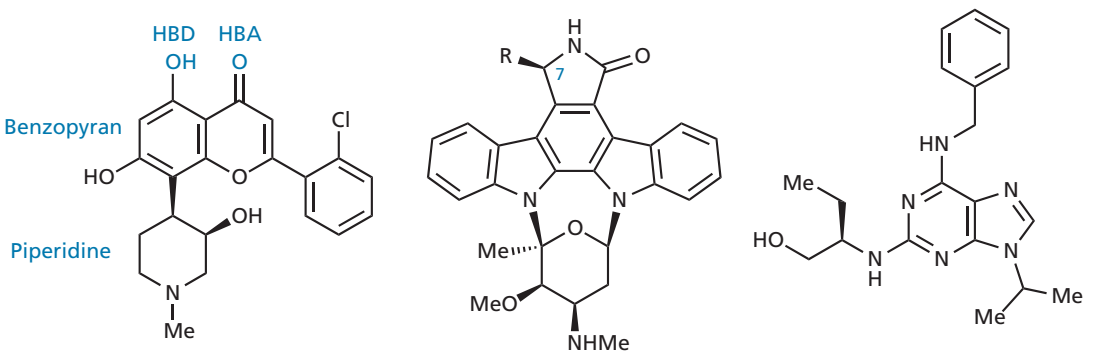
21.6.2.4 Other kinase targets

There are many other types of protein kinase which have been found to be overexpressed in various types of cancer cells, and research is being carried out to find selective inhibitors for these. Recently approved protein kinase inhibitors include **vemurafenib**, **ruxolitinib**, and **crizotinib** (Fig. 21.58; see also Box 13.4).

Studies have also been carried out to find kinase inhibitors that target signalling pathways that are unique to proposed cancer stem cells, such as the curiously named **hedgehog signalling pathway**. **Vismodegib** is the first of these kinase inhibitors to reach the market and was approved in 2012.

21.6.2.5 Multi-tyrosine receptor kinase inhibitors

As the title indicates, **multi-tyrosine receptor kinase inhibitors** (**mTRKIs**) are agents that are designed to be selective against a number of tyrosine receptor kinase targets, all of which have some bearing on the generation and survival of cancer cells. One author has described them as being selectively non-selective! In other words, they should be non-selective in inhibiting a number of kinases that contribute to a cancer, but selective in the sense that they do not inhibit kinases that would lead to side effects—a difficult goal to achieve. The big advantage of an mTRKI is that drug resistance is less likely to occur. If one of the drug's targets mutates and becomes resistant, the other targets are still vulnerable. An mTRKI can be viewed as a combination therapy wrapped up within a single drug. Such drugs are sometimes referred to as promiscuous as they affect a variety of different targets.



Flavopiridol

Staurosporine; R=H
7-Hydroxystaurosporin; R=OH

R-Roscovitine

FIGURE 21.57 Inhibitors of cyclin-dependent kinases.

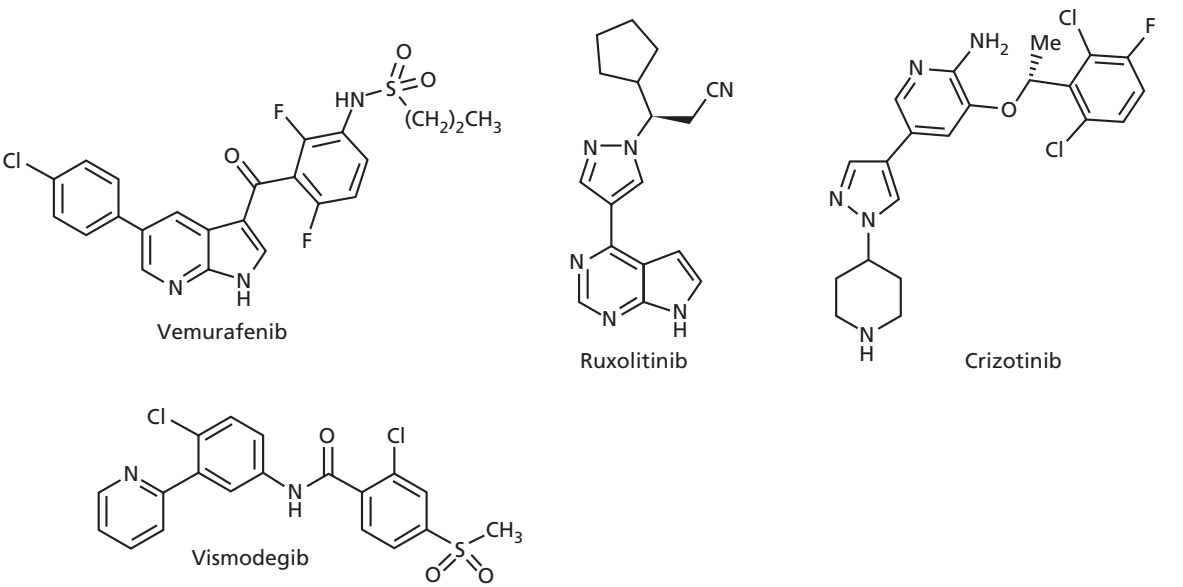


FIGURE 21.58 Newly approved kinase inhibitors.

mTRKIs are likely to be particularly promising agents for the treatment of cancers which are driven by several abnormalities. In truth, none of the kinase inhibitors approved for the market to date are 100% selective for one kinase target and could all, in principle, be defined as mTRKIs. However, it is generally recognized that some inhibitors are more promiscuous than others, and it is those that are defined as mTRKIs. The least promiscuous of the mTRKIs is **lapatinib**, which has already been described (section 21.6.2.1), while the most promiscuous is **sunitinib**, described later.

The mTRKIs that have currently reached the market target the kinases associated with angiogenesis, as well as another kinase associated with the tumour itself (for example **c-KIT**). These include **sorafenib**, which was developed from a urea lead compound using a mixture of traditional medicinal chemistry strategies and multiple point variations (Box 21.10). Sunitinib (Fig. 21.59), which was approved in 2006, is another example. Sunitinib binds mostly to the region of the active site normally occupied by ATP and has little interaction with the hydrophobic pockets that could confer selectivity for

BOX 21.10 Design of sorafenib

In order to find the lead compound for sorafenib, high throughput screening of 200,000 compounds was carried out against recombinant **Raf-1 kinase** (also called **c-Raf**). This led to the identification of a urea (I) with micromolar activity. Substituents and rings were altered in a systematic fashion and it was found that a *para* methyl group on the phenyl ring resulted in a 10-fold increase in activity (Fig. 1). However, despite the synthesis of many more analogues, no further improvement in activity could be obtained. Up to this point, conventional medicinal chemistry strategies had been followed which involved altering one group at a time. This allows one to rationalize any alterations in activity that result from the change of any ring or substituent. It was then decided to use parallel synthesis to produce 1000 analogues having all possible combinations of the different substituents and rings that had been studied to date. This led to the discovery of a urea (IV) having slightly improved activity over structure (II). The curious thing about this structure is that it deviates from the SAR results obtained by single point modifications. Structure IV has a phenoxy substituent and an isoxazole ring, but neither of these groups would be considered good for activity based on the initial SAR. For example, structure III has the phenoxy substituent while structure VI has the isoxazole ring, but both structures have low activity compared with the lead compound. Conventionally, this would be taken to imply that neither group is good for activity.

However, it does not take into account the synergistic effects that two or more modifications might have. The strategy of multiple point modifications allows the identification of such synergistic effects and demonstrates that there are limitations to simple SAR analyses.

(*Continued*)

BOX 21.10 Design of sorafenib (*Continued*)

Structure IV was now adopted as the new lead compound. Replacing the phenyl ring with a pyridine ring led to structure (V) and a fivefold increase in activity, as well as improving aqueous solubility and cLogP. Conventional optimization strategies then led to sorafenib which is 1000-fold more active than the original lead compound.

The urea functional group serves as an anchor group in a similar manner to the amide group present in imatinib. It forms two hydrogen bonding interactions to the catalytic aspartate and glutamate residues in the active site, and orientates the molecule such that each half of the molecule is positioned into two selectivity regions. The atoms coloured in blue are involved in important hydrogen bonding interactions (Fig. 1).

Regorafenib is a closely related structure that is undergoing clinical trials.

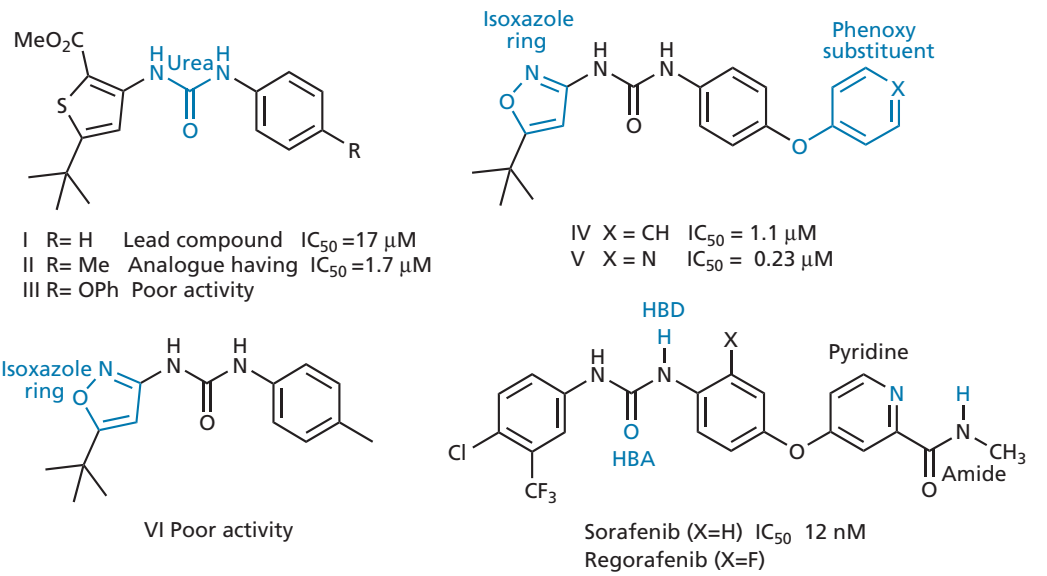


FIGURE 1 From lead compound to sorafenib.

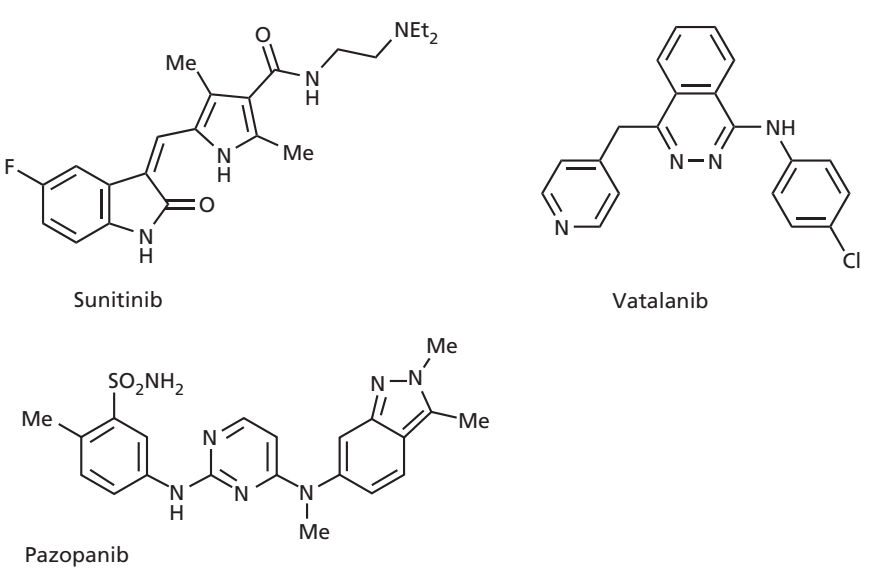


FIGURE 21.59 Multi-tyrosine receptor kinase inhibitors (mTRKIs).

BOX 21.11 Clinical aspects of kinase inhibitors

Kinase inhibitors of the EGF-receptor

The EGFR family of kinases has four members—EGFR, HER2, HER3, and HER4. Overexpression of these kinases is associated with a variety of cancers in the breast, lung, brain, prostate, gastrointestinal tract, and ovaries. The first clinically approved kinase inhibitor for the EGFR family was **gefitinib**, which is used for the treatment of refractory lung cancers. This was followed by **erlotinib**, which is approved for the treatment of non-small-cell lung cancer. Both agents act on tumours resulting from a mutation in EGFR where Leu-853 is replaced with arginine, resulting in destabilization of inactive conformations of the enzyme and an increased level of the active conformation. The mutation also weakens affinity for ATP while increasing affinity for the inhibitors, such that the latter compete effectively for the active site. Unfortunately, drug-resistant tumours caused by a second mutation where the gatekeeper residue Thr-790 is altered to methionine often emerge within a year of initiating treatment. This restores the enzyme's affinity for ATP such that competitive inhibitors are much less effective, despite their ability to still bind to the active site. **Lapatinib** was approved in 2007 for the treatment of patients with advanced or metastatic breast tumours which overexpress HER2. Lapatinib is given orally in combination with **capecitabine**. However, it should only be administered if the standard first-line treatment consisting of **trastuzumab** and a taxane has become ineffective because of tumour resistance. Tumours that have resistance to trastuzumab are unlikely to have resistance to lapatinib as the former drug binds to the extracellular region of the receptor and the latter binds to the intracellular kinase active site. Lapatinib is seen to have several advantages over trastuzumab. Whereas trastuzumab inhibits only HER2, lapatinib inhibits both HER2 and EGFR. Inhibition of two proteins is seen as being more effective than inhibiting either one alone. Moreover, drug resistance is less likely to appear if two targets are affected. Lapatinib is also able to cross the blood–brain barrier (unlike trastuzumab) and can combat any breast tumour cells that have reached the brain as a result of metastasis. Finally, lapatinib has less cardiac toxicity compared with trastuzumab. The drug is currently undergoing clinical trials for a range of other cancer treatments, such as those involving the head, neck, and kidney. **Vandetanib** was approved in 2011 for the treatment of medullary thyroid cancer. It acts in two ways by inhibiting both the VEGF and EGFR receptor kinase active sites, thus inhibiting angiogenesis and cell growth respectively.

Kinase inhibitors of Bcr-Abl and c-Kit

Imatinib was introduced for the treatment of a rare blood cancer called chronic myeloid leukaemia (CML), which accounts for 15–20% of all cases of adult leukaemia in Western populations. The cancer cells involved contain an abnormal protein kinase which is not found in normal cells. The protein kinase concerned is a member of the tyrosine kinase family and has been named **Bcr-Abl**. This name is derived from the genes which code for the protein (i.e. the c-*abl* and *bcr* genes). In normal cells these genes are distinct and on different chromosomes so they code for separate proteins. In the cancer cells associated with CML, part of one chromosome has been transferred to another, resulting in a shortened chromosome called the **Philadelphia chromosome**—a characteristic feature of this type of cancer. The result of this genetic transfer is the formation of a hybrid gene (*bcr–abl*) which is not properly regulated and which codes for excessive levels of the hybrid protein kinase. In turn, this leads to excessive quantities of white blood cells (leukocytes). Imatinib has been successful in 90% of patients, but tumour resistance can result in many cases. Imatinib also inhibits a tyrosine kinase called c-Kit and has been approved for the treatment of stomach cancers where this kinase is altered or overexpressed. The c-Kit receptor (also called CD117 or KIT) is a cytokine receptor expressed on the surface of stem cells and is activated by stem cell factor. Unfortunately, mutations in c-KIT can result in tumour resistance. Imatinib also inhibits the platelet-derived growth factor receptor (PDGF-R), and the drug is currently approved for the treatment of 10 different types of cancer.

Nilotinib and dasatinib have been approved for the treatment of CML when imatinib therapy is unsuccessful because of drug resistance. Dasatinib is also being considered for metastatic melanoma. The greater binding affinities of these agents means that they still bind sufficiently strongly if a mutation should result in the loss of one binding interaction. The exception is the T315I mutant, where the interaction with threonine is particularly important.

Other kinase inhibitors

Sorafenib has been approved as a treatment of liver and kidney cancers. The agent inhibits the kinase activity of the membrane-bound receptors VEGFR, PDGFR, c-KIT, and RET, as well as an intracellular target (B-RAF). **Sunitinib** was approved in 2006 for the treatment of gastrointestinal stromal tumours (a rare cancer) and advanced renal cell carcinoma (a common kidney cancer). The agent is a simultaneous inhibitor of VEGF-R2 and PDGF-Rβ, and there is evidence that this is more effective than inhibition of either of these targets alone. It also inhibits c-KIT and Flt3.

Pazopanib was approved in 2009 for the treatment of renal cell carcinoma. It inhibits VEGFR-1, VEGFR-2, VEGFR-3, PDGFR, and c-kit.

(*Continued*)

BOX 21.11 Clinical aspects of kinase inhibitors (*Continued*)

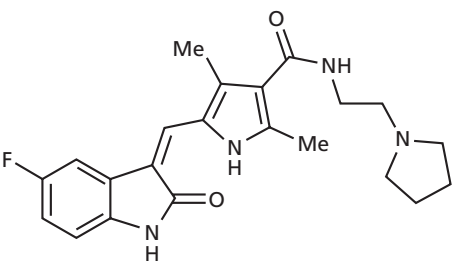
Vemurafenib was approved in 2011 for the treatment of late-stage melanoma, and targets abnormal B-Raf protein kinases.

Ruxolitinib was approved in 2011 for the treatment of myelofibrisosis, and targets **janus associated kinases** (Jak).

Crizotinib was approved in 2011 for the treatment for certain lung tumours, and targets an abnormal form of the anaplastic lymphoma kinase receptor (ALK or CD246). **Vismodegib** was approved in 2012 for the treatment of skin cancers, and targets a signalling pathway that is unique to stem cells called the **Hedgehog pathway**.

Temsirolimus is an analogue of the antibiotic **rapamycin** and was approved in 2007 for the treatment of advanced renal cell carcinoma. **Everolimus** is a similar structure and was approved in 2009.

Finally, **toceranib** is the first anticancer drug approved by the FDA for the treatment of tumours in dogs. It inhibits kit tyrosine kinase.



Toceranib.

different kinases. **Vatalanib** (Fig. 21.59) is another multi-kinase inhibitor undergoing clinical trials, while **pazopanib** was approved in 2009.

The idea of targeting several targets with the one drug is described as **polypharmacology**. Drugs acting in this way may have potentially enhanced anti-tumour activity relative to more selective kinase inhibitors. An alternative polypharmacology approach is to use a 'cocktail' of different selective kinase inhibitors.

21.6.2.6 Kinase inhibitors derived from natural products

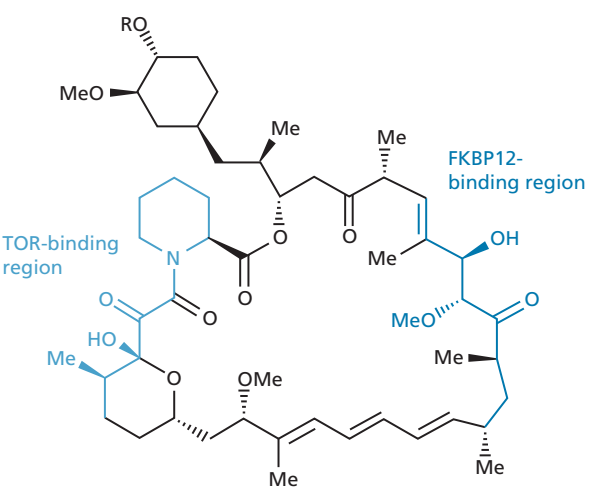
Temsirolimus and **everolimus** (Fig. 21.60) are analogues of a natural product called **sirolimus** (**rapamycin**)—a macrolide which is produced by the bacterium *Streptomyces hygroscopicus*. Sirolimus is used as an immunosuppressant during kidney transplants. However, the more polar temsirolimus and everolimus have been approved for the treatment of certain types of tumour where a kinase enzyme called **mTOR** (or **FRAP**) is excessively active. mTOR triggers a signal transduction process leading to cell growth and so its inhibition serves to inhibit tumour development. The mechanism of inhibition is rather unusual, compared with those mentioned so far.

The mechanism starts with part of the macrolide structure binding to an immunopholin protein called **FKBP12** (also called the **FK506-binding protein**). Once this drug–protein complex has been formed, a different part of the macrolide ring structure binds to mTOR to form a ternary structure consisting of the two proteins with the drug sandwiched between. In essence, the drug promotes the dimerization of the two proteins, such that they interact with each other. This interaction results in inhibition of mTOR and the signal transduction process that it normally triggers.

It has also been proposed that inhibition of mTOR might reverse the resistance of some tumours to certain other anticancer agents.

KEY POINTS

- Many cancers produce abnormal or overexpressed proteins that are involved in the signalling pathways which stimulate cell growth and division. Agents which act selectively against these targets are less likely to have the serious side effects associated with traditional cytotoxic agents.



Sirolimus (rapamycin); R = H
Temsirolimus; R = CO-C(Me)(CH_2OH)$_2$
Everolimus; R = $(CH_2)_2OH$

FIGURE 21.60 Sirolimus and analogues.

- An abnormal form of the Ras protein is permanently active and is associated with many cancers. Inhibiting the farnesyl transferase (FT) enzyme prevents the Ras protein becoming attached to the cell membrane and prevents it interacting with other elements of the signal transduction process. The observed anticancer effects of FT inhibitors may be due to their effect on a variety of proteins other than just Ras.
- FT inhibitors contain a group that acts as a ligand for the zinc cofactor in the enzyme. Early inhibitors were modelled on the end tetrapeptide moiety of Ras. Newer agents are less peptide-like and are smaller molecules with improved pharmacokinetic properties.
- Protein kinases are enzymes which use ATP to phosphorylate hydroxyl or phenol groups in protein substrates. Protein kinase receptors are proteins in the cell membrane which play a dual role of receptor and enzyme, and which are activated by growth factors. Both the messengers and the receptors have been implicated in various cancers by being overexpressed or abnormal in nature. Anticancer agents have been designed to act as inhibitors of the kinase active site of these proteins. Most bind to the ATP binding region, as well as other regions of the active site. Kinase inhibitors with different selectivities can be designed because there are variations in the amino acids present in the active sites of different kinases.

21.7 Miscellaneous enzyme inhibitors

In previous sections, we looked at enzyme inhibitors associated with DNA synthesis and function, as well as enzymes involved in signal transduction. In this section, we look at the inhibition of enzymes which have not been covered so far, but which have important roles to play in angiogenesis, metastasis, and apoptosis.

21.7.1 Matrix metalloproteinase inhibitors

Matrix metalloproteinases (**MMPs**) are zinc-dependent enzymes which play an important role in the invasiveness and metastasis of cancer cells—processes that have few anticancer agents acting against them. The MMPs are extremely destructive enzymes involved in the normal turnover and remodelling of the extracellular matrix or connective tissue (section 21.1.9). This process is usually tightly controlled by natural protein inhibitors. However, excessive activity can result in various problems, including chronic degenerative diseases, inflammation, and tumour invasiveness. There are four main groups of MMPs—**collagenases**, **gelatinases**, **stromelysins**, and **membrane type** (MT)—and a number of these are implicated in tumour growth, invasion, metastasis, and angiogenesis.

Matrix metalloproteinase inhibitors (**MMPIs**) can be used to inhibit the breakdown of the extracellular matrix to make it more difficult for cancer cells to escape and metastasize. They can also be used to inhibit angiogenesis by blocking the release of VEGF from storage depots in the extracellular matrix. A variety of first-generation inhibitors have been developed which are based on the natural protein substrates for the collagenase enzymes of this family. These enzymes catalyse the cleavage of peptide bonds between glycine and isoleucine (or leucine) in protein substrates (Fig. 21.61). The substrate is thought to bind such that the carbonyl oxygen of glycine coordinates to the zinc cofactor, while the neighbouring NH acts as a hydrogen bond donor to the peptide backbone of an alanine residue. A water molecule is held between zinc and a glutamate residue, and acts as the nucleophile to hydrolyse the peptide bond, assisted by the negatively charged glutamate residue and the zinc cofactor.

A variety of peptide-based inhibitors have been designed. In general, they have the following features:

- replacement of the susceptible peptide bond with a moiety which is stable to hydrolysis;

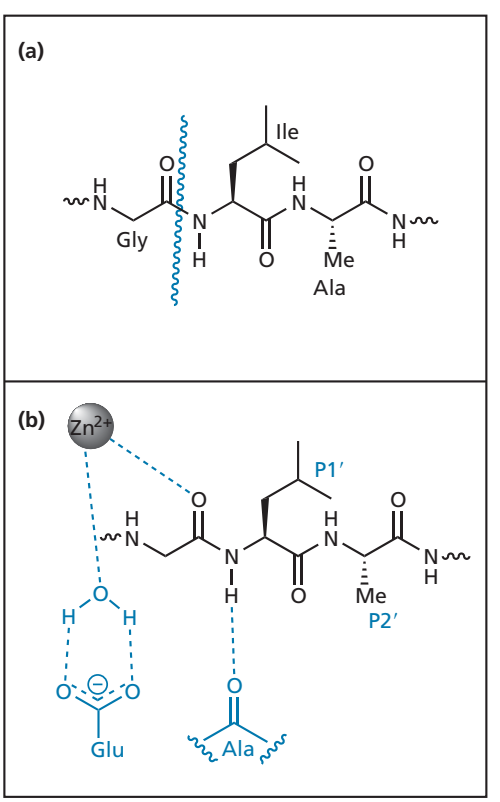


FIGURE 21.61 (**a**) Peptide bond cleaved by matrix metalloproteinases. (**b**) Binding interactions.

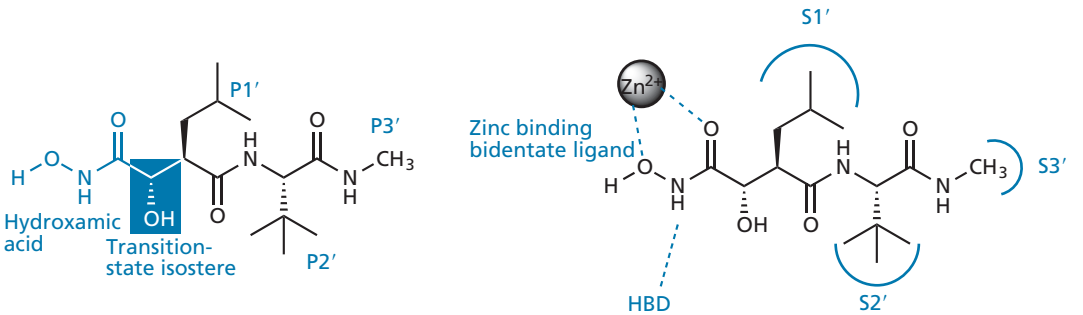


FIGURE 21.62 Structure and binding interactions of marimastat.

- one or more substituents that can fit into the enzyme subsites and form van der Waals interactions. The subsites normally accept the amino acid residues of the substrate;
- at least one functional group capable of forming a hydrogen bond to the enzyme backbone;
- a group capable of strong interactions with the zinc ion cofactor, such as a thiol, carboxylate, or hydroxamic acid.

Early work showed that inhibitors mimicking the substrate groups P1′ and P2′ to the right of the scissile peptide link (right-hand-side inhibitors) were more effective than those mimicking the left-hand side. It was also discovered that a hydroxamate group was a particularly good ligand for the zinc cofactor, as it can act as a bidentate ligand using both oxygen atoms, while the NH group forms a hydrogen bond interaction with a carbonyl oxygen on the enzyme backbone.

These features can be seen in **marimastat** (Fig. 21.62), which is an orally active, synthetic compound that reached phase III clinical trials for breast and prostate cancer. A hydroxamic acid group is present to form a strong bidentate interaction with zinc. Substituents (P1′–P3′) are also present to fit three binding pockets in the active site. The NH moiety of the amide bond normally between glycine and isoleucine has been replaced by a hydroxymethylene group which prevents the normal hydrolysis reaction taking place and acts as a transition state isostere. The hydroxyl group is also beneficial for inhibitory activity and aqueous solubility. Binding studies suggest that the hydroxyl group is directed away from the protein surface and is hydrogen bonded to water. The *t*-butyl substituent (P2′) also serves as a steric shield to protect the terminal amide from hydrolysis.

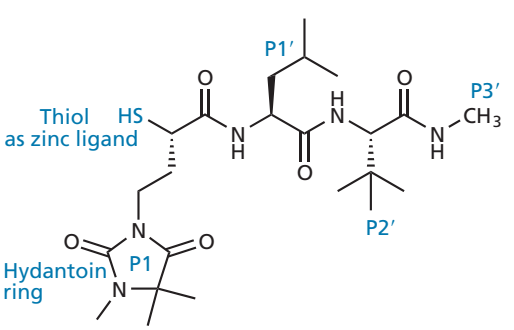


FIGURE 21.63 BMS 275291 (D 2163).

The nature of the P1′ group can be varied, and determines the activity and selectivity of the inhibitors against the various metalloproteinases. The nature of the P2′ group can also be varied, but it is beneficial to have a bulky group to act as a steric shield to protect the peptide bonds. It also serves to desolvate the peptide bonds such that energy is not expended on desolvation prior to binding.

Another peptide-like structure to reach clinical trials is **BMS 275291** (Fig. 21.63), which has a thiol group as the ligand for zinc. The hydantoin ring is thought to access the S1 subsite. The right half of the structure is identical to marimastat.

Unfortunately, inhibitors such as marimastat lack selectivity and produce side effects such as tendinitis. They also have poor pharmacokinetic properties owing to their peptide nature. For example, they have poor aqueous solubility and show susceptibility to peptidases in the gastrointestinal tract.

Therefore, work is now under way to develop a second generation of non-peptide-like metalloproteinase inhibitors which are more selective in their action (Fig. 21.64). Examples include **CGS 27023A** and **prinomastat**, which both reached clinical trials. These structures have decreased peptide character, but still suffer from a lack of selectivity, resulting in undesired side effects. CGS 27023A contains an isopropyl group which appears to protect the hydroxamic acid group from metabolism. Activity increased if the isopropyl group was incorporated into a ring in order to restrain the number of possible conformations. Further extension of the P1′ substituent then led to prinomastat. Second-generation inhibitors, such as **BAY 12-9655**, have a carboxylate group as the zinc ligand. Currently, research is looking

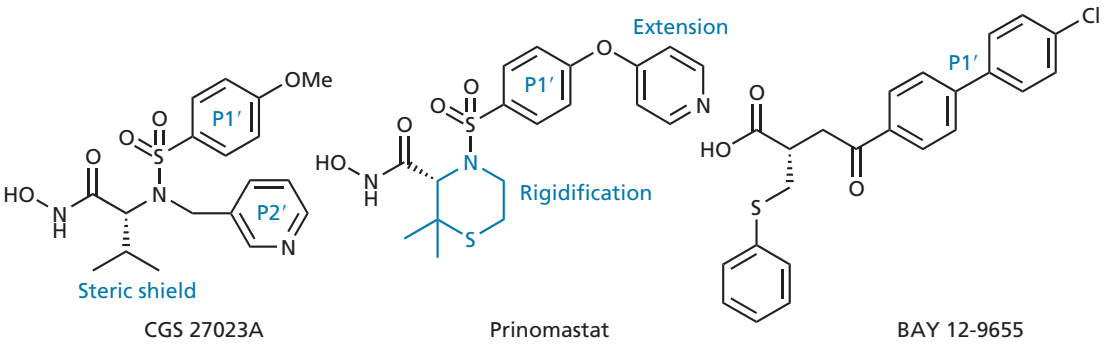


FIGURE 21.64 Second-generation matrix metalloproteinase inhibitors.

at more selective inhibitors that target MMPs, such as MMP2, MMP9, and MMP25.

21.7.2 Proteasome inhibitors

The proteasome is a complex structure which can be viewed as the cell's rubbish disposal unit. It is an ATP-dependent multi-catalytic protease that destroys proteins. Its prime role is to eliminate damaged or misfolded proteins and to degrade key regulatory proteins. Considering the destructive power of this structure, it is important that it destroys only defective proteins and not normal ones. Therefore, cells mark their defective proteins with a molecular label so that they can be recognized by this protein killing machine. This molecular label is a protein called **ubiquitin**.

As the proteasome is so destructive, one might think it would be best to boost its activity in tumour cells. In fact, the opposite strategy is adopted and research is looking at agents that inhibit its action. The rationale lies in the fact that the proteasome removes regulatory proteins which have 'done their job'. Blocking the proteasome will result in an accumulation of various regulatory proteins, which leads to a cellular crisis and triggers apoptosis. One of the proteins that accumulates as a result of proteasome inhibition is the apoptosis promoter **Bax** (section 21.1.7).

Bortezomib (Fig. 21.65) is a boronic acid dipeptide that inhibits the proteasome, possibly by forming a boron–threonine bond at active sites. It became the first proteasome inhibitor to be approved for the treatment of multiple myeloma. Unlike most anticancer drugs, bortezomib is not prone to multidrug resistance. **Aclarubicin** (**aclacinomycin A**) (Fig. 21.65) is an anthraquinone which affects proteasomes by inhibiting the chymotrypsin activity of the structure. The tetracyclic moiety and the three sugar rings are all necessary for activity.

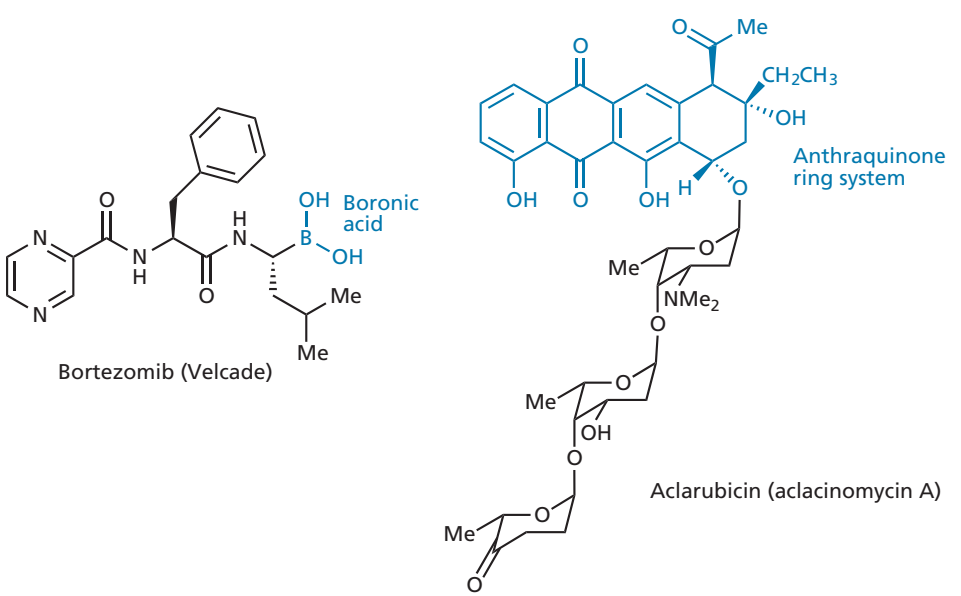


FIGURE 21.65 Proteasome inhibitors.

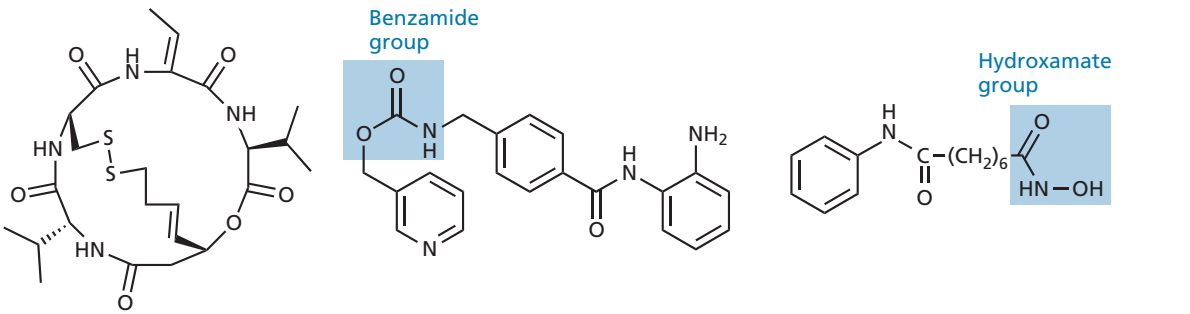


FIGURE 21.66 Histone deacetylase inhibitors.

21.7.3 Histone deacetylase inhibitors

Chromatins (Fig. 21.5) are structures where DNA is wrapped around proteins, most of which are **histones**. The histones assist in DNA packaging and also have a regulatory role. There is a repeating pattern of 8 histone proteins along the length of the chromatin structure, with each octet associated with about 200 base pairs of DNA. Each of these repeating units is known as a **nucleosome**.

Histone acetylase is an enzyme that adds acetyl groups to the lysine residues of histone tails which stick out from the chromatin structure. Acetylation neutralizes the positive charge normally associated with the lysine side chain and weakens the ionic interactions between the histones and the negatively charged sugar phosphate backbone of DNA, leading to a less compact structure. The more open structure allows **transcription factors** to access the promoter regions of various genes. **Histone deacetylase** is an enzyme that removes the acetyl groups leading to a more compact structure and prevents transcription factors accessing the promoter regions. This causes gene silencing, but can also lead to decreased DNA repair, resulting in an increased chance of cancer (see also section 21.8.4).

Several inhibitors of histone deacetylase have been studied (Fig. 21.66). **Romidepsin** (**depsipeptide**) is a natural product derived from a bacterial strain and was approved in 2009 for the treatment of some lymphomas. The disulphide bond is reduced inside cells to give a dithiol, which can then bind to a zinc cofactor present in the enzyme. The resulting enzyme inhibition promotes apoptosis and inhibits cell proliferation and angiogenesis. Synthetic agents are also being investigated which contain functional groups capable of acting as ligands for the zinc cofactor. For example, **entinostat** contains a benzamide group and is undergoing clinial trials, while **vorinostat** contains a hydroxamate group and was approved in 2006 for the treatment of cutaneous T-cell lymphoma. Clinical trials are also being carried out using vorinostat in combination with the tyrosine kinase inhibitor **erlotinib**—an example of **polypharmacology** where different drugs are administered to affect different targets.

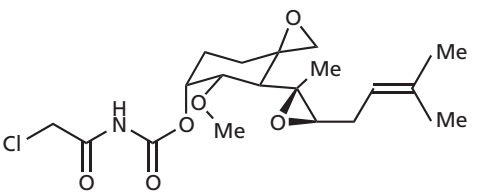


FIGURE 21.67 TNP 470.

21.7.4 Other enzyme targets

There are many other enzymes which are being studied as potential targets for anticancer agents. For example, inhibiting the **telomerase** enzyme should prevent cells becoming immortal and be useful in anticancer therapy. Several powerful inhibitors have been developed, although no telomerase inhibitor has reached the clinic to date.

The inhibition of regulatory enzymes may be useful in shutting down a biosynthetic pathway that is too active. One example is inhibition of **tyrosine hydroxylase** (section 23.12.1).

Methionine aminopeptidase is an enzyme that plays a key role in endothelial cell proliferation, and blocking it should inhibit angiogenesis. **TNP-470** (Fig. 21.67) is an analogue of a fungal product called **fumagillin** which acts as an inhibitor of this enzyme and is being studied as an anti-angiogenesis agent.

The activation of **caspases** to induce apoptosis is another possible approach to novel anticancer agents.

21.8 Miscellaneous anticancer agents

The field of anticancer research is a vast one with a wide diversity of novel structures being investigated. The following are examples of various structures which act at different targets or whose targets have not been identified.

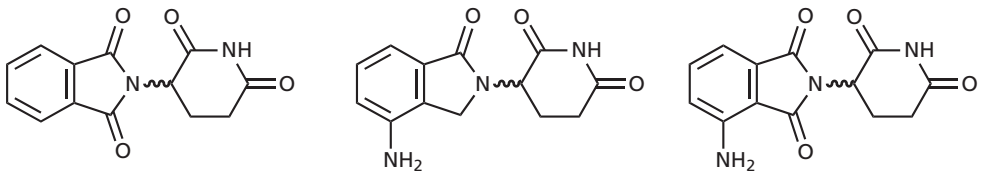


FIGURE 21.68 Thalidomide and thalidomide analogues.

21.8.1 Synthetic agents

Thalidomide (Fig. 21.68) was originally marketed as a safe, non-toxic sedative and anti-emetic in the 1950s, and rapidly became popular to counter the effects of morning sickness during pregnancy. Unfortunately, it was instrumental in one of the major medical disasters of modern times when it produced teratogenic effects in developing fetuses and led to babies being born with stunted limbs and other developmental deformities—the so-called **thalidomide babies**. As thalidomide was considered such a safe drug at the time, few suspected that it was the cause of the problem and, by the time it was linked to the deformities and withdrawn in 1961, 8000–12,000 babies were affected. Consequently, thalidomide gained a lasting notoriety which was instrumental in a significant tightening of the regulations surrounding the testing of drugs. Despite its notorious past, there has been continuing interest in thalidomide as it has some remarkable properties which indicate a wide variety of clinical uses. Early on, it was recognized that thalidomide has an anti-inflammatory property which was useful in treating leprosy. This activity was eventually linked to thalidomide's ability to inhibit the synthesis of the pro-inflammatory endogenous cytokine **TNF-α**, which is produced by monocytes. In 1998, the drug was approved for the treatment of leprosy. However, thalidomide has a raft of other properties. For example, it is an immunosuppressant and could possibly be used for the treatment of autoimmune diseases or in countering the immune response to allow organ transplants to be accepted by the host. Interest in thalidomide as an anticancer agent started when it was found that it inhibited angiogenesis by an unknown mechanism. Tests showed that thalidomide did, indeed, have anticancer activity and it entered phase III clinical trials for the treatment of renal cancer and multiple myeloma on that basis. Since then, it has been discovered that the anticancer properties of thalidomide are more complex than its effect on angiogenesis alone. In some patients, thalidomide can boost the immune system by a variety of mechanisms rather than suppress it, and this too may account for its anticancer activity. As thalidomide can suppress or boost the immune system depending on individual circumstances, it is known as an **immunomodulator**. Thalidomide also appears to arrest the growth of cells and promote apoptosis directly.

Analogues of thalidomide have been synthesized with the aim of removing its teratogenic properties. **Lenalidomide** (**Revlimid**) and **actimid** (Fig. 21.68) are

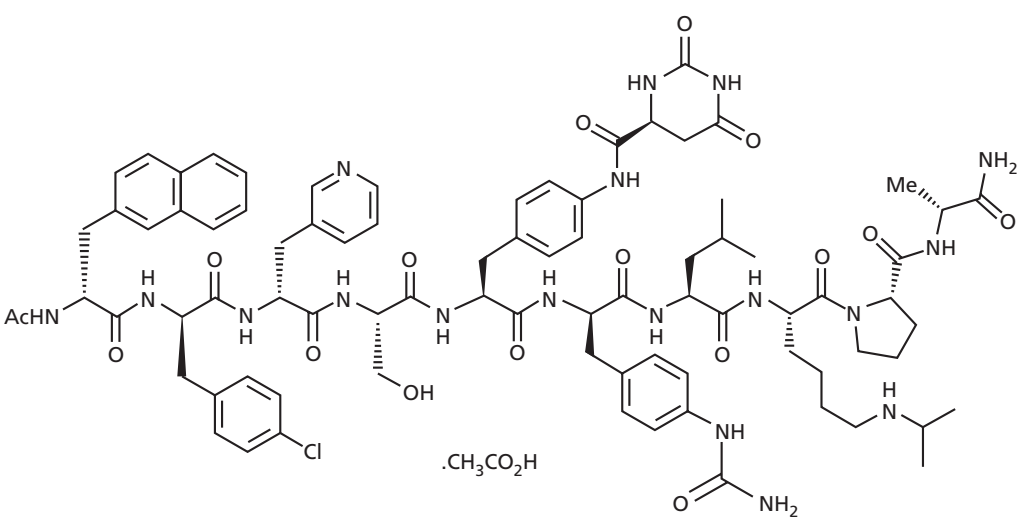


FIGURE 21.69 Degarelix acetate.

two such examples. Both contain an amino substituent on the aromatic ring which is found to be crucial in producing a safer drug. Revlimid entered clinical trials in the year 2000 and was given orphan drug status in 2001 for the treatment of the then incurable disease of multiple myeloma. In 2003, it entered phase III clinical trials and was given fast-track status. It is now approved for the treatment of multiple myeloma.

Arsenic trioxide is an orphan drug used for a variety of leukaemias. It is thought to promote cell suicide by targeting the cell's mitochondria. The compound has been used for many centuries in traditional Chinese medicine.

Degarelix acetate (Fig. 21.69) was approved in 2009 for the treatment of prostate cancer. It acts as an antagonist of **gonadotrophin-releasing hormone**.

21.8.2 Natural products

Pancratistatin (Fig. 21.70) is a natural product isolated from a plant called *Pancratium littoralis* which belongs to the genus *Narcissus*. Records show that extracts from plants in this genus were used by Hippocrates in 200 BC to treat breast cancer. The drug also inhibits angiogenesis and its phosphate prodrug shows potential as an anticancer drug. The exact mechanism of action is still to be determined. One or more of the hydroxyl groups at positions C-2, C-3, and C-4 are thought to be important.

Bryostatin 1 (Fig. 21.70) is a natural product which was isolated from a marine invertebrate off the coast of California in 1981. It was shown to boost the immune system and make it more effective against cancers. It is undergoing clinical trials and promises to be effective—either on its own or in combination with other established anticancer drugs, such as taxol, vincristine, fludarabine, or cisplatin.

Dolastatins are natural products which were isolated from the marine sea hare off the island of Mauritius in the Indian Ocean. A full synthesis has been developed to produce **dolastatin 10**, **auristatin PE**, and **dolastatin 15** (Fig. 21.71), which are all undergoing clinical trials.

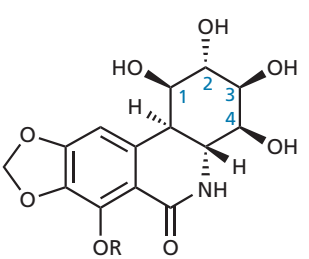
Cephalostatin 1 (Fig. 21.71) is a very potent anticancer agent which was isolated from a marine worm. Its mechanism of action has not been established, although it has been suggested that it spans the lipid bilayer of cells and disrupts membrane structure. It has not yet entered clinical trials and there is a need to develop an efficient synthesis of the compound to obtain sufficient quantities.

21.8.3 Protein therapy

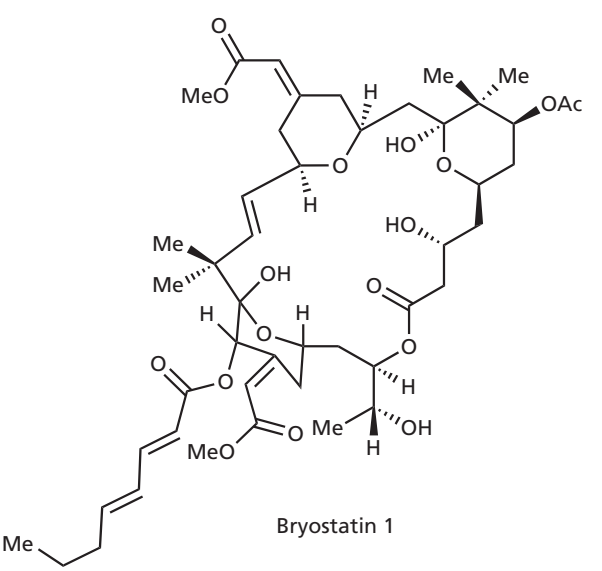
A variety of proteins are being considered as **anti-angiogenesis agents**. For example, **angiostatin** and **endostatin** are two naturally occurring proteins in the body which inhibit the formation of new blood vessels and are being studied in cancer therapy. α-**Interferon** inhibits the release of growth factors such as VEGF and is in phase III clinical trials for various cancers.

Cancer cells are more sensitive than normal cells to a natural death-inducing protein with the catchy title of **tumour necrosis factor-related apoptosis inducing ligand** (**TRAIL**), which stimulates apoptosis. Injecting purified TRAIL *in vivo* might selectively stimulate increased death rates in cancer cells. This is currently being studied in animals.

A variety of proteins are being considered as immunostimulants, including γ-**interferon** and **aldesleukin** (a preparation of **interleukin-2**).



Pancratistatin; R=H
Pancratistatin prodrug; R=phosphate



Bryostatin 1

FIGURE 21.70 Miscellaneous natural products that have anticancer activity.

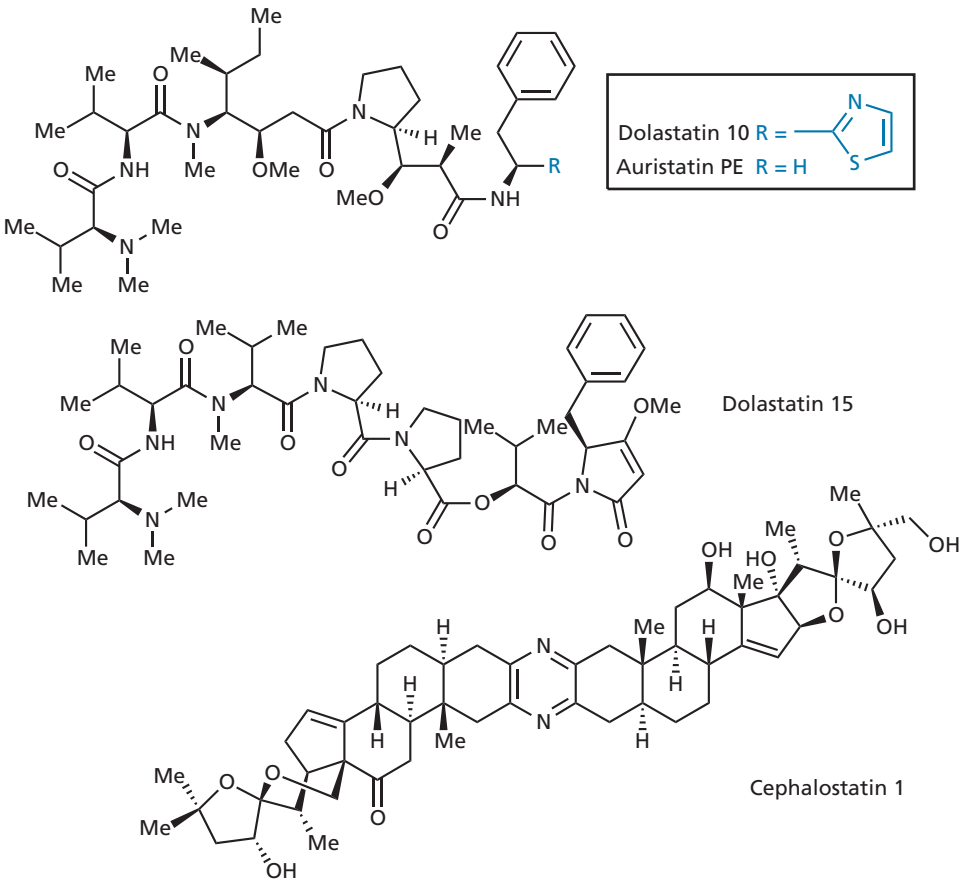


FIGURE 21.71 Natural products with anticancer activity.

Some cancer cells have lost the ability to carry out normal synthetic routes as a result of gene mutation and the production of inactive enzymes. For example, some leukaemia cells lose the capacity to synthesize the amino acid asparagine and have to obtain it from the blood supply. The enzyme **asparaginase** can catalyse the degradation of asparagine, so providing that enzyme should break down asparagine in the blood supply and starve the cancer cells of this amino acid. A preparation of the asparaginase enzyme (**crisantaspase**) is used to treat certain cancers of this type (see also section 14.8.2).

21.8.4 Modulation of transcription factor–co-activator interactions

Research is taking place to try and find anticancer agents that work by interacting with transcription factors in order to affect gene transcription. There are already examples of clinically useful agents that act as ligands for nuclear receptor transcription factors. Work is in progress to find small molecules that might disrupt the interaction between a transcription factor and a co-activator protein, and thus prevent the formation of the complex required to signal the start of transcription (Box 10.2).

In a similar vein, drug-like molecules are being investigated as potential ligands for a protein binding region called a **bromodomain**. This is a region that is capable of binding an acetylated lysine residue of another protein and plays an important role in protein–protein interactions (section 21.7.3). Therefore, drug-like molecules capable of interacting with bromodomains may act as protein–protein binding inhibitors (section 10.5). Contrary to what a chemist might assume, bromodomains do not contain bromine. Instead, their name refers to the *Drosphila* gene *brahma*, the sequence of which first identified the existence of bromodomains.

KEY POINTS

- Matrix metalloproteinases (MMPs) are zinc-dependent enzymes which degrade the extracellular matrix and encourage the processes of angiogenesis, tumour propagation, and metastasis.

- Inhibitors of MMPs have been based on small peptide sequences of the protein substrates. In general, the susceptible peptide bond has been replaced by a stable bond, substituents have been incorporated to fit binding subsites and a ligand for the zinc cofactor has been included.
- Second-generation inhibitors of MMPs are non-peptide in nature and are more selective in their action.
- The proteasome is a destructive enzyme complex that breaks down proteins. Inhibition leads to a build-up of conflicting regulatory proteins, which triggers apoptosis.
- Histone acetylases and deacetylases are involved in the regulation of transcription. Inhibitors of histone deacetylase are in clinical trials.
- A variety of other enzymes are potential targets for novel anticancer agents.
- A large number of synthetic structures and natural products have anticancer properties by unknown mechanisms. Others appear to work by having several different mechanisms.
- Protein therapy has proved useful in the treatment of certain cancers.

21.9 Antibodies, antibody conjugates, and gene therapy

21.9.1 Monoclonal antibodies

Cancer cells have unusual shapes and altered plasma membranes that contain distinctive antigens which have been over expressed. This allows the possibility of using antibodies against the disease. Although the antigens concerned are likely to be present in some normal cells, they are likely to be present to a greater extent on the cancer cells making the latter more vulnerable. Monoclonal antibodies (sections 10.7.2 and 14.8.3) have been produced for numerous tumour-associated antigens and a few have reached the clinic as anticancer agents (Box 21.12). These serve to activate the body's immune response to direct killer cells against the tumour. Alternatively, if the antigen is an overexpressed receptor, the antibody may bind to it and block the chemical messenger from binding. In this case, the antibody acts as a receptor antagonist. A new approach has been to develop monoclonal antibodies capable of binding to two different antigens, one on the target tumour cell and one on a normal T-cell of the immune system. In this way, the antibody directs the body's immune system against the tumour. **Blinatumomab** is one such monoclonal antibody that is undergoing clinical trials with the aim of targeting malignant B-cells.

21.9.2 Antibody–drug conjugates

Some monoclonal antibodies have an anticancer activity in the 'naked' form (i.e. without a drug attached), but the level of activity is usually too low to be effective and so a better strategy is to attach an anticancer drug to the antibody (an antibody–drug conjugate) such that the drug is delivered selectively to the cancer cell.

One of the original aims in designing antibody–drug conjugates was to deliver anticancer agents to tumour cells in greater concentrations than was possible by conventional therapy. There is often a narrow therapeutic window between the levels of drug that are effective and the levels leading to unacceptable toxicity. Antibody–drug conjugates were seen as a means of avoiding this problem, as it was anticipated that targeting would lead to higher concentrations of the drug at tumour cells. The first generation of such conjugates involved antibodies linked to anticancer agents such as **methotrexate**, **the vinca alkaloids**, and **doxorubicin**, but the results were disappointing. The anticancer activity achieved was less than using the drug itself and yet the toxicity problem remained the same.

It was later realized that the lifetime of the antibody–drug conjugate was substantially greater than that of the drug itself, which contributed to the toxicity problem. Furthermore, delivery to the tumour and penetration into it was limited owing to the size of the conjugate. This meant that the concentration of antibody–drug reaching the tumour was actually less than when the drug itself was used. As the rate of delivery and penetration is determined by the antibody, there is little that can be done to improve it. Therefore, it was realized that highly potent anticancer agents should be attached to the antibodies in order to be effective at the levels attained at the tumour cell. This rules out anticancer drugs such as **doxorubicin**, **etoposide**, **5-fluorouracil**, and **cisplatin**. More potent anticancer drugs are available, but if they should become detached from the antibody during circulation they are likely to cause severe toxicity. Therefore, it is important that any such drug is attached to the antibody by a stable bond and remains bound until it enters the cancer cell.

The requirements for antibody–drug conjugates now include the following:

- the antibody has to be humanized to avoid an immune response;
- it has to show selectivity for an antigen which is overexpressed in cancer cells rather than normal cells;
- it then needs to be internalized into the cell by receptor-mediated endocytosis such that the antibody–drug can be delivered into the cell;
- the link between the antibody and the drug should be stable until cell entry has taken place and then cleaved to release and activate the drug.

BOX 21.12 Clinical aspects of antibodies and antibody–drug conjugates

Antibodies

Trastuzumab (Herceptin) is a humanized monoclonal antibody which targets the extracellular region of the HER-2 growth factor receptor and was approved in 1998 for the standard first-line treatment of HER-2-positive metastatic breast cancer in combination with paclitaxel. HER-2 is a member of the EGF-R family of tyrosine kinase receptors, which is overexpressed in 25% of breast cancers. When the antibody binds to the receptor, it induces the immune response to attack the specified cell. It also promotes internalization and degradation of the receptor. Trastuzamab is given by injection. Unfortunately, the drug cannot cross the blood–brain barrier and is ineffective against any tumour cells that have metastasized to the brain. Drug resistance and cardiac toxicity are further problems which can arise.

Alemtuzumab is a humanized antibody that lyses B lymphocytes and is used for B cell chronic lymphocytic leukaemia where other therapies have not worked. The antibody binds to a receptor (the CD52 antigen) which is found both on normal and cancerous immune cells (B- and T-lymphocytes). Although the agent shows no selectivity for cancer cells over normal cells, normal cells recover quicker after treatment.

Rituximab is a chimeric antibody targeting the CD20 receptor on B lymphocytes and was approved in 1997 for the treatment of diffuse B-cell non-Hodgkin's lymphoma and follicular lymphoma. It causes lysis of B lymphocytes. Patients should be monitored very closely as there are reported fatalities relating to the release of cytokines. In 2010, it was approved by the FDA for the treatment of chronic lymphocytic leukaemia.

Cetuximab is a chimeric monoclonal antibody that targets the extracellular domain of the EGF-receptor and blocks EGF from binding. It is used alongside irinotecan for the treatment of metastatic colorectal tumours which express EPGF-R and which have proved resistant to previous chemotherapy that has included irinotecan. The antibody is also used alongside radiotherapy for the treatment of locally advanced squamous cell cancer of the head and neck.

Bevacizumab is a humanized monoclonal antibody that is given intravenously and disables the growth factor VEGF required for angiogenesis. It is used in the first-line treatment of metastatic colorectal cancer along with fluorouracil and folinic acid (leucovorin). It also used in the first-line treatment of metastatic breast cancer alongside paclitaxel.

Ofatumumab targets a different epitope of the CD20 receptor from rituximab and was approved in October 2009. It is used to treat leukaemia that cannot be controlled by other forms of chemotherapy.

Panitumumab is a fully humanized monoclonal antibody that targets the epidermal growth factor receptor and has been approved for the treatment of colorectal cancer.

Antibody–drug conjugates

Gemtuzumab ozogamicin (Box 21.13) was approved for the treatment of acute myeloid leukaemia (AML) but was withdrawn in 2010.

Ibritumomab was the first approved drug involving radio-immunotherapy for the treatment of non-Hodgkin's lymphoma.

Tositumomab was approved in 2003 for the treatment of non-Hodgkin's lymphoma that was refractory to rituximab.

Brentuximab vedotin was approved in 2011 for the treatment of Hodgkin's lymphoma and systematic anaplastic large cell lymphoma. The antibody targets a cell membrane protein called CD30, and is linked to the antitumour agent **monomethyl auristatin E** (MMAE or **vedotin**), which is a synthetic analogue of the naturally occurring auristatins.

There are various ways in which a drug can be linked to an antibody. For example, there are lysine residues present throughout the whole molecule which contain a nucleophilic primary amino group. A number of drug molecules could be added to the one antibody by acylating or alkylating these groups. There is a problem with this approach, however, as it is quite possible for a molecule to be attached to the region responsible for 'recognizing' the antigen. This would prevent antibody–antigen binding. Moreover, the masking of polar amino groups may lead to precipitation of the antibody–drug complex.

A better approach is to reduce the four intrastrand disulphide links at the hinge region of the antibody (Fig. 21.72) to produce eight thiol groups and to attach drugs to these by alkylation or via a disulphide linkage. This has the advantage that it can be carried out in a controlled fashion and the drugs do not mask the antigen recognition site. The disadvantage is that a maximum of only eight drugs can be added to any one antibody molecule. One way round this may be to add a linker molecule to the antibody which could itself bear several drug molecules.

Another method of attaching the drug to the antibody is to take advantage of the carbohydrate region between the two heavy chains. Mild oxidation of vicinal diols in the sugar rings produces aldehyde groups to which drugs can be linked through an imine functional group (Fig. 21.73). Further reduction can then be carried out to form more stable amine linkages.

It is important that the linker is cleaved once the antibody–drug complex enters the cancer cell. Various

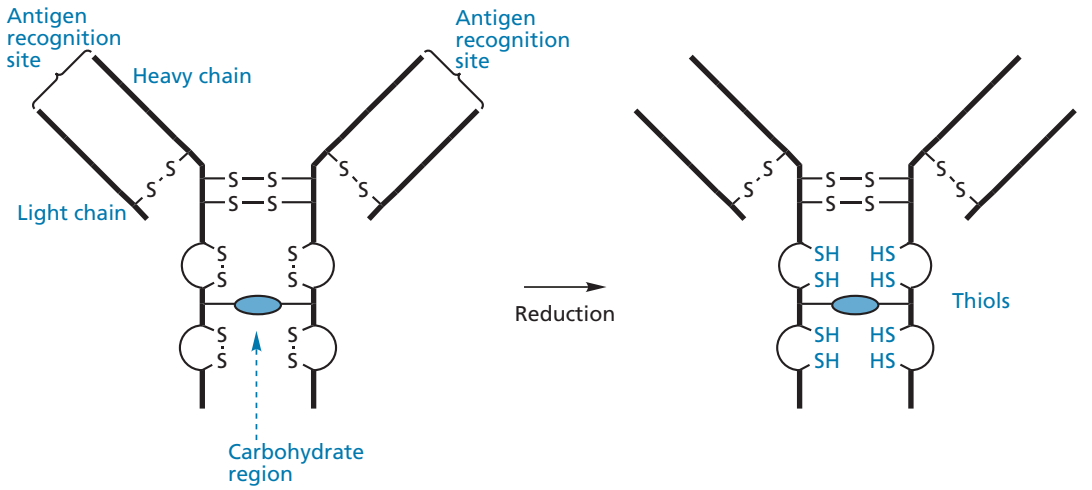


FIGURE 21.72 Reduction of disulphide links.

linkers have been tried such as acid-labile linkers, peptidase-labile linkers, and disulphide linkers. The disulphide linker can be cleaved by disulphide exchange with an intracellular thiol such as glutathione, which has a higher concentration within cells than in plasma.

The drug itself needs to be highly potent ($IC_{50} < 10^{-10}$ M) which involves the use of drugs that are 100–1000 times more cytotoxic than conventional cytotoxic drugs. A variety of agents are being investigated including **radioactive isotopes**, **ricin**, **diphtheria toxin**, *Pseudomonas aeruginosa* **exotoxin A**, **maytansinoids**, **adozelesin**, **calicheamicin** γ_1, **auristatins**, highly potent **taxoids**, and highly potent **doxorubicin analogues**.

Ibritumomab and **tositumomab** are conjugated murine antibodies which carry radioactive isotopes to an antigen called **CD20** on the surface of B-lymphocytes. These cells grow uncontrollably in non-Hodgkin's lymphoma. Ibritumomab carries ^{90}Y and tositumomab carries ^{131}I.

21.9.3 Antibody-directed enzyme prodrug therapy (ADEPT)

Antibody-directed enzyme prodrug therapy involves two steps. The first is the administration of an antibody–enzyme complex. The antibody is raised against tumour-selective antigens and is linked to an enzyme, such as **bacterial carboxypeptidase**. This complex then gets bound to the tumour. Unlike antibody–drug conjugates, the antibody–enzyme complex needs to remain attached to the surface of the cell and should not be internalized. A certain period of time needs to elapse to give the complex time to bind to target cells and for unbound complex to be cleared from the blood supply. A prodrug of a cytotoxic drug is then administered. The prodrug is designed such that it will be stable in the blood supply and can only be cleaved and activated by the enzyme complexed to the antibody. This means that the toxic drug is only produced at the tumour and can be administered in higher doses than the parent drug. **CJS 149** is an example of one such prodrug which is activated by a bacterial carboxypeptidase (Fig. 21.74). An advantage of ADEPT over antibody–drug conjugates is that the enzyme is catalytic and can generate a large number of active drug molecules at the site of the tumour. These can then diffuse into the tumour and affect cells which might not have any antibody attached to them.

A lot of research has been carried out on ADEPT using bacterial enzymes such as **carboxypeptidase G2**, **penicillin G acylase**, and **β-lactamase**. The advantage of using a 'foreign' enzyme is that enzymes can be chosen that are not present in the mammalian cell, and so there is no chance of the prodrug being activated by

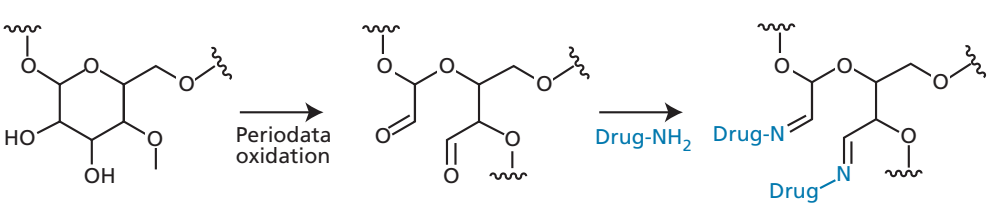


FIGURE 21.73 Linking drugs to carbohydrates.

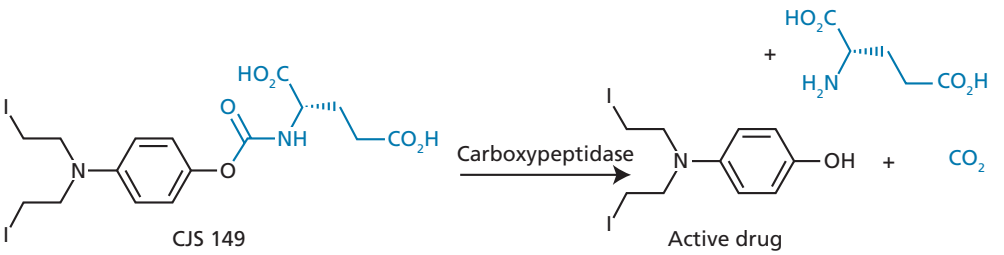


FIGURE 21.74 Activation of a prodrug by carboxypeptidase.

mammalian enzymes during its circulation round the body. It is also possible to use foreign enzymes which have counterparts in the body, as long as the latter are only present in low levels in the blood and/or they are structurally distinct. Prodrugs can be designed that react selectively with the foreign enzyme rather than the mammalian version. Examples of enzymes in this category include β-**glucuronidase** and **nitroreductase**.

Many studies have been carried out on ADEPT. One example is an antibody–β-lactamase complex capable of

BOX 21.13 Gemtuzumab ozogamicin: an antibody–drug conjugate

A humanized antibody called gemtuzumab has been linked to the highly potent anticancer drug **calicheamicin**. The trisulphide group normally present in calicheamicin was first modified to a disulphide with a hydrazide linker attached, while the antibody was treated with periodate to generate aldehyde groups at the carbohydrate region. The two molecules were then linked up by reacting the hydrazine group on the drug with the aldehyde groups on the antibody (Fig. 1). The resulting conjugate is called gemtuzumab ozogamicin and was approved for the treatment of acute myeloid leukaemia (AML) from 2000–2010.

When the antibody–drug complex reaches the target leukemic cell, the antibody attaches itself to a **CD33 antigen** and the antibody–drug complex is then taken into the cell by endocytosis. It is thought that the drug is then released from the antibody in lysosomes or endosomes by acidic hydrolysis of the hydrazone, and that reduction of the disulphide group occurs later in the cell nucleus to produce the active thiol (Fig. 2).

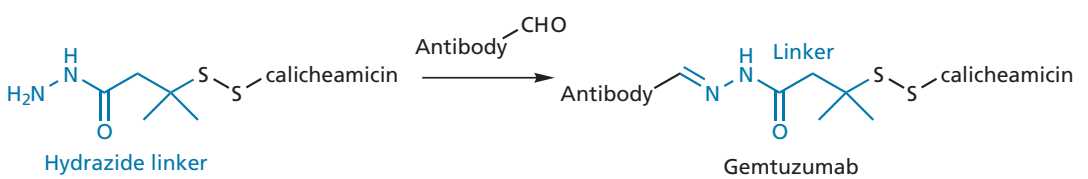


FIGURE 1 Linking calicheamicin to an antibody.

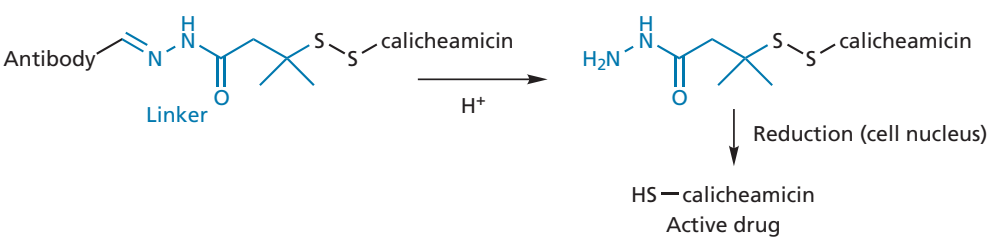


FIGURE 2 Release and activation of calicheamicin.

reacting with the cephalosporin prodrug of an alkylating agent (Fig. 21.75). This takes advantage of the mechanism by which cephalosporins react with β-lactamase to eliminate a leaving group (section 19.5.2.1).

One of the problems associated with ADEPT is the possibility of an immune response to the antibody–enzyme complex since the enzyme is a foreign protein. For this reason, it may be preferable to use human enzymes along with prodrugs that are already approved for anticancer use. Research has been carried out on human enzymes, such as **alkaline phosphatase**, **carboxypeptidase A**, and **β-glucuronidase**. The advantage of using a human enzyme is the decreased chance of an immune response, but the disadvantage is the increased risk of prodrug activation occurring during circulation in the blood supply.

Another problem may be insufficient enzyme activity. For example, the activation of **irinotecan** has been achieved using a particularly active isozyme of **human carboxylesterase** enzyme isolated from the liver. The isozyme concerned (hCE-2) was 26 times more active than another isozyme hCE-1, but was still too low to be effective for ADEPT. Nevertheless, the isozyme may be suitable for gene therapy (section 21.9.5) where greater concentrations of the isozyme could be achieved within the cell than could be brought to the cell by antibodies.

The time gap between the administration of the antibody–enzyme complex and the prodrug is critical. Enough time must be provided to ensure that unbound complex has dropped to low levels, otherwise the prodrug will be activated in the blood supply; however, the longer the time gap, the more chance the levels of the antibody–enzyme complex will drop at the tumour. One way to tackle this problem is a three-stage ADEPT strategy. The antibody–enzyme complex is administered as before. Sufficient time is given for the complex to concentrate at the tumour, then a second antibody is administered which targets the conjugate and speeds up its clearance from the blood supply. The second antibody can be galactosylated to speed up its clearance rate such that it only has time to target circulating conjugate and does not survive long enough to penetrate the tumour. Finally, the prodrug is added as before.

21.9.4 Antibody-directed abzyme prodrug therapy (ADAPT)

Abzymes are antibodies which have a catalytic property. It is possible that prodrugs could be designed that act as antigens for these antibodies and are activated by the abzyme's catalytic properties. This can be done by immunizing mice with a transition-state analogue of the reaction that is desired, followed by isolation of the monoclonal antibodies by hybridization techniques. As the antibody targets the prodrug rather than antigens on the cancer cell, this fails to target drugs to cancer cells. However, it should be possible to construct hybrid antibodies where one arm recognizes antigens on cancer cells while the other arm recognizes the prodrug and activates it. This approach is still in its early stages, but it has several potential advantages over ADEPT. For example, it should be possible to design catalytic mechanisms that do not occur naturally, allowing highly selective activation of prodrugs at tumours. It also removes the risk of an immune response due to foreign enzymes. At present, the catalytic activity of abzymes is too low to be useful and much more research has to be carried out.

21.9.5 Gene-directed enzyme prodrug therapy (GDEPT)

Gene-directed enzyme prodrug therapy involves the delivery of a gene to the cancer cell. Once delivered, the gene codes for an enzyme capable of transforming

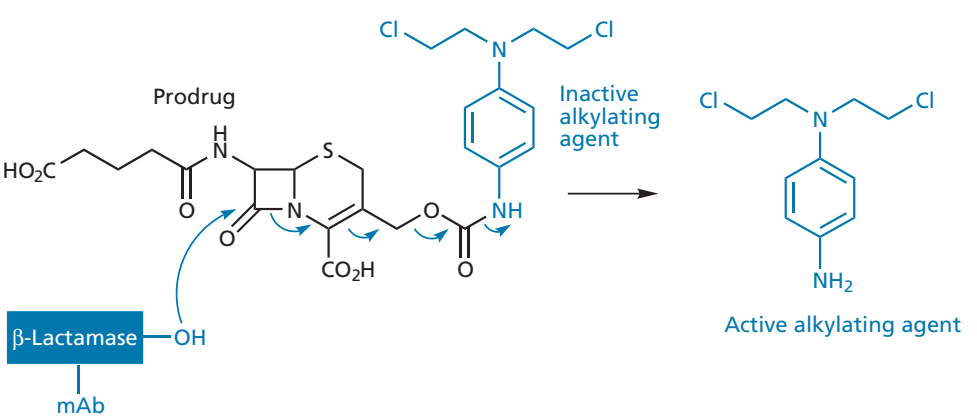


FIGURE 21.75 ADEPT strategy to release an alkylating agent.

a prodrug into an active drug. As the enzyme will be produced inside the cell, the prodrug is required to enter the cell.

The main challenge in GDEPT is delivering the gene selectively to tumour cells. In one method, the gene is packaged inside a virus, such as a retrovirus or adenovirus. In the case of **adenoviruses**, the desired genes could be spliced into the viral DNA such that the virus inserts into host cell DNA on infection. The virus is also modified genetically such that it is no longer virulent and can do no harm to normal cells. Non-viral vectors have also been tried, such as cationic lipids and peptides. So far, it has not been possible to achieve the required selectivity for cancer cells over normal cells, and so the delivery vector has to be administered directly to the tumour.

The enzymes which are ultimately produced by the introduced genes should not be present in normal cells, so that prodrug activation only occurs in tumour cells. One advantage of GDEPT over ADEPT is the fact that foreign enzymes could be generated inside cancer cells and hidden from the immune response. The **thymidine kinase** enzyme produced by herpes simplex virus has been studied intensively in GDEPT. This enzyme activates the antiviral drugs **aciclovir** and **ganciclovir** (section 20.6.1). As these drugs are poor substrates for mammalian thymidine kinase, activation will only be significant in the tumour cells containing the viral form of the enzyme. Several clinical trials have been carried out using this approach.

One problem associated with GDEPT is that it is unlikely that all tumour cells will receive the necessary gene to activate the prodrug. It is therefore important that the anticancer drug is somehow transferred between cells in the tumour—a so-called **bystander effect**. This may occur by a variety of means, such as release of the activated drug from the infected cell, direct transfer through intercellular gap junctions, or by the release of drug-carrying vesicles following cell death.

GDEPT has been used to introduce the genes for the bacterial enzymes **nitroreductase** and **carboxypeptidase G2** into cancer cells. Prodrugs were then administered which were converted to alkylating agents by the resulting enzymes. One of the problems with carboxypeptidase G2 is the difficulty some of the prodrugs have in crossing cell membranes. In order to overcome this problem, the gene was modified such that the resulting enzyme was incorporated into the cell membrane with the active site revealed on the outer surface of the cell.

Gene therapy aimed at activating the prodrug **irinotecan** is being explored to try and improve the process by which the urethane is hydrolysed to the active drug (section 21.2.2.2). This could involve the introduction of a gene encoding a more active carboxypeptidase enzyme into tumour cells. For example, **rabbit liver carboxypeptidase** is 100–1000 times more efficient than the human form of the enzyme.

21.9.6 Other forms of gene therapy

Gene therapy could also be used to introduce the genes coding for regulatory proteins which have been suppressed in cancer cells. For example, attempts have been made to introduce the gene for the **p53 protein** via a virus vector.

KEY POINTS

- Monoclonal antibodies have been targeted against antigens which are over expressed in certain cancer cells. They are useful in the treatment of breast cancers, colorectal cancer, and lymphomas.
- Antibody–drug conjugates involve the linking of a highly potent drug or radioisotope to an antibody. The conjugate is designed to target specific cancer cells and then be enveloped by the cell such that the drug can be released inside the cell.
- Antibodies should be humanized to avoid the immune response.
- Drugs can be attached to antibodies via lysine residues. Alternatively, the antibody can be modified to produce thiol or aldehyde groups to which drugs can be attached.
- ADEPT involves an antibody–enzyme conjugate which is targeted to specific cancer cells. Once the antibody has become attached to the outer surface of cancer cells, a prodrug is administered which is activated by the enzyme at the tumour site.
- ADAPT involves an antibody which has catalytic activity designed to activate a prodrug. At present, the activity of such abzymes is too low to be useful.
- GDEPT involves the delivery of a gene into a cancer cell. The gene codes for an enzyme capable of activating an anticancer prodrug.

21.10 Photodynamic therapy

Conventional prodrugs are inactive compounds which are normally metabolized in the body to their active form. A variation of the prodrug approach is the concept of a sleeping agent. This is an inactive compound which is converted to the active drug by some form of external influence. The best example of this approach is the use of photosensitizing agents such as **porphyrins** or **chlorins** in cancer treatment—photodynamic therapy (PDT). Porphyrins occur naturally in chlorophyll in plants and haemoglobin in red blood cells. They usually complex a metal ion in the centre of the molecule (magnesium in chlorophyll and iron in haemoglobin). In this form, they are non-toxic.

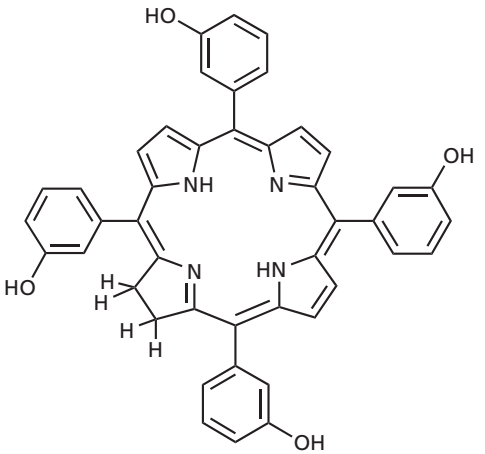


FIGURE 21.76 Temoporfin.

However, if they lack the central ion, they have the potential to do great damage. Given intravenously, these agents accumulate within cells and have some selectivity for tumour cells. By themselves, the agents have little effect, but if the cancer cells are irradiated with red light or a red laser, the porphyrins are converted to an excited state and react with molecular oxygen to produce highly toxic singlet oxygen. Singlet oxygen can then attack proteins and unsaturated lipids in the cell membrane leading to the formation of hydroxyl radicals which further react with DNA leading to cell destruction. **Temoporfin (Foscan)** (Fig. 21.76) is an example of a chlorin photosensitizing agent which is used to treat advanced head and neck tumours that do not respond to other treatments.

Unfortunately, the porphyrin structures used for PDT are inherently hydrophobic, which makes them difficult to formulate. Encapsulation using liposomes, oils, or polymeric micelles is one method of avoiding this problem, and has the advantage that tumours engulf and retain macromolecules more readily than would be the case with normal tissue. This is because the blood vessels nourishing tumours are leaky (see section 21.1.9) and release larger molecules than would be released from normal blood vessels.

Despite this, problems still remain. For example, the liposomes which carry the agent can be engulfed and

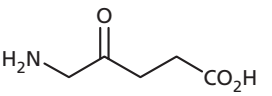


FIGURE 21.77 5-Aminolevulinic acid.

destroyed by cells of the reticuloendothelial system. The most serious disadvantage with PDT, however, is photosensitivity. Once the drug has been released from liposomes and activated, it is free to circulate round the body and accumulate in the eyes and skin, leading to phototoxic side effects which render the patient highly sensitive to light. Indeed, it is this property that first highlighted the possibility of using porphyrins in PDT. **Porphyria** is a disease where porphyrins accumulate in the skin and result in photosensitization and disfigurement. Victims are unable to tolerate sunlight, and disfigurements can include erosion of the gums to reveal red, fang-like teeth. It is likely that sufferers of this disease may have inspired the medieval vampire legends. Indeed, it is interesting to note that victims would have been averse to garlic, as components of garlic exacerbate the symptoms and cause an agonizing reaction. It was the observation that porphyrins could break down cells that led to the idea that these agents could be used to break down cancer cells.

Problems such as photosensitivity have limited the application of PDT, but research is underway to find improved methods of delivering the agent.

5-Aminolevulinic acid (Fig. 21.77) is used as a photosensitiser to treat skin blemishes that may turn cancerous. The compound is a biosynthetic precursor for porphyrins and is applied to the blemishes several hours before photodynamic therapy is carried out. This gives sufficient time for a build-up of porphyrins in the affected tissue.

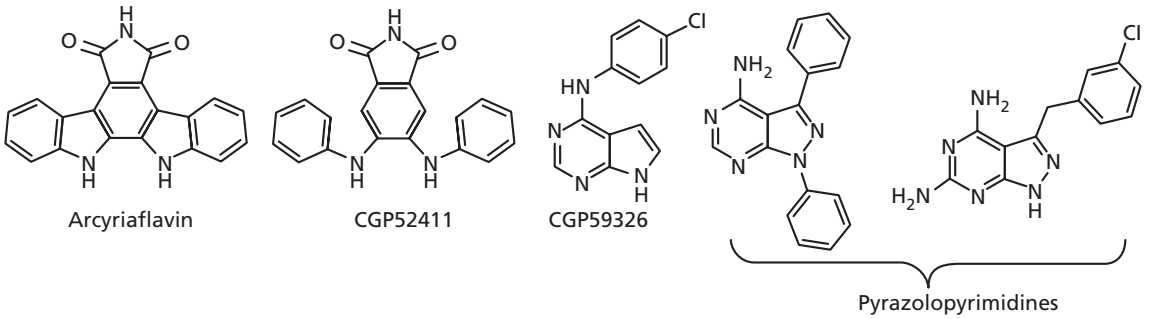
KEY POINTS

- Photodynamic therapy involves the irradiation of tumours containing porphyrin photosensitizers. This produces reactive oxygen species which are fatal to the cell.
- Photosensitivity is a serious problem, as the porphyrins can accumulate in the eyes and skin where they become activated by daylight.

QUESTIONS

1. Sch-226374 (Fig. 21.46) contains an aromatic ring which protects an imidazole ring from metabolism. Why do you think this imidazole ring is so susceptible to metabolism?
2. Do you think sulphonamides would be suitable antibacterial agents to treat opportunistic infections in cancer patients?
3. As esters are commonly used as prodrugs, esterases would be suitable enzymes to use in ADEPT. What are your thoughts on this statement?
4. Staurosporin (Fig 21.57) is a kinase inhibitor that shows no selectivity, but is a useful lead compound for potential anti-tumour agents. A simplification

strategy resulted in arcyriaflavin, which is selective for PKC. There are three reasons why this molecule is simpler. Explain what they are and why simplification is desirable.



5. CGP 52411 is a further simplification of arcyriaflavin A. Remarkably, this compound is inactive against PKC and is selective for the EGF-R kinase active site. Suggest why there might be such a drastic change in selectivity.
6. Further studies showed that CGP 52411 was bound to the ATP binding site of the kinase active site (Fig. 21.47). Suggest how this structure might bind. SAR studies show that substitution on any of the NH groups or the aromatic rings is bad for activity.
7. CGP 59326 and the pyrazolopyrimidine structures shown are also useful inhibitors of the kinase active site of the EGF-R. Suggest how they might be bound to the active site.
8. In the development of imatinib, a conformational blocker was introduced (Fig. 21.52). Suggest a conformation which would be feasible in the lead compound that would be prevented by the conformational blocker.
9. Imatinib has a pyrimidine ring where one of the nitrogens is involved in an important hydrogen bond interaction. It has been suggested that it should be possible to produce an analogue where the pyrimidine ring is replaced by a pyridine ring. What are your thoughts on this suggestion?
10. Suggest a mechanism by which CC-1065 and adozelesin act as alkylating agents.
11. ZD9331 (Fig. 21.21) has a tetrazole ring as part of its structure. What purpose does this serve?

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Titles for general further reading are listed on p. 763

For additional material see Web article 23: histone deacetylase inhibitors in medicinal chemistry.

For additional material see Web article 24: current metallodrugs in cancer.

22 Cholinergics, anticholinergics, and anticholinesterases

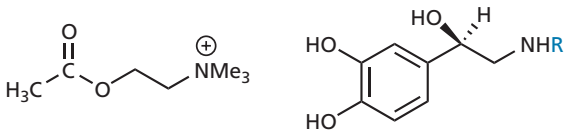
In this chapter, we shall concentrate on drugs that have an effect on the cholinergic nervous system. There are several clinically important drugs in this category which act in the peripheral and/or the central nervous system.

22.1 The peripheral nervous system

The peripheral nervous system (PNS) is so called because it is peripheral to the central nervous system (CNS; the brain and spinal column). There are many divisions and subdivisions of the peripheral system that can lead to confusion. The first distinction to make is between **sensory** and **motor nerves**:

- sensory nerves take messages from the body to the CNS;
- motor nerves carry messages from the CNS to the rest of the body.

An individual nerve cell is called a **neuron** (Appendix 4) and neurons must communicate with each other in order to relay messages. However, neurons are not physically connected. Instead, there are gaps which are called **synapses** (Fig. 22.1). If a neuron is to communicate its message to another neuron (or a target organ), it can only do so by releasing a chemical that crosses the synaptic gap and binds to receptors on the target cell. This interaction between neurotransmitter and receptor can then stimulate other processes, which, in the case of a second neuron, continues the message. As these chemicals effectively carry a message from a neuron, they are known as chemical messengers or **neurotransmitters**. There are a large number of neurotransmitters in the body, but the important ones in the peripheral nervous system are **acetylcholine** and **noradrenaline** (Fig. 22.2). The very fact that neurotransmitters are chemicals allows the medicinal chemist to design and synthesize organic compounds which can mimic (**agonists**) or block **(antagonists)** their action.



Acetylcholine

R = H Noradrenaline
R = Me Adrenaline

FIGURE 22.2 Acetylcholine, noradrenaline, and adrenaline.

22.2 Motor nerves of the PNS

In this chapter, we are concerned primarily with drugs that influence the activity of motor nerves. Motor nerves take messages from the CNS to various parts of the body, such as skeletal muscle, smooth muscle, cardiac muscle, and glands (Figs 4.1 and 22.3). The message travelling along a single neuron is often compared to an electrical pulse, but the analogy with electricity should not be taken too far as the pulse is a result of ion flow across the

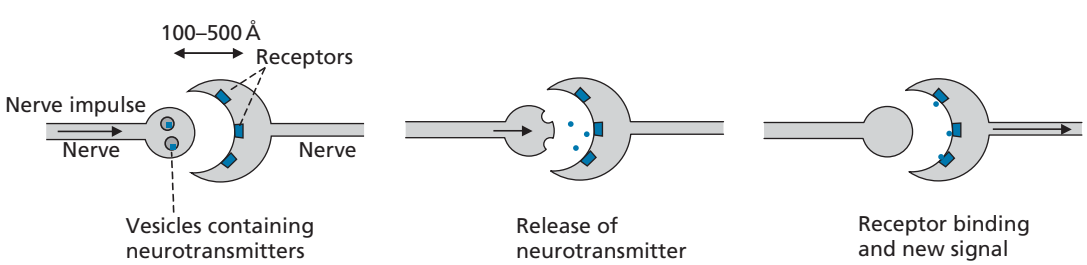


FIGURE 22.1 Signal transmission at a synapse.

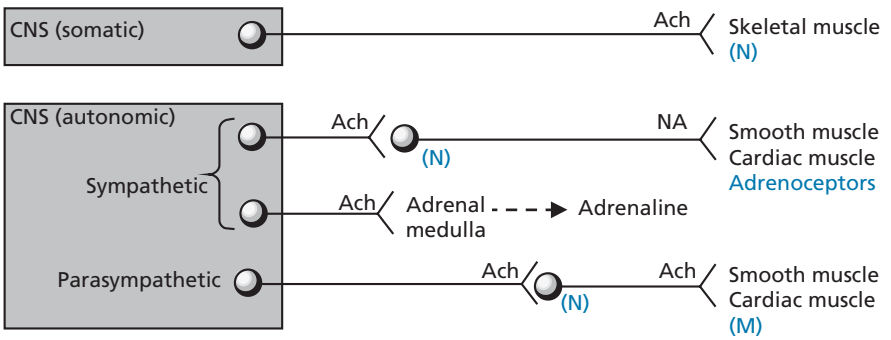


FIGURE 22.3 Motor nerves of the peripheral nervous system. N = nicotinic receptor; M = musarinic receptor; AcH = acetylcholine; NA = noradrenaline.

membranes of neurons and not a flow of electrons (see Appendix 4).

It should be evident that the workings of the human body depend crucially on an effective motor nervous system. Without it, we would not be able to operate our muscles and we would end up as flabby blobs, unable to move or breathe. We would not be able to eat, digest, or excrete our food because the smooth muscle activity of the gastrointestinal tract (GIT) and the urinary tract is controlled by motor nerves. We would not be able to control body temperature, as the smooth muscle controlling the diameter of our peripheral blood vessels would cease to function. Finally, our heart would resemble a wobbly jelly rather than a powerful pump. In short, if the motor nerves failed to function, we would be in a mess! Let us now look at the motor nerves in more detail.

The motor nerves of the PNS have been classified into three subsystems: the **somatic motor nervous system**, the **autonomic motor nervous system**, and the **enteric nervous system**. These are considered in the following sections.

22.2.1 The somatic motor nervous system

The somatic motor nerves carry messages from the CNS to the skeletal muscles. There are no synapses en route and the neurotransmitter at the neuromuscular junction is **acetylcholine**. Acetylcholine binds to cholinergic receptors within the cell membranes of muscle cells and the final result is contraction of skeletal muscle.

22.2.2 The autonomic motor nervous system

The autonomic motor nerves carry messages from the CNS to smooth muscle, cardiac muscle, and the adrenal medulla. This system can be divided into the **sympathetic** and **parasympathetic** nervous systems.

Sympathetic neurons leave the CNS and synapse almost immediately with a second neuron using acetylcholine as neurotransmitter. The second neuron then proceeds to various tissues and organs around the body. **Noradrenaline** is the neurotransmitter released from the second neuron, and this interacts with adrenergic receptors present in target cells and organs. At the heart, the action of noradrenaline leads to contraction of cardiac muscle and an increase in heart rate. Elsewhere, it relaxes smooth muscle and reduces the contractions of the gastrointestinal and urinary tracts. It also reduces salivation and the dilatation of the peripheral blood vessels. In general, the sympathetic nervous system promotes the '**fight or flight**' response by shutting down the body's housekeeping roles (digestion, defecation, urination, etc.), while stimulating the heart.

There are some neurons in the sympathetic nervous system which do not synapse with a second neuron, but go directly to a gland called the **adrenal medulla**. Acetylcholine is the neurotransmitter released by these neurons and it stimulates the adrenal medulla to release the hormone **adrenaline**, which then circulates through the blood system. **Adrenaline** reinforces the actions of noradrenaline by activating adrenergic receptors throughout the body, whether they are supplied directly with nerves or not.

Parasympathetic neurons leave the CNS, travel some distance, then synapse with a second neuron using acetylcholine as neurotransmitter. The second neuron then proceeds to synapse with the same target tissues and organs as the sympathetic neurons. However, acetylcholine acts as the neurotransmitter, rather than noradrenaline, and activates cholinergic receptors on the target cells. The resulting effects are the opposite to those caused by activation of adrenergic receptors. For

example, cardiac muscle is relaxed, whereas the smooth muscle of the digestive and urinary tracts is contracted.

As the sympathetic and parasympathetic nervous systems oppose each other in their actions, they can be looked upon as acting like a brake and an accelerator on the different tissues and organs around the body. The analogy is not quite apt because both systems are always operating and the overall result depends on which effect is the stronger.

22.2.3 The enteric system

The third constituent of the PNS is the enteric system, which is located in the walls of the GIT. It receives messages from **sympathetic** and **parasympathetic nerves**, but it also responds to local effects to provide local reflex pathways which are important in the control of GIT function. A large variety of neurotransmitters are involved including **serotonin**, **neuropeptides**, and **ATP**. **Nitric oxide** (**NO**) is also involved as a chemical messenger.

22.2.4 Defects in motor nerve transmission

Defects in motor nerve transmission would clearly lead to a large variety of ailments involving the heart, skeletal muscle, GIT, urinary tract, and many other organs. Such defects might be the result of either a deficit or an excess of neurotransmitter. Therefore, treatment involves the administration of drugs which can act as agonists or antagonists, depending on the problem. There is a difficulty with this approach, however. Usually, the problem we wish to tackle occurs at a certain location where there might, for example, be a lack of neurotransmitter. Application of an agonist to make up for low levels of neurotransmitter at the heart might solve the problem there, but would lead to problems elsewhere in the body where the levels of neurotransmitter would be normal. At those areas, the agonist would cause too much activity and cause unwanted side effects. Therefore, drugs showing selectivity for different parts of the body would, clearly, be preferred. This selectivity has been achieved to a great extent with both cholinergic and adrenergic agents. In this chapter, we concentrate on cholinergic agents (adrenergic agents are covered in Chapter 23).

22.3 The cholinergic system

22.3.1 The cholinergic signalling system

Let us look first at what happens at synapses involving acetylcholine as the neurotransmitter. Figure 22.4 shows the synapse between two neurons and the events involved when a message is transmitted from one neuron to another. The same general process takes place when a message is passed from a neuron to a muscle cell.

1. The first stage involves the biosynthesis of acetylcholine (Fig. 22.5). Acetylcholine is synthesized from **choline** and **acetyl coenzyme A** at the end of the presynaptic neuron. The reaction is catalysed by the enzyme **choline acetyltransferase**.
2. Acetylcholine is incorporated into membrane-bound vesicles by means of a specific transport protein.
3. The arrival of a nerve signal leads to an opening of calcium ion channels and an increase in intracellular calcium concentration. This induces the vesicles to fuse with the cell membrane and release the transmitter into the synaptic gap.
4. Acetylcholine crosses the synaptic gap and binds to the cholinergic receptor, resulting in stimulation of the second neuron.
5. Acetylcholine moves to an enzyme called **acetylcholinesterase**, which is situated on the postsynaptic neuron, and which catalyses the hydrolysis of acetylcholine to produce choline and acetic acid (ethanoic acid).
6. Choline is taken up into the presynaptic neuron by a transport protein to continue the cycle.

The most important thing to note is that there are several stages where it is possible to use drugs to either promote or inhibit the overall process. The greatest success so far has been with drugs targeted at stages 4 and 5 (i.e. the cholinergic receptor and the acetylcholinesterase enzyme). These are considered in more detail in subsequent sections.

22.3.2 Presynaptic control systems

Cholinergic receptors (called **autoreceptors**) are present at the terminus of the presynaptic neuron (Fig. 22.6). The purpose of these receptors is to provide a means of local control over nerve transmission. When acetylcholine is released from the neuron, some of it will find its way to these autoreceptors and switch them on. This has the effect of inhibiting further release of acetylcholine.

The presynaptic neuron also contains receptors for **noradrenaline**, which act as another control system for acetylcholine release. Branches from the sympathetic nervous system lead to the cholinergic synapses and when the sympathetic nervous system is active, noradrenaline is released and binds to these receptors. Once again, the effect is to inhibit acetylcholine release. This indirectly enhances the activity of noradrenaline at target organs by lowering cholinergic activity.

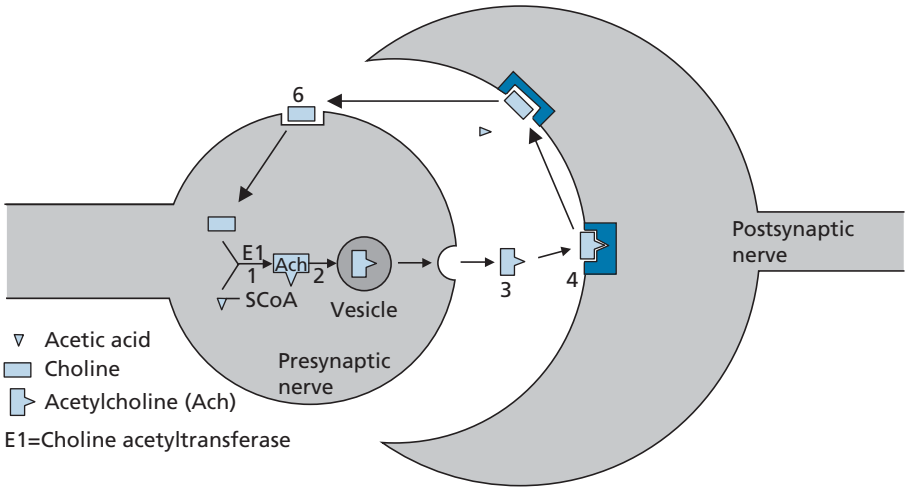


FIGURE 22.4 Synapse with acetylcholine acting as the neurotransmitter.

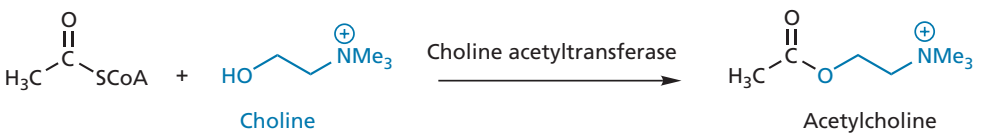


FIGURE 22.5 Biosynthesis of acetylcholine.

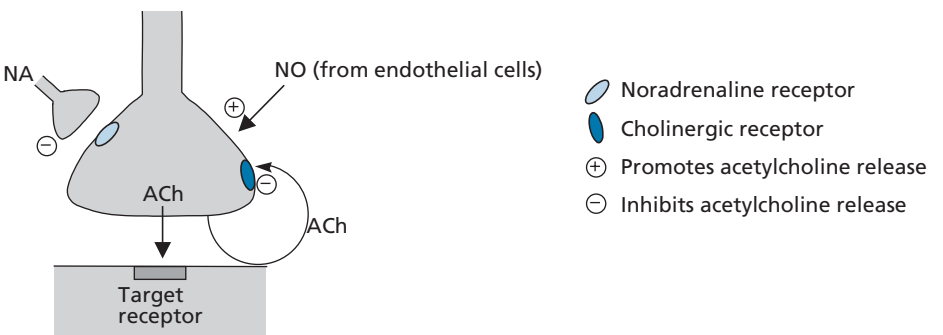


FIGURE 22.6 Presynaptic control systems.

The chemical messenger **nitric oxide** (**NO**) can also influence acetylcholine release, but, in this case, it promotes release. A large variety of other chemical messengers including **co-transmitters** (see below) are also implicated in presynaptic control. The important thing to appreciate is that presynaptic receptors offer another possible drug target to influence the cholinergic nervous system.

22.3.3 Co-transmitters

Co-transmitters are messenger molecules released along with acetylcholine. The particular co-transmitter released depends on the location and target cell of the neurons. Each co-transmitter interacts with its own receptor on the postsynaptic cell. Co-transmitters have a variety of structures and include peptides, such as **vasoactive intestinal peptide** (VIP), **gonadotrophin-releasing hormone** (GnRH), and **substance P**. The roles of these agents appear to be as follows:

- they are longer-lasting and reach more distant targets than acetylcholine, resulting in longer-lasting effects;
- the balance of co-transmitters released varies under different circumstances (e.g. presynaptic control) and so can produce different effects.

22.4 Agonists at the cholinergic receptor

One point might have occurred to you. If there is a lack of acetylcholine acting at a certain part of the body, why not just administer more acetylcholine? After all, it is easy enough to make in the laboratory (Fig. 22.7).

There are three reasons why this is not feasible.

- Acetylcholine is easily hydrolysed in the stomach by acid catalysis and cannot be given orally.
- Acetylcholine is easily hydrolysed in the blood by esterase enzymes (esterases).
- There is no selectivity of action. Additional acetylcholine will switch on all cholinergic receptors in the body.

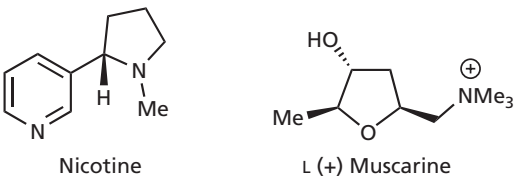
Therefore, we need analogues of acetylcholine that are more stable to hydrolysis and more selective with respect to where they act in the body. We shall look at selectivity first.

There are two ways in which selectivity can be achieved. Firstly, some drugs may be distributed more efficiently to one part of the body than another. Secondly, there are different types of cholinergic receptor, which vary in the way they are distributed in tissues. It is possible to design synthetic agents that show selectivity for these receptors and, hence, have tissue selectivity.

This is not just a peculiarity of cholinergic receptors. Differences have been observed for other types of receptors, such as those for dopamine, noradrenaline, and serotonin, and there are many types and subtypes of receptor for each chemical messenger (see Chapter 4).

The first indications that different types of cholinergic receptor existed came from the action of natural compounds. It was discovered that the compounds **nicotine** (present in tobacco) and **muscarine** (the active principle of a poisonous mushroom) (Fig. 22.8) were both cholinergic agonists, but that they had different physiological effects.

Nicotine showed selectivity for cholinergic receptors present on skeletal muscle or at the synapses between different neurons, whereas muscarine showed selectivity for cholinergic receptors present on smooth muscle and cardiac muscle. From these results, it was concluded that there was one type of cholinergic receptor on skeletal muscles and at nerve synapses (the **nicotinic receptor**), and a different type of cholinergic receptor on smooth muscle and cardiac muscle (the **muscarinic receptor**) (Fig. 22.3).



Nicotine L (+) Muscarine

FIGURE 22.8 Nicotine and muscarine.

Muscarine and nicotine were the first compounds to indicate that receptor selectivity was possible, but they are unsuitable as medicines because they have undesirable side effects resulting from their interactions with other receptors. In the search for a good drug it is important to gain selectivity for one class of receptor over another (e.g. the cholinergic receptor in preference to an adrenergic receptor) and selectivity between receptor types (e.g. the muscarinic receptor in preference to a nicotinic receptor). It is also preferable to gain selectivity for particular subtypes of a receptor. For example, not every muscarinic receptor is the same throughout the body. At present, five subtypes of the muscarinic receptor have been discovered (M1–M5) and ten subtypes of the nicotinic receptor (α1–α10).

The principle of selectivity was proven with nicotine and muscarine, and so the race was on to design novel drugs which had the selectivity of nicotine or muscarine, but not the side effects.

KEY POINTS

- The cholinergic nervous system involves nerves which use the neurotransmitter acetylcholine as a chemical messenger. These include the motor nerves which innervate skeletal muscle, nerves which synapse with other nerves in the peripheral nervous system (PNS), and the parasympathetic nerves innervating cardiac and smooth muscle.
- There are two types of cholinergic receptor. Muscarinic receptors are present in smooth and cardiac muscle. Nicotinic receptors are present in skeletal muscle and in synapses between neurons.
- Acetylcholine is hydrolysed by the enzyme acetylcholinesterase when it departs the cholinergic receptor. The hydrolytic product choline is taken up into presynaptic neurons and

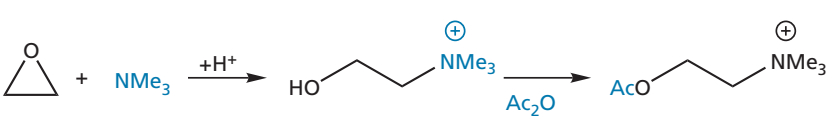


FIGURE 22.7 Synthesis of acetylcholine

acetylated back to acetylcholine. The cholinergic receptor and the enzyme acetylcholinesterase are useful drug targets.

- Acetylcholine cannot be used as a drug, because it is rapidly hydrolysed by acid and enzymes. It shows no selectivity for different types and subtypes of cholinergic receptor.

22.5 Acetylcholine: structure, structure–activity relationships, and receptor binding

The first stage in any drug development is to study the lead compound and to find out which parts of the molecule are important to activity so that they can be retained in future analogues [i.e. structure–activity relationships (SARs)]. These results also provide information about what the binding site of the cholinergic receptor looks like and help decide what changes are worth making in new analogues.

In this case, the lead compound is acetylcholine itself. The results described below are valid for both the nicotinic and muscarinic receptors, and were obtained by the synthesis of a large range of analogues.

- The positively charged nitrogen atom is essential to activity. Replacing it with a neutral carbon atom eliminates activity.
- The distance from the nitrogen to the ester group is important.
- The ester functional group is important.
- The overall size of the molecule cannot be altered much. Bigger molecules have poorer activity.
- The ethylene bridge between the ester and the nitrogen atom cannot be extended (Fig. 22.9).
- There must be two methyl groups on the nitrogen. A larger, third alkyl group is tolerated, but more than one large alkyl group leads to loss of activity.
- Bigger ester groups lead to a loss of activity.

Clearly, there is a tight fit between acetylcholine and its binding site, which leaves little scope for variation. The

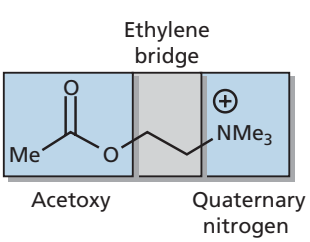


FIGURE 22.9 Acetylcholine.

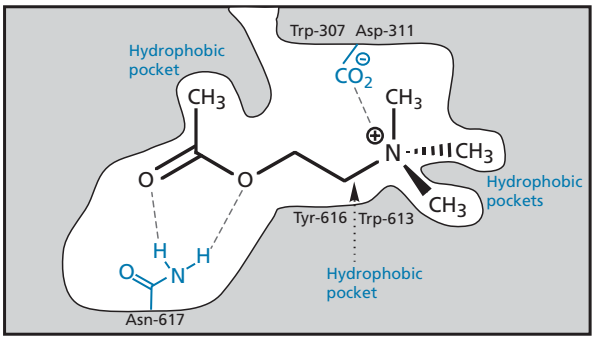


FIGURE 22.10 Muscarinic receptor binding site.

findings listed tally with a receptor binding site as shown in Fig. 22.10.

It is proposed that important hydrogen bonding interactions exist between the ester group of acetylcholine and an asparagine residue. It is also thought that a small hydrophobic pocket exists which can accommodate the methyl group of the ester, but nothing larger. This interaction is thought to be more important in the muscarinic receptor than the nicotinic receptor.

The evidence suggests that the NMe_3^+ group is placed in a hydrophobic pocket lined with three aromatic amino acids. It is also thought that the pocket contains two smaller hydrophobic pockets, which are large enough to accommodate two of the three methyl substituents on the NMe_3^+ group. The third methyl substituent on the nitrogen is positioned in an open region of the binding site and so it is possible to replace it with other groups. A strong ionic interaction has been proposed between the charged nitrogen atom and the anionic side group of an aspartate residue. The existence of this ionic interaction represents the classical view of the cholinergic receptor, but there is an alternative suggestion which states that there may be an induced dipole interaction between the NMe_3^+ group and the aromatic residues in the hydrophobic pocket.

There are several reasons for this. Firstly, the positive charge on the NMe_3^+ group is not localized on the nitrogen atom, but is spread over the three methyl groups (compare section 17.7.1). Such a diffuse charge is less likely to be involved in a localized ionic interaction and it has been shown by model studies that NMe_3^+ groups can be stabilized by binding to aromatic rings. It might seem strange that a hydrophobic aromatic ring should be capable of stabilizing a positively charged group, but it has to be remembered that aromatic rings are electron-rich, as shown by the fact they can undergo reaction with electrophiles. It is thought that the diffuse positive charge on the NMe_3^+ group is capable of distorting the π electron cloud of aromatic rings to induce a dipole moment (section 1.3.4). Induced ion–dipole interactions between the NMe_3^+ group and an aromatic residue such as tyrosine would then account for

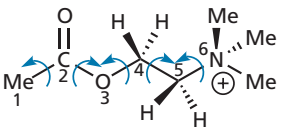


FIGURE 22.11 Bond rotations in acetylcholine leading to different conformations.

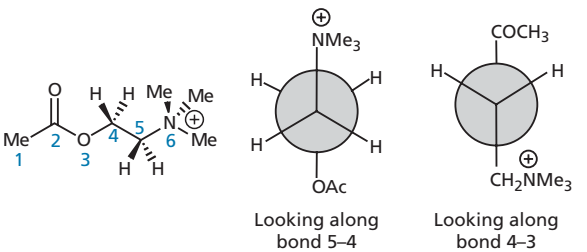


FIGURE 22.12 The sawhorse and Newman projections of acetylcholine.

the binding. The fact that three aromatic amino acids are present in the pocket adds weight to the argument.

Of course, it is possible that both types of binding interactions are taking place, which will please both parties!

A large amount of effort has been expended trying to identify the active conformation of acetylcholine, i.e. the shape adopted by the neurotransmitter when it binds to the cholinergic receptor. This has been no easy task, as acetylcholine is a highly flexible molecule (Fig. 22.11) where bond rotation along the length of its chain can lead to many possible stable conformations (or shapes).

In the past, it was assumed that a flexible neurotransmitter would adopt its most stable conformation when binding. In the case of acetylcholine, that would be the conformation represented by the sawhorse and Newman projections shown in Fig. 22.12. However, there is not a massive energy difference between alternative stable conformations such as the gauche conformation shown in Figure 22.13. The stabilization energy gained from binding interactions within the binding site could more than compensate for any energy penalties involved in adopting a slightly less stable conformation.

In order to try and establish the active conformation of acetylcholine, rigid cyclic molecules have been studied which contain the skeleton of acetylcholine within their structure; for example muscarine and the analogues shown in Fig. 22.14. In these structures, the portion of the acetylcholine skeleton which is included in a ring is locked into a particular conformation because bonds within rings cannot rotate freely. If such molecules bind to the cholinergic receptor, this indicates that this particular conformation is 'allowed' for activity.

Test your understanding and practise your molecular modelling with Exercise 22.2.

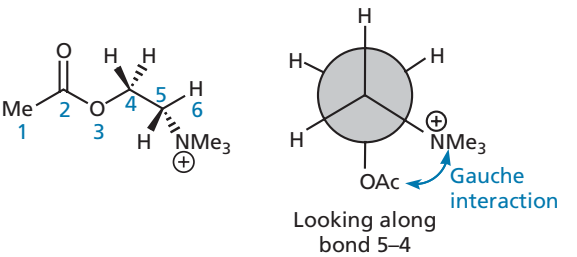


FIGURE 22.13 A gauche conformation for acetylcholine.

Many such structures have been prepared, but it has not been possible to identify one *specific* active conformation for acetylcholine. This probably indicates that the cholinergic receptor has a certain amount of latitude and can recognize the acetylcholine skeleton within the rigid analogues, even when it is not in the ideal active conformation. Nevertheless, such studies have shown that the separation between the ester group and the quaternary nitrogen is important for binding, and that this distance differs for the muscarinic and the nicotinic receptor (Fig. 22.15).

Having identified the binding interactions and pharmacophore of acetylcholine, we shall now look at how acetylcholine analogues were designed with improved stability.

Test your understanding and practise your molecular modelling with Exercises 22.1 and 22.2.

22.6 The instability of acetylcholine

As described previously, acetylcholine is prone to hydrolysis. This is explained by considering one of the conformations that the molecule can adopt (Fig. 22.16). In this conformation, the positively charged nitrogen interacts with the carbonyl oxygen and has an electron-withdrawing effect. To compensate, the oxygen atom pulls electrons from the neighbouring carbon atom and makes that carbon atom electron deficient and more prone to nucleophilic attack. Water is a poor nucleophile, but, because the carbonyl group is more electrophilic, hydrolysis takes place relatively easily. This influence of the nitrogen ion is known as **neighbouring group participation** or **anchimeric assistance**.

We shall now look at how the problem of hydrolysis was overcome, but it should be appreciated that we are doing this with the benefit of hindsight. At the time the problem was tackled the SAR studies were incomplete and the format of the cholinergic receptor binding site was unknown.

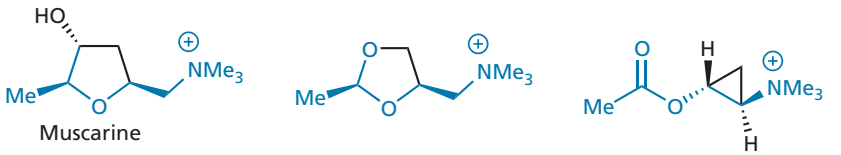


FIGURE 22.14 Rigid molecules incorporating the acetylcholine skeleton (C–C–O–C–C–N).

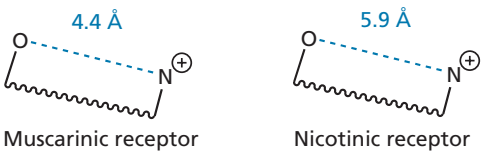


FIGURE 22.15 Pharmacophore of acetylcholine.

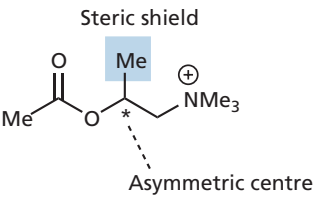


FIGURE 22.17 Methacholine (racemic mixture).

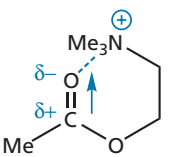


FIGURE 22.16 Neighbouring group participation. The arrow indicates the inductive pull of oxygen which increases the electrophilicity of the carbonyl carbon (see Molecular modelling exercise 22.1).

22.7 Design of acetylcholine analogues

There are two possible approaches to tackling the inherent instability of acetylcholine: steric shields and electronic stabilization.

22.7.1 Steric shields

The principle of steric shields was described in section 14.2.1 and can be demonstrated with **methacholine** (Fig. 22.17). Here, an extra methyl group has been placed on the ethylene bridge as a steric shield to protect the carbonyl group. The shield hinders the approach of any potential nucleophile and also hinders binding to esterase enzymes, thus slowing down chemical and enzymatic hydrolysis. As a result, methacholine is three times more stable to hydrolysis than acetylcholine.

The obvious question now is why not put on a bigger alkyl group like an ethyl group or a propyl group? Alternatively, why not put a bulky group on the acyl half of the molecule, as this would be closer to the carbonyl centre and have a greater shielding effect?

In fact, these approaches were tried. They certainly increased stability but they lowered cholinergic activity. We should already know why—the fit between acetylcholine and its receptor is so tight that there is little scope for enlarging the molecule. The extra methyl group is acceptable, but larger substituents hinder the molecule binding to the cholinergic receptor and decrease its activity.

Introducing a methyl steric shield has another useful effect. It was discovered that methacholine has significant muscarinic activity, but very little nicotinic activity. Therefore, methacholine shows good selectivity for the muscarinic receptor. This is perhaps more important than the gain in stability.

Selectivity for the muscarinic receptor can be explained if we compare the proposed active conformation of methacholine with muscarine (Fig. 22.18), as the methyl group of methacholine occupies the same position as a methylene group in muscarine. This is only possible for the *S*-enantiomer of methacholine and when the two enantiomers of methacholine were separated, it was found that the *S*-enantiomer was, indeed, the more active enantiomer. It is not used therapeutically, however.

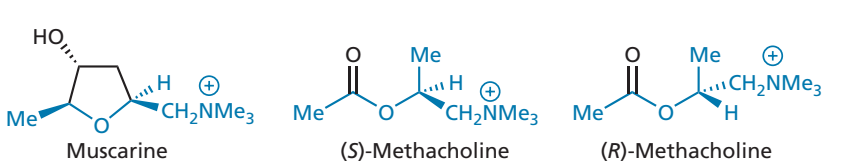


FIGURE 22.18 Comparison of muscarine and the *R*- and *S*-enantiomers of methacholine.

22.7.2 Electronic effects

The use of electronic factors to stabilize functional groups was described in sections 14.2.2 and 14.2.3, and was used in the design of **carbachol** (Fig. 22.19)—a long-acting cholinergic agent which is resistant to hydrolysis. Here, the acyl methyl group has been replaced by NH_2 which means that the ester has been replaced by a urethane or carbamate group. This functional group is more resistant to hydrolysis because the lone pair of electrons on nitrogen can interact with the neighbouring carbonyl group and lower its electrophilic character (Fig. 22.20).

The tactic worked, but it was by no means a foregone conclusion that it would. Although the NH_2 group is equivalent in size to the methyl group, the former is polar and the latter is hydrophobic, and it was by no means certain that a polar NH_2 group would be accepted into a hydrophobic pocket in the binding site. Fortunately, it is and activity is retained, which means that the amino group acts as a **bioisostere** for the methyl group. A bioisostere is a group which can replace another group without affecting the pharmacological activity of interest (sections 13.3.7 and 14.2.2). Thus, the amino group is a bioisostere for the methyl group as far as the cholinergic receptor is concerned, but not as far as the esterase enzymes are concerned.

The inclusion of the electron-donating amino group greatly increases chemical and enzymatic stability. Unfortunately, carbachol shows very little selectivity between the muscarinic and nicotinic receptors. Nevertheless, it is used clinically for the treatment of glaucoma where it can be applied locally, thus avoiding the problems of receptor selectivity. Glaucoma arises when the aqueous contents of the eye cannot be drained. This raises the pressure on the eye and can lead to blindness. Agonists cause the eye muscles to contract and allow drainage, thus relieving the pressure.

22.7.3 Combining steric and electronic effects

We have seen that the β-methyl group of methacholine increases stability and introduces receptor selectivity. Therefore, it made sense to add a β-methyl group to carbachol. The resulting compound is **bethanechol** (Fig. 22.21) which is both stable to hydrolysis and selective in its action. It is occasionally used therapeutically in stimulating the GIT and urinary bladder after surgery.

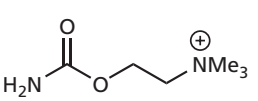


FIGURE 22.19 Carbachol.

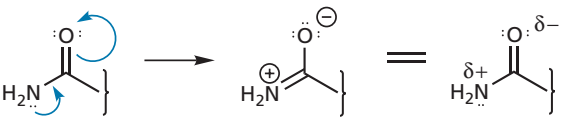
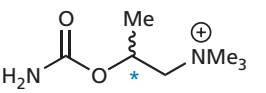


FIGURE 22.20 Resonance structures of carbachol.



* Asymmetric centre

FIGURE 22.21 Bethanechol.

Both these organs are 'shut down' with drugs during surgery (section 22.9).

22.8 Clinical uses for cholinergic agonists

22.8.1 Muscarinic agonists

A possible future use for muscarinic agonists is in the treatment of Alzheimer's disease. However, current clinical uses include:

- treatment of glaucoma;
- 'switching on' the GIT and urinary tract after surgery;
- treatment of certain heart defects by decreasing heart muscle activity and heart rate.

Pilocarpine (Fig. 22.22) is an example of a muscarinic agonist which is used in the treatment of glaucoma. It is an alkaloid obtained from the leaves of shrubs belonging to the genus *Pilocarpus*. Although there is no quaternary ammonium group present in pilocarpine, it is assumed that the drug is protonated before it interacts with the muscarinic receptor. Molecular modelling shows that pilocarpine can adopt a conformation having the correct pharmacophore for the muscarine receptor; i.e. a separation between nitrogen and oxygen of 4.4 Å.

Pilocarpine is also being considered for the treatment of Alzheimer's disease, as are other muscarinic agonists such as **oxotremorine** and various **arecoline** analogues (Fig. 22.22). At present, anticholinesterases are used clinically for the treatment of this disease (section 22.15).

22.8.2 Nicotinic agonists

Nicotinic agonists are used in the treatment of myasthenia gravis. This is an autoimmune disease where the body has produced antibodies against its own cholinergic

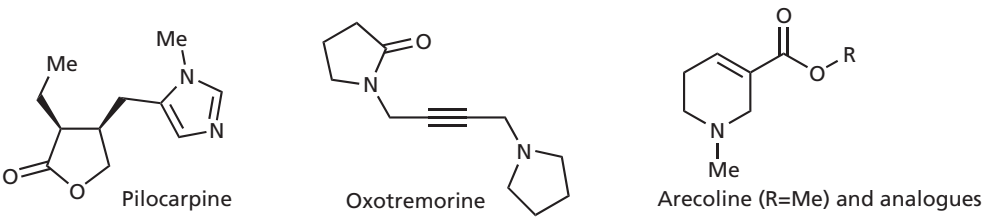


FIGURE 22.22 Examples of muscarinic agonists.

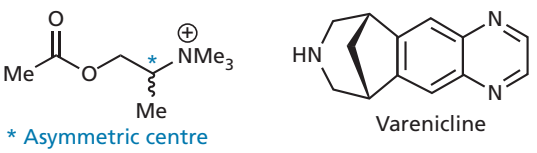


FIGURE 22.23 Examples of selective nicotinic agonists.

receptors. As a result, the number of available receptors drops and so fewer messages reach the muscle cells. In turn, this leads to severe muscle weakness and fatigue. Administering an agonist increases the chance of activating what few receptors remain. An example of a selective nicotinic agonist is the first structure shown in Fig. 22.23. This agent is very similar in structure to methacholine, and differs only in the position of the methyl substituent. This is sufficient, however, to completely alter receptor selectivity. Despite that, this particular compound is not used clinically and anticholinesterases (section 22.15.1.2) are the preferred treatment. **Varenicline** *is* used clinically, however. It is a partial agonist at nicotinic receptors and was approved in 2006 as an aid to stop smoking.

KEY POINTS

- Acetylcholine fits snugly into the binding site of cholinergic receptors and there is little scope for variation. Two of the *N*-methyl groups and the acyl methyl group fit into hydrophobic pockets. The ester is involved in hydrogen bonding, and the quaternary nitrogen is involved in ionic interactions and/or induced dipole interactions.
- Rigid analogues of acetylcholine have been used to try and identify the active conformation.
- Acetylcholine is unstable to acid because of neighbouring group participation. Stable analogues have been designed using steric shields and/or electronic effects.

22.9 Antagonists of the muscarinic cholinergic receptor

22.9.1 Actions and uses of muscarinic antagonists

Antagonists of the cholinergic receptor are drugs which bind to the receptor but do not 'switch it on'. By binding to the receptor, an antagonist acts like a plug at the receptor binding site and prevents acetylcholine from binding (Fig. 22.24). The overall effect on the body is the same as if there was a lack of acetylcholine. Therefore, antagonists have the opposite clinical effect from agonists.

The antagonists described in this section act only at the muscarinic receptor and therefore affect nerve transmissions to glands, the CNS, and the smooth muscle of the GIT and urinary tract. The clinical effects and uses of these antagonists reflect this.

The clinical effects of muscarinic antagonists are:

- reduced saliva and gastric secretions;
- reduced motility of the GIT and urinary tract by relaxation of smooth muscle;
- dilatation of eye pupils;
- CNS effects

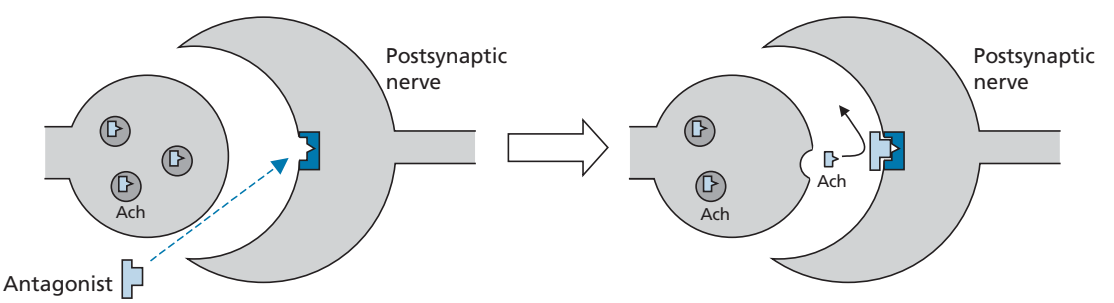


FIGURE 22.24 Action of an antagonist to block a receptor.

The clinical uses are:

- shutting down the GIT and urinary tract during surgery;
- ophthalmic examinations;
- relief of peptic ulcers;
- treatment of Parkinson's disease;
- treatment of anticholinesterase poisoning;
- treatment of motion sickness;
- a potential use for M2 antagonists is in the treatment of Alzheimer's disease.

22.9.2 Muscarinic antagonists

The first antagonists to be discovered were natural products—in particular alkaloids (nitrogen-containing compounds derived from plants).

22.9.2.1 Atropine and hyoscine

Atropine (Fig. 22.25) is present in the roots of *Atropa belladonna* (deadly nightshade) and is included in a root extract which was once used by Italian women to dilate their eye pupils. This was considered to enhance beauty, hence the name belladonna. Clinically, atropine has been used to decrease gastrointestinal motility and to counteract anticholinesterase poisoning.

Atropine has an asymmetric centre but exists as a racemate. Usually, natural products exist exclusively as one enantiomer. This is also true for atropine, which is present in the plants of the genus Solanaceae as a single enantiomer called **hyoscyamine**. As soon as the natural product is extracted into solution, however, racemization takes place. The asymmetric centre in atropine is easily racemized as it is next to a carbonyl group and an aromatic ring. This makes the proton attached to the asymmetric centre acidic and easily removed.

Hyoscine (or scopolamine) (Fig. 22.25) is obtained from the thorn apple (*Datura stramonium*) and is very similar in structure to atropine. It has been used in the treatment of motion sickness.

These two compounds bind to the cholinergic receptor, but, at first sight, they do not look anything like acetylcholine. If we look more closely though, we can see that a basic nitrogen and an ester group are present, and if we superimpose the acetylcholine skeleton on to the atropine skeleton, the distance between the ester and the nitrogen groups is similar in both molecules (Fig. 22.26). There is, of course, the problem that the nitrogen in atropine is uncharged, whereas the nitrogen in acetylcholine has a full positive charge. This implies that the nitrogen atom in atropine must be protonated and charged when it binds to the cholinergic receptor.

Therefore, atropine has two important binding features shared with acetylcholine—a charged nitrogen when protonated and an ester group. It is able to bind to the receptor, but why is it unable to switch it on? Because atropine is a larger molecule than acetylcholine, it is capable of binding to other binding regions within the binding site which are not used by acetylcholine itself. As a result, it interacts differently with the receptor and does not induce the same conformational changes (induced fit) as acetylcholine. This means that the receptor is not activated.

Test your understanding and practise your molecular modelling with Exercise 22.3.

As both atropine and hyoscine are tertiary amines rather than quaternary salts, they are able to cross the blood–brain barrier as the free base. Once they are in the brain, they can become protonated and antagonize muscarinic receptors which causes CNS effects; for example hallucinogenic activity is brought on with high doses, and both hyoscine and atropine were used by witches in past centuries to produce that very effect. Other CNS effects observed in atropine poisoning are restlessness, agitation, and hyperactivity.

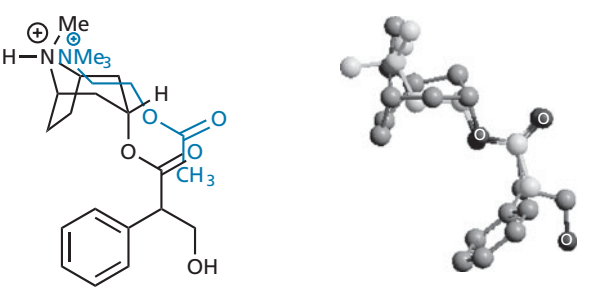


FIGURE 22.26 Acetylcholine skeleton superimposed on to the atropine skeleton.

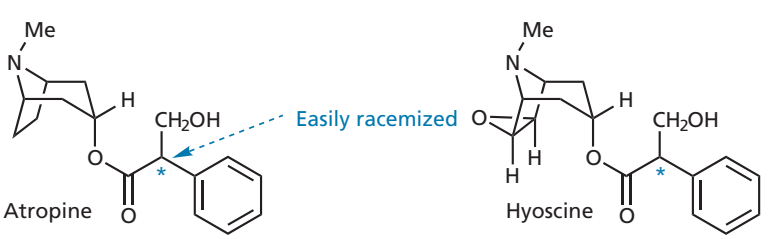


FIGURE 22.25 Atropine and hyoscine.

In recent times, the disorientating effect of scopolamine has seen it being used as a truth drug for the interrogation of spies and so it is no surprise to find it cropping up in various novels. An interesting application for scopolamine was described in Jack Higgins' novel *Day of Judgement* where it was used in association with **suxamethonium** (Fig 22.33) to torture one hapless victim. Suxamethonium was applied to the conscious victim in order to create initial convulsive muscle spasms, followed by paralysis, inability to breathe, agonizing pain, and a living impression of death. Scopolamine was then used to erase the memory of this horror, so that the impact would be just as bad when the process was repeated!

22.9.2.2 Structural analogues based on atropine

In order to reduce CNS side effects, quaternary salts of atropine and atropine analogues are used clinically (Fig. 22.27). For example, **ipratropium** is used as a bronchodilator in chronic obstructive pulmonary disease. Atropine methonitrate acts at the intestine to relieve spasm.

A large number of different analogues of atropine were synthesized to investigate the SAR of atropine, revealing the importance of the aromatic ring, the ester group, and the basic nitrogen (which is ionized).

It was further discovered that the complex ring system was not necessary for antagonist activity, so simplification could be carried out. For example, **amprotropine** (Fig. 22.28) is active and has an ester group separated from an amine by three carbon atoms.

Chain contraction to two carbon atoms can be carried out without loss of activity, and a large variety of active antagonists have been prepared having the general formula shown in Fig 22.29, for example **tridihexethyl chloride** and **propantheline bromide**.

These studies came up with the following generalizations:

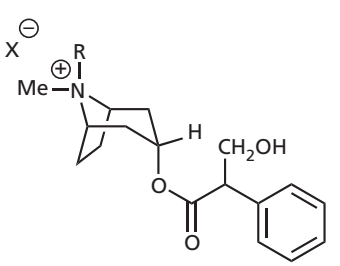
- the alkyl groups (R) on nitrogen can be larger than methyl (in contrast to agonists);
- the nitrogen can be tertiary or quaternary, whereas agonists must have a quaternary nitrogen. Note, however, that the tertiary nitrogen is probably charged when it interacts with the receptor;
- very large acyl groups are allowed (R^1 and R^2 = aromatic or heteroaromatic rings). This is in contrast to agonists where only the acetyl group is permitted.

This last point appears to be the most crucial in determining whether a compound will act as an antagonist or not. The acyl group has to be bulky, but it also has to have that bulk arranged in a certain manner; in other words, there must be some sort of branching in the acyl group.

The conclusion that can be drawn from these results is that there must be hydrophobic binding regions next to the normal acetylcholine binding site. The overall shape of the acetylcholine binding site plus the extra binding regions would have to be T- or Y-shaped in order to explain the importance of branching in antagonists (Fig. 22.30). A structure such as **propantheline**, which contains the complete acetylcholine skeleton, as well as the hydrophobic acyl side chain binds more strongly to the receptor than acetylcholine itself. The extra binding interactions mean that the conformational changes induced in the receptor will be different from those induced by acetylcholine and will fail to induce the secondary biological response. As long as the antagonist is bound, acetylcholine is unable to bind and pass on its message.

For additional material see Web article 8: photoaffinity labelling

A large variety of antagonists have proved to be useful medicines (Fig. 22.31), with many showing selectivity for specific organs. For example, **tropicamide** and **cyclopentolate** are used in eye drops to dilate pupils for ophthalmic examination, while **trihexyphenidyl and benzatropine** are used centrally to counteract movement disorders caused by Parkinson's disease. Some agents act selectively to decrease gastric secretion; others are useful in ulcer therapy. The selectivity of action for these drugs owes more to their distribution properties than to receptor selectivity. In other words, the compounds can reach some parts of the body more easily than others. Having said that, the antagonist **pirenzepine**, which is used in some countries for the treatment of peptic ulcers, is a selective M_1 antagonist with no activity against M_2 receptors.



R = Me, X = NO_3^-; Atropine methonitrate
R = ^{i}Pr, X = Br^-; Ipratropium

FIGURE 22.27 Structural analogues of atropine.

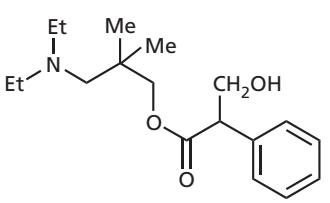
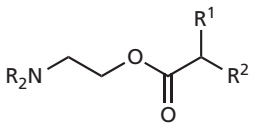
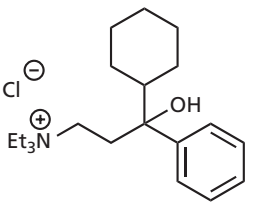


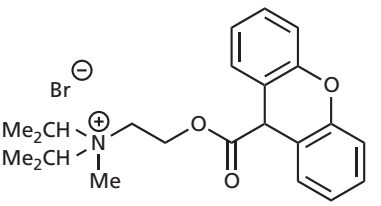
FIGURE 22.28 Amprotropine.



R^1 and R^2 = Aromatic or heteroaromatic



Tridihexethyl chloride



Propanthreline bromide

FIGURE 22.29 Simplified analogues of atropine.

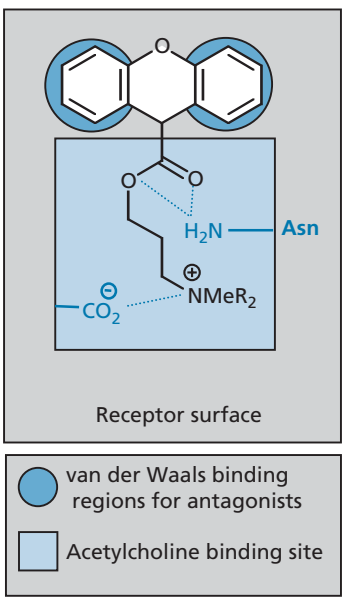
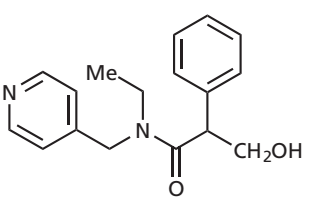


FIGURE 22.30 The binding of propantheline to the muscarinic receptor.

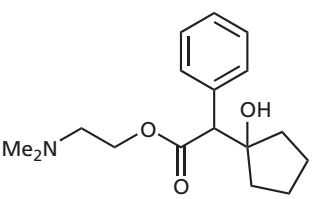
22.10 Antagonists of the nicotinic cholinergic receptor

22.10.1 Applications of nicotinic antagonists

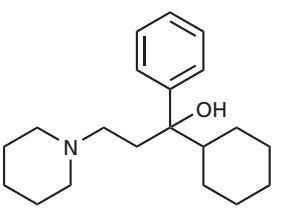
Nicotinic receptors are present in nerve synapses at ganglia, as well as at the neuromuscular synapse. However, drugs are able to show a level of selectivity between these two sites, mainly because of the distinctive routes which have to be taken to reach them. Antagonists of ganglionic nicotinic receptor sites are not therapeutically useful because they cannot distinguish between the ganglia of the sympathetic nervous system and the ganglia of the parasympathetic nervous system (both use nicotinic receptors) (Fig. 22.3). Consequently, they have many side effects. However, antagonists of the neuromuscular junction are therapeutically useful and are known as **neuromuscular blocking agents**.



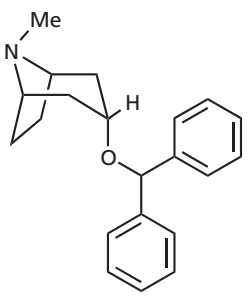
Tropicamide



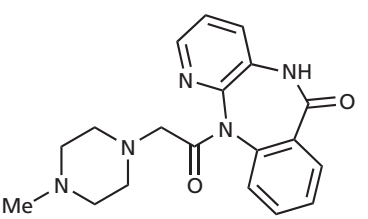
Cyclopentolate



Trihexyphenidyl



Benzatropine



Pirenzepine

FIGURE 22.31 Some examples of clinically useful cholinergic antagonists.

22.10.2 Nicotinic antagonists

22.10.2.1 Curare and tubocurarine

Curare was first identified in the sixteenth century when Spanish soldiers in South America found themselves under attack by indigenous people using poisoned arrows. It was discovered that the Indians were using a crude, dried extract from a plant called *Chondrodendron tomentosum,* which stopped the heart and also caused paralysis. Curare is a mixture of compounds, but the active principle is a cholinergic antagonist that blocks nerve transmissions from nerve to muscle.

It might seem strange to consider such a compound for medicinal use, but at the right dose levels and under proper control, there are useful applications for this sort of action. The main application is in the relaxation of abdominal muscles in preparation for surgery. This allows the surgeon to use lower levels of general anaesthetic than would otherwise be required and increase the safety margin for operations.

As mentioned previously, curare is actually a mixture of compounds, and it was not until 1935 that the active principle (**tubocurarine**) was isolated. The determination of the structure took even longer, and it was not established until 1970 (Fig. 22.32). Tubocurarine was used clinically as a neuromuscular blocker, but it had undesirable side effects as it also acted as an antagonist at the nicotinic receptors of the autonomic nervous system (Fig. 22.2). Better agents are now available.

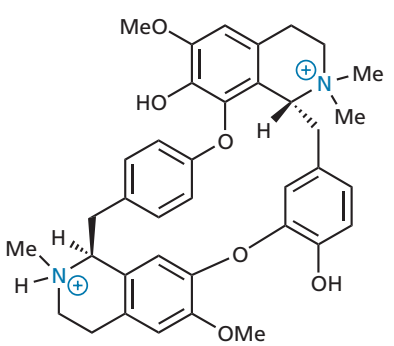


FIGURE 22.32 Tubocurarine.

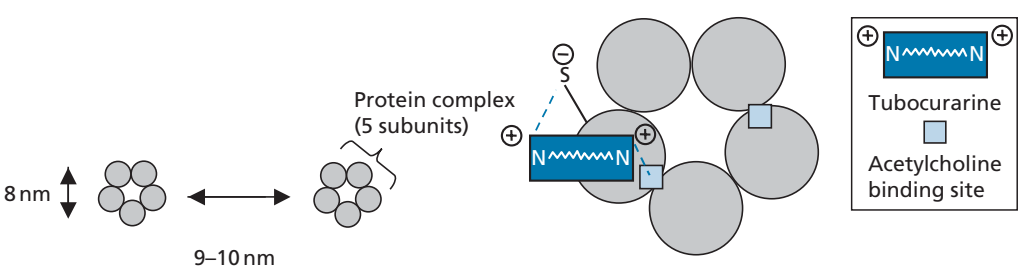
The structure of tubocurarine presents a problem to our theory of receptor binding. Although it has a couple of charged nitrogen centres, there is no ester to interact with the acetyl binding region. Studies on the compounds discussed so far show that the positively charged nitrogen on its own is not sufficient for good binding, so why should tubocurarine bind to the nicotinic receptor?

Test your understanding and practise your molecular modelling with Exercise 22.4.

The answer lies in the fact that the molecule has *two* positively charged nitrogen atoms (one tertiary, which is protonated, and one quaternary). Originally, it was believed that the distance between the two centres (1.15 nm) might be equivalent to the distance between two separate cholinergic receptors and that the tubocurarine molecule could bridge the two binding sites, and act as a steric shield for both. However pleasing that theory may be, the dimensions of the nicotinic receptor make this impossible. The nicotinic receptor is a protein dimer made up of two identical protein complexes separated by 9–10 nm—far too large to be bridged by the tubocurarine molecule (Fig. 22.33 and section 22.11).

Another possibility is that the tubocurarine molecule bridges two acetylcholine binding sites within the one protein complex. As there are two such sites within the complex, this appears to be an attractive theory. However, the two sites are more than 1.15 nm apart and so this too has to be ruled out. It has now been proposed that one of the positively charged nitrogens on tubocurarine binds to the anionic binding region of an acetylcholine binding site, while the other binds to a nearby cysteine residue 0.9–1.2 nm away (Fig. 22.33).

Despite the uncertainty surrounding the binding interactions of tubocurarine, it seems highly probable that two ionic binding regions are involved. Such an interaction is extremely strong and would more than



(a) Receptor dimer **(b)** Interaction with tubocurarine

FIGURE 22.33 Tubocurarine binding to the cholinergic receptor.

make up for the lack of the ester binding interaction. It is also clear that the distance between the two positively charged nitrogen atoms is crucial to activity. Therefore, analogues that retain this distance should also be good antagonists. Strong evidence for this comes from the fact that the simple molecule decamethonium is a good antagonist (section 22.10.2.2).

22.10.2.2 Decamethonium and suxamethonium

Decamethonium (Fig. 22.34) is as simple an analogue of tubocurarine as one could imagine. It is a flexible, straight-chain molecule and is capable of a large number of conformations. The fully extended conformation places the nitrogen atoms 1.4 nm apart, but there are other more folded conformations that position the nitrogen centres 1.14 nm apart, which compares well with the equivalent distance in tubocurarine (1.15 nm) (see also Box 17.4 and Molecular modelling exercise 22.4).

The drug binds strongly to cholinergic receptors and has proved a useful clinical agent, but it suffers from several disadvantages. For example, when it binds initially to nicotinic receptors, it acts as an agonist rather than an antagonist. In other words, it switches on receptors such that sodium ion channels open up to depolarize muscle cell membranes and cause brief contractions of the muscle. Because the drug is not rapidly hydrolysed in the same way as acetylcholine, it remains bound to the receptor leading to persistent depolarization and subsequent desensitization of the end plate. At that stage, it can be viewed as an antagonist as it no longer stimulates muscle contraction and blocks access to acetylcholine. (A theory of how such an effect might take place is described in section 8.6.) Another disadvantage is that it binds too strongly, so patients take a long time to recover from its effects.

We now face the opposite problem from the one faced when designing cholinergic agonists. Instead of stabilizing a molecule, we need to introduce some instability—a sort of timer control whereby the molecule can be inactivated more quickly. Success was first achieved with **suxamethonium** (Fig. 22.34) where two ester groups are incorporated into the chain in such a way that the distance between the charged nitrogens remains the same. The ester groups are susceptible to chemical and enzymatic hydrolysis and, once this takes place, the molecule can no longer bridge the two binding regions on the receptor and is inactivated. The ester groups are also introduced such that suxamethonium mimics two acetylcholine molecules linked end on. Suxamethonium has a fast onset and short duration of action (5–10 minutes), but suffers from various side effects. Furthermore, about one person in every 2000 lacks the plasma cholinesterase enzyme which hydrolyses suxamethonium. Nevertheless, it is still used clinically in short surgical procedures, such as the insertion of tracheal tubes.

Both decamethonium and suxamethonium are classed as depolarizing neuromuscular blockers and have effects on the autonomic ganglia, which explains some of their side effects. Decamethonium also lacks total selectivity for the neuromuscular junction and has an effect on cholinergic receptors in the heart. This leads to an increased heart rate and a fall in blood pressure.

22.10.2.3 Steroidal neuromuscular blocking agents

The design of **pancuronium**, **vecuronium**, and **rocuronium** (Fig. 22.35) was based on tubocurarine, but involved a steroid nucleus acting as a spacer between the two nitrogen groups. The distance between the quaternary nitrogens is 1.09 nm compared with 1.15 nm in tubocurarine. Acyl groups were also added to introduce one or two acetylcholine skeletons into the molecule in order to improve affinity for the receptor sites. These compounds have a faster onset of action than tubocurarine and do not affect blood pressure. They are not as rapid in onset as suxamethonium and have a longer duration of action (45 minutes). Their main advantage is that they have fewer side effects and so they are widely used clinically. Unlike decamethonium and suxamethonium, these agents have no agonist activity and act as pure antagonists, so they have no depolarizing effect on target muscle cells. The neuromuscular blocking activity of rocuronium can be reversed with a cyclodextrin called s**ugammadex** (Box 10.3).

22.10.2.4 Atracurium and mivacurium

The design of atracurium (Fig. 22.36) was based on the structures of tubocurarine and suxamethonium. It is

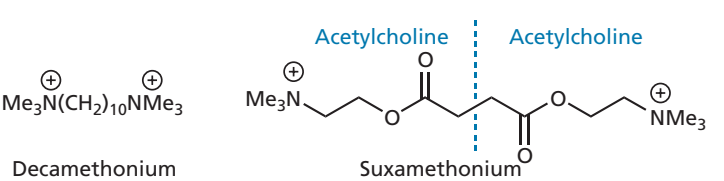


FIGURE 22.34 Decamethonium and suxamethonium.

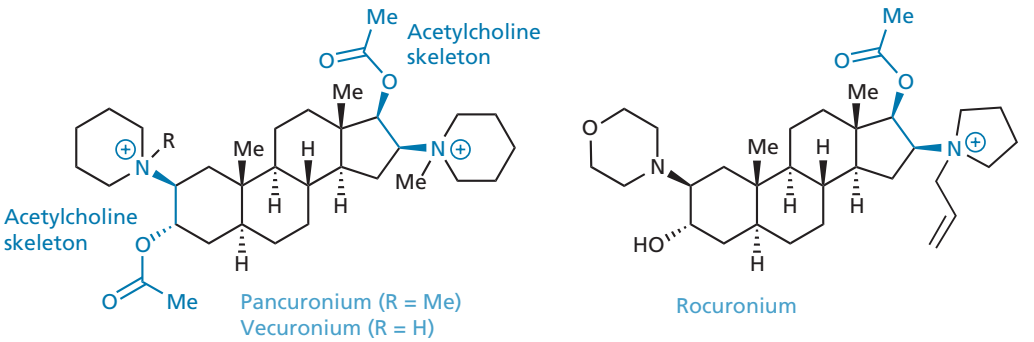


FIGURE 22.35 Steroidal neuromuscular blocking agents.

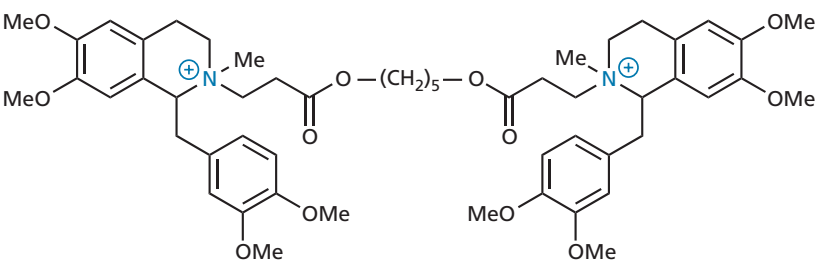


FIGURE 22.36 Atracurium.

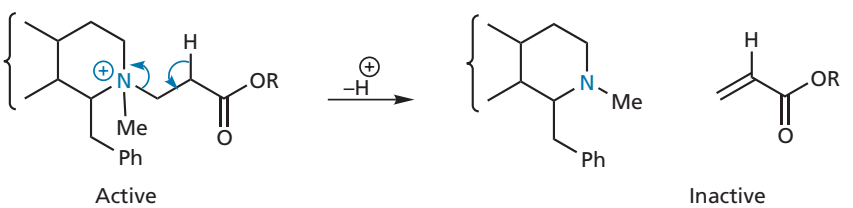


FIGURE 22.37 Hofmann elimination of atracurium.

superior to both as it lacks cardiac side effects and is rapidly broken down in blood. This rapid breakdown allows the drug to be administered as an intravenous drip.

The rapid breakdown is due to a self-destruct mechanism. At the slightly alkaline pH of blood (pH = 7.4), the molecule can undergo a **Hofmann elimination** (Fig. 22.37). Once this happens, the compound is inactivated because the positive charge on the nitrogen is lost and the molecule is split in two. It is a particularly clever example of drug design in that the very element responsible for the molecule's biological activity promotes its deactivation.

The important features of atracurium are:

- *the spacer*—a 13-atom chain connects the two quaternary centres;
- *the blocking units*—the cyclic structures at either end of the molecule which block the binding site from acetylcholine;
- *the quaternary centres*—these are essential for receptor binding. If one is lost through Hofmann elimination, the binding interaction is too weak and the antagonist leaves the binding site;
- *the Hofmann elimination*—the ester groups within the spacer chain are crucial to the rapid deactivation process. Hofmann eliminations normally require strong alkaline conditions and high temperatures—hardly normal physiological conditions. However, if a good electron-withdrawing group is present on the carbon that is *beta* to the quaternary nitrogen centre, it allows the reaction to proceed under the much milder alkaline conditions present in blood (pH 7.4). The electron-withdrawing ester group increases the acidity of the hydrogen on the *beta*-carbon such that it is easily lost. The Hofmann elimination does not occur at acid pH, and so the drug is stable in solution at a pH of 3–4 and can be stored safely in a refrigerator.

Because the drug acts very briefly (approximately 30 minutes), it is added intravenously for as long as it is needed. As soon as surgery is over, the intravenous drip is stopped and antagonism ceases almost instantaneously.

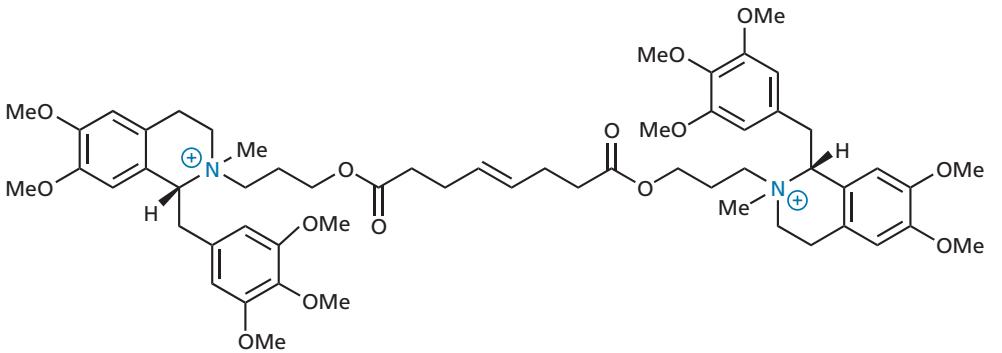


FIGURE 22.38 Mivacurium.

Another major advantage is that the drug does not require enzymes to become deactivated and so deactivation occurs at a constant rate between patients. With previous neuromuscular blockers, deactivation depended on metabolic mechanisms involving enzymic deactivation and/or excretion. The efficiency of these processes varies from patient to patient and is particularly poor for patients with kidney failure or with low levels of plasma esterases.

Mivacurium (Fig. 22.38) is a newer drug which is similar to atracurium and is inactivated rapidly by plasma enzymes, as well as by the Hofmann elimination. It has a faster onset (about 2 minutes) and shorter duration of action (about 15 minutes), although the duration is longer if the patients have liver disease or enzyme deficiencies.

22.10.2.5 Other nicotinic antagonists

Local anaesthetics and barbiturates appear to prevent the changes in ion permeability which would normally result from the interaction of acetylcholine with the nicotinic receptor. They do not, however, bind to the cholinergic binding site. It is believed that they bind instead to the part of the receptor which is on the inside of the cell membrane, perhaps binding to the ion channel itself and blocking it.

Certain snake toxins have been found to bind irreversibly to the nicotinic receptor, thus blocking cholinergic transmissions. These include toxins such as **α-bungarotoxin** from the Indian cobra. The toxin is a polypeptide containing 70 amino acids which cross-links the α- and β-subunits of the cholinergic receptor (section 22.11).

Finally, the antidepressant and antismoking drug **bupropion** (section 23.12.4) has been shown to be a nicotinic antagonist, as well as a reuptake inhibitor of noradrenaline and dopamine. It is possible that the drug's effectiveness as an antismoking aid may be related to its blockage of neuronal nicotinic receptors in the brain.

KEY POINTS

- Cholinergic antagonists bind to cholinergic receptors but fail to activate them. They block binding of acetylcholine and have a variety of clinical uses.
- Muscarinic antagonists normally contain a tertiary or quaternary nitrogen, a functional group involving oxygen, and a branch point containing two hydrophobic ring substituents.
- Nicotinic antagonists are useful as neuromuscular blockers in surgery.
- The pharmacophore for a nicotinic antagonist consists of two charged nitrogen atoms separated by a spacer molecule such that the centres are a specific distance apart.
- One of the charged nitrogens binds to the cholinergic binding site; the other interacts with a nucleophilic group neighbouring the binding site.
- Neuromuscular blockers should have a fast onset of action, minimal side effects, and a short duration of action to allow fast recovery. The lifetime of neuromuscular blockers can be decreased by introducing ester groups which are susceptible to enzymatic hydrolysis.
- Neuromuscular blockers which degrade chemically by means of the Hofmann elimination are not dependent on metabolic reactions and are more consistent from patient to patient.

22.11 Receptor structures

The nicotinic receptor has been isolated successfully from the electric ray (*Torpedo marmorata*)—a fish found in the Atlantic and the Mediterranean—allowing the receptor to be studied carefully. As a result, a great deal is known about its structure and operation. It is a protein complex made up of five subunits, two of which are the same. The five subunits (two α, one β, one γ, and one δ) form a cylindrical or barrel shape which traverses the cell membrane

(section 4.6.2). The centre of the cylinder acts as an ion channel for sodium, and a gating or lock system is controlled by the interaction of the nicotinic receptor with acetylcholine. In the absence of acetylcholine, the gate is shut. When acetylcholine binds, the gate is opened. The binding site for acetylcholine is situated mainly on the α-subunit and there are two binding sites per ion channel complex. It is usually found that nicotinic receptors occur in pairs, linked together by a disulphide bridge between the δ-subunits.

This is the make up of the nicotinic receptor at neuromuscular junctions. The nicotinic receptors at ganglia and in the CNS are more diverse in nature involving different α- and β-subunits. This allows drugs to act selectively on neuromuscular, rather than neuronal, receptors. For example, decamethonium is only a weak antagonist at autonomic ganglia, whereas **epibatidine** (extracted from a South American frog) is a selective agonist for neuronal receptors. The snake toxin **α-bungarotoxin** is specific for receptors at neuromuscular junctions.

Muscarinic receptors belong to the superfamily of G-protein-coupled receptors (section 4.7) which operate by activation of a signal transduction process (sections 5.1–5.3). Five subtypes of muscarinic receptors have been identified and are labelled M_1–M_5. These subtypes tend to be concentrated in specific tissues. For example, M_2 receptors occur mainly in the heart, whereas M_4 receptors are found mainly in the CNS. M_2 receptors are also used as the autoreceptors on presynaptic cholinergic neurons (section 22.3.2).

The M_1, M_3, and M_5 receptors are associated with a signal transduction process involving the secondary messenger **inositol triphosphate** (IP_3) (section 5.3). The M_2 and M_4 receptors involve a process which inhibits the production of the secondary messenger **cyclic-AMP** (section 5.2). Lack of M_1 activity is thought to be associated with dementia.

KEY POINTS

- The nicotinic receptor is an ion channel consisting of five protein subunits. There are two binding sites for each ion channel.
- The muscarinic receptor is a G-protein-coupled receptor. Various subtypes of muscarinic receptor predominate in different tissues.

22.12 Anticholinesterases and acetylcholinesterase

22.12.1 Effect of anticholinesterases

Anticholinesterases are inhibitors of acetylcholinesterase—the enzyme that hydrolyses acetylcholine (section 22.3.1). If acetylcholine is not destroyed, it can return to reactivate the cholinergic receptor and increase cholinergic effects (Fig. 22.39). Therefore, an acetylcholinesterase inhibitor will have the same biological effect as a cholinergic agonist.

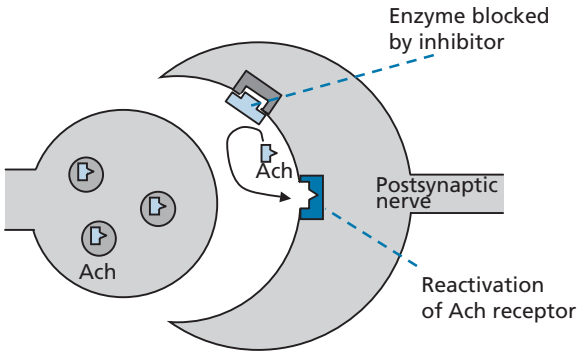


FIGURE 22.39 Effect of anticholinesterases (Ach = acetylcholine).

22.12.2 Structure of the acetylcholinesterase enzyme

The acetylcholinesterase enzyme has a fascinating tree-like structure (Fig. 22.40). The trunk of the tree is a collagen molecule which is anchored to the cell membrane. There are three branches with disulphide bridges that lead off from the trunk, each of which holds the acetylcholinesterase enzyme above the surface of the membrane. The enzyme itself is made up of four protein subunits, each of which has an active site. Therefore, each enzyme tree has 12 active sites. The trees are rooted immediately next to the cholinergic receptors such that they efficiently capture acetylcholine as it departs the receptor. In fact, the acetylcholinesterase enzyme is one of the most efficient enzymes known. A soluble cholinesterase enzyme called **butyrylcholinesterase** is also present in various tissues and plasma. This enzyme has a broader substrate specificity than acetylcholinesterase and can hydrolyse a

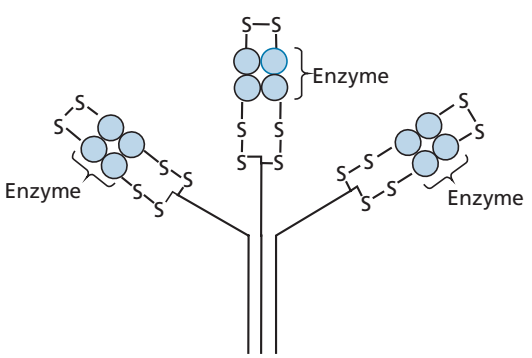


FIGURE 22.40 The acetylcholinesterase enzyme.

variety of esters. Its physiological function is not totally clear, but it has been found to catalyse the hydrolysis of toxic esters, such as cocaine, and appears to have a non-catalytic role in cell differentiation and development. It is also more effective than acetylcholinesterase at hydrolysing high levels of acetylcholine when the acetylcholinesterase enzyme itself becomes substrate inhibited.

22.12.3 The active site of acetylcholinesterase

The design of anticholinesterases depends on the shape of the enzyme's active site, the binding interactions involved with acetylcholine, and the mechanism of hydrolysis. The active site itself is at the foot of a narrow gorge (Fig. 22.41a) and, at the entrance to the gorge, there is a peripheral binding site. It is believed that this site plays a crucial role in recognizing acetylcholine as the substrate. One of the key interactions is a weak π-cation interaction between the heteroaromatic ring of a tryptophan residue and the charged quaternary nitrogen of acetylcholine (Fig. 22.41b). After acetylcholine has been 'captured' it is rapidly transferred down the gorge to the active site (Fig. 22.41c). This process is aided by the fact that the gorge is lined with 14 conserved aromatic residues, which can also form π-cation interactions with acetylcholine and thus channel the substrate down the gorge into the active site. Once acetylcholine enters the active site, another tryptophan residue forms yet another π-cation interaction (Fig. 22.41d). An electrostatic gradient running down the gorge encourages the movement of acetylcholine. The gradient is due to several negatively charged amino acid residues in the active site, which create a dipole that points down the gorge to serve as an electronic steering mechanism for the positively charged substrate. The tryptophan residues in the peripheral binding site and the active site are 12A apart and this is

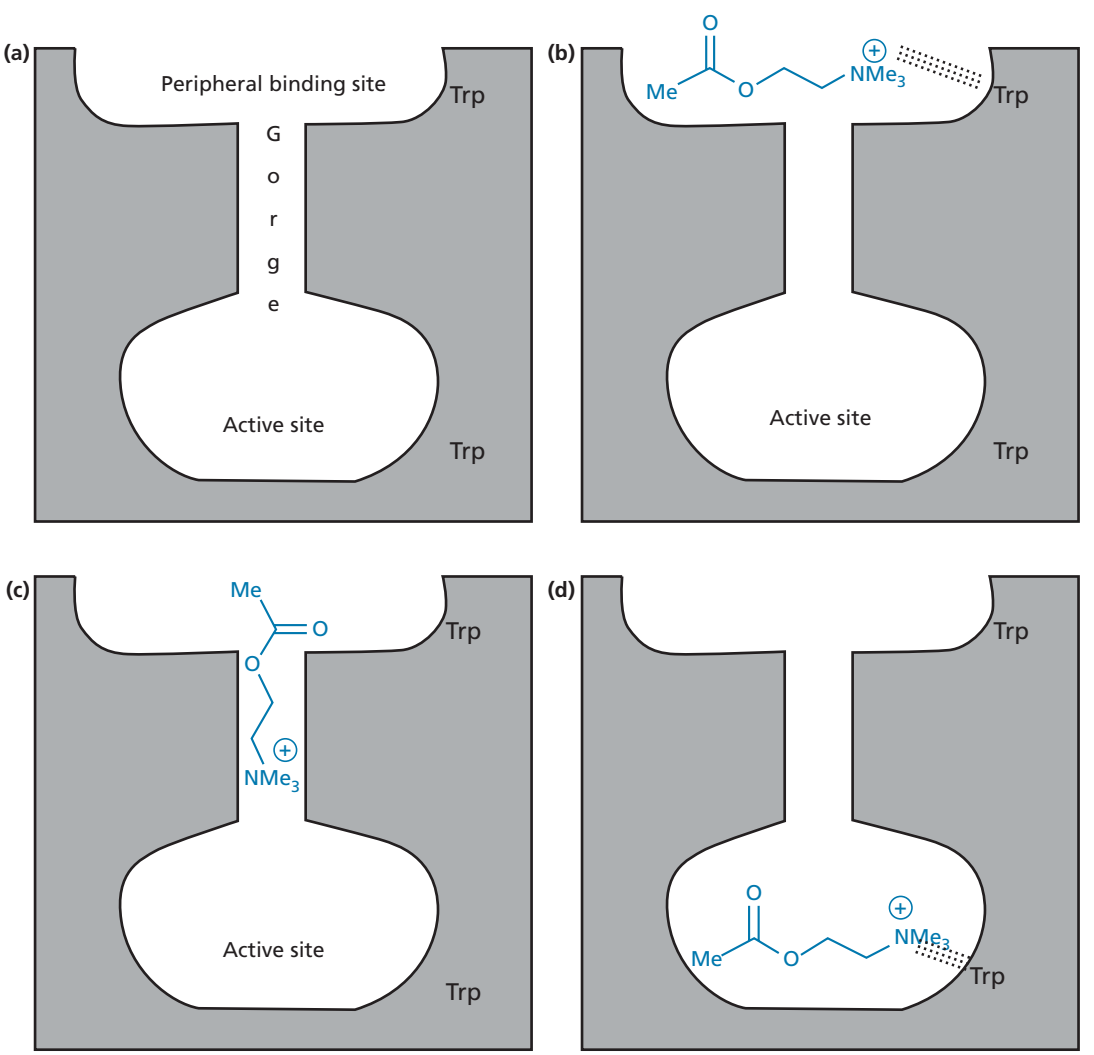


FIGURE 22.41 Process by which acetylcholine is recognized and bound.

significant when it comes to designing potential **dual-action drugs** (section 22.15.2).

22.12.3.1 Crucial amino acids within the active site

The important amino acids within the active site are those which bind acetylcholine, as well as those involved in the mechanism of hydrolysis. As far as binding is concerned, several amino acids are thought to be involved, but a key interaction is the interaction between a tryptophan residue and the quaternary nitrogen atom (Fig. 22.42). The key amino acid residues involved in the catalytic mechanism are serine, histidine, and glutamate.

22.12.3.2 Mechanism of hydrolysis

The histidine residue acts as an acid–base catalyst throughout the mechanism, while serine acts as a nucleophile. This is not a particularly good role for serine, as an aliphatic alcohol is a poor nucleophile and is unable to hydrolyse an ester, but the acid/base catalysis provided by histidine overcomes that disadvantage. The glutamate residue interacts with the histidine residue and serves to orientate and activate the ring (compare chymotrypsin—section 3.5.3). There are several stages to the mechanism (Fig. 22.43):

1. Acetylcholine approaches and binds to the active site. Serine acts as a nucleophile and uses a lone pair of electrons to form a bond to the ester of acetylcholine. Nucleophilic addition to the ester takes place and opens up the carbonyl group
2. The histidine residue catalyses this reaction by acting as a base and removing a proton, thus making serine more nucleophilic
3. Histidine now acts as an acid catalyst and protonates the alkoxy (OR) portion of the intermediate, turning it into a much better leaving group
4. The carbonyl group reforms and expels the alcohol portion of the ester (i.e. choline)
5. The acyl portion of acetylcholine is now covalently bound to the active site. Choline leaves the active site and is replaced by water
6. Water acts as a nucleophile and uses a lone pair of electrons on oxygen to attack the acyl group
7. Water is normally a poor nucleophile, but, histidine aids the process again by acting as a basic catalyst and removing a proton
8. Histidine acts as an acid catalyst by protonating the intermediate
9. The carbonyl group is reformed and the serine residue is released. Because it is now protonated, it is a much better leaving group
10. Ethanoic acid leaves the active site and the cycle can be repeated.

The enzymatic process is remarkably efficient owing to the close proximity of the glutamate residue (not shown), the serine nucleophile, and the histidine acid–base catalyst. As a result, hydrolysis by acetylcholinesterase is 10^8 (one hundred million) times faster than in its absence. The process is so efficient that acetylcholine is hydrolysed within a 100 μs of reaching the enzyme.

22.13 Anticholinesterase drugs

Anticholinesterase drugs act as inhibitors of the enzyme acetylcholinesterase. This inhibition can be either reversible or irreversible depending on how the drug interacts with

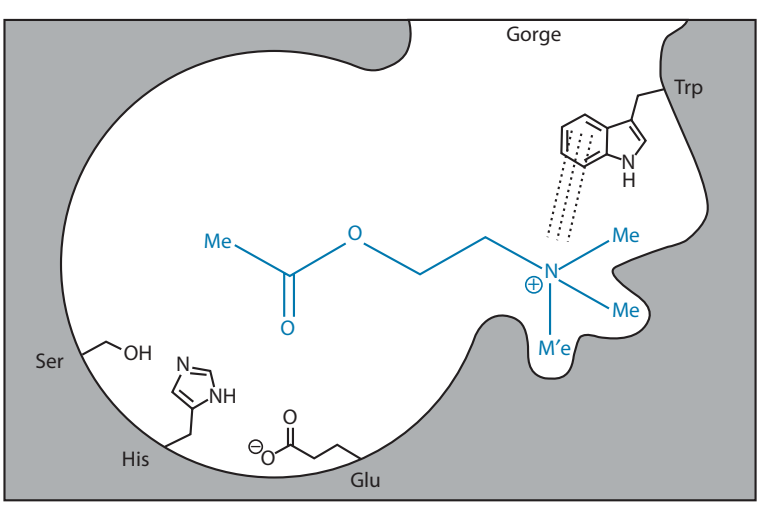


FIGURE 22.42 Key amino acid residues within the active site.

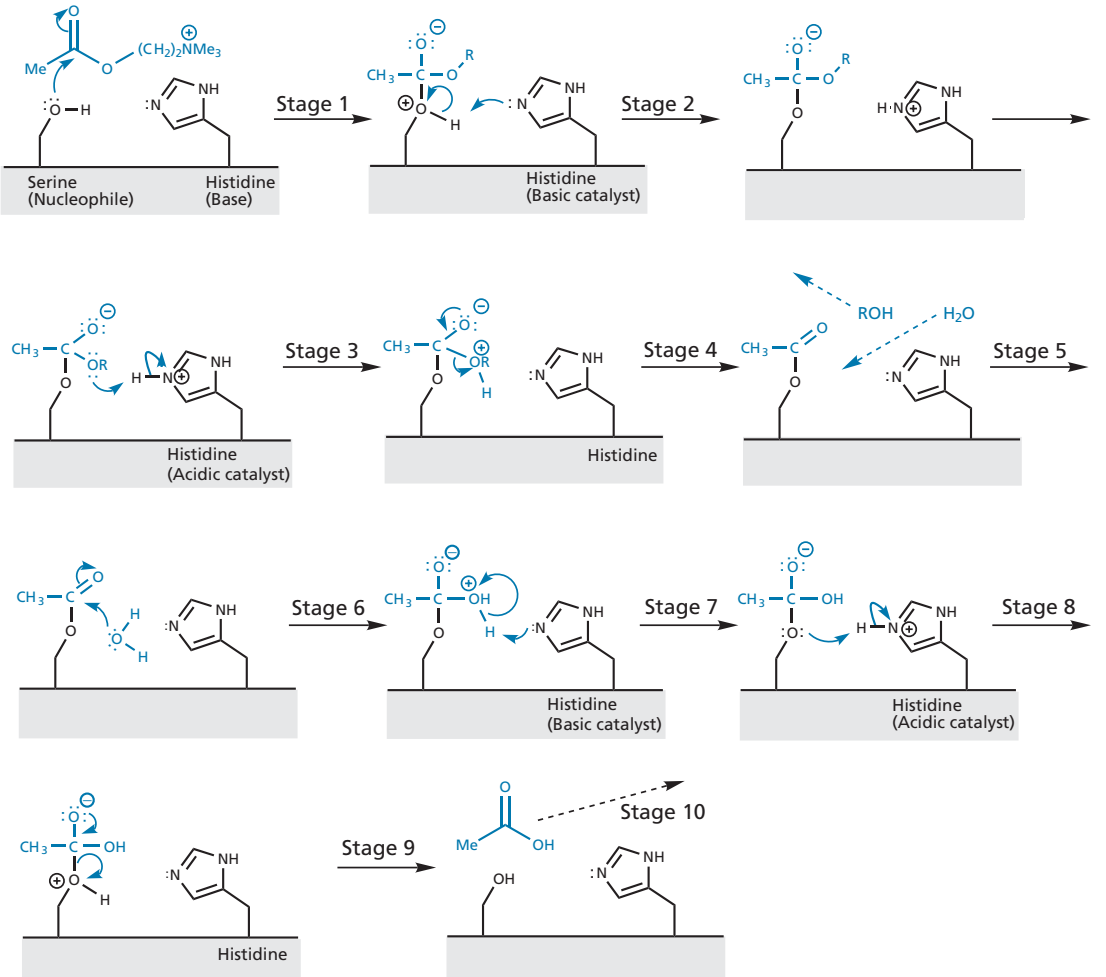


FIGURE 22.43 Mechanism of hydrolysis for the acetylcholinesterase enzyme (the glutamate component of the catalytic triad is not shown).

the active site. Two main groups of acetylcholinesterases are considered here—carbamates and organophosphorus agents.

22.13.1 Carbamates

22.13.1.1 Physostigmine

As in so many fields of medicinal chemistry, it was a natural product that provided the lead for the carbamate inhibitors. The natural product was **physostigmine** (Fig. 22.44) (also called **eserine**) which was discovered in 1864 as a product of the poisonous **calabar bean** (the ordeal bean, *Physostigma venenosum*) from West Africa. Extracts of these beans were fed to criminals to assess whether they were guilty or innocent. Death indicated a guilty verdict. The structure was established in 1925 and physostigmine is still used clinically to treat glaucoma.

SAR studies of physostigmine demonstrate that:

- the carbamate group is essential to activity;
- the benzene ring is important;
- the pyrrolidine nitrogen is important and is ionized at blood pH.

Working backwards, the positively charged pyrrolidine nitrogen is important because it binds to the anionic binding region of the enzyme. The benzene ring may be involved in some extra hydrophobic bonding

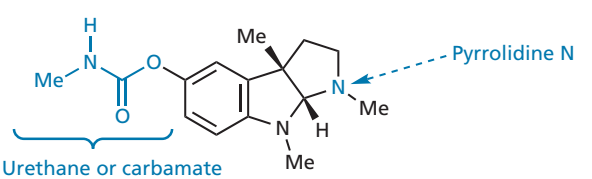


FIGURE 22.44 Physostigmine.

with the active site. Alternatively, it may be important in the mechanism of inhibition as it provides a good leaving group. The carbamate group is the crucial group responsible for physostigmine's inhibitory properties. To understand why, we must look at what happens when physostigmine acts as the substrate for acetylcholinesterase (Fig. 22.45).

The first four stages proceed as normal, with histidine catalysing the nucleophilic attack of the serine residue on physostigmine (stages 1 and 2). The leaving group (this time a phenol) is expelled with the aid of acid catalysis from histidine (stages 3 and 4) and departs the active site to be replaced by a water molecule.

The next stage turns out to be extremely slow. Despite the fact that histidine can still act as a basic catalyst, water finds it difficult to attack the carbamoyl intermediate. This step becomes the rate-determining step for the whole process and the overall rate of hydrolysis of physostigmine is 40×10^6 times slower than that of acetylcholine. As a result, the cholinesterase active site becomes blocked and is unable to react with acetylcholine.

The final stage is slow because of the stability of the carbamoyl–enzyme complex. This is because the nitrogen can feed a lone pair of electrons into the carbonyl group and drastically reduce its electrophilic character (Fig. 22.46) (cf. section 22.9.2).

22.13.1.2 Analogues of physostigmine

Physostigmine has limited medicinal use because of serious side effects, and it has only been used in the treatment of glaucoma or as an antidote for atropine poisoning. Simpler analogues, however, have been used in the treatment of myasthenia gravis and as an antidote to curare poisoning.

Miotine (Fig. 22.47) still has the necessary carbamate, aromatic, and tertiary aliphatic nitrogen groups. It is active as an antagonist but it also has disadvantages: it is susceptible to chemical hydrolysis and it can cross the blood–brain barrier (section 11.4.5) as the free base, resulting in side effects due to its action in the CNS.

Neostigmine and **pyridostigmine** (Fig. 22.47) were designed to deal with both these problems. Firstly, a quaternary nitrogen atom is present and so there is no chance of the free base being formed. As the molecule is permanently charged, it cannot cross the blood–brain barrier

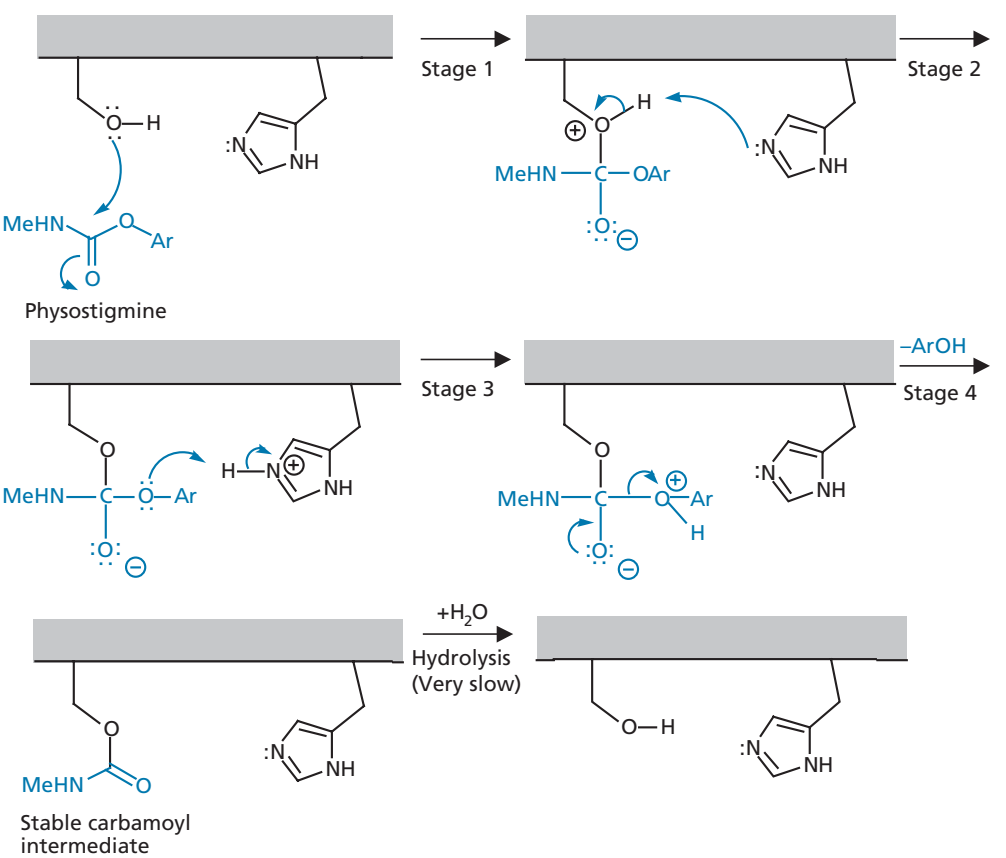


FIGURE 22.45 Mechanism of inhibition by physostigmine (Ar represents the tricyclic system of physostigmine).

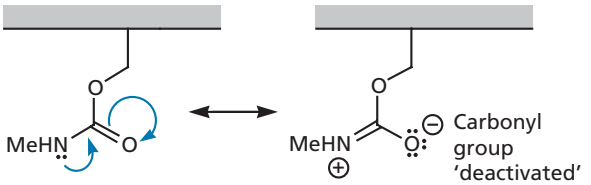


FIGURE 22.46 Stabilization of the carbamoyl–enzyme intermediate.

and so the drug is free of CNS side effects. Increased stability is achieved by using a dimethylcarbamate group rather than a methylcarbamate group. Two further points to note about neostigmine are:

- the quaternary nitrogen is 4.7 Å away from the carbamate group;
- the direct bonding of the quaternary centre to the aromatic ring reduces the number of conformations that the molecule can adopt. This is an advantage if the active conformation is retained because the molecule is more likely to be in the active conformation when it approaches the active site.

Both neostigmine and pyridostigmine are in use today. They are given intravenously to reverse the actions of neuromuscular blockers or used orally in the treatment of myasthenia gravis. Pyridostigmine was one of the drugs used in the chemical cocktail provided to allied troops in Iraq during **Operation Desert Shield**. The agent was present to help protect against possible exposure to **organophosphate nerve gases**. **Edrophonium** is a similar agent used to reverse neuromuscular blocking and is also used as a treatment of myasthenia gravis.

22.13.2 Organophosphorus compounds

The potential of organophosphorus compounds as nerve agents was first recognized by German scientists in the 1920s and 1930s, and research was carried out to investigate their potential as weapons of war. When World War II broke out, governments in the UK, USA, Sweden, and Russia recognized the danger of Germany perfecting these weapons and started their own research efforts during the 1940s. In the UK, this was carried out at the **Porton Down Defence Centre**. Fortunately, these agents were never used, but researchers in different countries continued work to find suitable antidotes that would protect troops from a possible attack. It has not been proved whether the organophosphate nerve gases have ever been used in combat, but many believe that they were part of the chemical weapons arsenal that was used against the Kurds by the Iraqi government. It has also been proposed that **sarin** (Fig. 22.48) may have been released when Iraqi chemical plants and ammunition dumps were bombed during the period 1990–91, and that this might be a possible cause of the mystery illness that afflicted many of the veterans of that war—**Gulf War syndrome**. Bosnians, Serbs, and Croats have also been accused of using nerve agents during the break-up of Yugoslavia in the 1990s. Certainly, nerve agents have been used by terrorist groups: the most notorious example was the release of sarin in the Tokyo subway during 1995.

The organophosphate nerve agents are examples of the weapons of mass destruction which several Western countries feared might be used by Iraq on its neighbours or supplied to extremist groups. The invasion of Iraq in

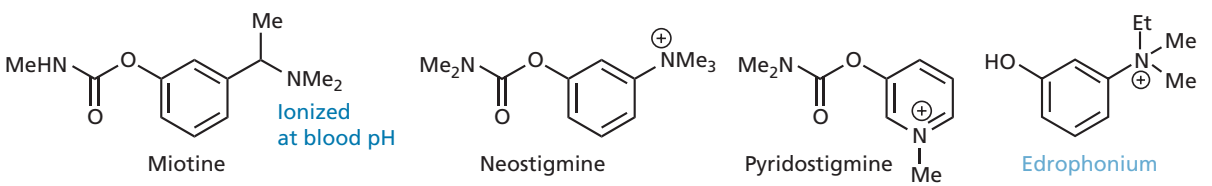


FIGURE 22.47 Analogues of physostigmine. Miotine is a chiral molecule that has been studied as a racemate.

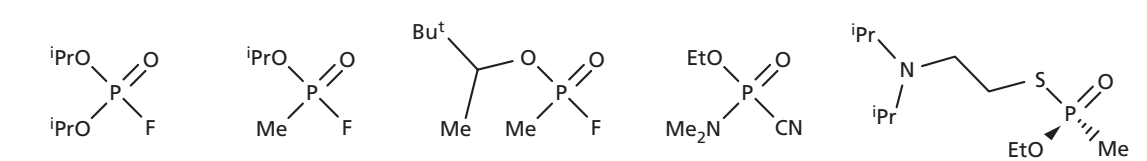


FIGURE 22.48 Examples of nerve agents.

2003 was designed to combat this threat, but subsequent searches failed to reveal any such weapons.

It would be wrong to give the impression that the only use for organophosphates is as weapons of war and terror. They are also extremely important insecticides used in agriculture and animal husbandry, and have a variety of uses in medicine. We shall consider these aspects in the following sections.

22.13.2.1 Nerve agents

The nerve gases **dyflos** and **sarin** (**GB**) (Figure 22.48) were discovered and perfected long before their mode of action was known. Both agents inhibit acetylcholinesterase by irreversibly phosphorylating the serine residue at the active site (Fig. 22.49).

The early part of the mechanism is similar to the normal mechanism, but the phosphorylated adduct which is formed is extremely resistant to hydrolysis. Consequently, the enzyme is permanently inactivated. As acetylcholine cannot be hydrolysed, the cholinergic system is continually stimulated. This results in permanent contraction of skeletal muscle, resulting in death.

Other nerve agents include **tabun** (**GA**), **soman** (**GD**), and **VX**. VX is the most toxic of the nerve agents, having an LD_{50} of 10 mg through skin contact. It was discovered at Porton Down in the UK in 1954 then traded to the USA in exchange for technological information on nuclear weapons. The USA produced several tons of the material for its chemical warfare programme, but decided to dispose of its stockpiles in the late 1960s—a process that was only completed in 2008. Much of the nerve agent now lies at the bottom of the Atlantic Ocean.

22.13.2.2 Medicines

Once the mechanism of action of nerve agents was discovered, compounds such as **ecothiopate** (Fig. 22.50) were designed to fit the active site more effectively by including a quaternary amine to bind with the anionic region. This meant that lower doses would be more effective. Ecothiopate is used medicinally in the form of eye drops for the treatment of glaucoma and has advantages over dyflos, which has also been used in this way. Unlike dyflos, ecothiopate slowly hydrolyses from the enzyme over a matter of days.

22.13.2.3 Insecticides

The insecticides **parathion**, **malathion**, and **chlorpyrifos** (Fig. 22.50) are good examples of how a detailed knowledge of biosynthetic pathways can be useful in drug design. These agents are relatively non-toxic compared with nerve gases because the P = S double bond prevents inhibition of the acetylcholinesterase enzymes. In contrast, the equivalent compounds containing a P = O double bond are highly lethal.

Fortunately, there are no metabolic pathways in mammals which can convert the P = S double bond to a P = O double bond. In insects, however, the insecticides act as prodrugs and are metabolized by oxidative desulphurization. The resulting anticholinesterases prove lethal.

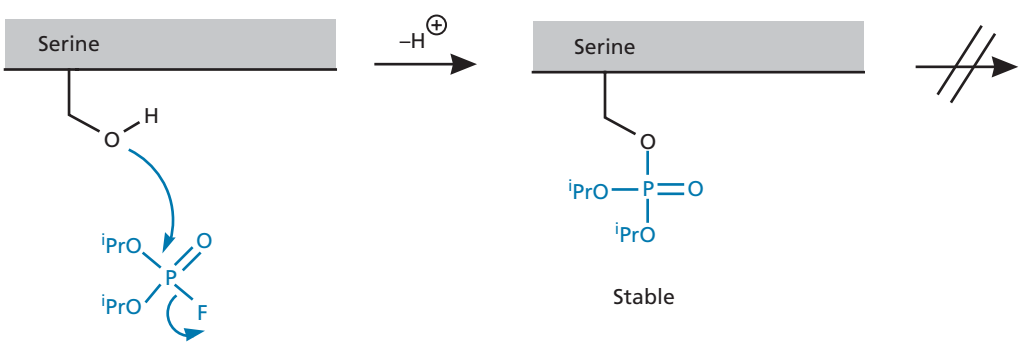


FIGURE 22.49 Simplified mechanism of action of dyflos at the active site of acetylcholinesterase.

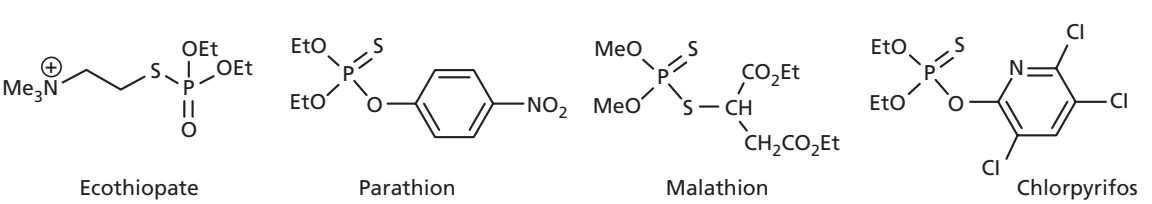


FIGURE 22.50 Organophosphates used as medicines and insecticides.

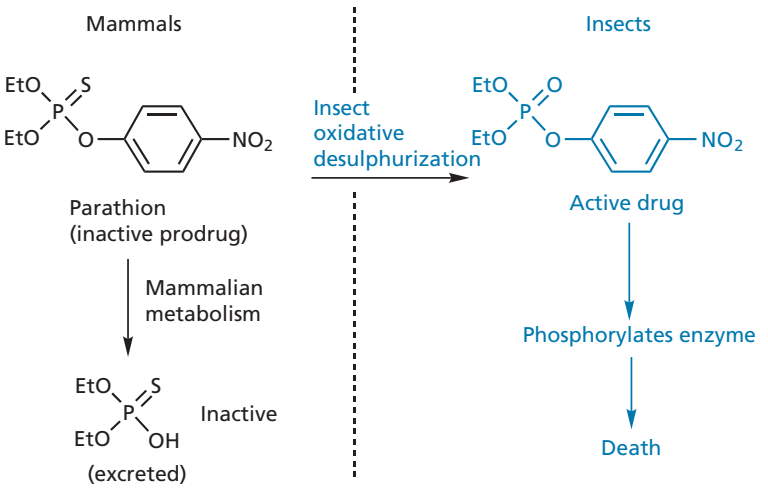


FIGURE 22.51 Metabolism of insecticides in mammals and insects.

In mammals, the same compounds are metabolized in a different way to give inactive compounds which are then excreted (Fig. 22.51). Despite this, organophosphate insecticides are not totally safe and prolonged exposure to them can cause serious side effects if they are not handled with care. Parathion has high lipid solubility and is absorbed easily through mucous membranes, and can also be absorbed through the skin. Preparations of malathion are used medicinally for the treatment of head lice, crab lice, and scabies, but should not be used too frequently or over prolonged periods.

22.14 Pralidoxime: an organophosphate antidote

Pralidoxime (Fig. 22.52) is an antidote to organophosphate poisoning and represents one of the early examples of rational drug design, Any antidote for organophosphate poisoning has to displace the organophosphate moiety from serine by hydrolysing the phosphate–serine bond. However, this is a strong bond and not easily broken. Therefore, a strong nucleophile is required.

The literature revealed that phosphates can be hydrolysed with hydroxylamine (Fig. 22.53). This proved too toxic a compound to be used on humans, so the next stage was to design an equally reactive nucleophilic group which would specifically target the acetylcholinesterase enzyme. If such a compound could be designed, then there was less chance of the antidote taking part in toxic side reactions.

The designers' job was made easier by the knowledge that the organophosphate group does not fill the active site, and the anionic binding region is vacant. The obvious thing to do was to find a suitable group to bind to this anionic centre and attach a hydroxylamine moiety to it. Once positioned in the active site, the hydroxylamine group could react with the phosphate ester (Fig. 22.52).

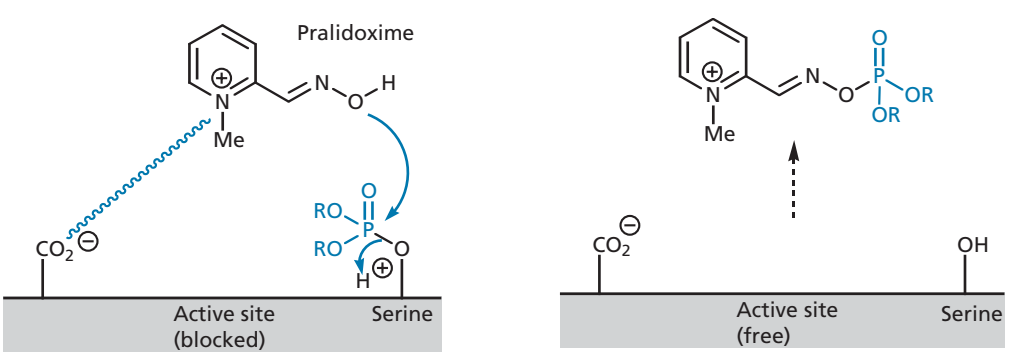


FIGURE 22.52 Pralidoxime as an antidote for organophosphate poisoning.

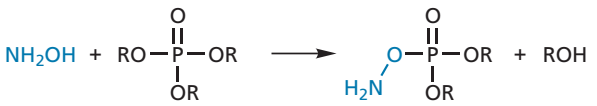


FIGURE 22.53 Hydrolysis of phosphates.

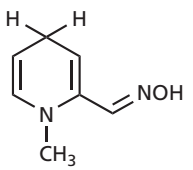


FIGURE 22.54 ProPAM.

Pralidoxime was the result. The positive charge is provided by a methylated pyridine ring and the nucleophilic side group is attached to the *ortho* position, as it was calculated that this would place the nucleophilic hydroxyl group in exactly the correct position to react with the phosphate ester. The results were spectacular, with pralidoxime showing a potency as an antidote 10^6 times greater than hydroxylamine.

Because pralidoxime has a quaternary nitrogen, it is fully charged and cannot pass through the blood–brain barrier into the CNS. This means that the antidote cannot work on any enzymes that have been inhibited in the brain. Pro-2-PAM (Fig. 22.54) is a prodrug of pralidoxime which avoids this problem. As a tertiary amine it can pass through the blood–brain barrier and is oxidized to pralidoxime once it has entered the CNS.

22.15 Anticholinesterases as 'smart drugs'

22.15.1 Acetylcholinesterase inhibitors

Acetylcholine is an important neurotransmitter in the CNS, as well as in the PNS. It has been proposed that the memory loss, intellectual deterioration, and personality changes associated with Alzheimer's disease may, in part, be due to the destruction of cholinergic nerves in the brain. Such damage is associated with the appearance of extracellular protein plaques and intracellular protein tangles in nerve fibres. These aberrant protein structures are neurotoxic and responsible for the destruction of neurons.

Although Alzheimer's disease is primarily a disease of the elderly, it can strike victims as young as 30 years of age and is the fourth leading cause of death in the developed world, affecting nearly 50% of those aged 85 years or more. It has been predicted that there will be 70 million sufferers worldwide by 2050, representing 1.2% of the total population.

The destruction of cholinergic nerves results in a drop in both cholinergic receptors and acetylcholine levels in the brain. Therefore, research has been carried out into the use of anticholinesterases for the treatment of Alzheimer's disease—the so-called **smart drugs**. There is no evidence that these compounds assist general memory improvement and so they are not a student's answer to exam cramming! The treatment does not offer a cure for Alzheimer's disease either, but it can alleviate the symptoms by increasing the duration of action of acetylcholine such that activation of the cholinergic receptors remaining is prolonged. Unlike anticholinesterases acting in the periphery, 'smart drugs' have to cross the blood–brain barrier and so structures containing quaternary nitrogen atoms are not suitable. Tests with physostigmine were carried out in 1979, but the compound was not ideal as it does not enter the brain sufficiently well and shows short-lived, non-selective inhibition. The first drug to be approved for the treatment of Alzheimer's disease was **tacrine** (Fig. 22.55) in 1993. However, this is an extremely toxic drug and is only beneficial for about a year. Other agents which have subsequently been introduced include **donepezil** in 1997, **rivastigmine** in 2000, and **galantamine** (obtained from daffodils or snowdrop bulbs) in 2001. Rivastigmine (an analogue of physostigmine) was the first drug to be approved in all countries of the European Union. It shows selectivity for the brain and has beneficial effects on cognition, memory, concentration, and functional abilities, such as day-to-day tasks or hobbies. The drug has a short half-life, reducing the risk of accumulation or drug–drug interactions. **Metrifonate** (an organophosphate) and **anabaseine** (from ants and marine worms) have also been tested for the treatment of Alzheimer's disease. Herbal medicines have been used in the past to treat the symptoms of Alzheimer's disease and may provide useful lead compounds for further research (Box 22.1).

The anticholinesterase drugs have been shown to be beneficial in the early stages of Alzheimer's disease, but are of less benefit when the disease has become advanced. One disadvantage with the long-term use of these agents is the fact that they increase acetylcholine levels all round the body and not just in the brain; this leads to gastrointestinal side effects. Another problem is that the increased acetylcholine levels result in an increased activation of presynaptic cholinergic receptors which act as a feedback control to lower the amounts of acetylcholine released. As a result, there has been research into finding selective cholinergic agonists that could be used to treat the symptoms of the disease.

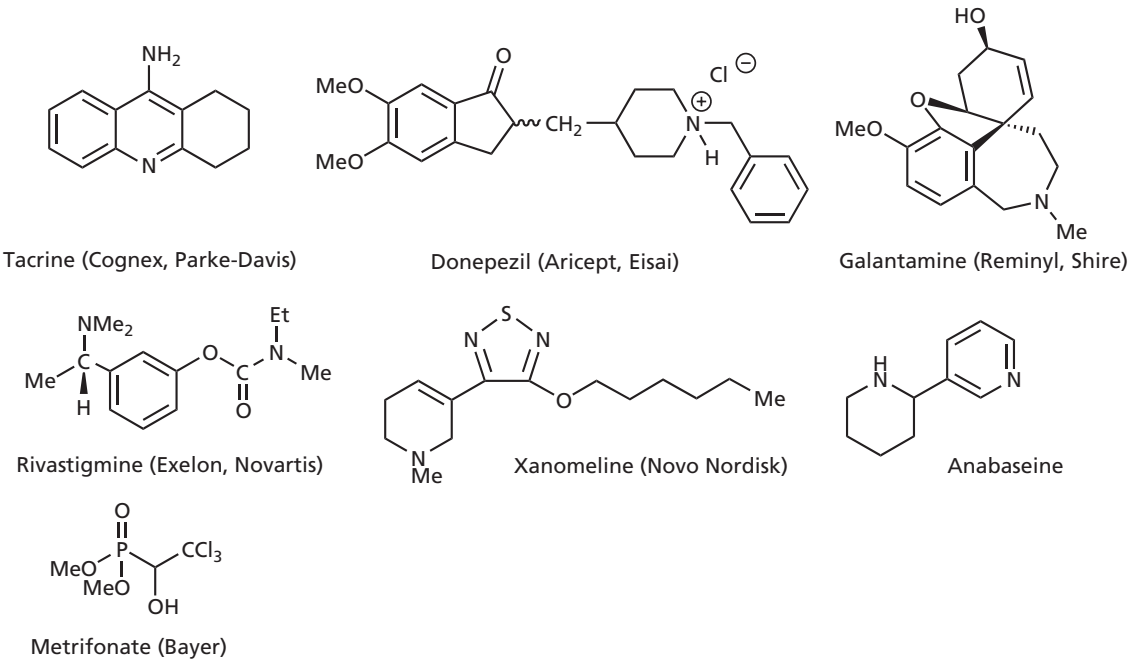
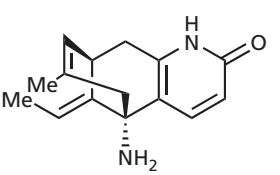


FIGURE 22.55 'Smart drugs'.

BOX 22.1 Mosses play it smart

An extract from the club moss *Huperzia serrata* has been used for centuries in Chinese herbal medicine to treat ailments varying from confusion in Alzheimer's disease to schizophrenia. The extract contains a novel alkaloid called **huperzine A**, which acts as an anticholinesterase. Binding is very specific and so the drug can be used in small doses, thus minimizing the risk of side effects. Huperzine A has been approved for clinical use in China and has been shown to have memory-enhancing effects.

A synthetic route to the natural product has been worked out which has allowed the synthesis of different analogues, but none of these is as active as the natural product. The tricyclic ring system seems to be necessary for good activity, ruling out the possibility of significant simplification. All the functional groups in the molecule are also required for good activity.



Huperzine A.

22.15.2 Dual-action agents acting on the acetylcholinesterase enzyme

In recent years, it has been discovered that the acetylcholinesterase enzyme appears to do more than just catalyse the hydrolysis of acetylcholine. Under normal conditions, the enzyme plays a non-catalytic role in neural development, cell adhesion, and differentiation. Protein–protein interactions involving the interaction of the peripheral binding site of acetylcholinesterase with other proteins promote these processes, with the tryptophan residue described previously (section 22.12.3) playing a crucial role.

Unfortunately, it has also been discovered that the enzyme can play an active role in promoting the deposits of aberrant protein that are found in the brain of Alzheimer's sufferers. Studies have shown that the peripheral binding site of the enzyme is capable of binding **β-amyloid**

protein, which is normally soluble and has an antioxidant role. However, on binding to acetylcholinesterase, the protein undergoes a conformational change which causes it to become insoluble, leading to the appearance of the protein plaques and tangles associated with Alzheimer's disease. The enzyme has been described as a **pathological chaperone** for this process and becomes associated with the protein deposits. Moreover, soluble oligomers of the protein are also formed within cells, which disrupt mitochondria function and increase oxidative stress, resulting in cell toxicity and cell death. These, indeed, may be more relevant to the disease than the visible extracellular plaques.

There is an exciting possibility that drugs might be developed which could halt the progression of the disease by preventing the binding of β-amyloid protein to the peripheral binding site of acetylcholinesterase. Research is currently in progess aimed at designing dual-action drugs that are capable of inhibiting this process, as well as acting as acetylcholinesterase inhibitors. **Donepezil** (Fig. 22.55) is one currently used inhibitor that can span the gorge to interact with both the peripheral binding site and the active site. It has also been shown to have an inhibitory effect on protein aggregation. However, much of the early work has looked at tacrine dimers. **Tacrine** (Fig. 22.55) is believed to enter the active site of the enzyme in a similar manner to acetylcholine; in other words, it is protonated and binds initially to the peripheral binding site. It is then transferred down the gorge into the active site (Fig. 22.41). A dimer was designed where two tacrine molecules were linked by a hydrocarbon chain of sufficient length to allow one tacrine moiety to bind to the active site while the other interacted simultaneously with the peripheral binding site. Different lengths of linker were tried and it was found that a seven-carbon chain was ideal—**bis(7)-tacrine** (Fig. 22.56). This compound was found to be 150–1000 times more potent as an enzyme inhibitor, depending on the source of enzyme studied. Studies have shown that the key tryptophan residues in the active site and the peripheral binding site can form π-cation interactions with each of the tacrine components. The linker can also form van der Waals interactions with the gorge and there is an entropy gain achieved by the displacement of water from the gorge. However, there is an entropy penalty resulting from the restriction in flexibility of the linker once it is constrained within the gorge. The tricyclic hydrophobic nature of the tacrine moieties is also important as there is only a small desolvation penalty involved when the structure binds. Stronger π-cation interactions would be possible if one of the tacrine ring systems was replaced with a simpler amine, but the latter would be strongly solvated and would require a higher desolvation penalty.

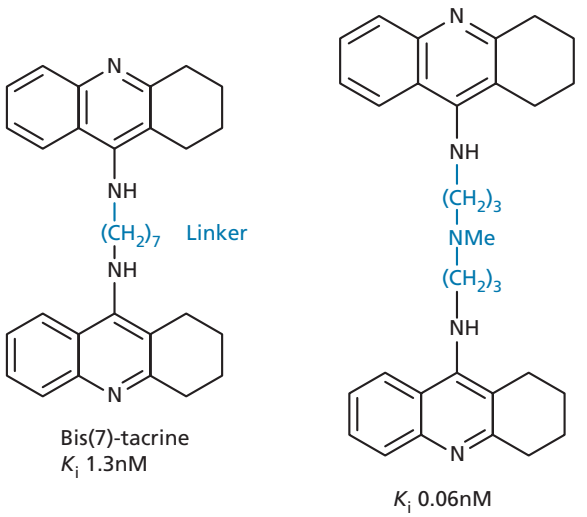


FIGURE 22.56 Tacrine dimers as dual-action agents.

Test your understanding and practise your molecular modelling with Exercises 22.5 and 22.6.

The introduction of an *N*-methyl group into the linker resulted in further binding interactions and increased potency. The *N*-methyl group is protonated when the dimer binds and so it can form π-cation interactions with the aromatic residues lining the gorge. Following on from this work, a large number of structures were synthesized including homodimers of **galantamine** and **huperzine B**, as well as heterodimers containing two different acetylcholinesterase inhibitors. Other dual-action structures have been prepared consisting of a standard acetylcholinesterase inhibitor linked to a moiety designed to bind more effectively with the peripheral binding site. Many of these have been shown to inhibit both the catalytic activity of acetylcholinesterase, as well as protein aggregation. Nevertheless, none of these compounds has entered the clinic to date.

There is a good chance that a dual-action agent will eventually reach the clinic. However, there are many factors involved in Alzheimer's disease and so drugs interacting with acetylcholinesterase alone are unlikely to provide a total cure. Attention is now turning to treatments that can address more than one of the various targets implicated in Alzheimer's disease. These treatments could involve a cocktail of different drugs acting at different targets. An alternative approach is to use an agent that can interact with different targets in a predictable way (**multiple-target directed ligands**) (see also section 13.3.14). For example, dual-action agents that inhibit the acetylcholinesterase enzyme have been designed which have one or more of the following properties;

- antioxidant activity and/or the ability to chelate metals;

- the ability to inhibit enzymes, such as butyrylcholinesterase, monoamine oxidase, or BACE1;
- antagonist activity at α_2-adrenoceptors, $5HT_3$ receptors, *N*-methyl-D-aspartate (NMDA) receptors, muscarinic (M_2) receptors, or H_3 receptors;
- inhibition of serotonin reuptake from nerve synapses;
- the blockade of calcium ion channels.

22.15.3 Multi-targeted agents acting on the acetylcholinesterase enzyme and the muscarinic M_2 receptor

As an example of one area of research into multi-targeted directed ligands, we shall consider agents that have been designed to act as dual-action agents at the acetylcholinesterase enzyme (AChE), as well as antagonists of the M_2 receptor. The M_2 receptor is an autoreceptor present on presynaptic cholinergic neurons. Activation of the autoreceptor inhibits the release of acetylcholine from the presynaptic neuron (section 22.3.2 and 22.11) and so M_2 antagonists will increase acetylcholine release and help to raise acetylcholine levels. The lead compound for this work was a polyamine structure called **benextramine** (Fig. 22.57). This is an irreversible α-adrenoceptor antagonist, but it also shows activity as an anticholinesterase and M_2 receptor antagonist. Polyamines have been identified as good lead compounds for multi-targeted directed ligands as the protonated nitrogens present have the capability of forming π-cation interactions with aromatic residues in virtually any protein target. Moreover, the flexible linear structure allows the polyamine to adopt a huge number of different conformations, making it more likely that suitable conformations are present that allow interaction with different targets. Such compounds are defined as **promiscuous ligands** (section 12.2.7).

Studies showed that the 2-methoxybenzyl group was important to activity, but not the disulphide bridge. Varying the chain length led to **methoctramine**, which had improved M_2 activity, while retaining good acetylcholinesterase (AChE) activity. It was also shown that a diamine diamide backbone retained affinity for M_2 and so the two 'internal' amines were replaced with amides in order to improve lipophilicity. This decreased affinity for M_2 receptors and butyrylcholinesterase (BuChE), but increased affinity for AChE. *N*-Methylation further increased affinity for AChE resulting in the discovery of **caproctamine**.

Compared with benextramine, caproctamine was found to be 42 times more active as an AChE inhibitor and two times less active as a BuChE inhibitor, while retaining affinity for the M_2 receptor. It was also demonstrated that the structure could bind simultaneously to the tryptophan residues in the active and peripheral binding sites, while the linker formed hydrophobic interactions with aromatic residues in the gorge. However, caproctamine showed very little ability to inhibit AChE-induced Aβ aggregation, which demonstrated that the ability to interact with the peripheral binding site does not necessarily block protein aggregation.

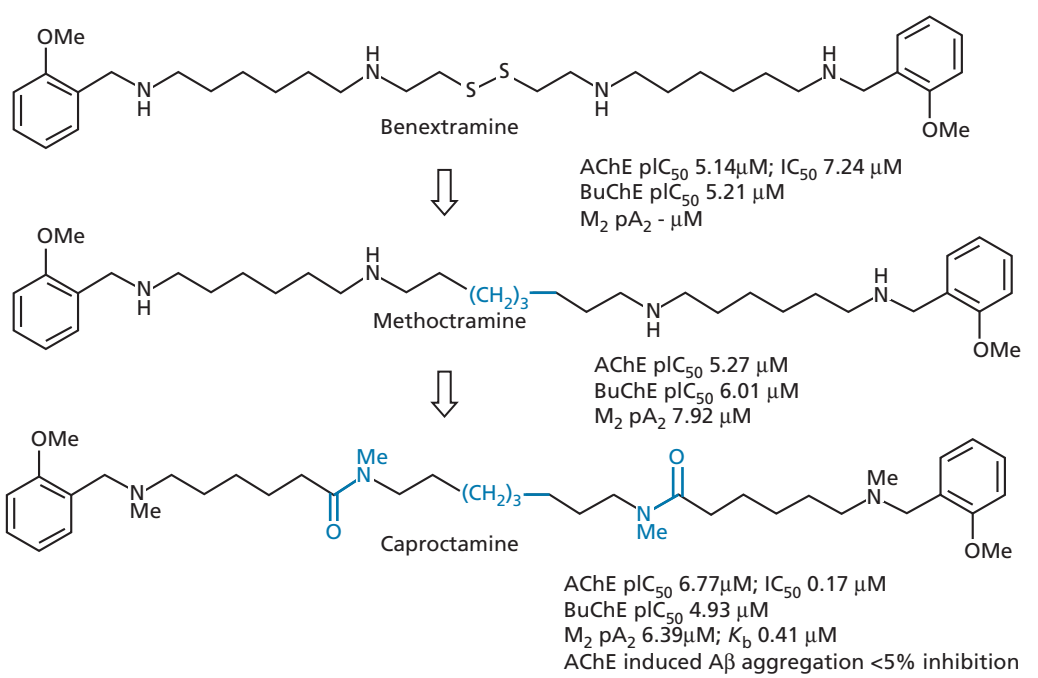


FIGURE 22.57 Development of caproctamine from benextramine.

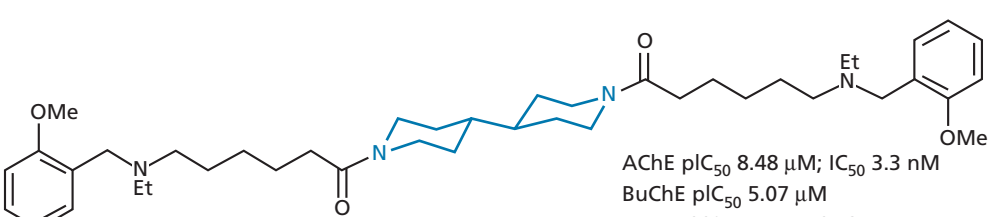
Further work was done to introduce some rigidity into the linker chain by introducing piperidine rings (Fig. 22.58). This resulted in increased anticholinesterase activity and M_2 antagonism, as well as inhibition of AChE-induced Aβ aggregation.

A more substantial aromatic system was introduced into the middle of the linker because it was believed that this would form π–π interactions with aromatic residues in the gorge of AChE and would also allow the structure to interact directly with Aβ proteins to inhibit self-induced aggregation of the protein. This led to the structure shown in Fig. 22.59, which proved to have nanomolar activity as an AChE inhibitor. The structure also proved more active in its ability to inhibit AChE-induced Aβ aggregation. Its activity as an M_2 antagonist was not reported, however.

Docking experiments indicated that the structure could bind to the key tryptophan residues in the catalytic and peripheral binding sites, while the central tetracyclic ring system interacts by π–π or van der Waals interactions with aromatic residues in the gorge. Hydrogen bonding is possible between the methoxy groups and a tyrosine residue in the active site. It remains to be seen whether further development of this compound will result in a clinically useful agent.

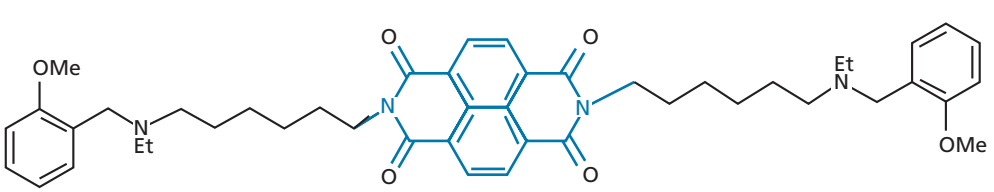
KEY POINTS

- Anticholinesterases inhibit the enzyme acetylcholinesterase and have the same clinical effects as cholinergic agonists.
- The active site for acetylcholinesterase is similar to the binding site for the cholinergic receptor, but also includes a catalytic triad of amino acids—histidine, serine, and glutamate.
- Histidine acts as an acid–base catalyst, while serine acts as a nucleophile during the hydrolytic mechanism. Glutamate orientates and activates histidine.
- The carbamate inhibitors are derived from the lead compound physostigmine. They react with acetylcholinesterase to produce a carbamoyl-bound intermediate which is stable and slow to hydrolyse.
- Organophosphorus agents have been used as nerve gases, medicines, and insecticides. They irreversibly phosphorylate serine in the active site.
- Pralidoxime was designed as an antidote for organophosphate poisoning. It can bind to the active site of phosphorylated enzymes and displace the phosphate group from serine.
- Anticholinesterases have been used as smart drugs in the treatment of Alzheimer's disease. They have to cross the blood–brain barrier and cannot be permanently charged.
- Dual-action agents have been designed as potential drugs for the treatment of Alzheimer's disease. They are designed to bind simultaneously to the active site and the peripheral binding site.
- Multi-target agents have been deigned that target acetylcholinesterase and other targets that have been implicated in Alzheimer's disease.



AChE pIC_{50} 8.48 μM; IC_{50} 3.3 nM
BuChE pIC_{50} 5.07 μM
M_2 K_b 660 nM; pA_2 6.18 μM
Aβ(AChE) 41% at 100 μM

FIGURE 22.58 Rigidification of caproctamine.

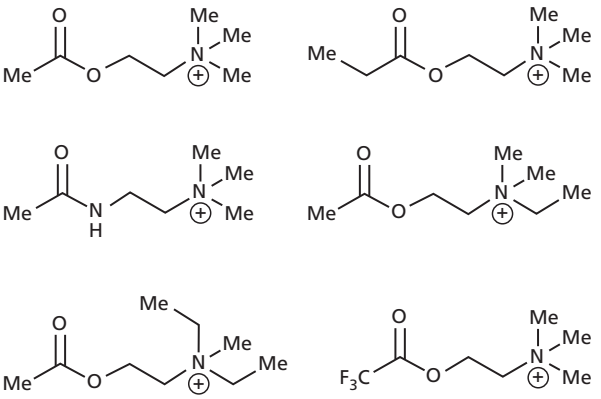


hAChE IC_{50} 0.37nM; AChE-induced Aβ aggregation >90% inhibition;
Self-induced Aβ aggregation 54.5% inhibition.

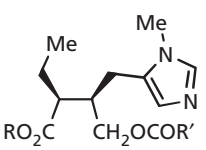
FIGURE 22.59 Further rigidified analogue of caproctamine.

QUESTIONS

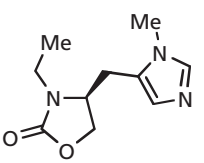
1. Based on the binding site described in Fig. 22.10, suggest whether the following structures are likely to act as agonists or not.



2. Suggest a mechanism by which atropine is racemized.
3. A fine balance of binding interactions is required of a neurotransmitter. What do you think is meant by this and what consequences does it have for drug design?
4. Suggest how the binding interactions holding acetylcholine to the active site of acetylcholinesterase might aid in the hydrolysis of acetylcholine.
5. Explain how the following diester could act as a prodrug for pilocarpine.

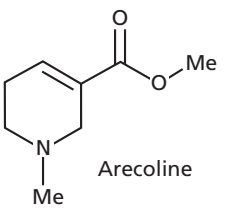


Diester prodrug for pilocarpine



Pilocarpine analogue

6. What advantage do you think the pilocarpine analogue shown might have over pilocarpine itself, and why?
7. Arecoline has been described as a cyclic 'reverse ester' bioisostere of acetylcholine. What is meant by this and what similarity is there, if any, between arecoline and acetylcholine?
8. Arecoline has a very short duration of action. Why do you think this is?
9. Suggest analogues of arecoline that might have better properties, such as a longer duration of action.



Arecoline

10. Neuromuscular blocking activity for tubocurarine is associated with a pharmacophore where the distance between two charged nitrogen atoms is 1.15 nm. Decamethonium can adopt a folded conformation where the N–N separation is 1.14 nm. Octamethonium is an analogue of decamethonium which contains an eight-carbon bridge between the charged nitrogens. The fully extended conformation is the most stable conformation and corresponds to a N–N distance of 1.157 nm. Discuss whether octamethonium is likely to be more active than decamethonium.
11. An electrostatic gradient has been proposed that guides acetylcholine into the active site of the acetylcholinesterase enzyme. Can you foresee any problems associated with the presence of such a gradient? It has also been proposed that there may be a 'back door' into the active site. What do you think this means, how could it occur, and why would it be necessary?
12. Research is being carried out to design Alzheimer's drugs that will inhibit both acetylcholinesterase and butyrylcholinesterase, despite the fact that the former enzyme is more effective at catalysing the hydrolysis of acetylcholine. Why do you think this approach is considered relevant? What might be the disadvantages of such an approach?

FURTHER READING

Hardman, J. G., Limbird, L. E., Molinoff, P. B., Ruddon, R. W., and Goodman Gilman, A. (eds) (1996) Anticholinesterase agents. In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 9th edn. McGraw-Hill, New York, pp. 161–176.

Quinn, D. M. (1987) Acetylcholinesterase. *Chemical Reviews* **87**, 955–975.

Roberts, S. M. and Price, B. J. (eds) (1985) Atracurium design and function. In: *Medicinal Chemistry – The Role of Organic Research in Drug Research*. Academic Press, London.

Teague, S. J. (2003) Implications of protein flexibility for drug discovery. *Nature Reviews Drug Discovery* **2**, 527–541.

Titles for general further reading are listed on p. 763.

23 Drugs acting on the adrenergic nervous system

23.1 The adrenergic nervous system

23.1.1 Peripheral nervous system

In Chapter 22, we studied the cholinergic system and the important role it plays in the peripheral nervous system (PNS). **Acetylcholine** is the crucial neurotransmitter in the cholinergic system and has specific actions at various synapses and tissues. The other important player in the PNS (sections 22.1 and 22.2) is the adrenergic system, which makes use of the chemical messengers **adrenaline** and **noradrenaline**. Noradrenaline (also called **norepinephrine**) is the neurotransmitter released by the sympathetic nerves which feed smooth muscle and cardiac muscle, whereas adrenaline (**epinephrine**) is a hormone released along with noradrenaline from the **adrenal medulla**.

The action of noradrenaline at various tissues is the opposite to that of acetylcholine, which means that tissues are under a dual control. For example, if noradrenaline has a stimulant activity at a specific tissue, acetylcholine has an inhibitory activity at that same tissue. Both the cholinergic and adrenergic systems have a 'background' activity, so the situation is analogous to driving a car with one foot on the brake and one foot on the accelerator. The overall effect on the tissue depends on which effect is predominant.

The adrenergic nervous system has a component that the cholinergic system does not have—the facility to release adrenaline during times of danger or stress. This is known as the **fight or flight** response. Adrenaline is carried by the blood supply round the body and activates adrenergic receptors in preparation for immediate physical action, whether that be to fight the perceived danger or to flee from it. This means that the organs required for physical activity are activated, while those that are not important are suppressed. For example, adrenaline stimulates the heart and dilates the blood vessels to muscles so that the muscles are supplied with sufficient blood for physical activity. At the same time, smooth muscle activity in the gastrointestinal tract is suppressed as digestion is not an immediate priority. This fight or flight response is clearly an evolutionary advantage and stood early humans in good stead when faced with an unexpected encounter with a grumpy old bear. Nowadays, it is unlikely that you will meet a grizzly bear on your way to the supermarket, but the fight or flight response is still functional when you are faced with modern dangers such as crazy drivers. It also functions in any situation of stress such as an imminent exam, important football game, or public performance. In general, the effects of noradrenaline are the same as those of adrenaline, although noradrenaline constricts blood vessels to skeletal muscle rather than dilates them.

23.1.2 Central nervous system

There are also adrenergic receptors in the central nervous system (CNS) and noradrenaline is important in many functions of the CNS, including sleep, emotion, temperature regulation, and appetite. However, the emphasis in this chapter is on the peripheral role of adrenergic agents.

23.2 Adrenergic receptors

23.2.1 Types of adrenergic receptor

In Chapter 22, we saw that there are two types of cholinergic receptor, with subtypes of each. The same holds true for adrenergic receptors. The two main types of adrenergic receptor are called the **α** and **β-adrenoceptors**. Both the α and the β-adrenoceptors are G-protein-coupled receptors (section 4.7), but differ in the type of G-protein with which they couple (G_o for α-adrenoceptors; G_s for β-adrenoceptors).

For each type of receptor, there are various receptor subtypes with slightly different structures. The α-adrenoceptor consists of α_1- and α_2-subtypes, which differ in the type of secondary message produced. The α_1 receptors activate **inositol triphosphate** (IP_3) and **diacylglycerol** (DG) as secondary messengers (section 5.3), whereas the α_2-receptors inhibit the production of the secondary messenger **cyclic-AMP** (section 5.2.3). The β-adrenoceptor consists of β_1-, β_2-, and β_3-subtypes, all of which activate the formation of cyclic-AMP. To complicate matters slightly further, both the α_1- and α_2-adrenoceptors have further subcategories (α_{1A}, α_{1B}, α_{1D} α_{2A}, α_{2B}, α_{2C}).

All of these adrenergic receptor types and subtypes are 'switched on' by adrenaline and noradrenaline, but the fact that they have slightly different structures means that it should be possible to design selective agonists that can distinguish between them. This is crucial in developing drugs that have minimal side effects and act at specific organs in the body, for, as we shall see, the various adrenoceptors are not evenly distributed in different tissues. By the same token, it should be possible to design selective antagonists with minimal side effects that switch off particular types and subtypes of adrenoceptor.

23.2.2 Distribution of receptors

The various adrenoceptor types and subtypes vary in their distribution, with certain tissues containing more of one type of adrenoceptor than another. Table 23.1 describes various tissues, the types of adrenoceptor which predominate in these tissues, and the effect of activating these receptors (see also Box 23.1).

A few points are worth highlighting here:

- activation of α-receptors generally contracts smooth muscle (except in the gut), whereas activation of β-receptors generally relaxes smooth muscle. This latter effect is mediated through the most common of the β-adrenoceptors—the β_2-receptor. In the heart, the β_1-adrenoceptors predominate and activation results in contraction of muscle;
- different types of adrenoceptor explain why adrenaline can have different effects at different parts of the body. For example, the blood vessels supplying skeletal muscle have mainly β_2-adrenoceptors and are dilated by adrenaline, whereas the blood vessels elsewhere have mainly α-adrenoceptors and are constricted by adrenaline. As more blood vessels are constricted than are dilated in the system, the overall effect of adrenaline is

TABLE 23.1 Distribution and effects of adrenoceptors in different parts of the body

Organ or tissue	Predominant adrenoceptors	Effect of activation	Physiological effect
Heart muscle	β_1	Muscle contraction	Increased heart rate and force
Bronchial smooth muscle	α_1	Smooth muscle contraction	Closes airways
	β_2	Smooth muscle relaxation	Dilates and opens airways
Arteriole smooth muscle (not supplying muscles)	α	Smooth muscle contraction	Constricts arterioles and increases blood pressure (hypertension)
Arteriole smooth muscle (supplying muscle)	β_2	Smooth muscle relaxation	Dilates arterioles and increases blood supply to muscles
Veins	α	Smooth muscle contraction	Constricts veins and increases blood pressure (hypertension)
	β_2	Smooth muscle relaxation	Dilates veins and decreases blood pressure (hypotension)
Liver	α_1 & β_2	Activates enzymes which metabolize glycogen and deactivates enzymes which synthesize glycogen	Breakdown of glycogen to produce glucose
Gastrointestinal tract smooth muscle	α_1, α_2, and β_2	Relaxation	'shuts down' digestion
Kidney	β_2	Increases renin secretion	Increases blood pressure
Fat cells	β_3	Activates enzymes	Fat breakdown

BOX 23.1 Clinical aspects of adrenergic agents

The main clinical use for adrenergic agonists is in the treatment of asthma. Activation of β_2-adrenoceptors causes the smooth muscles of the bronchi to relax, thus widening the airways. Agonists acting selectively on α_1-adrenoceptors cause vasoconstriction and can be used alongside local anaesthetics in dentistry to localize and prolong the effect of the anaesthetic at the site of injection. They are also used as nasal decongestants. Selective α_2-agonists are used in the treatment of glaucoma, hypertension, and pain.

The main uses for adrenergic antagonists are in treating angina and hypertension. Agents which act on the α-receptors of blood vessels cause relaxation of smooth muscle, dilatation of the blood vessels, and a drop in blood pressure. Selective α_1-antagonists are now preferred for the treatment of hypertension and are also being investigated as potential agents for the treatment of benign prostatic hyperplasia. Selective α_2-antagonists are being studied for the treatment of depression. Agents that block β_1-receptors in the heart (β-blockers) slow down the heart rate and reduce the force of contractions. β-blockers also have a range of effects in other parts of the body, which combine to lower blood pressure.

to increase the blood pressure, while at the same time providing sufficient blood for the muscles in the fight or flight response.

23.3 Endogenous agonists for the adrenergic receptors

The term **endogenous** refers to any chemical which is present naturally in the body. As far as the adrenergic system is concerned, the body's endogenous chemical messengers are the neurotransmitter noradrenaline and the hormone adrenaline. Both act as agonists and switch on adrenoceptors. They belong to a group of compounds called the **catecholamines**—so-called because they have an alkylamine chain linked to a **catechol** ring (the 1,2-benzenediol ring) (Fig. 23.1).

Test your understanding and practise your molecular modelling with Exercise 23.1.

23.4 Biosynthesis of catecholamines

The biosynthesis of noradrenaline and adrenaline starts from the amino acid **L-tyrosine** (Fig. 23.2). The enzyme

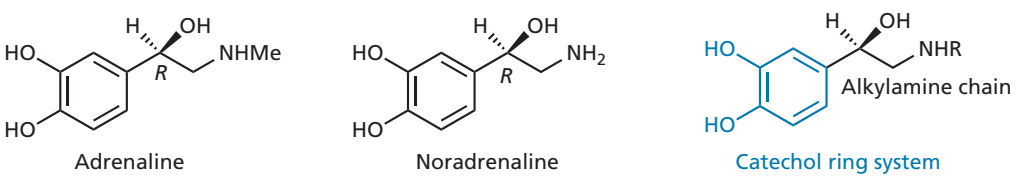


FIGURE 23.1 Adrenergic transmitters.

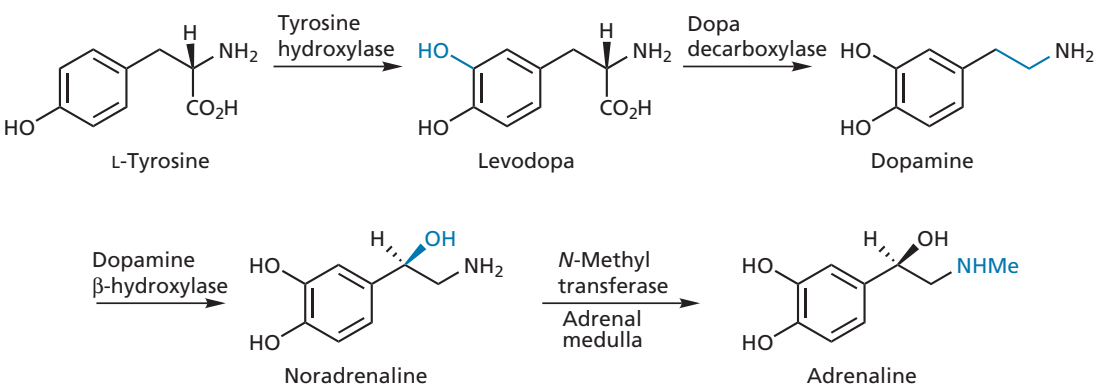


FIGURE 23.2 Biosynthesis of noradrenaline and adrenaline.

tyrosine hydroxylase catalyses the introduction of a second phenol group to form **levodopa** (L-dopa) which is then decarboxylated by **aromatic L-aminoacid decarboxylase** (**dopa decarboxylase**) to give **dopamine**—an important neurotransmitter in its own right. Dopamine is then hydroxylated to **noradrenaline**, which is the end product in adrenergic neurons. In the adrenal medulla, however, noradrenaline is *N*-methylated to form **adrenaline**. The biosynthesis of the catecholamines is controlled by regulation of **tyrosine hydroxylase**—the first enzyme in the pathway. This enzyme is inhibited by noradrenaline—the end product of biosynthesis, thus allowing self-regulation of catecholamine synthesis and control of catecholamine levels.

23.5 Metabolism of catecholamines

Metabolism of catecholamines in the periphery takes place within cells and involves two enzymes—**monoamine oxidase** (**MAO**) and **catechol *O*-methyltransferase** (**COMT**). MAO converts catecholamines to their corresponding aldehydes. These compounds are inactive as adrenergic agents and undergo further metabolism (as shown in Fig. 23.3 for noradrenaline). The final carboxylic acid is polar and excreted in the urine.

An alternative metabolic route is possible which results in the same product. This time the enzyme COMT catalyses the methylation of one of the phenolic groups of the catecholamine. The methylated product is oxidized by MAO then converted to the final carboxylic acid and excreted (Fig. 23.4).

Metabolism in the CNS is slightly different, but still involves MAO and COMT as the initial enzymes.

23.6 Neurotransmission

23.6.1 The neurotransmission process

The mechanism of neurotransmission is shown in Fig. 23.5 and applies to adrenergic neurons innervating smooth or cardiac muscle, as well as synaptic connections within the CNS.

Noradrenaline is biosynthesized in a presynaptic neuron then stored in membrane-bound vesicles. When a nerve impulse arrives at the terminus of a neuron, it stimulates the opening of calcium ion channels and promotes the fusion of the vesicles with the cell membrane to release noradrenaline. The neurotransmitter then diffuses to adrenergic receptors on the target cell where it binds and activates the receptor, leading to the signalling process which will eventually result in a cellular response. After the message has been received, noradrenaline departs the receptor and is taken back into the presynaptic neuron by a transport protein. Once in the cell, noradrenaline is repackaged into the vesicles. Some of the noradrenaline is metabolized before it is repackaged, but this is balanced out by noradrenaline biosynthesis.

23.6.2 Co-transmitters

The process of adrenergic neurotransmission is actually more complex than that illustrated in Fig. 23.5. For example, noradrenaline is not the only neurotransmitter released during the process. **Adenosine triphosphate** (**ATP**) and a protein called **chromogranin A** are released from the vesicles along with noradrenaline and

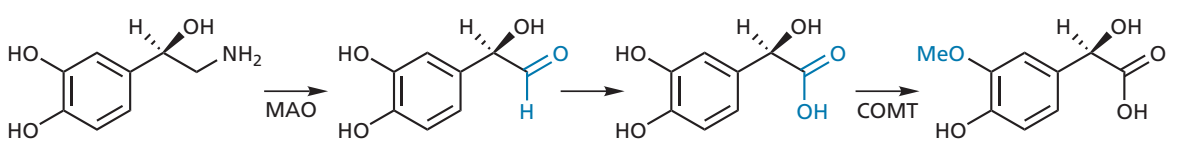


FIGURE 23.3 Metabolism of noradrenaline with monoamine oxidase (MAO) then catechol *O*-methyltransferase (COMT).

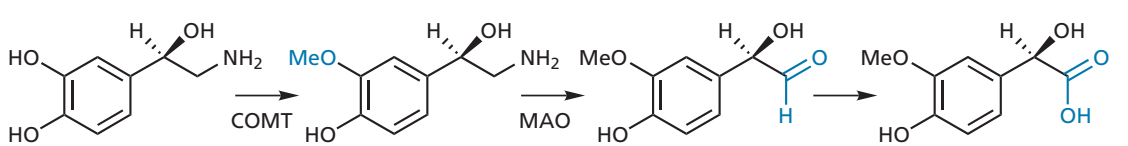


FIGURE 23.4 Metabolism of noradrenaline with catechol *O*-methyltransferase (COMT) then monoamine oxidase (MAO).

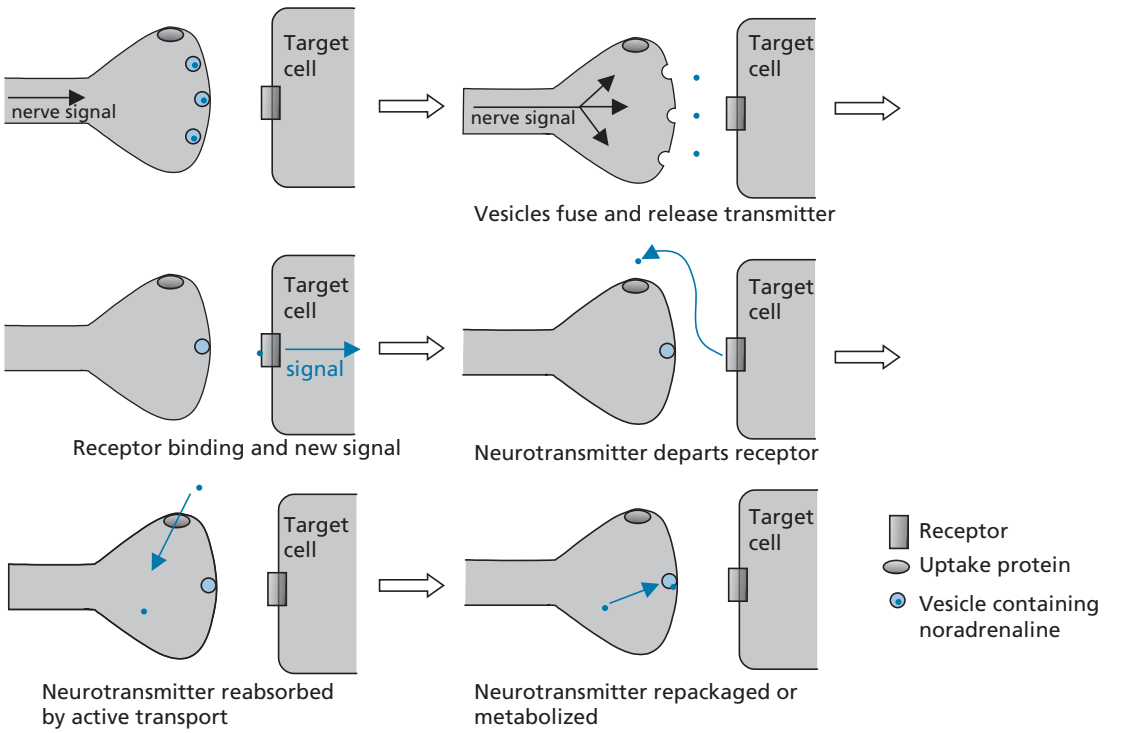


FIGURE 23.5 Transmission process for noradrenaline.

act as co-transmitters. They interact with their own specific receptors on the target cell and allow a certain variation in the speed and type of message which the target cell receives. For example, ATP leads to a fast response in smooth muscle contraction.

23.6.3 Presynaptic receptors and control

A further feature of the neurotransmission process not shown in Fig. 23.5 is the existence of presynaptic receptors which have a controlling effect on noradrenaline release (Fig. 23.6). There are a variety of these receptors, each of which responds to a specific chemical messenger. For example, there is an adrenergic receptor (the **α_2-adrenoceptor**) which interacts with released noradrenaline and has an inhibitory effect on further release of noradrenaline. Thus, noradrenaline acts to control its own release by a negative feedback system.

There are receptors specific for **prostaglandins** released from the target cell. For example, the prostaglandin **PGE_2** appears to inhibit transmission, whereas **$PGF_{2\alpha}$** appears to facilitate it. Thus, the target cell itself can have some influence on the adrenergic signals coming to it.

There are presynaptic muscarinic receptors that are specific for **acetylcholine** and serve to inhibit release of noradrenaline. These receptors respond to side branches of the cholinergic nervous system which synapse on to the adrenergic neuron. This means that when the cholinergic system is active, it sends signals along its side branches to inhibit adrenergic transmission. Therefore, as the cholinergic activity to a particular tissue increases, the adrenergic activity decreases, both of which enhance the overall cholinergic effect (cf. section 22.5.2).

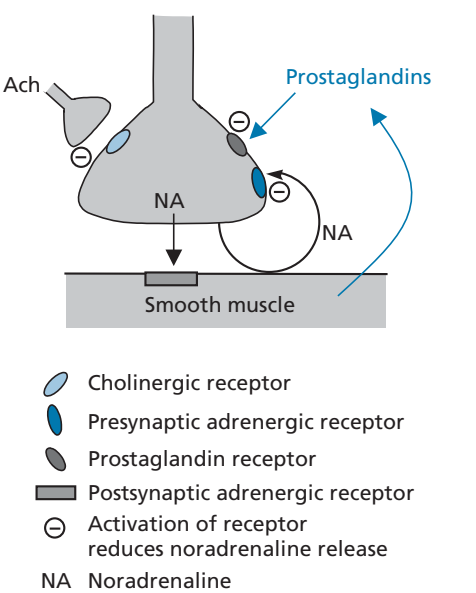


FIGURE 23.6 Presynaptic receptors on a neuron releasing noradrenaline as neurotransmitter.

23.7 Drug targets

Having studied the nerve transmission process, it is now possible to identify several potential drug targets which will affect the process (Fig. 23.7):

1. The biosynthetic enzymes involved in the synthesis of noradrenaline within presynaptic neurons (section 23.4)
2. The vesicle carriers which package noradrenaline within the presynaptic neuron prior to release
3. The process of exocytosis where vesicles fuse with the cell membrane and release noradrenaline into the synaptic gap when the neuron is active
4. Adrenergic receptors in the postsynaptic neuron which are activated by noradrenaline to generate a signal in that neuron
5. The transport proteins which are responsible for the reuptake of noradrenaline from the synaptic gap
6. The metabolic enzymes which metabolize noradrenaline (section 23.5)
7. The presynaptic adrenergic receptors which regulate noradrenaline release (section 23.6.3).

In the next section, we concentrate on the adrenergic receptors. In later sections, we will consider some of the other possible drug targets.

KEY POINTS

- The neurotransmitter involved in the adrenergic nervous system is noradrenaline. Adrenaline is a hormone which is released by the adrenal medulla at times of stress and activates adrenergic receptors.
- The sympathetic nerves innervating smooth muscle and cardiac muscle release noradrenaline.

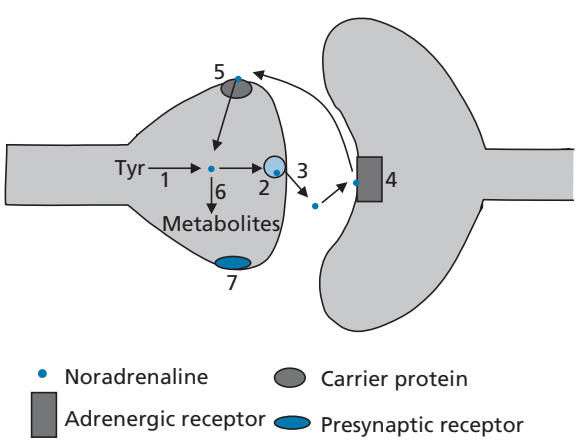


FIGURE 23.7 Drug targets affecting noradrenaline transmission.

- Adrenergic receptors are G-protein-coupled receptors. There are two main types: the α- and the β-adrenoceptors. There are various subtypes of each.
- The different types and subtypes of adrenoceptor predominate in different tissues. Drugs which show receptor selectivity also show tissue selectivity.
- The major use of adrenergic agonists is in the treatment of asthma. The major use of adrenergic antagonists is in cardiovascular medicine.
- Adrenaline, noradrenaline, and dopamine are catecholamines.
- The biosynthesis of catecholamines starts from tyrosine and involves levodopa as an intermediate.
- Catecholamines are metabolized by monoamine oxidase and catechol *O*-methyltransferase.
- Noradrenaline is synthesized in presynaptic neurons, and packaged in vesicles prior to release. Once released, it activates receptors on target cells. It is then is taken up into presynaptic neurons by a transport protein and repacked into vesicles. A certain percentage of noradrenaline is metabolized.
- Adrenergic receptors are the main targets for adrenergic drugs.

23.8 The adrenergic binding site

The adrenergic receptors are G-protein-linked receptors which consist of seven transmembrane (TM) helices (section 4.7). In order to study the binding site of a receptor, one would ideally crystallize it with a ligand bound to the binding site. X-ray crystallography would then be used to determine the crystal structure and identify how the ligand binds. Unfortunately, membrane-bound receptors are very difficult to crystallize, and it was only in 2007 that the β_2-adrenoceptor was crystallized (section 17.14.1). Unfortunately, the crystal structure obtained does not reveal how an agonist binds to the ligand binding site. Therefore, a knowledge of the binding site is based on mutagenesis studies and molecular modelling. Mutagenesis studies involve mutating amino acids to see which ones are crucial for ligand binding, while molecular modelling involves the construction of a model binding site based on the structures of similar proteins whose structures are known (section 17.14.1). From these studies, it has been proposed that three of the transmembrane helices (TM3, TM5, and TM6) are involved in the binding site, illustrated for the β-adrenoceptor in Fig. 23.8. Mutagenesis studies have indicated the importance of an aspartic acid residue (Asp-113), a phenylalanine residue (Phe-290), and two serine residues (Ser-207 and Ser-204). Modelling studies indicate that these groups can bind to adrenaline or noradrenaline as shown in

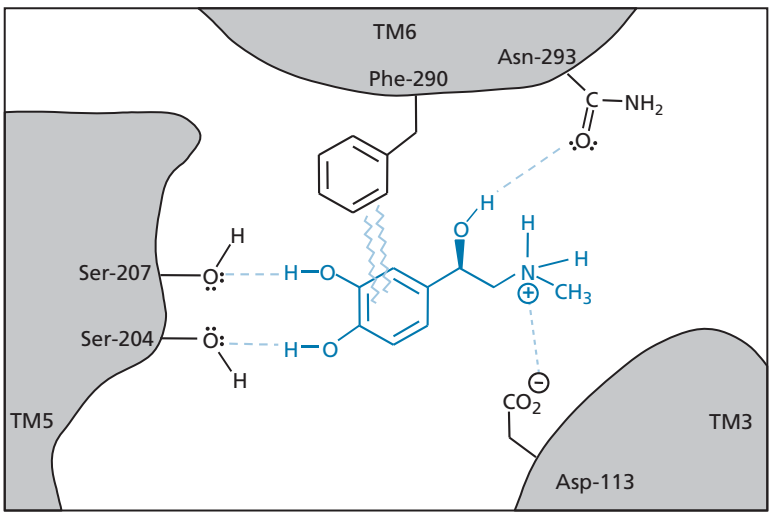


FIGURE 23.8 Adrenergic binding site.

the figure. The serine residues interact with the phenolic groups of the catecholamine via hydrogen bonding. The aromatic ring of Phe-290 interacts with the catechol ring by van der Waals interactions, while Asp-113 interacts with the protonated nitrogen of the catecholamine by ionic bonding. There is also a proposed hydrogen bonding interaction between Asn-293 and the alcohol function of the catecholamine.

23.9 Structure–activity relationships

23.9.1 Important binding groups on catecholamines

Support for the above binding site interactions is provided by studies of structure–activity relationships (SAR) on catecholamines. These emphasize the importance of having the alcohol group, the intact catechol ring system with both phenolic groups unsubstituted, and the ionized amine (Fig. 23.9).

Some of the evidence supporting these conclusions is as follows:

- **the alcohol group**—the *R*-enantiomer of noradrenaline is more active than the *S*-enantiomer, indicating that the secondary alcohol is involved in a hydrogen bonding interaction. Compounds lacking the hydroxyl group (e.g. dopamine) have a greatly reduced interaction. Some of the activity *is* retained, indicating that the alcohol group is important, but not essential;
- **the amine** is normally protonated and ionized at physiological pH. This is important as replacing nitrogen with carbon results in a large drop in activity. Activity is also affected by the number of substituents on the nitrogen. Primary and secondary amines have good adrenergic activity, whereas tertiary amines and quaternary ammonium salts do not;

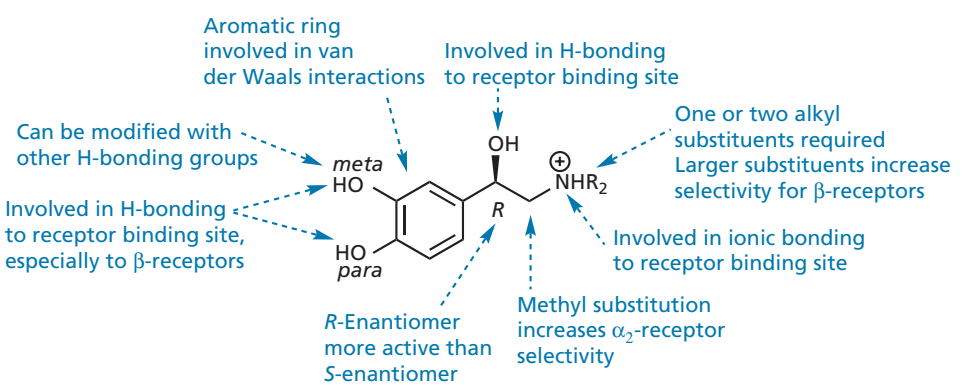


FIGURE 23.9 Important binding groups for adrenergic agents.

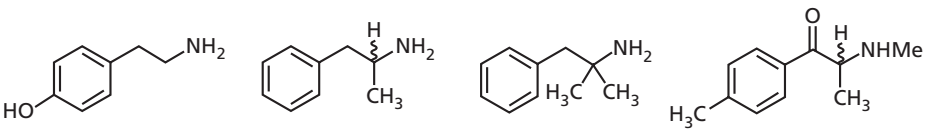


FIGURE 23.10 Agents that have no affinity for the adrenergic receptor.

- **both phenol substituents** are important. For example, **tyramine**, **amphetamine**, **phentermine**, and the banned substance **mephedrone** (Fig. 23.10) have little, or no, affinity for adrenoceptors, although they do have an effect on the adrenergic system through other mechanisms (section 23.12.4). Having said that, the phenol groups can be replaced by other groups capable of interacting with the binding site by hydrogen bonding. This is particularly true for the *meta* phenol group, which can be replaced by groups such as CH_2OH, CH_2CH_2OH, NH_2, NHMe, NHCOR, NMe_2, and $NHSO_2R$;
- **alkyl substitution** on the side chain linking the aromatic ring to the amine decreases activity at both α- and β-adrenergic receptors. This may be a steric effect which blocks hydrogen bonding to the alcohol or which prevents the molecule adopting the active conformation.

23.9.2 Selectivity for α- versus β-adrenoceptors

SAR studies demonstrate certain features which introduce a level of receptor selectivity between the α- and β-adrenoceptors.

- *N*-**Alkyl substitution**: it was discovered that adrenaline has the same potency for both types of adrenoceptor, whereas noradrenaline has a greater potency for α-adrenoceptors than for β-adrenoceptors. This indicates that an *N*-alkyl substituent has a role to play in receptor selectivity. Further work demonstrated that increasing the size of the *N*-alkyl substituent resulted in loss of potency at the α-receptor but an increase in potency at β-receptors. For example, the synthetic analogue **isoprenaline** (Fig. 23.11) is a powerful β-stimulant devoid of α-agonist activity. The presence of a bulky *N*-alkyl group, such as isopropyl or *tertiary*-butyl, is particularly good for β-adrenoceptor activity. These results indicate that the β-adrenoceptor has a hydrophobic pocket into which a bulky alkyl group can fit, whereas the α-adrenoceptor does not (Fig. 23.12).
- **Phenol groups** seem particularly important for β-receptors. If they are absent, activity drops more significantly for β-receptors than for α-receptors.
- **α-Methyl substitution**: addition of an α-methyl group (e.g. α-methylnoradrenaline; Fig. 23.13) increases α_2-receptor selectivity.
- **Extension**: as mentioned earlier, isopropyl or *t*-butyl substituents on the amine nitrogen are particularly good for β-selectivity. Increasing the length of the alkyl chain offers no advantage, but if a polar functional group is placed at the end of the alkyl group, the situation changes. In particular, adding a phenol group to the end of a C_2 alkyl chain results in a dramatic rise in activity, demonstrating that an extra polar binding region has been accessed which can take part in hydrogen bonding. For example, the activity of the extension analogue shown in Fig. 23.13 is increased by a factor of 800.

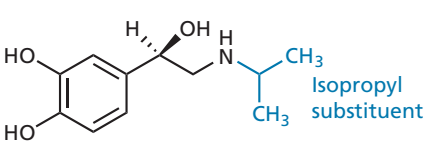


FIGURE 23.11 (*R*)-Isoprenaline.

23.10 Adrenergic agonists

23.10.1 General adrenergic agonists

Adrenaline is an obvious agonist for the overall adrenergic system and it is frequently used in emergency situations, such as cardiac arrest or anaphylactic reactions. The latter can be caused by hypersensitivity to certain foodstuffs (e.g. nuts) or foreign chemicals, such as a bee sting or penicillin. Individuals who have a high risk of suffering a severe anaphylactic reaction should carry a pre-assembled syringe carrying adrenaline which can be injected intramuscularly (**Anapen** or **Epipen**). Adrenaline is also administered with local anaesthetics in order to constrict blood vessels and prolong local anaesthetic activity at the site of injection.

Adrenaline is fast acting which makes it ideal for emergency situations, but it has a short duration of action and is rapidly cleared from the system. Moreover, it switches on all possible adrenergic receptors, leading

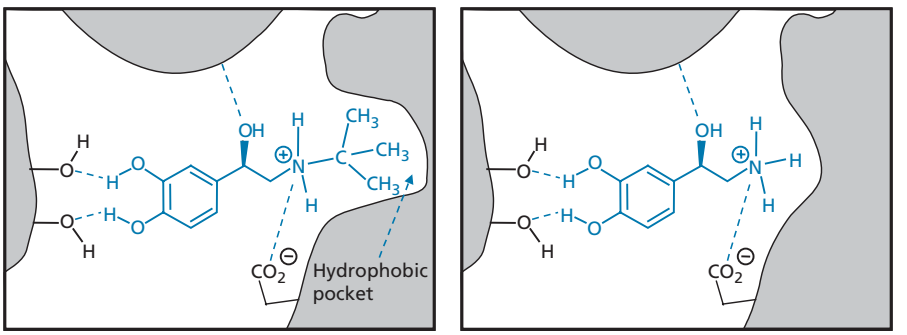


FIGURE 23.12 Comparison of β- and α-adrenoceptor binding sites.

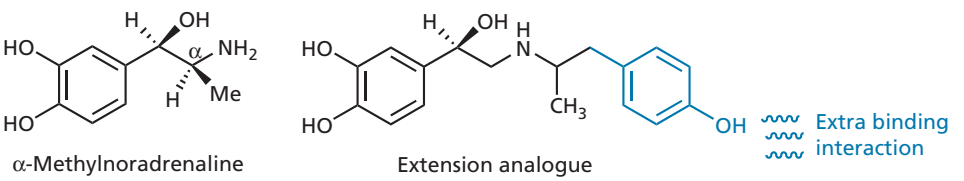


FIGURE 23.13 α-Methylnoradrenaline and extension analogue of noradrenaline.

to a whole range of side effects, including nausea, tachycardia, arrhythmias, hypertension, palpitations, anxiety, tremor, headache, restlessness, sweating, and dizziness. Therefore, if long-term medication is required, it is preferable to have agonists which are selective for specific adrenoceptors.

Ephedrine (Fig. 23.14) is a natural product present in various plants which have been used in folk medicine for many years. There are two asymmetric centres, and ephedrine exists as a racemate of the *R, S* and *S, R* stereoisomers. It activates both α– and β-adrenoceptors and has been used extensively in non-prescription preparations as a bronchodilator. It has also been used as a vasopressor and cardiac stimulant. As it lacks the phenolic groups of adrenaline, it is not susceptible to metabolism by catechol *O*-methyltransferase. It is also more lipophilic, and so it can cross the blood–brain barrier and act as a stimulant. Ephedrine is the active constituent of herbal remedies that contain the dried plant material ***ma-huang***.

Pseudoephedrine (Fig. 23.14) occurs naturally in certain plant species and is the *S,S* diastereomer of ephedrine. It is used as a nasal decongestant in preparations such as **Sudafed**, **Benylin**, and **Lemsip**. Unfortunately, it can be used in the illicit manufacture of amphetamines and so many pharmaceutical firms are starting to replace it with alternative decongestants.

23.10.2 α_1-, α_2-, β_1-, and β_3-Agonists

In general, there is limited scope for agonists at these receptors, although there is potential for anti-obesity drugs which act on the β_3-receptor. The β_1-agonist **dobutamine** (Fig. 23.15) is used to treat cardiogenic shock. Agonists acting on the α-adrenoceptors are less useful because these agents constrict blood vessels, raise blood pressure, and can cause cardiovascular problems. However, selective α_1 and α_2 agonists have found a number of uses as described in Box 23.1. **Clonidine** is a selective α_2-agonist which is used for the treatment of

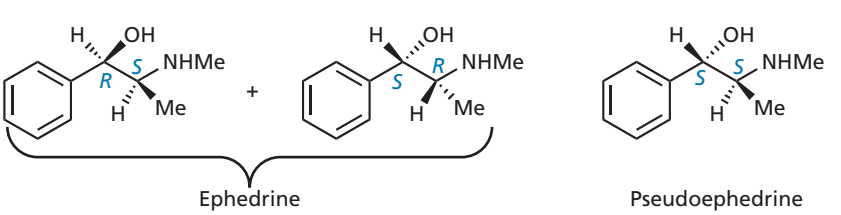
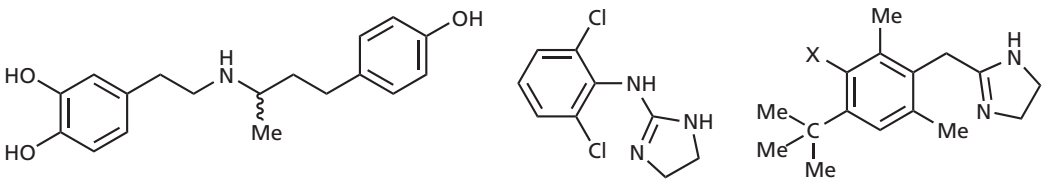


FIGURE 23.14 Ephedrine and pseudoephedrine.



Dobutamine | Clonidine | Oxymetazoline; X = OH, Xylometazoline; X = H

FIGURE 23.15 Adrenergic agonists.

hypertension. There is also strong evidence that it acts as an analgesic, especially if it is injected directly into the spinal cord. Selective α_1-agonists such as **oxymetazoline** and **xylometazoline** act as vasoconstrictors, and are used widely as topical medicines for the treatment of nasal congestion and bloodshot eyes.

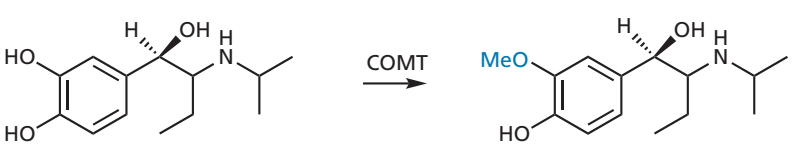
23.10.3 β_2-Agonists and the treatment of asthma

The most useful adrenergic agonists in medicine today are the β_2-agonists. These can be used to relax smooth muscle in the uterus to delay premature labour, but they are more commonly used for the treatment of asthma. Activation of the β_2-adrenoceptor results in smooth muscle relaxation and, as β_2-receptors predominate in bronchial smooth muscle, this leads to dilatation of the airways.

Adrenaline is often used to dilate the airways in emergency situations, but it is not suitable for long-term use because of its short duration of action and cardiovascular side effects (section 23.10.1). These side effects result from adrenaline interacting with all available adrenergic receptors and so a more selective agent for β_2-receptors is preferable.

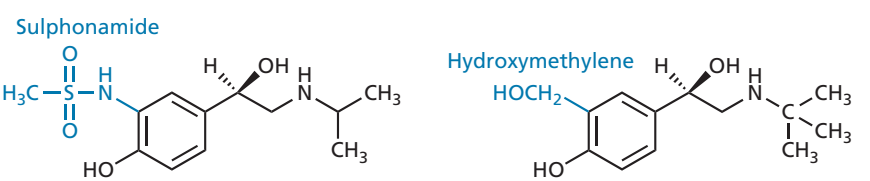
Isoprenaline (Fig. 23.11) shows some selectivity for β-receptors over α-receptors because of its bulky *N*-alkyl substituent. It was used for some time as an anti-asthmatic agent, but showed no selectivity between the different subtypes of β-receptors. Therefore, isoprenaline also activated the β_1-receptors of the heart, leading to unwanted cardiovascular effects. The search was then on to find a selective agonist for β_2-receptors which could be inhaled and have a long duration of action. Further research demonstrated that selectivity between different types of β-receptors could be obtained by introducing alkyl substituents to the side chain linking the aromatic ring and the amine, and/or varying the alkyl substituents on the nitrogen. For example, **isoetharine** (Fig. 23.16) was shown to be selective for β_2-receptors. Unfortunately, it was short lasting.

This short duration of action occurs because drugs such as isoetharine and adrenaline are taken up by tissues and methylated by the metabolic enzyme **catechol-*O*-methyltransferase** (COMT) to form an inactive ether. In order to prevent this, attempts were made to modify the *meta* phenol group and make it more resistant to metabolism (Fig. 23.17). This was no easy task as the



Isoetharine (active) | Inactive metabolite

FIGURE 23.16 Metabolism of (*R*)- isoetharine (COMT = catechol *O*-methyltransferase).



R-Soterenol | *R*-Salbutamol

FIGURE 23.17 Selective β_2 agonists.

phenolic group is important to activity, so it was necessary to replace it with a group which could still bind to the receptor and retain biological activity, but would not be recognized by the metabolic enzyme.

Various functional groups were tried at the *meta* position with a sulphonamide group ($MeSO_2NH$) proving successful. This resulted in a long-lasting selective β_2-agonist called **soterenol** (Fig. 23.17). However, this compound was never used clinically because a better compound was obtained in **salbutamol** (known as **albuterol** in the USA) (Box 23.2). Here, the *meta* phenol group of the catecholamine skeleton was replaced by a hydroxymethylene group—an example of a *group shift* strategy (section 14.2.6). Salbutamol has the same potency as isoprenaline, but is 2000 times less active on the heart. It has a duration of four hours and is not taken up by transport proteins or metabolized by COMT. Instead, it is more slowly metabolized to a phenolic sulphate. Salbutamol was marketed as a racemate and soon became a market leader in 26 countries for the treatment of asthma. The *R* enantiomer is 68 times more active than the *S* enantiomer. Furthermore, the *S* enantiomer accumulates to a greater extent in the body and produces side effects. Consequently, the pure *R* enantiomer (**levalbuterol**) was eventually marketed—an example of **chiral switching** (section 15.2.1).

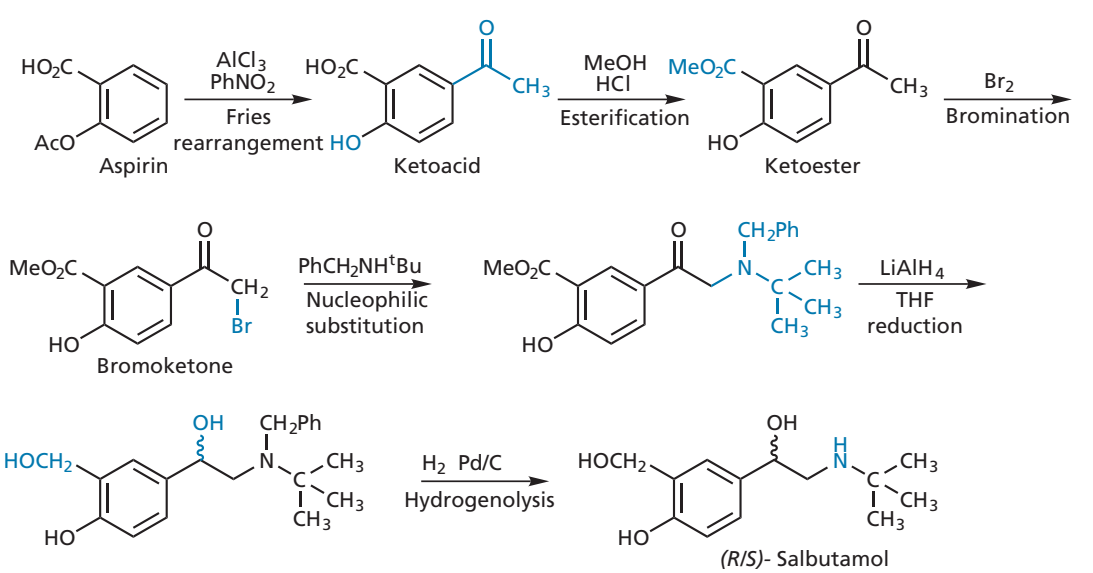
Several analogues of salbutamol have been synthesized to test whether the *meta* CH_2OH group could be modified further. These demonstrated the following requirements for the *meta* substituent:

- it has to be capable of taking part in hydrogen bonding—substituents such as $MeSO_2NHCH_2$, $HCONHCH_2$, and $H_2NCONHCH_2$ permitted this;
- substituents with an electron-withdrawing effect on the ring have poor activity (e.g. CO_2H);
- bulky *meta* substituents are bad for activity because they prevent the substituent adopting the necessary conformation for hydrogen bonding;
- the CH_2OH group can be extended to CH_2CH_2OH but no further.

Having identified the advantages of a hydroxymethyl group at the *meta* position, attention turned to the *N*-alkyl substituents. Salbutamol itself has a bulky t-butyl group. *N*-Arylalkyl substituents were added which would

BOX 23.2 Synthesis of salbutamol

Salbutamol is an important anti-asthmatic agent that can be synthesized from aspirin. **Fries rearrangement** of aspirin produces a ketoacid which is then esterified. A bromoketone is then prepared which allows the introduction of an amino group by nucleophilic substitution. The methyl ester and ketone are then reduced, and, finally, the *N*-benzyl protecting group is removed by hydrogenolysis.



Synthesis of salbutamol.

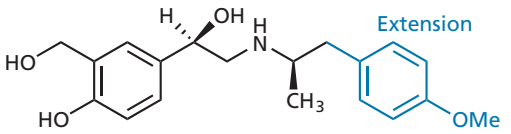


FIGURE 23.18 (*R*)- Salmefamol.

be capable of reaching the polar region of the binding site described earlier (*extension strategy*; section 23.9.2). For example, **salmefamol** (Fig. 23.18) is 1.5 times more active than salbutamol and has a longer duration of action (6 hours). The drug is given by inhalation, but in severe attacks it may be given intravenously.

Further developments were carried out to find a longer lasting agent in order to cope with nocturnal asthma—a condition which usually occurs at about 4 a.m. (commonly called the **morning dip**). It was decided to increase the lipophilicity of the drug because it was believed that a more lipophilic drug would bind more strongly to the tissue in the vicinity of the adrenoceptor and be available to act for a longer period. Increased lipophilicity was achieved by increasing the length of the *N*-substituent with a further hydrocarbon chain and aromatic ring. This led to **salmeterol** (Fig. 23.19), which has twice the potency of salbutamol and an extended action of 12 hours.

In 2009, **indacaterol** (Fig. 23.20) was approved in Europe for the treatment of chronic obstructive pulmonary disease and only needs to be taken once a day.

KEY POINTS

- The important binding groups in catecholamines are the two phenolic groups, the aromatic ring, the secondary alcohol, and the ionized amine.
- Placing a bulky alkyl group on the amine leads to selectivity for β-receptors over α-receptors.
- Extending the *N*-alkyl substituent to include a hydrogen-bonding group increases affinity for β-receptors.
- Agents which are selective for β_2-adrenoceptors are useful anti-asthmatic agents.
- Early β_2-agonists were metabolized by catechol-*O*-methyltransferase. Replacing the susceptible phenol group with a hydroxymethylene group prevented metabolism while retaining receptor interactions.
- Longer lasting anti-asthmatics have been obtained by increasing the lipophilic character of the compounds.

23.11 Adrenergic receptor antagonists

23.11.1 General α-/β-blockers

Carvedilol and **labetalol** are agents which act as antagonists at both the α– and β-adrenoceptors (Fig. 23.21). They have both been used as antihypertensives and carvedilol has been used to treat cardiac failure.

23.11.2 α-Blockers

Selective α_1-antagonists have been used to treat hypertension or to control urinary output. **Prazosin** (Fig. 23.22) was the first α_1-selective antagonist to be used for the treatment of hypertension, but it is short acting. Longer lasting drugs, such as **doxazosin** and **terazosin**, are better because they are given as once-daily doses. These agents relieve hypertension by blocking the actions of noradrenaline or adrenaline at the α_1-receptors of smooth muscle in blood vessels. This results in relaxation of the smooth muscle and dilatation of the blood vessels, leading to a lowering in blood pressure. These drugs have also been used for the treatment of patients with an enlarged prostate—a condition known as **benign prostatic hyperplasia**. The enlarged prostate

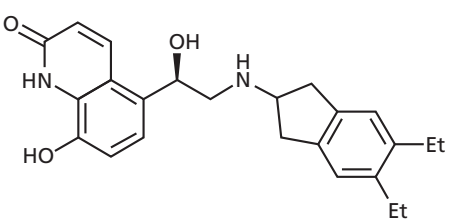


FIGURE 23.20 Indacaterol.

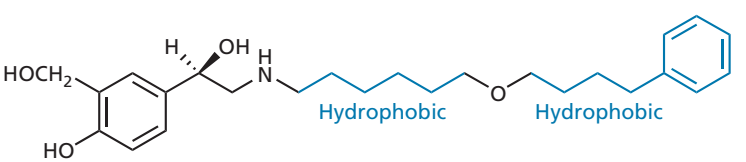


FIGURE 23.19 (*R*)- Salmeterol.

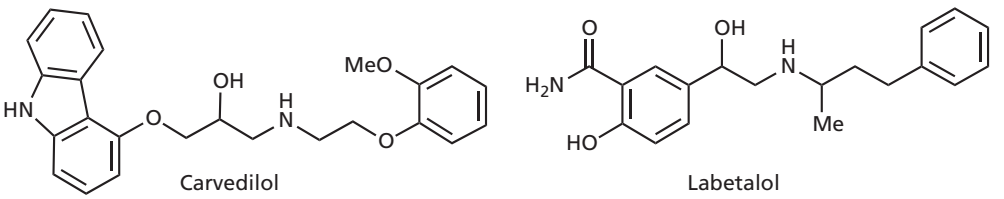


FIGURE 23.21 General α-/β-blockers.

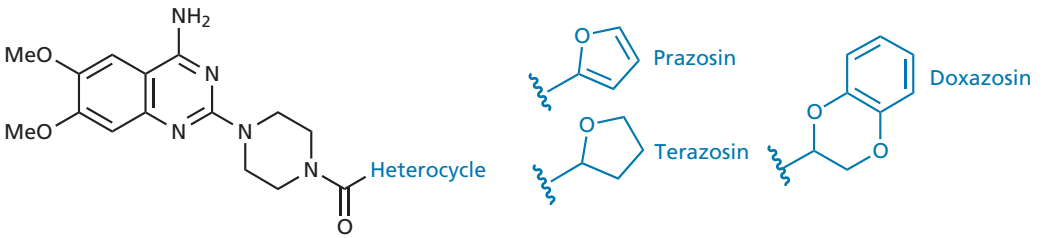


FIGURE 23.22 α_1-Selective antagonists.

puts pressure on the urinary tract and it becomes difficult to pass urine. The α_1-blockers prevent activation of the α_1-adrenoceptors that are responsible for smooth muscle contraction of the prostate gland, prostate urethra, and the neck of the bladder. This leads to smooth muscle relaxation at these areas, reducing the pressure on the urinary tract and helping the flow of urine. The agents are not a cure for the problem, but they relieve the symptoms.

α_2-Antagonists are being considered as antidepressants. Depression is associated with decreased release of noradrenaline and serotonin in the CNS, and antidepressants work by increasing the levels of one or both of these neurotransmitters. It may seem odd then, to consider an adrenergic antagonist as an antidepressant agent, but it makes sense when it is appreciated that the α_2-receptors are presynaptic adrenergic receptors or **autoreceptors** (section 23.6.3). Activation of these results in a decrease of noradrenaline released from the neuron, so blocking the autoreceptor will actually increase noradrenaline levels.

Mirtazepine (Fig. 23.23) is an antidepressant agent which blocks this receptor and increases the level of noradrenaline released. However, the α_2-receptor also controls the release of serotonin from serotonin nerve terminals, and so mirtazepine increases serotonin levels as well. It is not known for certain whether the antidepressant activity observed is due to increased noradrenaline levels or serotonin levels, or both. Current work is looking at the design of dual-action drugs which include the ability to block α_2-adrenoceptors (Case study 7).

Older antidepressants that are designed to increase noradrenaline and serotonin levels by different mechanisms can take 2–6 weeks before they have an effect. This delay in action is due to feedback control involving the α_2-receptors. When taken initially, the drugs certainly cause noradrenaline levels to increase, but feedback control counteracts this effect. It is only when the presynaptic receptors become desensitized that neurotransmitter levels increase sufficiently to have a clinical effect.

23.11.3 β-Blockers as cardiovascular drugs

23.11.3.1 First-generation β-blockers

The most useful adrenergic antagonists used in medicine today are the **β-blockers**, which were originally designed to act as antagonists at the β_1-receptors of the heart.

The first goal in the development of these agents was to achieve selectivity for β-receptors over α-receptors. **Isoprenaline** (Fig. 23.24) was chosen as the lead compound. Although this is an agonist, it is active at β-receptors and not α-receptors. Therefore, the goal

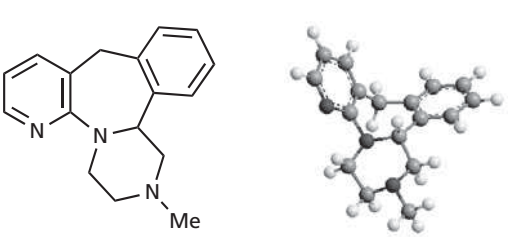


FIGURE 23.23 Mirtazepine.

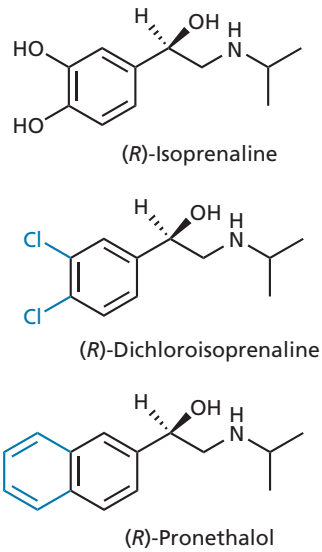


FIGURE 23.24 Partial β-agonists.

was to take advantage of this inherent specificity and modify the molecule to convert it from an agonist to an antagonist.

The phenolic groups are important for agonist activity, but this does not necessarily mean that they are essential for antagonist activity as antagonists can often block receptors by binding in a different way. Therefore, one of the early experiments was to replace the phenol groups with other substituents. Replacing the phenolic groups of isoprenaline with chloro substituents produced **dichloroisoprenaline** (Fig. 23.24). This compound was a partial agonist. In other words, it has some agonist activity, but it was weaker than a pure agonist. Nevertheless, dichloroisoprenaline blocks natural chemical messengers from binding and can therefore be viewed as an antagonist because it lowers adrenergic activity.

The next stage was to try to remove the partial agonist activity. A common method of converting an agonist into an antagonist is to add an extra aromatic ring. This can sometimes result in an extra hydrophobic interaction with the receptor which is not involved when the agonist binds. This, in turn, means a different induced fit between the ligand and the binding site, such that the ligand binds without activating the receptor. Therefore, the chloro groups of dichloroisoprenaline were replaced by an extra benzene ring to give a naphthalene ring system. The product obtained (**pronethalol**; Fig. 23.24) was still a partial agonist, but was the first β-blocker to be used clinically for angina, arrhythmia, and high blood pressure.

Research was carried out to see what effect extending the length of the chain between the aromatic ring and the amine would have. One of these projects involved the introduction of various linking groups between the naphthalene ring and the ethanolamine portion of the molecule (Fig. 23.25). At this stage, a chance event occurred. The researchers wanted to use β-naphthol as a starting material in order to introduce a linking group of X = O-CH_2 (Fig. 23.25). However, the stores had run out of the reagent and so α-naphthol was used instead to prepare the structure now known as **propranolol** (Fig. 23.25). In this structure, the chain was at the 1-position of the naphthalene ring rather than the 2-position, and nobody expected it to be active. To everyone's astonishment, propranolol was found to be a pure antagonist, having 10–20 times greater activity than pronethalol. It was introduced into the clinic for the treatment of angina and is now the benchmark against which all β-blockers are rated. Its contribution to medicine was so significant that its inventor, James Black, received the Nobel Prize in 1988. The *S*-enantiomer is the active form, although propranolol is used clinically as a racemate. When the original target structure from β-naphthol was eventually synthesized, it was similar in properties to pronethalol.

23.11.3.2 Structure–activity relationships of aryloxypropanolamines

Propranolol is an example of an aryloxypropanolamine structure (see Box 23.3). A large number of aryloxypropanolamines have been synthesized and tested, demonstrating the following SAR (Fig. 23.26):

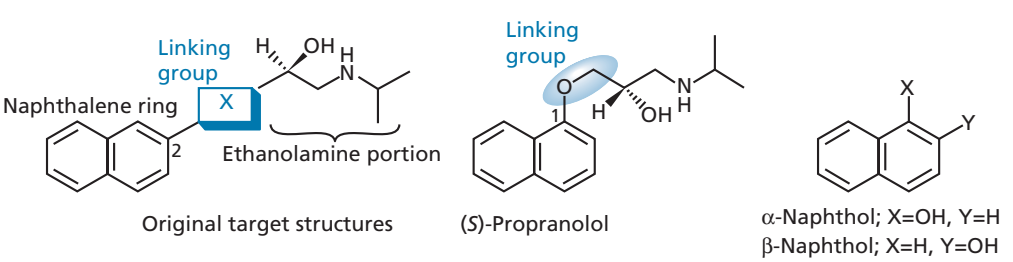


FIGURE 23.25 Chain extension tactics and the discovery of propranolol.

BOX 23.3 Synthesis of aryloxypropanolamines

Propranolol is a first-generation β-blocker and acts as an antagonist at β-adrenoceptors. The synthesis of propranolol is relatively simple and can be easily adapted to produce a large number of analogues. A phenol is reacted with 2-chloromethyloxirane such that nucleophilic substitution of the alkyl chloride takes place. The resulting product is then treated with an amine to ring-open the epoxide. This introduces the amine and generates the secondary alcohol at the same time. Because of the nature of the synthetic route, a huge variety of phenols and amines can be used to produce different analogues. There is an asymmetric centre in the final product, but it is only possible to synthesize the racemate using this route. A different, and more expensive, route would have to be used to synthesize the *R*- or the *S*-enantiomer.

Synthesis of aryloxypropanolamines.

- branched bulky *N*-alkyl substituents such as isopropyl and *t*-butyl groups are good for β-antagonist activity, suggesting an interaction with a hydrophobic pocket in the binding site (compare β-agonists);
- variation of the aromatic ring system is possible and heteroaromatic rings can be introduced, such as those in pindolol and timolol (Fig. 23.27);
- substitution on the side chain methylene group increases metabolic stability but lowers activity;
- the alcohol group on the side chain is essential for activity;
- replacing the ether oxygen on the side chain with S, CH_2, or NMe is detrimental, although a tissue-selective β-blocker has been obtained replacing O with NH;
- *N*-alkyl substituents longer than isopropyl or *t*-butyl are less effective (but see next point);
- adding an *N*-arylethyl group, such as -$CHMe_2$-CH_2Ph or CHMe-CH_2Ph, is beneficial (*extension*);
- the amine must be secondary.

23.11.3.3 Selective β_1-blockers (second-generation β-blockers)

Propranolol is a non-selective β-antagonist which acts as an antagonist at β_2-receptors, as well as β_1-receptors. Normally, this is not a problem, but it is serious if the patient is asthmatic as the propranolol could initiate an asthmatic attack by antagonizing the β_2-receptors in bronchial smooth muscle. This leads to contraction of bronchial smooth muscle and closure of the airways.

Practolol (Fig. 23.28) is not as potent as propranolol, but it is a selective cardiac β_1-antagonist which does not

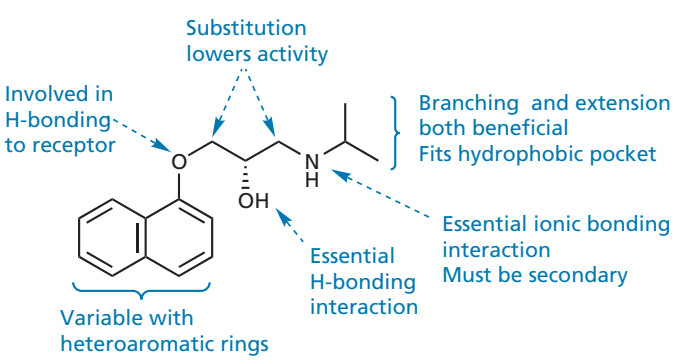


FIGURE 23.26 Structure–activity relationships of aryloxypropanolamines.

BOX 23.4 Clinical aspects of β-blockers

β-Blockers are used for the treatment of angina, myocardial infarction, arrhythmias, and hypertension. The effects of **propranolol** and other first-generation β-blockers depends on how active the patient is. At rest, propranolol causes little change in heart rate, output, or blood pressure. However, if the patient exercises or becomes excited, propranolol reduces the resulting effects of circulating adrenaline. The β-blockers were originally intended for use in angina, but they also had an unexpected antihypertensive activity (i.e. they lowered blood pressure). Indeed, the β-blockers are now more commonly used as antihypertensives rather than for the treatment of angina. The antihypertensive activity arises from the following effects:

- action at the heart to reduce cardiac output;
- action at the kidneys to reduce renin release; renin catalyses formation of angiotensin I, which is quickly converted to angiotensin II—a potent vasoconstrictor (Case study 2);
- action in the CNS to lower the overall activity of the sympathetic nervous system;

These effects override the fact that β-blockers block the β-receptors on blood vessels and would normally cause vasoconstriction.

First-generation β-blockers have various side effects, such as the following:

- bronchoconstriction in asthmatics—this is a dangerous side effect and the β-blockers are not recommended for patients with asthma;
- fatigue and tiredness of limbs due to reduced cardiac output;
- CNS effects (dizziness, nightmares, and sedation), especially with lipophilic β-blockers, such as **propranolol, pindolol**, and **oxprenolol**, all of which can cross the blood–brain barrier. More water-soluble agents, such as **nadolol**, are less likely to have such side effects (Fig. 1);
- coldness of the extremities;
- heart failure for patients on the verge of a heart attack—the β-blockers produce a fall in the resting heart rate and this may push some patients over the threshold;
- inhibition of noradrenaline release at synapses.

The second-generation β-blockers are more cardioselective and have fewer side effects. However, they still have some effect on bronchial smooth muscle and so they should only be used on asthmatic patients when there is no alternative treatment. Water-soluble β-blockers, such as **atenolol**, are less likely to enter the brain and so there is less risk of sleep disturbance or nightmares. β-Blockers which act as partial agonists (e.g. **acebutolol**) tend to cause less bradycardia and may also cause less coldness of the extremities. **Esmolol** is a short-acting β-blocker with a rapid onset of action. It is administered by slow intravenous injection during surgical procedures in order to treat any tachycardia (rapid heart rates) that might occur.

β-Blockers have a range of other clinical uses apart from cardiovascular medicine. They are used to counteract overproduction of catecholamines resulting from an enlarged thyroid gland or tumours of the adrenal gland. They can also be used to alleviate the trauma of alcohol and drug withdrawal, as well as relieving the stress associated with situations such as exams, public speaking, and public performances. There are some studies which suggest that propranolol might be a useful treatment for post-traumatic stress disorder and for the removal of traumatic memories. **Timolol** and **betaxolol** are used in the treatment of glaucoma (although their mechanism of action is not clear), while propranolol is used to treat anxiety and migraine.

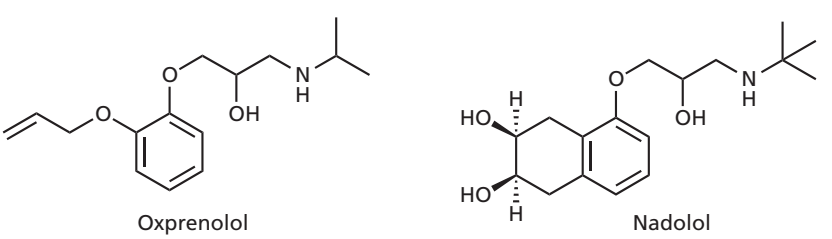


FIGURE 1 Oxprenolol and nadolol.

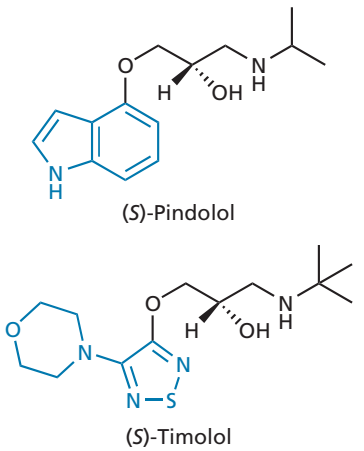


FIGURE 23.27 β_1-Antagonists containing heteroaromatic ring systems.

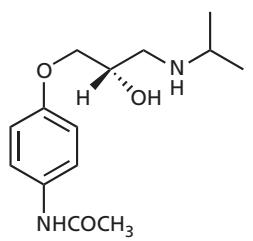


FIGURE 23.28 (*S*)-Practolol.

block vascular or bronchial β_2-receptors. It is much safer for asthmatic patients and, because it is more polar than propranolol, it has many fewer CNS effects.

Practolol was marketed as the first cardioselective β_1-blocker for the treatment of angina and hypertension, but after a few years it had to be withdrawn because of unexpected, but serious, side effects in a very small number of patients. These side effects included skin rashes, eye problems, and peritonitis.

Further investigations were carried out and it was demonstrated that the amido group had to be in the *para* position of the aromatic ring rather than the *ortho* or *meta* positions if the structure was to retain selectivity for the cardiac β_1-receptors. This implied that there was an extra hydrogen bonding interaction taking place with β_1-receptors (Fig. 23.29) which was not taking place with β_2-receptors.

Replacement of the acetamido group with other groups capable of hydrogen bonding led to a series of cardioselective β_1-blockers which included **acebutolol**, **atenolol**, **metoprolol**, and **betaxolol** (Fig. 23.30).

23.11.3.4 Short-acting β-blockers

Most clinically useful β-blockers should have a reasonably long duration of action such that they need only be taken once or twice a day. However, there is an advantage in having a very short-acting agent with a half-life measured in minutes rather than hours, because they can be administered during surgical procedures to treat any cardiac problems that may arise during the operation. **Esmolol** (Fig. 23.31) is one such agent. It has a rapid onset of action and is administered if the heart starts to beat too rapidly. Because it is a short-acting agent, its actions are quickly reversed once administration has been stopped.

Practolol was the lead compound used in the development of esmolol. The amide group was replaced with an ester, with the expectation that the ester would act as a bioisostere for the amide. Moreover, it was anticipated that the ester group would prove susceptible to esterase

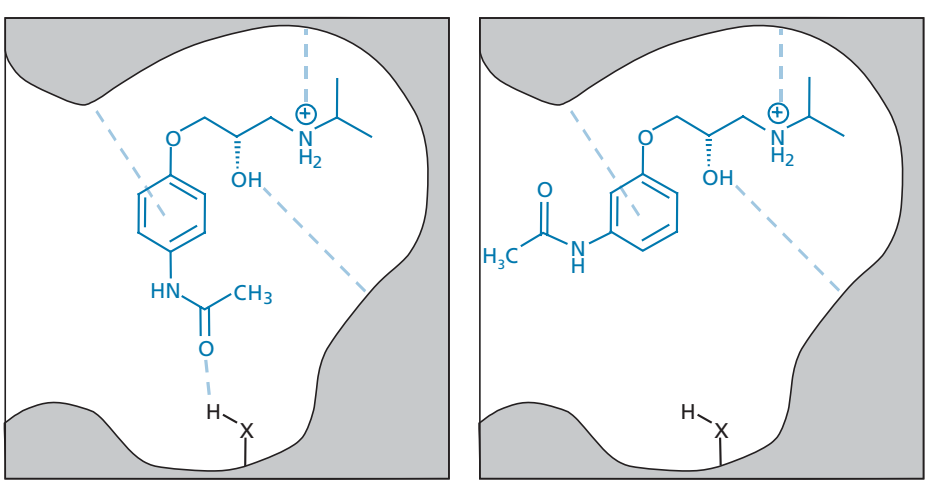


FIGURE 23.29 Binding interactions of antagonists with β_1-receptors.

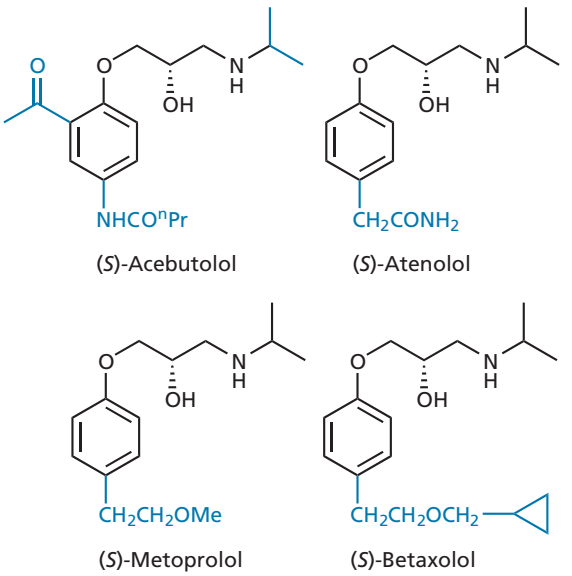


FIGURE 23.30 Second-generation β-blockers.

enzymes and be rapidly hydrolysed to an inactive metabolite. The aryl ester was indeed active as a β-blocker, but was not hydrolysed rapidly enough to be clinically useful. It was concluded that the aromatic ring was acting as a steric shield to the esterase enzymes, and so linker chains were inserted between the aromatic ring and the ester group to make the ester more 'exposed'. An ethylene linker proved ideal resulting in the discovery of esmolol. The structure is slightly more potent than practolol and is significantly more cardioselective. Once administration has been stopped, it takes 12 minutes to reach 80% recovery and 20 minutes to reach full recovery. The inactive carboxylic acid metabolite that is formed is rapidly conjugated and excreted.

KEY POINTS

- Antagonists of β-adrenoceptors are known as β-blockers.
- Replacing the catechol ring with a naphthalene ring changes an agonist into a partial agonist.
- Variation of the linking group between naphthalene and the ethanolamine moiety resulted in the first β-antagonists.
- SAR of aryloxypropanolamines reveal the importance of the ionized amine, the side chain alcohol, and the ether linkage. Substituents on the nitrogen can be varied. The naphthalene ring can be replaced by various heterocyclic rings.
- First-generation β-blockers inhibit all β-receptors and can induce asthma in susceptible patients.
- Second-generation β-blockers show selectivity for β_1-receptors over β_2-receptors. Aryloxypropanolamines bearing a hydrogen-bonding group at the *para* position of an aromatic ring show β_1-selectivity.
- Third-generation β-blockers bear an extended *N*-substituent, which includes a hydrogen-bonding group capable of an extra interaction with the β_1-adrenoceptor.

23.12 Other drugs affecting adrenergic transmission

In the previous sections, we discussed drugs which act as agonists or antagonists at adrenergic receptors. However, there are various other drug targets involved in the adrenergic transmission process which are important in controlling adrenergic activity. In this section, we briefly cover some of the most important aspects of these.

23.12.1 Drugs that affect the biosynthesis of adrenergics

In section 23.4, we identified **tyrosine hydroxylase** as the regulatory enzyme for catecholamine biosynthesis. This makes it a potential drug target. For example, **α-methyltyrosine** (Fig. 23.32) inhibits tyrosine hydroxylase and is sometimes used clinically to treat tumour cells which overproduce catecholamines.

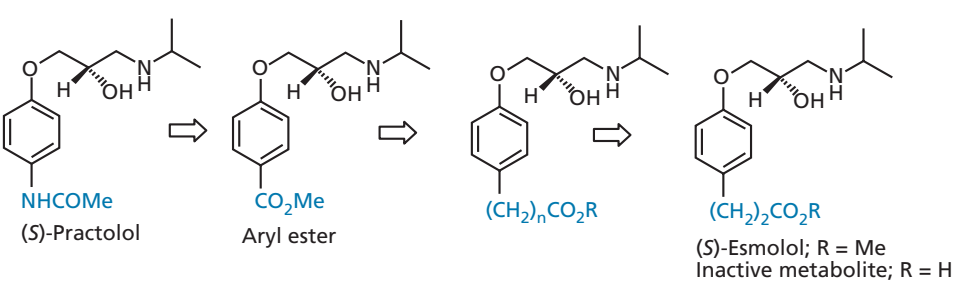


FIGURE 23.31 Development of short-acting β-blockers.

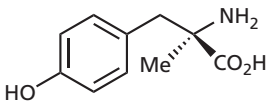


FIGURE 23.32 α-Methyltyrosine.

It is sometimes possible to 'fool' the enzymes of a biosynthetic process into accepting an unnatural substrate such that a false transmitter is produced and stored in the storage vesicles. For example, **α-methyldopa** is converted and stored in vesicles as **α-methylnoradrenaline** (Fig. 23.33) and displaces noradrenaline. Such false transmitters are less active than noradrenaline, so this is another way of down-regulating the adrenergic system. The drug has serious side effects, however, and is limited to the treatment of hypertension in late pregnancy.

A similar example is the use of **α-methyl-*m*-tyrosine** in the treatment of shock. This unnatural amino acid is accepted by the enzymes of the biosynthetic pathway and converted to **metaraminol** (Fig. 23.34).

23.12.2 Drugs inhibiting the uptake of noradrenaline into storage vesicles

The uptake of noradrenaline into storage vesicles can be inhibited by drugs. The natural product **reserpine** binds to the transport protein responsible for transporting noradrenaline into the vesicles and so noradrenaline accumulates in the cytoplasm where it is metabolized by monoamine oxidase (MAO). As noradrenaline levels drop, adrenergic activity drops. Reserpine was once prescribed as an antihypertensive agent, but it has serious side effects (e.g. depression). Therefore, it is no longer used.

23.12.3 Release of noradrenaline from storage vesicles

The storage vesicles are also the targets for the drugs **guanethidine** and **bretylium** (Fig. 23.35). Guanethidine is taken up into presynaptic neurons and storage vesicles by the same transport proteins as noradrenaline, and it displaces noradrenaline in the same way as reserpine. The drug also prevents exocytosis of the vesicle and so prevents release of the vesicle's contents into the synaptic gap. Guanethidine is an effective antihypertensive agent, but is no longer used in the clinic because of side effects resulting from non-specific inhibition of adrenergic nerve transmission. Bretylium works in the same way as guanethidine and is sometimes used to treat irregular heart rhythms.

23.12.4 Reuptake inhibitors of noradrenaline into presynaptic neurons

Once noradrenaline has interacted with its receptor, it is normally taken back into the presynaptic neuron by a transport protein. This transport protein is an important target for various drugs which inhibit noradrenaline uptake and thus prolong adrenergic activity. The tricyclic antidepressants, **desipramine**, **imipramine**, and **amitriptyline** (Fig. 23.36) work in this fashion in the CNS and were the principal treatment for depression from the 1960s to the 1980s.

It has been proposed that the tricyclic antidepressants (TCAs) are able to act as inhibitors because they are partly superimposable on noradrenaline. This can be seen in Fig. 23.37 where the aromatic ring and the nitrogen atoms of noradrenaline are overlaid with the nitrogen atom and one of the aromatic rings of desipramine.

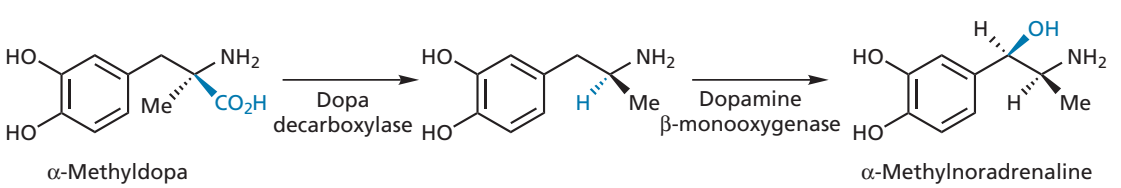


FIGURE 23.33 A false transmitter—α-methylnoradrenaline.

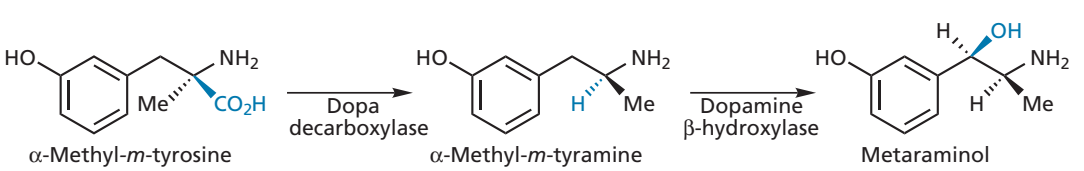


FIGURE 23.34 A false transmitter—metaraminol.

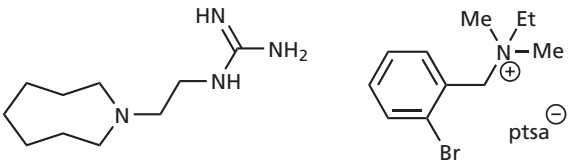


FIGURE 23.35 Agents that affect adrenergic activity (ptsa = *para*-toluenesulphonate).

Test your understanding and practise your molecular modelling with Exercise 23.2.

Note that the tricyclic system of desipramine is V-shaped, so that when the molecules are overlaid the second aromatic ring is held above the plane of the noradrenaline structure. Planar tricyclic structures would be expected to be less active as inhibitors, because the second aromatic ring would then occupy the space required for the amine nitrogen.

Unfortunately, the TCAs are not selective and interact with a variety of other targets, such as the reuptake protein for serotonin, the sodium and calcium ion channels in the heart, and the receptors for histamine, acetylcholine, and noradrenaline (mainly H_1, M_1 and α_1 respectively). Blockage of the transport protein for serotonin is beneficial to antidepressant activity, but interaction with ion channels and receptors results in various side effects including cardiotoxicity. Those agents containing tertiary amines (e.g. imipramine and amitriptyline) have the greatest side effects on the cholinergic system.

Newer antidepressant agents with better selectivity have now been developed and are termed **selective noradrenaline reuptake inhibitors** (SNRIs). **Reboxetine** (Fig. 23.38) is one such example and was marketed in 2003. It selectively inhibits noradrenaline uptake and has no appreciable action on cholinergic or α_1-adrenergic receptors. It also rapidly desensitizes presynaptic α_2-adrenergic receptors, which further enhances its activity and speeds up its onset of action. Dual noradrenaline and serotonin reuptake inhibitors such as **duloxetine** and **venlafaxine** (Fig. 23.38) are clinical agents which block the transport proteins for both noradrenaline and serotonin, but are more selective than the classical TCAs.

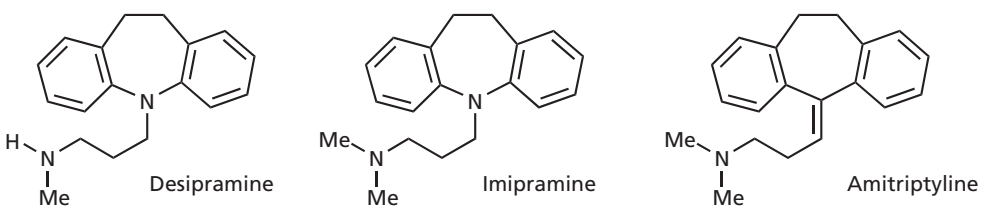


FIGURE 23.36 Tricyclic antidepressants.

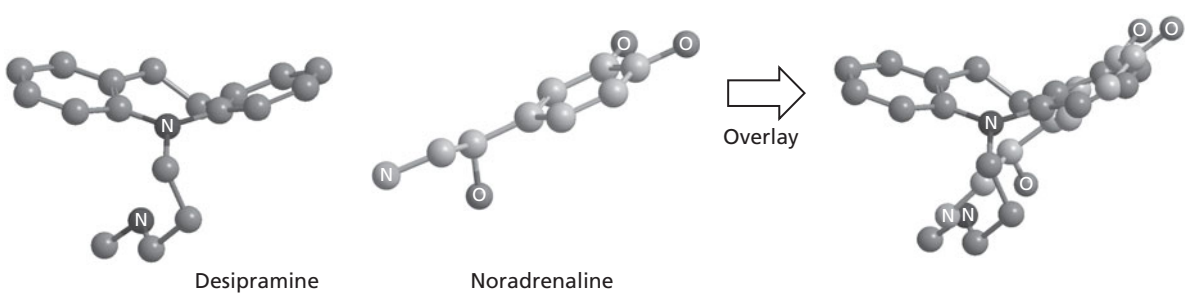


FIGURE 23.37 Overlay of desipramine and noradrenaline.

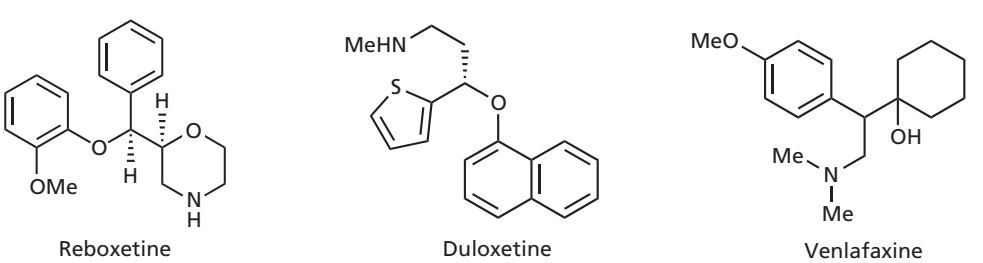


FIGURE 23.38 Reuptake inhibitors.

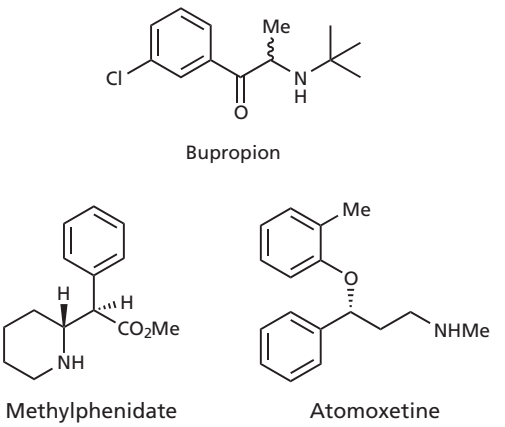


FIGURE 23.39 Adrenergic agents acting in the central nervous system.

Bupropion (**Zyban**; Fig. 23.39) inhibits the reuptake of both noradrenaline and dopamine. It has been used for the treatment of depression, and as an aid to giving up smoking (see also section 22.10.2.5). It is also being considered for the treatment of obesity in combination with the opioid antagonist naltrexone. This represents a massive market as it is predicted there will be 400 million obese people worldwide by 2015.

Stimulants acting as noradrenaline reuptake inhibitors have been used for the treatment of **attention deficit hyperactivity disorder**. This is the most commonly diagnosed childhood behavioural disorder and is associated with inattention, hyperactivity, and impulsivity. **Methylphenidate** (**Ritalin**; Fig. 23.39) is the most commonly prescribed medication for this disorder, while **atomoxetine** (Fig. 23.39) was approved in 2002. Both agents lead to increased levels of noradrenaline and dopamine in the brain.

Cocaine also inhibits noradrenaline uptake when it is chewed from coca leaves, but this time the inhibition is in the PNS rather than the CNS. Chewing coca leaves was well known to the Incas as a means of increasing endurance and suppressing hunger, and they would chew the leaves whenever they were faced with situations requiring long periods of physical effort or stamina. When the coca leaves are chewed, cocaine is absorbed into the systemic blood supply and acts predominantly on peripheral adrenergic receptors to increase adrenergic activity. Nowadays, cocaine abusers prefer to smoke or snort the drug, which allows it to enters the CNS more efficiently. There, it inhibits the uptake of dopamine rather than noradrenaline, resulting in its CNS effects.

Some amines such as **tyramine**, **amphetamine**, and **ephedrine** (Figs. 23.10 and 23.14) closely resemble noradrenaline in structure and are transported into the nerve cell by noradrenaline's transport proteins. Once in the cell, they are taken up into the vesicles. Because these amines are competing with noradrenaline for transport proteins, noradrenaline is more slowly reabsorbed into the nerve cells. Moreover, as the foreign amines are transported into the nerve cell, noradrenaline is transported out by those same transport proteins. Both of these facts mean that more noradrenaline is available to interact with its receptors. Therefore, amphetamines and similar amines have an indirect agonist effect on the adrenergic system.

Phentermine (Fig. 23.10) is very similar in structure to amphetamine, and causes increased levels of adrenaline and noradrenaline that result in hunger suppression. Consequently, it was approved in 1959 to suppress the appetite of obese patients. A combination of phentermine with the anticonvulsant and antimigraine drug **topiramate** is currently being considered as a treatment for obesity.

23.12.5 Inhibition of metabolic enzymes

Inhibition of the enzymes responsible for the metabolism of noradrenaline should prolong noradrenaline activity. We have seen how amines, such as tyramine, amphetamine, and ephedrine, inhibit the reuptake of noradrenaline into the presynaptic neuron. These amines also inhibit MAO, one of the important enzymes involved in the metabolism of noradrenaline. This, in turn, leads to a

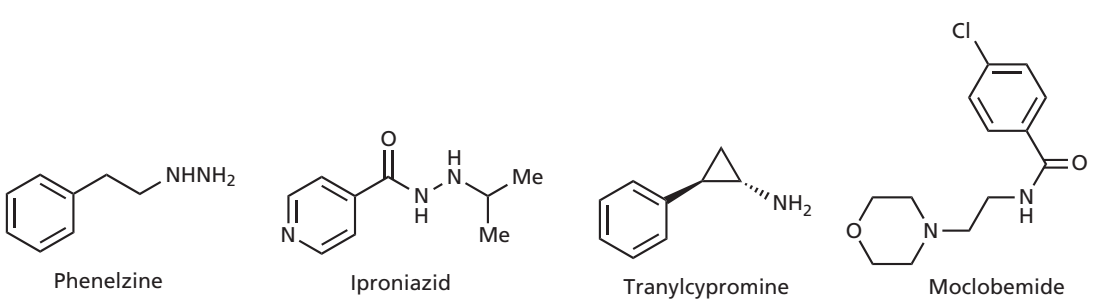


FIGURE 23.40 Monoamine oxidase inhibitors.

build-up in noradrenaline levels and an increase in adrenergic activity.

Monoamine oxidase inhibitors (**MAOIs**) such as **phenelzine**, **iproniazid**, and **tranylcypromine** (Fig. 23.40) have been used clinically as antidepressants, but other classes of compound such as the **tricyclic antidepressants** and **selective serotonin reuptake inhibitors** (SSRIs) are now favoured as they have fewer side effects. It is important to realize that the MAOIs affect the levels of all neurotransmitters that are normally metabolized by these enzymes, in particular, noradrenaline, dopamine, and serotonin. As a result of these widespread effects, it is difficult to be sure what mechanism is most involved in the antidepressant activity of these agents.

Another serious problem associated with MOAIs is their interaction with other drugs and food. A well-known example of this is the **cheese reaction**. Ripe cheese contains **tyramine** which is normally metabolized by MOAs in the gut wall and the liver, and so never enters the systemic circulation. If the MOAs are inhibited by MOAIs, tyramine is free to circulate round the body, enhancing the adrenergic system and leading to acute hypertension and severe headaches.

Better agents, such as **moclobemide** (Fig. 23.40), have been designed to act selectively on one of the isozymes of MAO (MAO-A; Box 7.4). They have also been designed to be reversible rather than irreversible in their action. This has the advantage that high levels of ingested tyramine will displace the inhibitor from MAO-A in the gut, allowing the enzyme to metabolize tyramine and prevent the high blood levels that would lead to toxic effects.

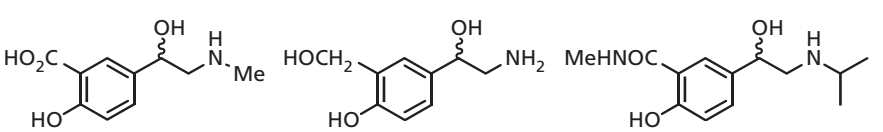
In recent years, there has been interest in using MAOIs as part of the treatment for Alzheimer's disease, as blocking MAO would lower the levels of free radical species present in the brain. A hybrid molecule with the ability to inhibit MAO and the cholinesterase enzymes has reached clinical trials (sections 13.3.14 and 22.15).

KEY POINTS

- Inhibitors of catecholamine biosynthesis affect adrenergic activity.
- Drugs that are similar to tyrosine may be converted by the catecholamine biosynthetic pathway to structures that act as false transmitters and lower adrenergic activity.
- The uptake and release of noradrenaline from storage vesicles can be inhibited by certain drugs.
- The tricyclic antidepressants inhibit the reuptake of noradrenaline into presynaptic neurons by blocking transport proteins. Adrenergic activity is increased in the CNS.
- Cocaine increases peripheral adrenergic activity by blocking noradrenaline reuptake. In the CNS it inhibits the reuptake of dopamine.
- Amphetamines compete with noradrenaline for the transport proteins responsible for transporting noradrenaline back into the presynaptic neuron. Adrenergic activity is increased in the CNS.
- Monoamine oxidase inhibitors (MAOIs) inhibit the metabolic enzyme monoamine oxidase (MAO) and result in increased levels of noradrenaline and other catecholamines.

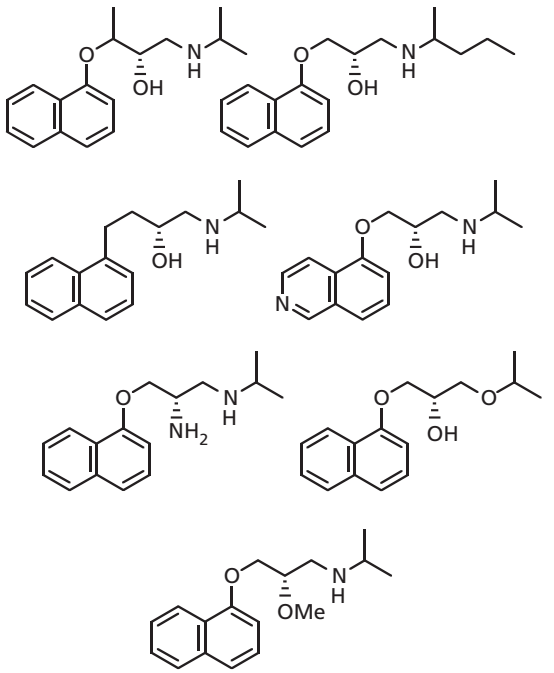
QUESTIONS

1. How would you synthesize the following structures to test their adrenergic agonist activity?



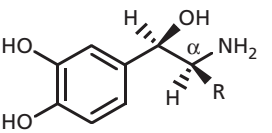
2. Suggest how you might synthesize the adrenergic antagonist, pindolol (Fig. 23.27).

3. Suggest whether the structures below are likely to have good or bad activity as β-blockers.



4. The catechol system is important for the binding of adrenergic agonists, yet is not required for adrenergic antagonists. Why should this be the case?

5. How would α-substitution affect the metabolism of adrenergic agents and why?



6. What synthetic complication arises from introducing an α-substituent as described in Question 5?

7. The active enantiomer of aryloxypropanolamines is the *S*-form, whereas the active enantiomer of arylethanolamines is the *R*-form. Does this imply that the two agents are binding differently to the binding site?

FURTHER READING

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Titles for general further reading are listed on p.763.

24 The opioid analgesics

24.1 History of opium

The search for a safe, orally active, and non-addictive analgesic based on the opiate structure is one of the oldest fields in medicinal chemistry, yet one where true success has proved elusive. The term **opiates** refers to narcotic analgesics that are structurally related to morphine, whereas **opioids** is the term used to cover all the synthetic, semi-synthetic, naturally occurring, and endogenous compounds that interact with opioid receptors in the body.

It is important to appreciate that the opioids are not the only compounds which are of use in the relief of pain: there are several other classes of analgesic, including **aspirin**. However, these compounds operate by different mechanisms from those used by the opioids and are effective against different types of pain.

The first opioids were extracted from opium—the sticky exudate obtained from the opium poppy (*Papaver somniferum*). Opium is, perhaps, the oldest herbal medicine known to humanity and has been used for myriad afflictions. It was particularly effective as a sedative and a treatment for diarrhoea. By the eighteenth and nineteenth centuries, preparations of opium known as **laudanum** had become extremely popular in Europe, not least in the British Royal Navy where the concoction was used by ships' surgeons as an analgesic and sedative. Laudanum also proved to be one of the first examples of a drug taken for 'recreational purposes'. A number of famous nineteenth-century authors and poets are known to have taken the drug on a regular basis, with several becoming addicted. Opium was also smoked in opium dens, which became widespread around the world—especially among Chinese communities. A growing realization of the long-term problems associated with taking opium led eventually to laws being introduced in the twentieth century that restricted its use to medical and scientific purposes.

For additional material see Web article 9: history of opium.

24.2 The active principle: morphine

24.2.1 Isolation of morphine

Opium contains a complex mixture of over 20 alkaloids. The principal alkaloid in the mixture, and the one responsible for opium's analgesic and sedative activity, is **morphine** (Fig. 24.1). Pure morphine was first isolated in 1803, but it was not until 1833 that chemists at the Edinburgh firm of Macfarlane and Co. (now Macfarlane–Smith) were able to isolate and purify it on a commercial scale. Because morphine was poorly absorbed orally, it was little used in medicine until the hypodermic syringe was invented in 1853. Injecting the drug directly into the blood supply revealed that morphine was a potent analgesic and sedative, and was far more effective than opium.

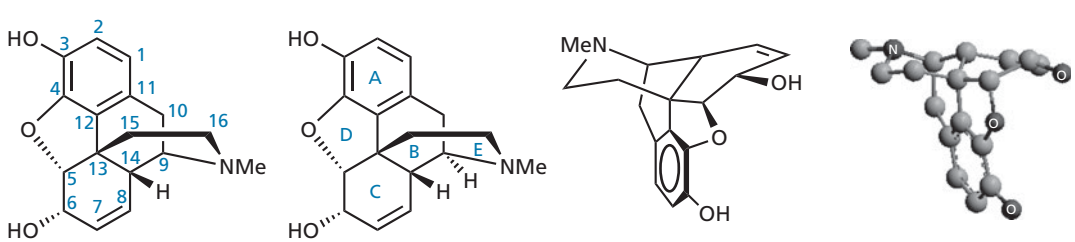


FIGURE 24.1 Structure of morphine showing different representations.

BOX 24.1 Clinical aspects of morphine

Morphine is still one of the most effective painkillers available to medicine and is currently the drug of choice in the treatment of severe pain. It is especially good for treating dull, constant pain, rather than sharp, periodic pain. It acts mainly in the brain and appears to work by elevating the pain threshold, thus decreasing the brain's awareness of pain. Unfortunately, it has a large number of side effects, which include depression of the respiratory centre, constipation, excitation, euphoria, nausea, vomiting, itching, pupil constriction, tolerance, and dependence.

Some side effects can be advantageous. For example, the observation that morphine causes constipation has led to the design of opioids which are used in the treatment of diarrhoea. Euphoria can be a useful side effect when treating pain in terminally ill patients. However, the effect is not observed in patients suffering severe pain. Moreover, the euphoric effects of morphine in healthy individuals can encourage people to take the drug for the wrong reasons. Other side effects, such as constipation, itching, and nausea may not appear serious, but they can become so uncomfortable that treatment has to be stopped.

The dangerous side effects of morphine are those of tolerance and dependence, allied with the effects that it can have on breathing. In fact, the most common cause of death from a morphine overdose is suffocation. This is caused by morphine decreasing the sensitivity of the respiratory centre in the brain to carbon dioxide. Tolerance and dependence in the one drug are particularly dangerous and lead to severe withdrawal symptoms when the drug is no longer taken.

Withdrawal symptoms associated with morphine include anorexia, weight loss, pupil dilation, chills, excessive sweating, abdominal cramps, muscle spasms, hyperirritability, lacrimation, tremor, increased heart rate, and increased blood pressure. No wonder addicts find it hard to kick the habit!

For additional material see Web article 10: clinical applications of opioids.

However, there was a price to be paid. The risks of addiction, tolerance, and respiratory depression (Box 24.1) were also greatly increased.

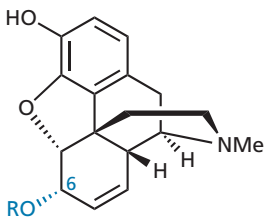
24.2.2 Structure and properties

Morphine was an extremely complex molecule by nineteenth-century standards, and identifying its structure posed a huge challenge to chemists. By 1881, the functional groups on morphine had been identified, but it took many more years to establish the full structure. In those days, the only way to find out the structure of a complicated molecule was to degrade the compound into simpler molecules that were already known and could be identified. For example, the degradation of morphine with a strong base produced methylamine, which established that there was a N-CH_3 fragment in the molecule. From such evidence, chemists would propose a structure. This would be like trying to work out the structure of a bombed cathedral from the rubble. Once a structure had been proposed, chemists would attempt to synthesize it. If the properties of the synthesized compound were the same as those of the natural compound, then the structure was proven. This was a long, drawn-out process made all the more difficult because nineteenth-century chemists had few of the synthetic reagents or procedures available today. As a result, it was not until 1925 that Sir Robert Robinson proposed the correct structure of morphine. A full synthesis was achieved in 1952 and the structure was finally proved by X-ray crystallography in 1968 (164 years after the original isolation). The molecule contains five rings labelled A–E and has a pronounced T shape. It is basic because of the tertiary amino group, but it also contains a phenol, alcohol, aromatic ring, ether bridge, and alkene double bond. The nitrogen atom of the amine can undergo inversion, which means that the *N*-methyl group can slowly interconvert between the axial and the equatorial positions.

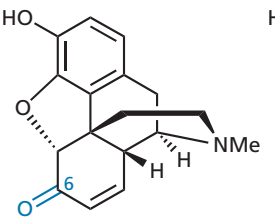
Test your understanding and practise your molecular modelling with Exercise 24.1.

24.3 Structure–activity relationships

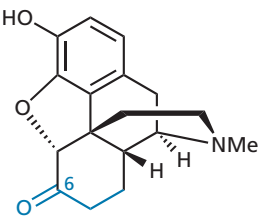
Following the discovery of morphine, it was natural for chemists to use the known reactions of the day to synthesize various analogues and to see whether these had analgesic activity or not. Different tests have been used to assess analgesic activity, which complicates the picture. Nevertheless, some conclusions can be made regarding the importance, or otherwise, of different functional groups. For example, **heterocodeine**, **6-ethylmorphine**, **6-acetylmorphine**, **6-oxomorphine**, **hydromorphone**, and **dihydromorphine** (Fig. 24.2) are examples of structures where the alkene or 6-hydroxy groups have been modified or removed. Analgesic activity is retained in these structures, indicating that neither of these groups is crucial to activity. However, analgesic activity drops significantly for **codeine**, **dihydrocodeine**, and **3-ethylmorphine**, indicating the importance of the phenolic group.



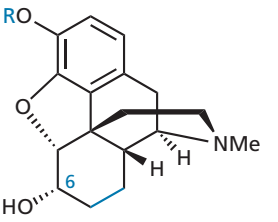
Heterocodeine; R = Me
6-Ethylmorphine; R = Et
6-Acetylmorphine; R = Ac



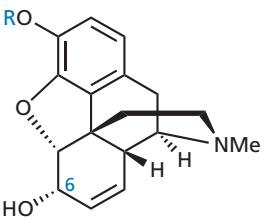
6-Oxomorphine



Hydromorphone



Dihydromorphine; R = H
Dihydrocodeine; R = Me



Codeine; R = Me
3-Ethylmorphine; R = Et
3-Acetylmorphine; R = Ac

FIGURE 24.2 Analogues of morphine.

For additional material see Web article 11: testing methods.

These, and other, results led to the conclusion that the important functional groups for analgesic activity are the phenol OH group, the aromatic ring, and the tertiary amine which is protonated and ionized when the drug interacts with its target binding site. These functional groups play an important role in binding the drug to the binding site by the intermolecular bonding forces indicated in Fig. 24.3.

At this stage, it is worth making some observations on the stereochemistry of morphine. Morphine is a chiral molecule containing several asymmetric centres and exists naturally as a single stereoisomer. When morphine was first synthesized, it was made as a racemic mixture of the naturally occurring enantiomer plus its mirror-image enantiomer (Fig. 24.4). It was noticeable that the activity of synthetic morphine was half that of natural morphine and separation of the enantiomers revealed that the unnatural enantiomer had no analgesic activity. This should come as no surprise as the macromolecules targeted by drugs are themselves asymmetric and are able to distinguish between the enantiomers of a chiral drug.

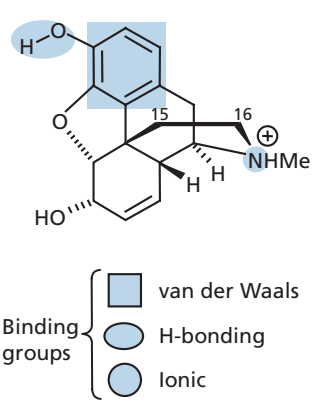
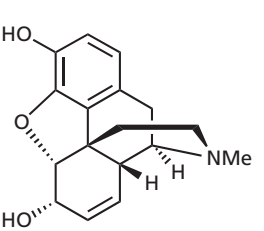
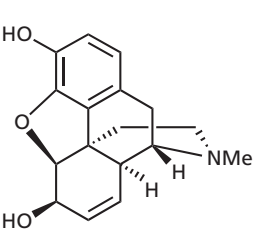


FIGURE 24.3 Important functional groups for analgesic activity in morphine.

Epimerization of a single asymmetric centre is not beneficial for activity either, as changing the stereochemistry of even one asymmetric centre can result in a drastic change of shape that could affect how the molecule binds to its target binding site. For example, epimerization of the asymmetric centre at position 14 results in a stereoisomer that has only 10% the activity of morphine (Fig. 24.5).



Natural morphine



Mirror image of morphine

FIGURE 24.4 Morphine and its mirror image.

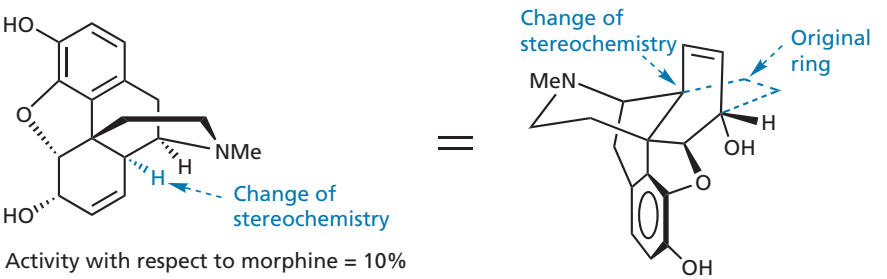


FIGURE 24.5 Epimerization of a single asymmetric centre.

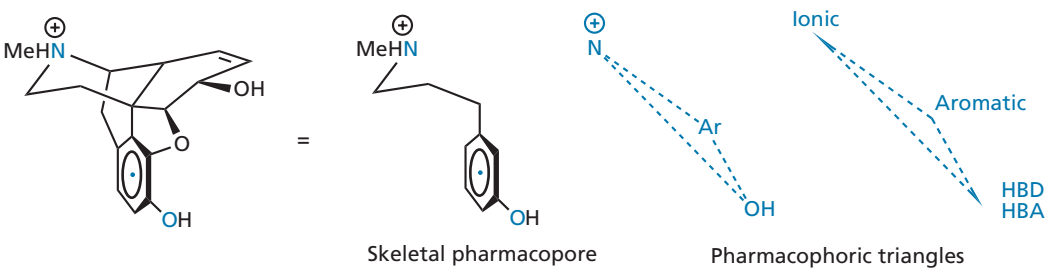


FIGURE 24.6 Opioid pharmacophores for morphine and related opioids.

To sum up, analgesic activity is not only related to the presence of the important functional groups defined earlier, but to their relative position with respect to each other—the **pharmacophore** (section 13.2). Opioid pharmacophores can be defined in different ways, either by defining a simple skeleton that links the important functional groups or by pharmacophoric triangles where the corners correspond to functional groups or binding interactions (Fig. 24.6).

Finally, a word of caution regarding the importance of the phenol group. There is no doubt that the phenol group is an important part of the opioid pharmacophore for receptor binding, but it is not necessarily as important when one considers the analgesic activity of different opioid structures *in vivo*. That is because pharmacokinetic factors also have an important role in the level of analgesic activity observed. As we will see, there are examples of opioid analgesics where the phenol group is masked or missing altogether. This has the advantage of making the molecule less susceptible to metabolism by phase II conjugation reactions (section 11.5.5). Moreover, the masking or absence of the phenol group increases hydrophobicity such that the molecule is absorbed from the gastrointestinal tract more easily and/or can cross the blood–brain barrier more efficiently. Consequently, the increased levels of opioid reaching the target receptors can compensate for weaker binding interactions. Some opioids with a masked phenol group also act as prodrugs, where the masking group is removed by metabolic enzymes. There are even instances where the phenol group is no longer required as part of the binding pharmacophore. Simpler, more flexible opioids are believed to interact with opioid receptors in a different way from morphine such that the phenol group becomes redundant (see sections 24.6.3.4 and 24.6.3.5). Alternatively, more complex opioids such as the orvinols contain additional binding groups that can compensate for the masking of the phenol group (section 24.6.4).

Test your understanding and practise your molecular modelling with Exercises 24.2 and 24.3.

KEY POINTS

- Morphine is extracted from opium and is one of the oldest drugs used in medicine.
- Morphine is a powerful analgesic but has various side effects, the most serious being respiratory depression, tolerance, and dependence.
- The structure of morphine consists of five rings forming a T-shaped molecule.
- The important binding groups on morphine are the phenol, the aromatic ring, and the ionized amine.

24.4 The molecular target for morphine: opioid receptors

Although morphine was isolated in the nineteenth century, it took many years to discover how it produced its

analgesic effect. It is now known that morphine activates analgesic receptors in the central nervous system (CNS) and that this leads to a reduction in the transmission of pain signals to the brain. There are three main types of analgesic or opioid receptor that are activated by morphine: the mu (μ), kappa (κ), and delta (δ) receptors. All of them are G-protein-coupled receptors which activate G_i or G_o signal proteins (section 4.7 and Chapter 5). Morphine acts as an agonist at all three types of receptor and activation leads to a variety of cellular effects depending on the type of receptor involved. These include the opening of potassium ion channels, the closing of calcium ion channels, or the inhibition of neurotransmitter release—all of which reduce the transmission of pain signals from one nerve cell to another. A newer form of terminology has now been introduced where the μ, κ, and δ receptors are called the **MOR**, **KOR**, and **DOR** receptors respectively. Nevertheless, we will continue to use the original nomenclature in this chapter as it is still more prevalent.

There are differences between the three opioid receptors in terms of their effects. Activation of the μ receptor results in sedation and the strongest analgesic effect, but this receptor is also associated with the strongest and most dangerous side effects of respiratory depression, euphoria, and addiction. Activation of the δ and κ receptors does not produce the same level of analgesia, but there are less serious side effects. For example, the δ receptor does not cause sedation, euphoria, or physical dependence, while the κ receptor has no effect on breathing, is free of euphoric effects, and has a low risk of physical dependence. The κ receptor is considered the safest of the three types of receptor and a lot of research has been carried out to develop κ-selective opioids. Unfortunately, it has been discovered that activation of the κ receptor can lead to sedation and psychological side effects, such as anxiety, depression, and psychosis. As a result, these agents have failed to fulfil their original promise.

A fourth opioid receptor was later identified in the 1990s which shows a lot of structural similarity to the classical opioid receptors. It was, therefore, referred to as the **opioid receptor-like receptor** (**ORL1**), but is now known as the **NOR** receptor. It was originally classed as an **orphan receptor** as its endogenous ligand was not known, but an endogenous ligand has now been identified as a polypeptide structure called **nociceptin**. Activation of the ORL1 receptor can either increase or decrease the sensitivity to pain depending on the location of the receptor and the method by which agonists are administered.

Morphine, and most of its analogues, bind strongly to the μ receptor, and less strongly to the κ or δ receptors. This explains why it has been so difficult to find a safe, powerful analgesic as the receptor with which they bind most strongly produces the most serious side effects.

Recently, it has been demonstrated that opioid receptors can occur as homomeric and heteromeric dimers. This has important consequences for drug design (section 24.9.2).

For additional material see Web article 12: opioid dimers and receptors.

24.5 Morphine: pharmacodynamics and pharmacokinetics

Pharmacodynamics refers to the manner in which a drug binds to its target and produces a pharmacological effect. The functional groups that are important to the activity of morphine act as binding groups in the following manner (Fig. 24.7):

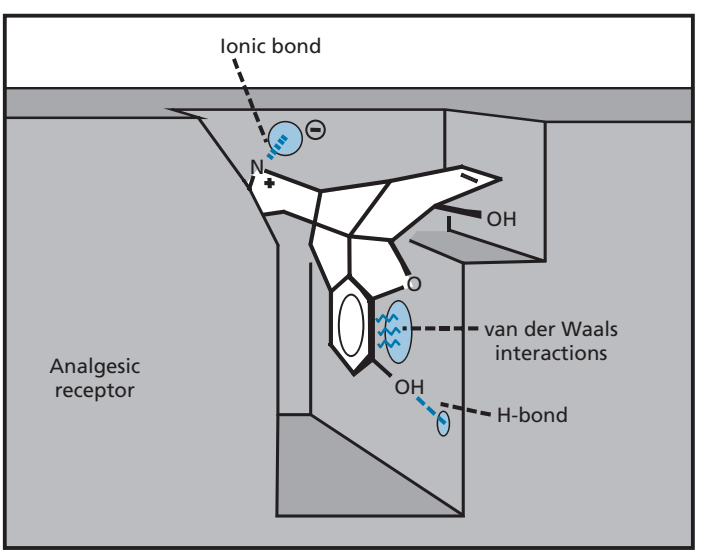


FIGURE 24.7 Binding interactions of morphine with the hypothetical binding site of an opioid receptor.

- the amine nitrogen is protonated and charged allowing it to form an ionic bond with a negatively charged region of the binding site;
- the phenol acts as a hydrogen bond donor and forms a hydrogen bond to a hydrogen bond acceptor in the binding site;
- the rigid structure of morphine means that its aromatic ring has a defined orientation with respect to the rest of the molecule, allowing van der Waals interactions with a suitable hydrophobic location in the binding site.

Pharmacokinetics refers to the ability of a drug to reach its target and to survive in the body. Morphine is relatively polar and is poorly absorbed from the gut, and so it is normally given by intravenous injection. However, only a small percentage of the dose administered actually reaches the analgesic receptors in the CNS because of the blood–brain barrier (section 11.4.5). This acts as a barrier to polar drugs and effectively prevents any ionized drug from crossing into the CNS. For example, the *N*-methyl quaternary salt of morphine (Fig. 24.8) is inactive when it is administered by intravenous injection because it is blocked by the blood–brain barrier. If this same compound is injected directly into the brain, however, it has a similar analgesic activity to morphine. If morphine was fully charged, it would not be able to enter the brain either. However, the amine group is a weak base and so morphine can exist both as the free base and ionized form. This means that morphine can cross the blood–brain barrier as the free base then ionize in order to interact with the opioid receptors. The p*Ka* values of useful analgesics should be 7.8–8.9 such that there is an approximately equal chance of the amine being ionized or un-ionized at physiological pH.

The extent to which different structures cross the blood–brain barrier plays an important role in analgesic activity. For example, **normorphine** (Fig. 24.8) has only 25% the activity of morphine. The secondary NH group is more polar than the original tertiary group, and so normorphine is less efficient at crossing the blood–brain barrier, leading to a drop in activity.

It is possible to get increased levels of morphine in the brain by using prodrugs (section 14.6) where some of the polar functional groups are masked. It is interesting to compare the activities of morphine, **6-acetylmorphine** (Fig. 24.2), and **diamorphine** (heroin) (Fig. 24.8). The most active (and the most dangerous) compound of the three is 6-acetylmorphine, which is four times more active than morphine. Diamorphine is also more active than morphine by a factor of two, but less active than 6-acetylmorphine. How do we explain this?

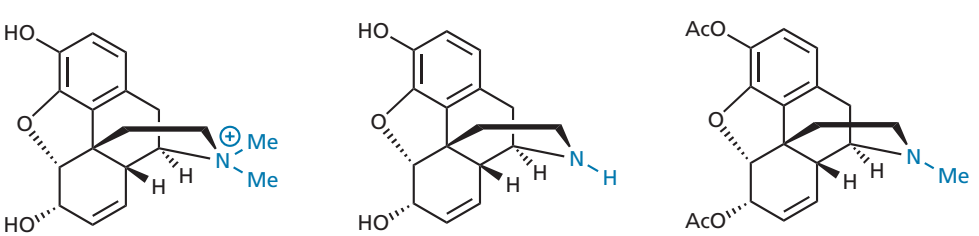
6-Acetylmorphine is less polar than morphine and will cross the blood–brain barrier into the CNS more quickly and in greater concentrations. The phenolic group is free and therefore it will interact immediately with the analgesic receptors.

Diamorphine has two masked polar groups, and so it is the most efficient compound of the three in crossing the blood–brain barrier. Before it can bind to the opioid receptors, however, the 3-acetyl group has to be removed by esterases in the CNS. This means that it is more powerful than morphine because of the ease with which it crosses the blood–brain barrier, but less powerful than 6-acetylmorphine because the 3-acetyl group has to be hydrolysed.

Diamorphine and 6-acetylmorphine are both more potent analgesics than morphine. Unfortunately, they also have greater side effects, as well as severe tolerance and dependence characteristics. Diamorphine is still used in Canada and the UK to treat terminally ill patients suffering chronic pain, but 6-acetylmorphine is considered so dangerous that its synthesis is banned in many countries.

The lifetime of morphine in the blood supply is quite short, with 90% of each dose being metabolized and excreted within 24 hours. The presence of the alcohol and phenol groups means that the molecule readily undergoes phase II conjugation reactions (section 11.5.5) and the resulting polar conjugates are quickly excreted.

Drug metabolism also plays an important role in the activity of different opioid structures. For example, **codeine** (Fig. 24.2) is the 3-methyl ether of morphine and has a binding affinity for the opioid receptor which is only



N-Methyl quaternary salt of morphine | Normorphine | Diamorphine

FIGURE 24.8 Analogues of morphine with differing abilities to cross the blood–brain barrier.

0.1% of morphine. It also has no analgesic activity when it is injected directly into the brain. This is not surprising as methylation of the phenol group would be expected to disrupt its ability to act as a binding group (section 13.1.1). What *is* surprising is the fact that codeine has an analgesic effect which is 20% that of morphine—much better than expected. Why is this? The answer lies in the fact that codeine is metabolized by *O*-demethylation in the liver to give morphine. Thus, codeine can be viewed as a prodrug for morphine. Codeine is present in opium and is used for treating moderate pain, coughs, and diarrhoea (see also section 11.5.6).

KEY POINTS

- The important binding interactions between morphine and opioid receptors are a hydrogen bonding interaction via a phenol group, an ionic interaction via a charged amine, and van der Waals interactions involving the aromatic ring.
- There are three different analgesic receptors (μ, κ, and δ) with which morphine interacts. All require the presence of a pharmacophore involving the phenol, aromatic ring, and ionized amine.
- Morphine binds most strongly to the μ receptor. This receptor is responsible for the serious side effects associated with morphine.
- The κ receptor is responsible for analgesia and sedation, and lacks serious side effects. However, activation causes psychological side effects which have prevented κ-selective opioids from reaching the market.
- The δ receptor is favoured by the enkephalins.
- The opioid receptors are G-protein-linked receptors.
- The ability of opioids to cross the blood–brain barrier plays an important role in analgesic activity.
- Some analgesics such as codeine and diamorphine act as prodrugs for morphine.

24.6 Morphine analogues

Considering the problems associated with morphine there is a need for novel analgesic agents which retain the analgesic activity of morphine, but which have fewer side effects and can be administered orally. The following sections illustrate how many of the classical drug design strategies described in Chapter 13 were effective in obtaining novel analgesic structures.

24.6.1 Variation of substituents

A series of alkyl substituents were placed on the phenolic group, but the resulting compounds were inactive or poorly active. We have already identified that the phenol group must be free for good analgesic activity.

The removal of the *N*-methyl group to give normorphine allowed a series of alkyl chains to be added to the basic centre (Box 24.2). These results are discussed in the next section.

24.6.2 Drug extension

The strategy of drug extension described in section 13.3.2 involves the addition of extra functional groups to a lead compound in order to probe for extra binding regions in a binding site. Many analogues of morphine containing extra functional groups have been prepared, but have rarely shown any improvement. There are two exceptions, however. The introduction of a hydroxyl group at position 14 (Fig. 24.9) increases activity for structures such as **oxymorphone** and **oxycodone**, and suggests that there might be an extra hydrogen bond interaction taking place with the binding site.

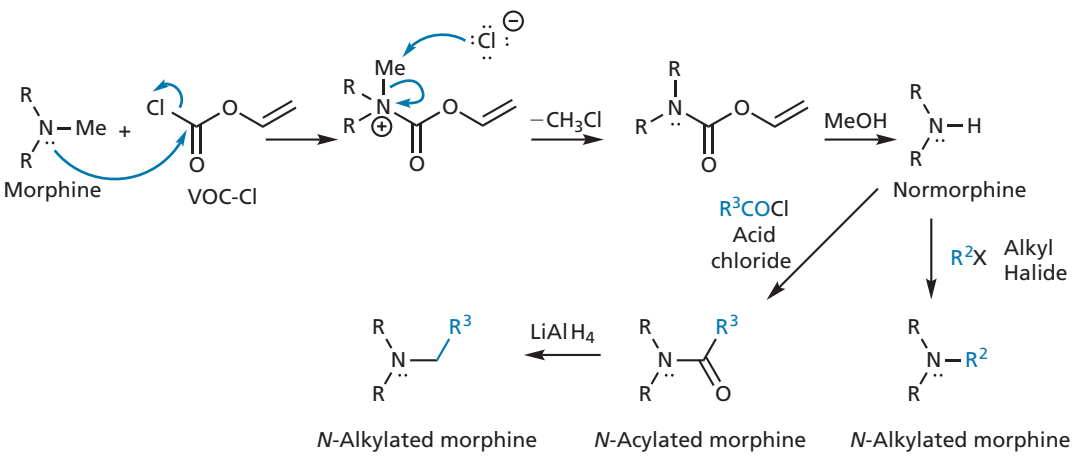
The other exception involves the variation of alkyl substituents on the nitrogen atom. As the alkyl group is increased in size from a methyl to a butyl group, the activity drops to zero (Fig. 24.10). With a larger group, such as a pentyl or a hexyl group, activity recovers slightly. None of this is particularly exciting, but when a phenethyl group is attached, the activity increases 14-fold relative to morphine—a strong indication that a hydrophobic binding region has been located which interacts favourably with the new aromatic ring (Fig. 24.9).

To conclude, the size and nature of the group on nitrogen is important to the activity spectrum. Drug extension can lead to better binding by making use of additional binding interactions.

Before leaving this subject, it is worth describing important results which occurred when an allyl or a cyclopropylmethyl group was attached to nitrogen (Fig. 24.11) (see also section 24.7). **Naloxone** and **naltrexone** have no analgesic activity at all, and **nalorphine** retains only weak analgesic activity. Not very exciting, you might think. What *is* important is that they act as antagonists to morphine, i.e. they bind to the analgesic receptors without 'switching them on' and then block morphine from binding. As a result, morphine can no longer act as an analgesic. One might be hard pushed to see an advantage in this and with good reason. If we are just considering analgesia, there is none. However, the fact that morphine is blocked from all its receptors means that none of its side effects are produced either, and it is the blocking of these effects that makes antagonists extremely useful. For example, accident victims are sometimes given an overdose of morphine. If this is not treated quickly, then the casualty may die of suffocation. Administering nalorphine means that the antagonist can block morphine from binding to opioid receptors and lead to recovery.

BOX 24.2 Synthesis of *N*-alkylated morphine analogues

The synthesis of *N*-alkylated morphine analogues is easily achieved by removing the *N*-methyl group from morphine to give **normorphine**, then alkylating the amino group with an alkyl halide. Removal of the *N*-methyl group was achieved originally by a von Braun degradation with cyanogen bromide, but is now more conveniently carried out using a chloroformate reagent such as vinyloxycarbonyl chloride. The final alkylation step can sometimes be profitably replaced by a two-step process involving an acylation to give an amide, followed by reduction.



Demethylation and alkylation of the basic centre.

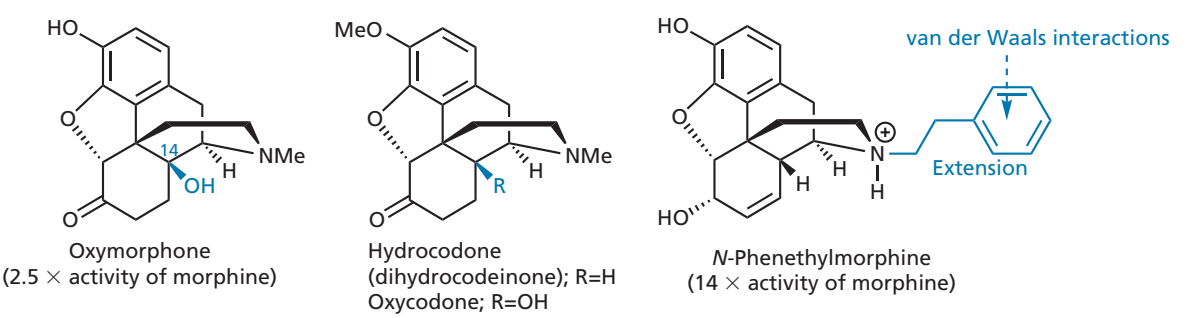


FIGURE 24.9 Extended analogues of morphine.

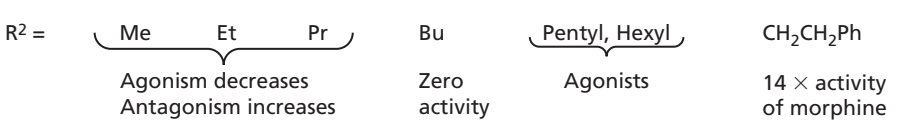


FIGURE 24.10 Change in activity with respect to alkyl group size.

The opioid antagonists have also proved useful in treating addictions. Naltrexone is eight times more active than naloxone as an antagonist and is given to drug addicts who have been weaned off morphine or heroin. Naltrexone blocks the opioid receptors, preventing the effects that addicts seek if they are tempted to restart their habit. As a result, they are more likely to remain abstinent. Naltrexone in combination with **bupropion** (section 23.12.4) is also being considered for the treatment of obesity. **Nalmefine** (Fig 24.11) is a close analogue which is currently undergoing clinical trials as an oral treatment for alcoholism. It binds more strongly than naltrexone to

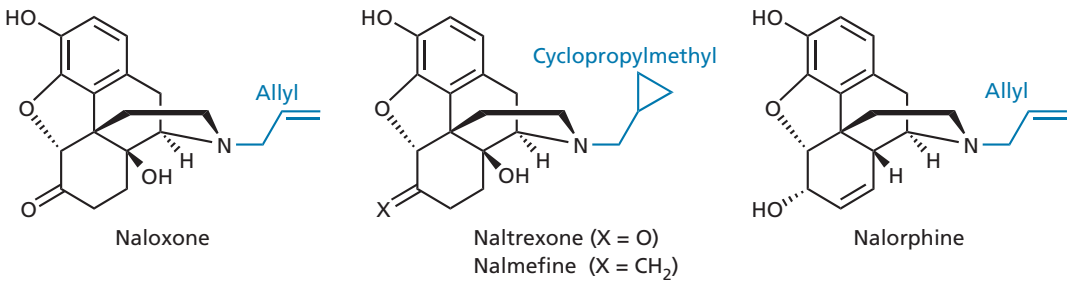


FIGURE 24.11 Antagonists to morphine.

opioid receptors and blocks the effects of natural opioids released as a result of drinking.

There is another interesting observation related to these antagonists. For many years, chemists had been trying to find a morphine analogue without serious side effects. There had been so little success in this search that many believed it would be impossible to separate the analgesic effects from the side effects. The fact that the antagonist naloxone blocks both the analgesic and side effects of morphine did nothing to change that view. However, the properties of nalorphine offered a glimmer of hope.

Nalorphine acts as an antagonist at the μ receptor and as a weak agonist at the κ receptor. Therefore, the slight analgesia observed with nalorphine is due to partial activation of the κ receptor. Moreover, this activity appears to be free of the undesired side effects associated with morphine. This was the first sign that a non-addictive, safe analgesic might be possible if structures were made that were selective for the κ receptor. Unfortunately, nalorphine has hallucinogenic and psychological side effects, which result from activation of the κ receptor.

24.6.3 Simplification or drug dissection

We turn now to more drastic alterations of the morphine structure and ask whether the complete carbon skeleton is really necessary. If the molecule could be simplified, it would be easier to synthesize analogues (section 13.3.8). The structure of morphine has five rings and five chiral centres (Fig. 24.12) and analogues were made to see whether structures with fewer rings and chiral centres were still active.

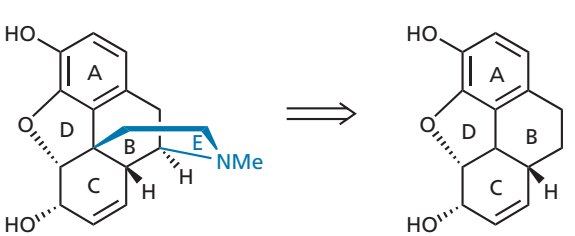


FIGURE 24.12 Removing ring E from morphine.

24.6.3.1 Removing ring E

Removing ring E leads to complete loss of activity. This emphasizes the importance of the basic nitrogen to analgesic activity.

24.6.3.2 Removing ring D

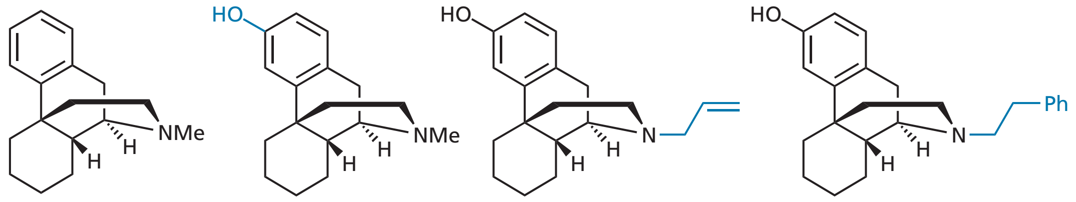
Removing the oxygen bridge, as well as the alcohol and alkene functional groups gives a series of tetracyclic compounds called the **morphinans** (Fig. 24.13), which have useful analgesic activity. This demonstrates that the oxygen bridge is not essential. The structures shown in Fig. 24.13 also have three asymmetric centres, rather than five.

N-**Methylmorphinan** was the first such compound tested and is only 20% as active as morphine, but as the phenolic group is missing, this is not surprising. The more relevant **levorphanol** structure is five times more active than morphine and, although side effects are also increased, levorphanol has a massive advantage over morphine in that it can be taken orally and lasts much longer in the body. This is because levorphanol is not metabolized in the liver to the same extent as morphine. As might be expected, the mirror image of levorphanol (**dextrorphan**) has insignificant analgesic activity.

The same strategy of drug extension already described for the morphine structures was tried on the morphinans, with similar results. For example, adding an allyl substituent on the nitrogen gives antagonists. Adding a phenethyl group to the nitrogen greatly increases potency. Adding a 14-hydroxyl group also increases activity.

To conclude:

- morphinans are more potent and longer-acting than their morphine counterparts, but they also have higher toxicity and comparable dependence characteristics;
- modifications carried out on the morphinans have the same structure–activity relationship (SAR) results as



N-Methylmorphinan (20% activity of morphine) | Levorphanol (5 × more potent than morphine) | Levallorphan (Antagonist 5 × more potent than nalorphine) | *N*-Phenethyllevorphanol (15 × more potent than morphine)

FIGURE 24.13 Examples of morphinans.

they do with morphine. This implies that morphine and morphinans are binding to the same receptors in the same way;

- the morphinans are easier to synthesize as they are simpler molecules with fewer rings and chiral centres.

24.6.3.3 Removing rings C and D

Removing both rings C and D gives an interesting group of compounds called the **benzomorphans** (Fig. 24.14), which retain analgesic activity. One of the simplest of these structures is **metazocine**, which has the same analgesic activity as morphine. Notice that the two methyl groups in metazocine are *cis* with respect to each other and represent the remnants of the C ring. It is important that these methyl groups are retained in order to obtain good activity.

The same chemical modifications carried out on the benzomorphans as described for the morphinans and morphine produce the same biological effects, implying a similar interaction with the analgesic receptors. For example, replacing the *N*-methyl group of metazocine with a phenethyl group gives **phenazocine**, which is four times more active than morphine and was the first compound discovered to have a useful level of analgesia without dependence properties.

Further developments led to **pentazocine** (Fig. 24.14), which has proved to be a useful long-term analgesic with a very low risk of addiction. Like nalorphine, pentazocine acts as an antagonist at the μ receptor but, unlike nalorphine, it is a full agonist at the κ receptor rather than a partial agonist. Pentazocine also acts as a weak agonist at the δ receptor.

Unfortunately, the compound has hallucinogenic and psychotomimetic side effects as a result of activating the κ receptor. A newer compound (**bremazocine**) has a longer duration, has 200 times the activity of morphine, appears to have no addictive properties, and does not depress breathing.

To conclude:

- rings C and D are not essential to analgesic activity;
- analgesia and addiction are not necessarily co-existent;

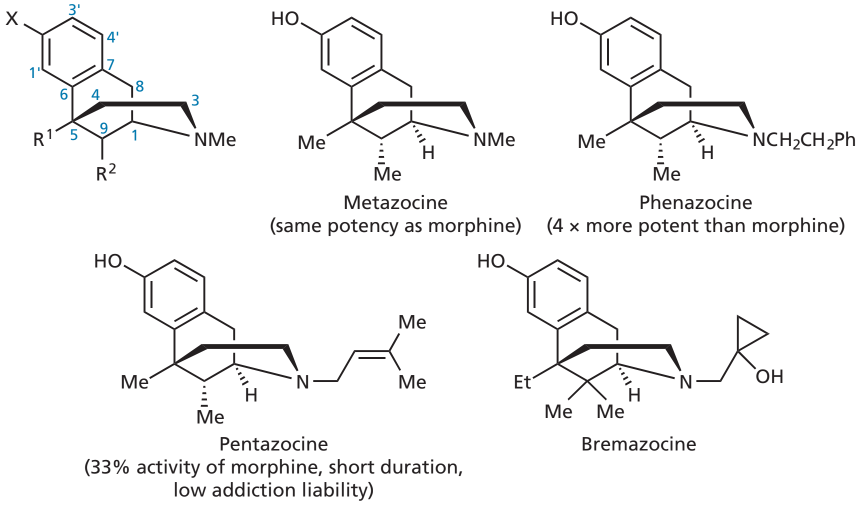


FIGURE 24.14 Benzomorphans.

- 6,7-benzomorphans are clinically useful compounds with reasonable analgesic activity, less addictive liability, and less tolerance;
- benzomorphans are simpler to synthesize than morphine and morphinans;
- benzomorphans bind to opioid receptors in the same manner as morphine and morphinans.

24.6.3.4 Removing rings B, C, and D

Removing rings B, C, and D gives a series of compounds known as **4-phenylpiperidines**. The analgesic activity of these compounds was discovered by chance in the 1940s when chemists were studying analogues of cocaine for antispasmodic properties. Their structural relationship to morphine was only identified when they were found to be analgesics—this is evident if the structure is drawn as shown in Fig. 24.15. Activity can be increased sixfold by introducing the phenolic group and altering the ester to a ketone to give **ketobemidone**.

Pethidine (**meperidine**) is a weaker analgesic than morphine, but shares the same undesirable side effects. On the plus side, it has a rapid onset and a shorter duration of action. As a result, it has been used as an analgesic in childbirth. The rapid onset and short duration of action mean that there is less chance of the drug depressing the baby's breathing once it is born. The structure was discovered in 1939 and was the first fully synthetic opioid analgesic to enter clinical practice.

The piperidines are more easily synthesized than any of the previous groups and a large number of analogues have been studied. There is some doubt as to whether they act in the same way as morphine at analgesic receptors, as some of the chemical adaptations we have already described do not lead to comparable biological results. For example, adding allyl or cyclopropyl groups does not give antagonists. The replacement of the methyl group of pethidine with a cinnamic acid residue increases the activity 30-fold, whereas putting the same group on morphine eliminates activity (Fig. 24.16).

These results might have something to do with the fact that the phenylpiperidines are more flexible molecules than the previous structures, and are likely to have different binding modes with opioid receptors (see section 24.8.3).

Fentanyl and its analogues (Fig. 24.17) represent a class of opioids known as the **4-anilinopiperidines** and are among the most potent agonists known for the μ receptor. These drugs lack a phenolic group and are very lipophilic. As a result, they can cross the blood–brain barrier more efficiently. Fentanyl itself is up to 100 times more active than morphine as a sedative and analgesic and it is thought that the Russian authorities used it in an attempt to incapacitate a group of terrorists during the infamous cinema siege of recent years. Apparently, the drug was introduced as a gas through the ventilation system into the auditorium and succeeded in rendering both terrorists and hostages unconscious. Unfortunately, the authorities waited too long to enter the building and

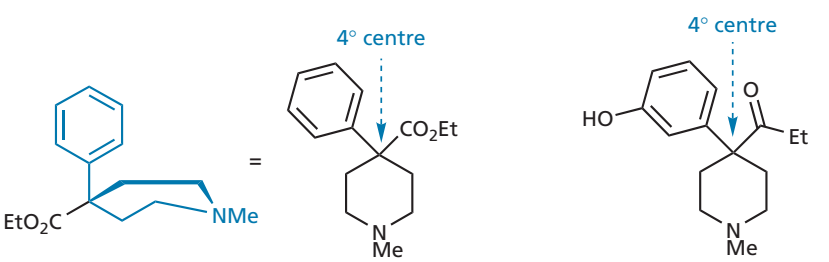


FIGURE 24.15 4-Phenylpiperidines.

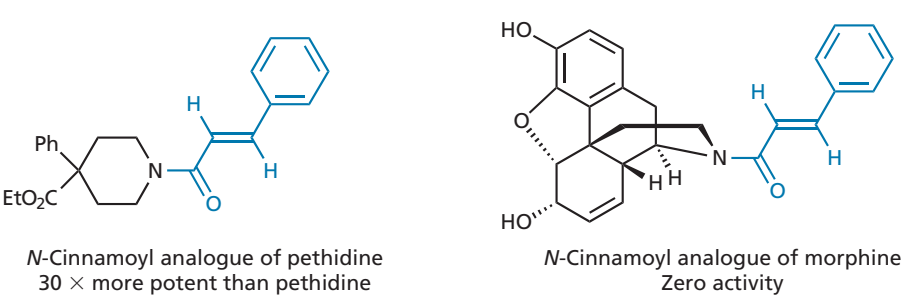


FIGURE 24.16 Effect of addition of a cinnamic acid residue (in blue) on meperidine and morphine.

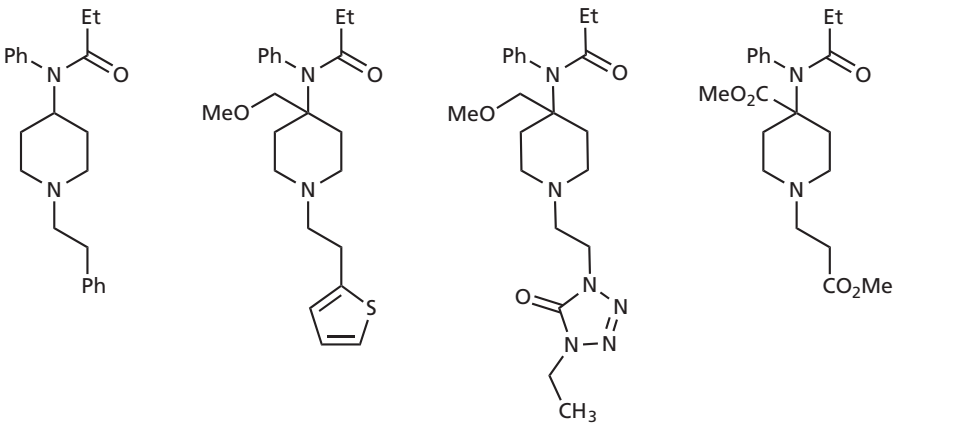


FIGURE 24.17 Fentanyl and analogues.

many innocent people died as a result of suffocation. Like morphine, an overdose of fentanyl can stop breathing by depressing the respiratory centre in the brain.

Fentanyl and the shorter lasting **alfentanil** and **remifentanil** are used during surgery for analgesia and to enhance anaesthesia. Remfentanil was designed to have a very short duration of action by introducing ester groups which are rapidly metabolized by non-specific esterase enzymes. It can be administered as an intravenous drip and does not accumulate in the body because of its rapid metabolism. This reduces the risk of serious side effects, such as depression of the respiratory centre.

To conclude:

- rings C, D, and E are not essential for analgesic activity;
- piperidines retain side effects, such as addiction and depression of the respiratory centre, because they are agonists at the μ receptor;
- piperidine analgesics are faster acting and have a shorter duration of action than morphine;
- the quaternary centre present in piperidines is usually necessary (fentanyl and its analogues are exceptions);
- the aromatic ring and basic nitrogen are essential to activity, but the phenol group is not;
- piperidine analgesics appear to bind with analgesic receptors in a different manner to previous structural classes.

For additional material see Web article 13: 4-anilinopiperidines

Test your understanding and practise your molecular modelling with Exercises 24.4–24.7

24.6.3.5 Removing rings B, C, D, and E

The analgesic **methadone** (Fig. 24.18) was discovered in Germany during World War II and is comparable in activity to morphine. It is orally active and has less severe emetic and constipation effects. Side effects such as sedation, euphoria, and withdrawal symptoms are also less severe, and so the compound has been given to drug addicts as a substitute for morphine or heroin in order to wean them off these drugs. This is not a complete cure, as it merely swaps an addiction to heroin or morphine for an addiction to methadone. This is considered less dangerous, though.

The molecule is a **diphenylpropylamine** structure containing a single asymmetric centre. When the molecule is drawn in the same manner as morphine, we would expect the *R*-enantiomer to be the more active enantiomer. This proves to be the case with the *R*-enantiomer being twice

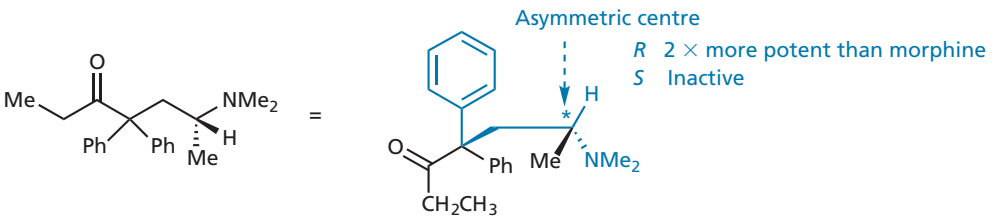


FIGURE 24.18 Methadone.

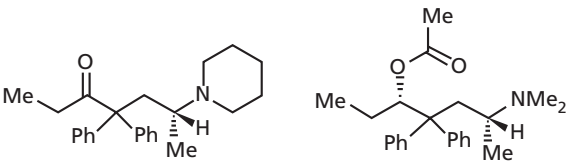


FIGURE 24.19 Dipipanone and L-α-acetylmethadol (LAAM).

as potent as morphine, whereas the *S*-enantiomer is inactive. This is quite a dramatic difference. Because the *R*- and *S*-enantiomers have identical physical properties and lipid solubility, they should both reach analgesic receptors to the same extent and so the difference in activity is most probably due to receptor–ligand interactions.

Many analogues of methadone have been synthesized, such as **dipipanone**, which is an oral analgesic, and **L-α-acetylmethadol** (LAAM) (Fig. 24.19). The latter has been used as a longer-acting alternative for maintenance therapy in opioid dependence (see also buprenorphine, section 24.6.4). A methadone-like structure has also been linked to the 4-phenylpiperidine skeleton to produce a useful agent for the treatment of diarrhoea (Box 24.3).

24.6.4 Rigidification

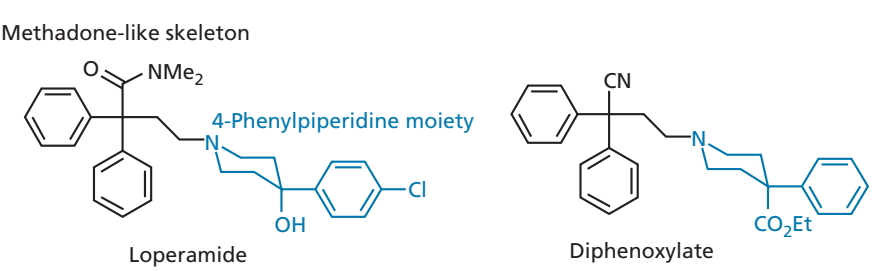
The strategy of rigidification is used to limit the number of conformations that a molecule can adopt. The aim is to retain the active conformation for the desired target and eliminate alternative conformations that might fit different targets (section 13.3.9). This should increase activity, improve selectivity, and decrease side effects. The best examples of this tactic in the analgesic field are the **orvinols** (or **oripavines**), which often show remarkably high activity. A comparison of these structures with morphine shows that an extra ring sticks out from what used to be the crossbar of the T-shaped morphine skeleton (Fig. 24.20).

For additional material see Web Article 14: orvinols

Some remarkably powerful orvinols have been obtained (Box 24.4). **Etorphine** (Fig. 24.21), for example, is 10,000 times more potent than morphine. This is a combination of its high hydrophobicity which allows it to cross the blood–brain barrier 300 times more easily than morphine, allied to a 20 times higher affinity for the analgesic receptor due to better binding interactions. At slightly higher doses than those required for analgesia, it can act as a 'knock-out' drug or sedative. It has a considerable margin of safety and is used to immobilize large animals, such as elephants. As the compound is so active, only very small doses are required and these can be dissolved in such small volumes (1 ml) that they can be placed in darts which can be fired into the hide of the animal. Reducing the double bond of etorphine increases activity more than 10-fold, and the resulting structure (**dihydroetorphine**) is one of the most potent

BOX 24.3 Opioids as anti-diarrhoeal agents

One of the main aims in drug design is to find agents that have minimal side effects, but, occasionally, it is possible to take advantage of a side effect. For example, one of the side effects of opioid analgesics is constipation. This is not very comfortable, but it is a useful property if you wish to counteract diarrhoea. The aim then is to design a drug such that the original side effect becomes the predominant feature. **Loperamide** is a successful anti-diarrhoeal agent which was first synthesized in 1969, approved by the US Food and Drugs Administration (FDA) in 1976, and marketed as **Imodium**. It can be viewed as a hybrid molecule involving a 4-phenylpiperidine and a methadone-like structure. The compound is lipophilic, slowly absorbed, and prone to metabolism, meaning that it acts as a selective agonist on opioid receptors in the gastrointestinal tract. It is also free from any euphoric effect, as it cannot cross the blood–brain barrier. All these features make it a safe medicine, free from the addictive properties of the opioid analgesics. **Diphenoxylate** is a structurally related agent that is also used in the treatment of diarrhoea.



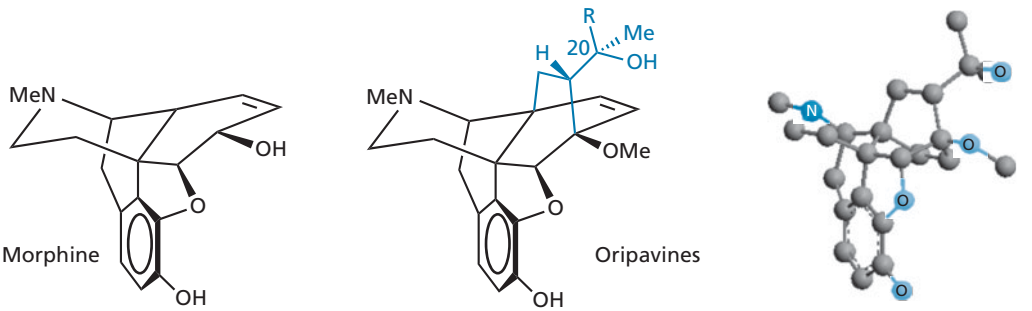


FIGURE 24.20 Comparison of morphine and orvinols.

analgesics ever reported. Dihydroetorphine is used in China as a strong analgesic and as a treatment for opioid addiction.

The presence of lipophilic groups at C20 (R in Fig. 24.20) is found to improve activity dramatically, indicating the existence of an extra hydrophobic binding region in the receptor binding site.* The group best able to interact with this region is a phenethyl substituent, and the product containing this group is even more active than etorphine. As one might imagine, these highly active compounds have to be handled very carefully in the laboratory.

Test your understanding and practise your molecular modelling with Exercises 24.7–24.11.

Because of their rigid structures, these compounds are highly selective agents for the analgesic receptors. Unfortunately, the increased analgesic activity is also accompanied by unacceptable side effects due to strong interactions with the μ receptor. It was, therefore, decided to see whether *N*-substituents, such as an allyl or cyclopropyl group, would give the oripavine equivalent of a pentazocine or a nalorphine—an agent acting as an antagonist at the μ receptor and an agonist at the κ receptor.

Adding a cyclopropyl group gives a very powerful antagonist called **diprenorphine** (Fig. 24.21), which is 100 times more potent than nalorphine and can be used to reverse the immobilizing effects of etorphine. Diprenorphine has no analgesic activity.

The related compound **buprenorphine** (Fig. 24.21) has similar clinical properties to drugs like nalorphine and pentazocine in that it has analgesic activity with a very low risk of addiction. It is a particularly safe drug because it has very little effect on respiration, and what little effect it does have actually decreases at high doses. Therefore, the risks of suffocation from a drug overdose are much smaller than with morphine. Buprenorphine has been used in hospitals to treat patients recovering from surgery, as well as those suffering from cancer. It has also been used as an alternative to methadone for weaning addicts off heroin. Its drawbacks include side effects such as nausea and vomiting, as well as the fact that it cannot be taken orally.

Buprenorphine has unusual receptor binding properties with respect to other opioids. It has a strong affinity for the μ receptor where it acts as a partial agonist, whereas it acts as an antagonist at the κ and δ receptors. Normally, one would expect compounds that act as antagonists at the μ receptor and agonists at the κ receptor to be safer analgesics, and so the clinical properties of buprenorphine are quite surprising. It is thought that the lack of serious side effects is related in some way to the rate at which buprenorphine interacts with the receptor. It is slow to bind, but once it has bound, it binds strongly and is slow to leave. As the effects of binding are gradual it means that there are no sudden changes in transmitter levels. Buprenorphine is the most lipophilic compound in the orvinol series of compounds and enters the brain very easily, and so the slow onset of binding has nothing to do with how easily it reaches the receptor. Because buprenorphine binds very strongly, less of it is required to interact with a certain percentage of analgesic receptors than morphine. However, buprenorphine is only a partial agonist and is less efficient at activating analgesic receptors. This means that it is unable to reach the maximum level of analgesia that can be acquired by morphine. Thus, buprenorphine can produce analgesia

* It has been proposed that the phenylalanine aromatic ring on enkephalins (see later) interacts with this same binding region.

BOX 24.4 Synthesis of the orvinols

The orvinols are synthesized from an alkaloid called **thebaine**, which is extracted from opium along with codeine and morphine. Although similar in structure to both these compounds, thebaine has no analgesic activity and is extremely toxic. There is a diene group present in ring C and when thebaine is treated with methyl vinyl ketone, a Diels–Alder reaction takes place to give an extra ring and increased rigidity to the structure (Fig. 1). As a ketone group has been introduced, it is now possible to try the strategy of drug extension by adding various groups to the ketone via a Grignard reaction. It is noteworthy that this reaction is stereospecific. The Grignard reagent complexes to both the 6-methoxy group and the ketone, and is then delivered to the less-hindered face of the ketone in an asymmetric reaction (Fig. 2). The final stage in the synthesis involves treatment with KOH and ethylene glycol to demethylate the methyl ether at position 3 without demethylating the methyl ether at position 6.

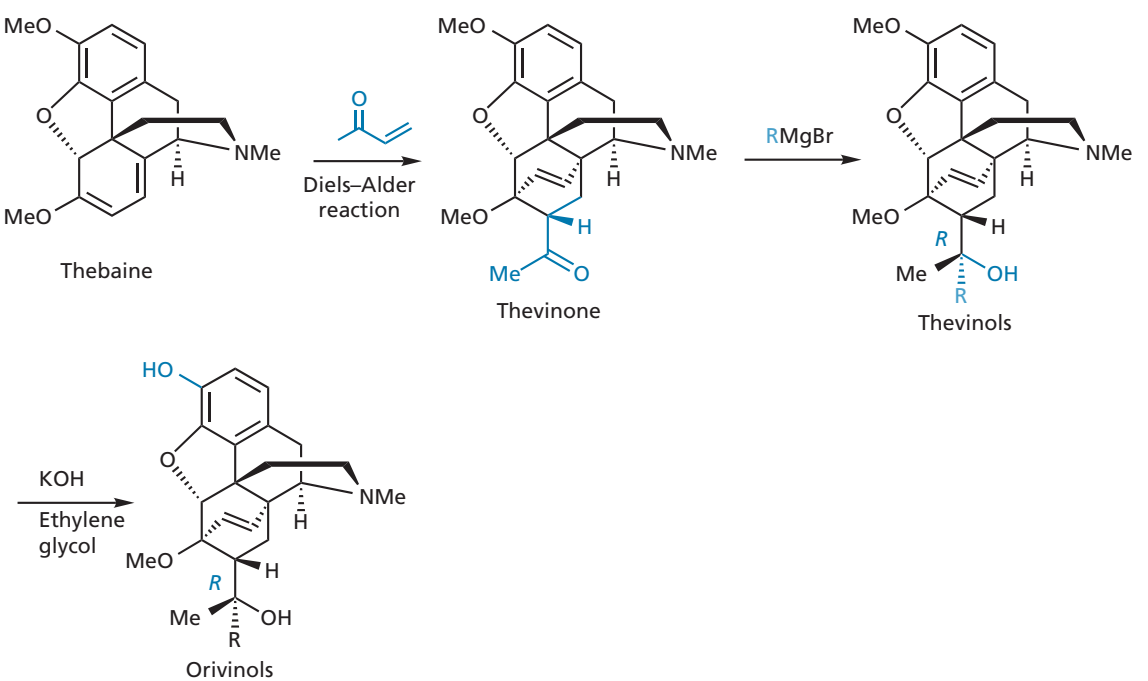


FIGURE 1 Formation of orvinols.

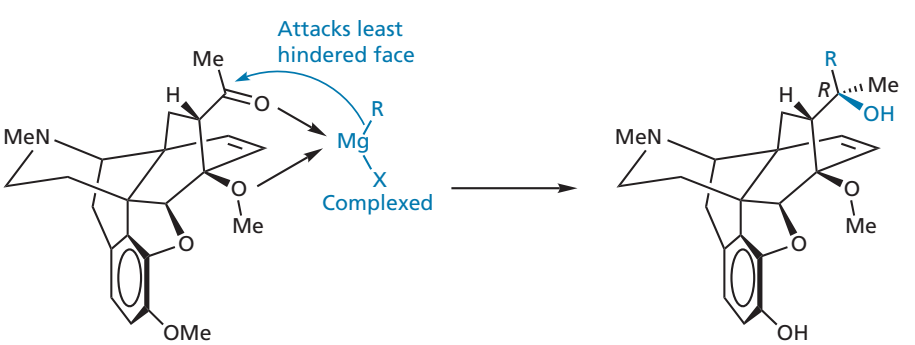


FIGURE 2 Grignard reaction leads to an asymmetric centre.

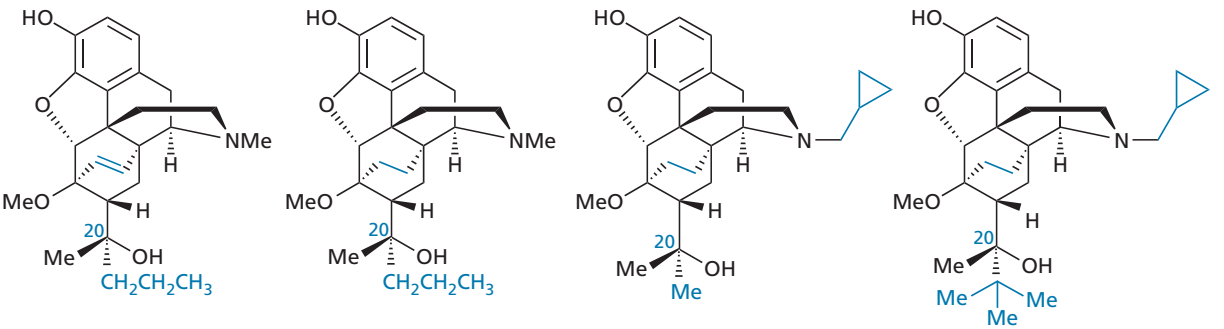


FIGURE 24.21 Etorphine and related structures.

at lower doses than morphine, but if the pain levels are high, buprenorphine is not as effective as morphine. Nevertheless, buprenorphine provides another example of an opioid analogue where analgesia has been separated from dangerous side effects.

KEY POINTS

- The addition of a 14-hydroxyl group or an *N*-phenethyl group usually increases activity as a result of interactions with extra binding regions.
- *N*-Alkylated analogues of morphine are easily synthesized by demethylating morphine to normorphine, then alkylating with alkyl halides.
- The addition of suitable *N*-substituents results in compounds which act as antagonists or partial agonists. Such compounds can be used as antidotes to morphine overdose, as treatment for addiction, or as safer analgesics.
- The morphinans and benzomorphans are analgesics which have a simpler structure than morphine and interact with analgesic receptors in a similar fashion.
- The 4-phenylpiperidines are a group of analgesic compounds which contain the analgesic pharmacophore present in morphine. They may bind to analgesic receptors slightly differently from analgesics of more complex structure.
- Methadone is a synthetic agent which contains part of the analgesic pharmacophore present in morphine. It is administered to drug addicts to wean them off heroin.
- Thebaine is an alkaloid derived from opium which lacks analgesic activity. It is the starting material for a three-stage synthesis of orvinols.
- Orvinols are extremely potent compounds owing to enhanced receptor interactions and an increased ability to cross the blood–brain barrier.
- The addition of *N*-cycloalkyl groups to the orvinols results in powerful antagonists or partial agonists, which can be used as antidotes for the treatment of addiction or as safer analgesics.

24.7 Agonists and antagonists

We return now to look at a particularly interesting problem regarding the agonist/antagonist properties of morphine analogues. Why should such a small change as replacing an *N*-methyl group with an allyl group result in such a dramatic change in biological activity, such that an agonist becomes an antagonist? Why should a molecule such as nalorphine act as an agonist at one analgesic receptor and an antagonist at another? How can different receptors distinguish between such subtle changes in a molecule?

We shall consider one possible explanation. Let us assume that an opioid receptor exists in an active or an inactive conformation (Fig. 24.22a). The active conformation is capable of binding G-proteins and triggering signal transduction, while the inactive conformation is not. Let us further assume that an equilibrium exists between the two conformations and that the equilibrium shifts depending on what type of ligand is bound. If the active conformation binds an agonist (Fig. 24.22b), the equilibrium shifts to the active form leading to increased signal transduction. If an antagonist binds to the inactive conformation, the opposite happens (Fig. 24.22c).

This argument assumes that the binding sites of the active and inactive forms of the receptor are capable of distinguishing between the structures of an agonist and an antagonist. This is quite feasible as the binding sites are likely to have different conformations. We shall assume that the binding regions required to bind the opioid pharmacophore are positioned identically in both binding sites (the blue regions in Figs. 24.23 and 24.24), but that an additional hydrophobic region is positioned closer to the ionic binding region in the inactive binding site than it is in the active binding site. Let us now consider the binding of the agonist *N*-phenethylmorphine (Fig. 24.23). Like morphine, it binds using its phenol, aromatic, and amine functional groups. The aromatic

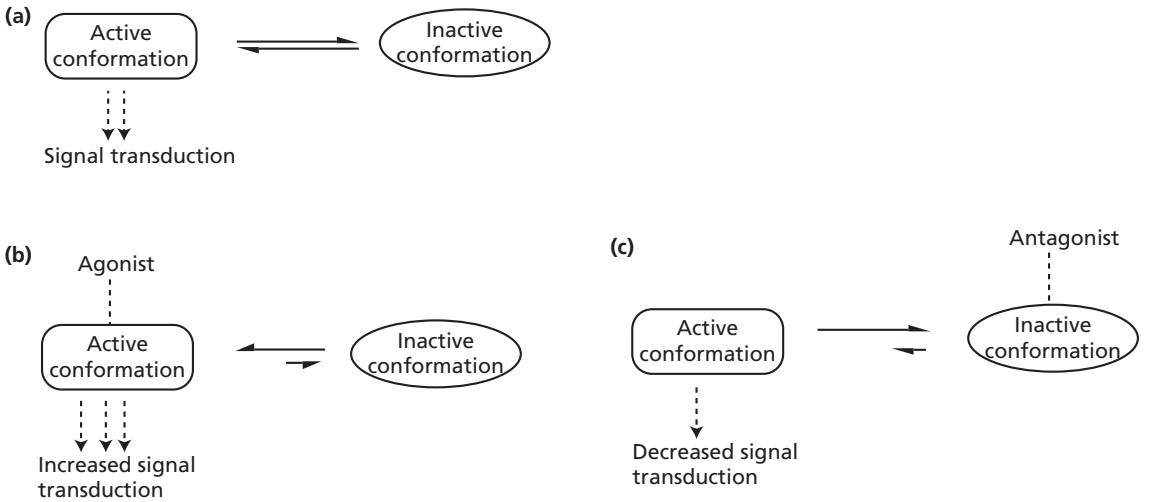


FIGURE 24.22 (**a**) Equilibrium between two receptor conformations. (**b**) Effect on adding an agonist. (**c**) Effect on adding an antagonist (see also Figure 8.16).

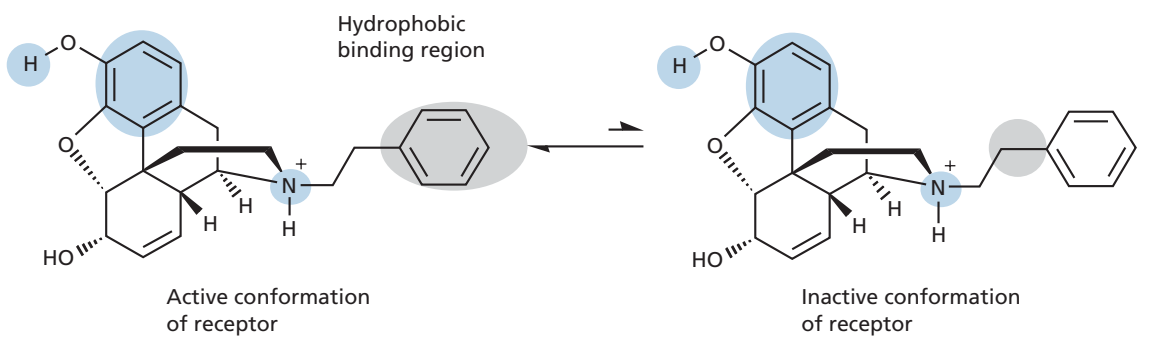


FIGURE 24.23 *N*-Phenethylmorphine binding interactions with the active and inactive conformations of the opioid receptor.

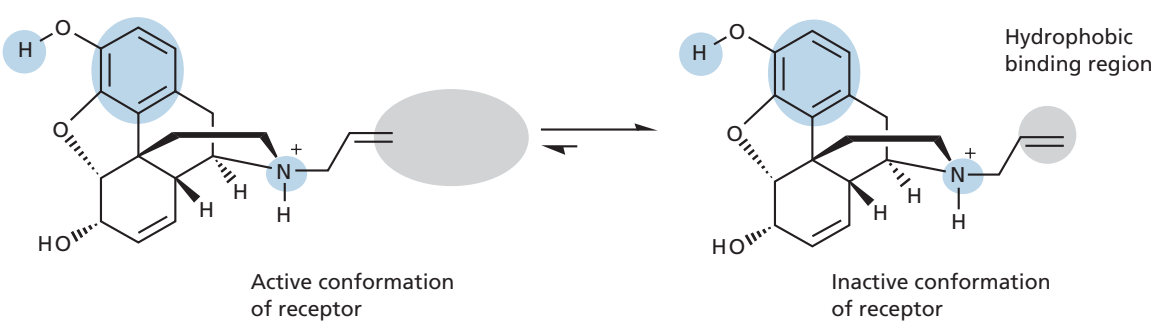


FIGURE 24.24 *N*-Allylmorphine binding interactions with the active and inactive conformations of the opioid receptor.

ring of the phenethyl group is quite far from the amine group. Therefore, it overlaps more effectively with the more distant hydrophobic region causing the equilibrium to shift to the active form of the receptor.

Now consider what happens if the phenethyl group is replaced by an allyl group (Fig. 24.24). The allyl group is much closer to the amine and interacts better with a closer hydrophobic region. Therefore, the equilibrium would shift to the inactive conformation.

How then do we explain the fact that some opioids act as an agonist with one type of opioid receptor, and as an antagonist at another? We could propose that the relative positions of the extra hydrophobic regions are different in the different types of receptor. In Figure 24.24, the

allyl group is almost overlapping with the hydrophobic binding region of the active conformation. If this binding region was slightly closer in a different type of receptor, it would permit the allyl group to form a better interaction and have agonist activity.

24.8 Endogenous opioid peptides and opioids

24.8.1 Endogenous opioid peptides

Morphine relieves pain by binding to analgesic receptors in the CNS, which implies that there must be endogenous chemicals which interact with these receptors. The search for these natural analgesics took many years, but led, ultimately, to the discovery of the **enkephalins**. The term enkephalin is derived from the Greek, meaning 'in the head', and that is exactly where the enkephalins are produced. There are two enkephalins: **Met-enkephalin** and **Leu-enkephalin** (Fig. 24.25). Both of the enkephalins are pentapeptides and have a slight preference for the δ receptor (Box 24.5). It has been proposed that enkephalins are responsible for the analgesic effects of acupuncture.

H-Tyr-Gly-Gly-Phe-Met-OH
Met-enkephalin

H-Tyr-Gly-Gly-Phe-Leu-OH
Leu-enkephalin

FIGURE 24.25 Enkephalins.

At least 15 endogenous peptides have now been discovered (the enkephalins, **dynorphins**, and the **endorphins**), varying in length from 5 to 33 amino acids. These compounds are thought to be neurotransmitters or neurohormones in the brain, and operate as the

BOX 24.5 A comparison of opioids and their effects on opioid receptors

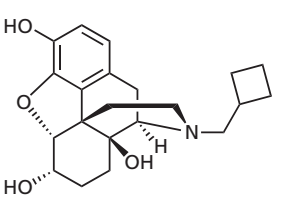
Table 24.1 shows the relative activities of different opioids as agonists, partial agonists, and antagonists at different opioid receptors. A plus sign indicates that the compound acts as an agonist, whereas a minus sign means that it acts as an antagonist. The number of plus signs or minus signs indicates the binding affinity. Plus signs in brackets indicate partial agonist activity.

The search for κ-selective agents has resulted in the clinically useful agents **nalbuphine** and **butorphanol** (Fig. 1). Unfortunately, many of the κ-selective agents are limited in their utility because they are partial agonists and are not potent enough to treat severe pain. Moreover, activation of the κ receptor has been associated with hallucinations and psychotomimetic side effects.

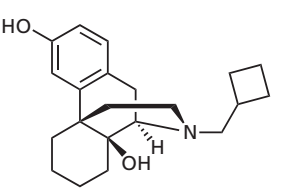
TABLE 24.1 Relative activities of opioids at opioid receptors

Receptor	Mor	Meth	Peth	Etor	Fent	Pent	Nal	Bup	Nalo	Nalt	Lenk	End	Dyn
μ	+++	+++	++	+++	+++	–	–	(+++)	–	–	+	+++	++
κ	+		+	+++		++	(++)	–	–	–		+++	+++
δ	+		+	+++	+	+			–	–	+++	+++	+

Mor = morphine; Meth = methadone; Peth = pethidine; Etor = etorphine; Fent = fentanyl; Pent = pentazocine; Nal = nalorphine; Bup = buprenorphine; Nalo = naloxone; Nalt = naltrexone; Lenk = Leu-enkephalin; End = β-endorphin; Dyn = dynorphin.



Nalbuphine



Butorphanol

FIGURE 1 Nalbuphine has the same activity as morphine, low addiction liability, no psychotomimetic activity, but is orally inactive. Butorphanol is also orally inactive.

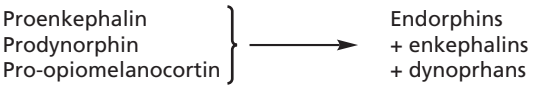


FIGURE 24.26 Production of the body's natural painkillers.

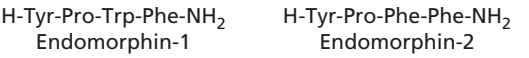


FIGURE 24.28 Endomorphins.

body's natural painkillers. They are mostly derived from three inactive precursor proteins—**pro-enkephalin**, **pro-dynorphin**, and **pro-opiomelanocortin** (Fig. 24.26).

All of these compounds have either the Met- or the Leu-enkephalin skeleton at their *N*-terminus, which emphasizes the importance of this pentapeptide structure towards analgesic activity. It has also been shown that tyrosine is essential to activity, and much has been made of the fact that there is a tyrosine skeleton in the morphine skeleton (Fig. 24.27).

If the crucial part of these molecules is the *N*-terminal pentapeptide, why should there be so many different peptides carrying out the same task? One suggestion is that the remaining peptide chain of each molecule is responsible for targeting each peptide to particular types of analgesic receptor. It is known that enkephalins show preference for the δ receptor, whereas dynorphins show selectivity for the κ receptor, and β-endorphins show selectivity to both the μ and δ receptors. This has led to a theory called the **message–address concept**, which proposes that part of a molecule is responsible for its pharmacological activity (the message) and another part is responsible for its target selectivity (the address) (see also sections 24.9.1 and 24.10).

The most recent endogenous opioid ligand was discovered in 1995 by two groups and was named **nociceptin** or **orphanin-FQ**. It is a heptadecapeptide derived from the protein **pronociceptin/orphanin FQ** and is a ligand for the **ORL1-receptor** (section 24.4). Curiously, the *N*-terminal amino acid is phenylalanine rather than tyrosine and it appears that this plays a crucial role in receptor selectivity. The endogenous opioids, such as the enkephalins, endorphins, and dynorphins, have tyrosine at the *N*-terminus and have no affinity for the ORL_1-receptor, whereas nociceptin/orphanin-FQ has negligible affinity for the μ, κ, and δ receptors.

The **endomorphins** (Fig. 24.28) have also been discovered recently and are unlike previous opioid peptides. For a start, they are tetrapeptides, whereas all other opioid peptides are pentapeptides or larger. Also, the second and third amino acids in their skeleton differ from glycine, another break from convention. Finally, they have a primary amide functional group at the C-terminus. They do, however, have the mandatory tyrosine and phenylalanine residues that are present in other opioid peptides. The endomorphins have a strong affinity and selectivity for the μ receptor. However, there is some doubt as to whether these are truly endogenous opioids or whether they are merely breakdown products resulting from the extraction process used to isolate proteins.

For additional material see Web article 15: the message-address concept.

24.8.2 Analogues of enkephalins and δ-selective opioids

SAR studies on the enkephalins have shown the importance of the phenol ring and amino group of the tyrosine residue. Without either, activity is lost. If tyrosine is replaced by another amino acid, activity is also lost—the only exception being D-serine.

It has been found that the enkephalins are inactivated by peptidase enzymes *in vivo*, with the most labile bond being the peptide link between tyrosine and glycine. Efforts have been made to synthesize analogues which are resistant towards this hydrolysis. It is possible to replace either, or both, of the glycine units with unnatural

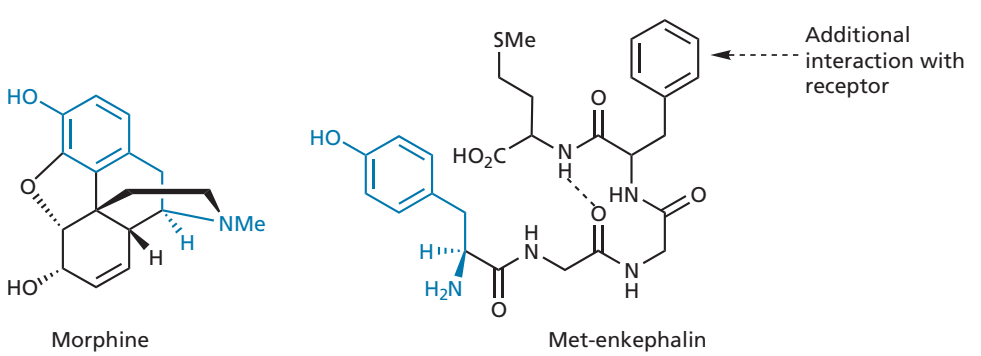


FIGURE 24.27 Comparison of morphine and Met-enkephalin (dashed line is a H-bond).

H-L-Tyr-Gly-Gly-L-Phe-L-Met-OH	Met-enkephalin—δ agonist and some μ activity.
H-L-Tyr-D-AA-Gly-NMe-L-Phe-L-Met-OH	Resistant to peptidase. Orally active.
N,N-Diallyl-L-Tyr-aib-aib-L-Phe-L-Leu-OH	Antagonist to δ receptor (aib = α-aminobutyric acid).
Longer enkaphalins/endorphins	Increase in κ activity. Slight increase in μ activity.

FIGURE 24.29 Tactics to stabilize the bond between the tyrosine and glycine residues.

D-amino acids, such as D-alanine. Since D-amino acids do not occur naturally in the human body, peptidases do not recognize the structure and the peptide bond is not attacked. The alternative tactic of replacing L-tyrosine with D-tyrosine is not possible as it completely alters the relative orientation of the tyrosine aromatic ring with respect to the rest of the molecule. As a result, the analogue is unable to bind to the analgesic receptor and is inactive.

N-Methylating the peptide link also blocks peptidase hydrolysis. Another tactic is to use unusual amino acids which are not recognized by peptidases, or prevent the molecule from fitting the peptidase active site (Fig. 24.29). Unfortunately, the enkephalins also have some activity at the μ-receptor and so the search for selective agents continues.

The first non-peptide structure to show selectivity for the δ-receptor was the antagonist **naltrindole** (Box 24.6).

BOX 24.6 Design of naltrindole

The message–address concept has been extremely useful in designing selective opioids. **Leu-enkephalin** shows selectivity for the δ receptor, and it has been shown that the tyrosine residue acts as the analgesic message, while the aromatic ring of phenylalanine acts as the address for the δ receptor (Fig. 1). It is believed that the observed selectivity is due to the aromatic ring interacting with a binding region that is unique to the δ receptor.

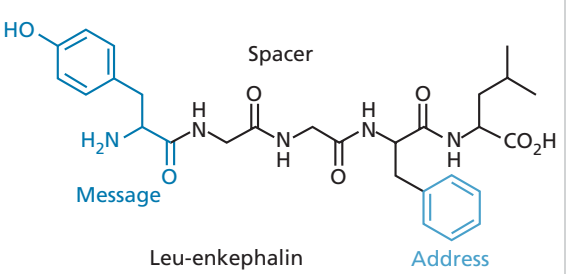


FIGURE 1 Leu-enkephalin.

In an attempt to obtain non-peptide, δ-selective opioids, an aromatic ring was fused to morphine-like structures to see whether it could act as an address segment. The position of the aromatic ring relative to the opioid message would be crucial, and success was achieved by fusing the aromatic ring to the C-ring of **naltrexone** to give **naltrindole**. Whereas naltrexone is a non-selective antagonist, naltrindole is a highly potent, δ-selective antagonist with 240 times more potency at the δ receptor. A molecular dynamics simulation demonstrated that Leu enkephalin could adopt a conformation where the relative positions of the aromatic rings of Tyr and Phe were reasonably similar to the corresponding rings in naltrindole (Fig. 2).

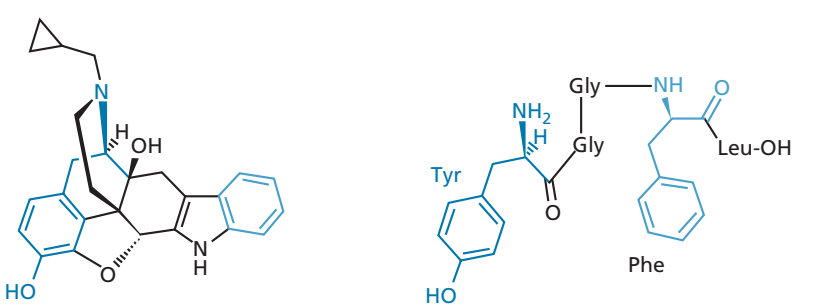


FIGURE 2 Comparison of naltrindole with Leu-enkephalin.

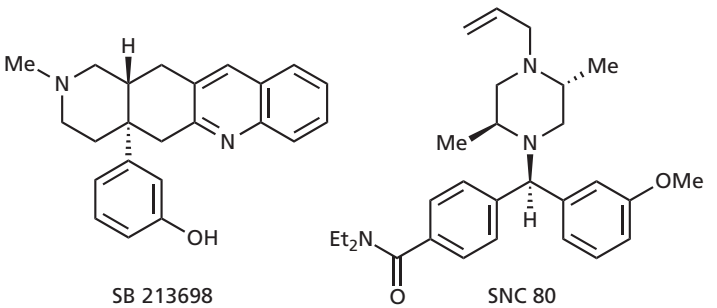


FIGURE 24.30 Non-peptide agonists that are selective for the δ receptor.

Several selective non-peptide agonists have since been developed, such as **SB 213698** and **SNC-80** (Fig. 24.30).

For additional material see Web article 15: the message-address concept.

24.8.3 Binding theories for enkephalins

It is clear from SAR studies on enkephalins that the tyrosine residue and the aromatic ring of phenylalanine are important for analgesic activity, which suggests that they act as important binding groups in their interaction with opioid receptors. This, in turn, implies that the receptor binding site contains two hydrophobic binding regions—one which interacts with the phenol ring of tyrosine (the T-binding region) and one which interacts with the aromatic ring of phenylanine (the P-binding region) (Fig. 24.31). The T-binding region is distinct from the P-binding region in terms of its position and the fact that is must contain a group capable of forming a hydrogen bond to the phenol group of the ligand.

It has also been suggested that the two hydrophobic binding regions may be approximately equidistant from the ionic binding region. This is supported by various studies on the conformations of enkephalins, which indicate that the Gly–Gly segment introduces a bend into the peptide backbone of the molecule such that it adopts a folded conformation. Assuming that the active conformation is similar in nature, this means that the T-binding region is likely to be closer to the P-binding region than one might have imagined.

The possibility of two hydrophobic binding regions roughly equidistant from the ionic binding region provides a possible explanation for the different SAR results obtained for simple opioids, such as pethidine, compared to rigid opioids, such as morphine. It makes sense that morphine should mimic the tyrosine residue of the enkephalins and interact with the T-binding region (Fig. 24.32). An alternative binding mode with the P-binding region might not be possible because of bad steric interactions.

In contrast, pethidine is a smaller, more flexible molecule and may well bind more easily to the P-binding region (Fig. 24.33). If so, this would explain why the activity of phenylpiperidines is not dependent on a phenol group, as the P-binding region lacks the necessary group to interact with it. The different binding mode would also explain why certain *N*-substituents on phenylpiperidines do not produce the same pharmacological results observed with rigid opioids. By interacting with the P-binding region, the phenylpiperidines would be orientated differently. This would mean that their

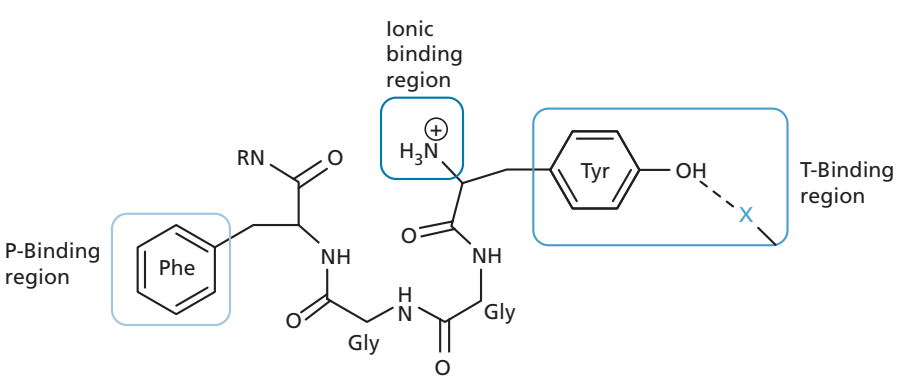


FIGURE 24.31 Proposed binding interactions of an enkephalin with its receptor binding site.

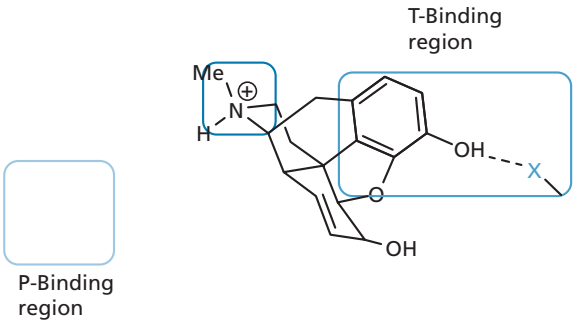


FIGURE 24.32 Interaction of morphine with proposed binding site.

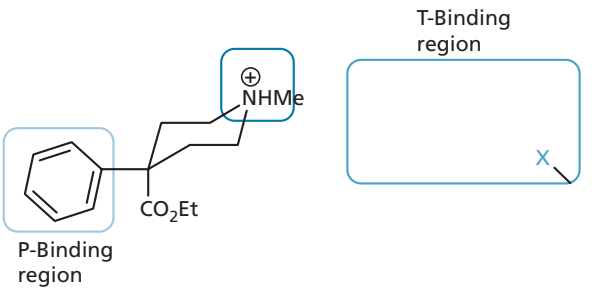


FIGURE 24.33 Interaction of pethidine with proposed binding site.

N-substituents would occupy a different region of space in the binding site and be unable to make the same kind of interactions.

24.8.4 Inhibitors of peptidases

An alternative approach to pain relief is to enhance the activity of natural enkephalins by inhibiting the peptidase enzymes which metabolize them (**enkephalinases**). Studies have shown that the enzyme responsible for metabolism has a zinc ion present in the active site, as well as a hydrophobic pocket which normally accepts the phenylalanine side chain present in enkephalins. A dipeptide (L-Phe–Gly) was chosen as the lead compound and a thiol group was incorporated to act as a binding group for the zinc ion (a similar strategy was used in the design of the ACE inhibitor captopril—Case study 2). The result was a structure called **thiorphan** (Fig. 24.34), which was shown to have analgesic activity. It remains to be seen whether agents such as these will prove useful as analgesics in the clinic. However, an enkephalinase inhibitor called **racecadotril** (or **acetorphan**) (Fig. 24.35) is used in some countries for the treatment of diarrhoea. The agent is actually a prodrug for thiorphan, which is formed after hydrolysis of the ester and thioester groups.

24.8.5 Endogenous morphine

For many years it was assumed that morphine itself could not possibly be an endogenous compound as the structure is an alkaloid produced by the poppy plant. Remarkably, morphine has now been identified as being present in tissues and body fluids, as have thebaine and codeine. It has also been demonstrated that human cells are capable of synthesizing morphine via a biosynthetic route similar to that used in the poppy plant. The levels of morphine are low and it is not yet clear what role it plays.

For additional material see Web article 16: morphine biosynthesis.

24.9 The future

24.9.1 The message–address concept

There has been a lot of research in recent years aimed at developing opioids that show selectivity for a particular type of opioid receptor. The message–address concept has been extremely useful in guiding this research. The basis behind the concept is that opioids have a pharmacophore (the message) that is responsible for its activity, whether that be as an agonist or an antagonist. In addition, selective agents have a feature (the address) that is responsible for its receptor selectivity. This feature

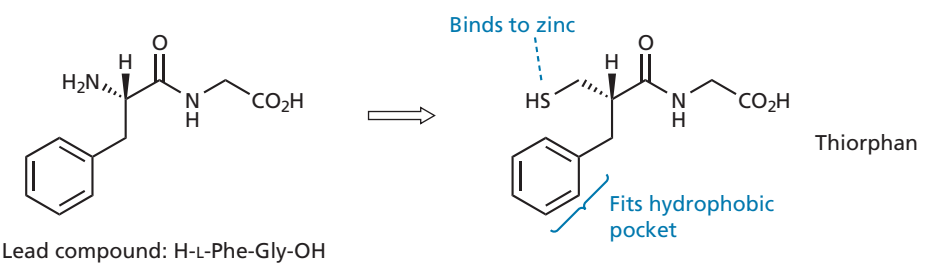


FIGURE 24.34 Development of thiorphan.

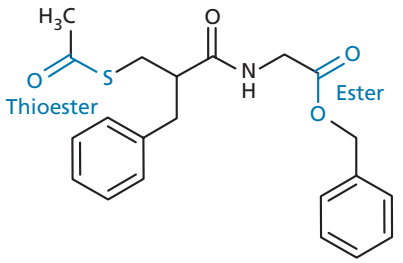


FIGURE 24.35 Racecadotril.

could be a functional group that interacts with a binding region that is unique to one type of receptor, resulting in increased affinity for that receptor. Alternatively, it could be a feature that acts as a steric shield and prevents the molecule from binding to some receptor types but not others. The message–address concept was first applied to endogenous opioids, such as the enkephalins (section 24.8.3), and has since been applied to the design of novel opioids (Box 24.6 and section 24.10).

For additional material see Web article 15: the message-address concept.

24.9.2 Receptor dimers

The opioids may be some of the oldest drugs used in medicine, but they are also some of the least understood. Investigations are still ongoing to try and discover the Holy Grail of opioid research—an opioid analgesic that is potent, orally active, and devoid of serious side effects. A better understanding of opioid receptors and the manner in which they interact with each other will help immensely in this ambitious goal.

There is now good evidence that opioid receptors form dimers in specific tissues (Fig. 24.36). These can exist as homodimers involving two identical opioid receptor types or as heterodimers, where the receptor types are different. It is thought that the transmembrane regions of the component receptors are intertwined, resulting in the equivalent of two hybrid receptors. Each hybrid receptor would have the same overall arrangement of seven transmembrane regions as in the monomeric receptor, but five of the transmembrane regions would be contributed by one of the receptor proteins, while the remaining two transmembrane regions would be contributed from the other receptor. Therefore, it is quite possible that the binding of a ligand to one of the hybrid receptors in a homodimer will affect the ability of the other hybrid receptor to bind a second ligand. For example, it is thought that an antagonist binding to one of the hybrid receptor binding sites will cause a conformational change in the dimeric complex that distorts the binding site of the second hybrid and prevent binding.

The picture becomes more complex when one considers heterodimeric receptors. Here, the hybrid receptors are not identical and so selective opioids will show selectivity for one of the hybrid receptors over the other, depending on which part of the receptor is most important in binding selectivity. For example, in κ receptors, there is a glutamic acid residue in the sixth transmembrane region that is important in binding κ-selective opioids. This means that κ-selective opioids will bind to receptor hybrid A in the complex shown in Fig. 24.36 if the light spheres represent a δ receptor and the dark spheres represent a κ receptor. Similarly, the extracellular loop three in the δ receptor is important in binding δ-selective opioids and so these agents would prefer to bind to receptor hybrid B. In either case, the binding of a selective antagonist to one of the hybrid receptors can result in antagonism at the other if the binding results in a conformational change over the whole complex. This can explain why selective opioid ligands appear to give contradictory results when

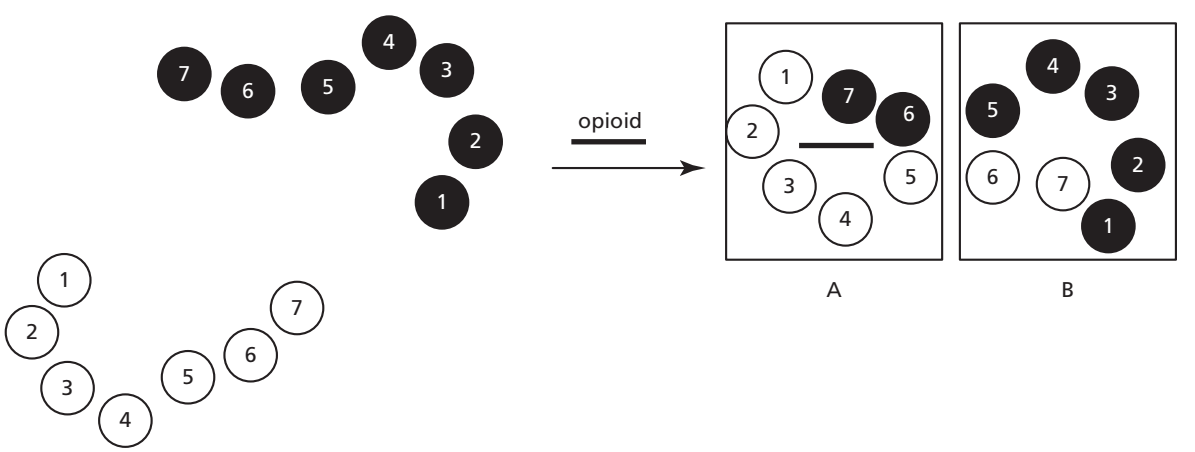


FIGURE 24.36 Formation of a receptor dimer with bound ligand.

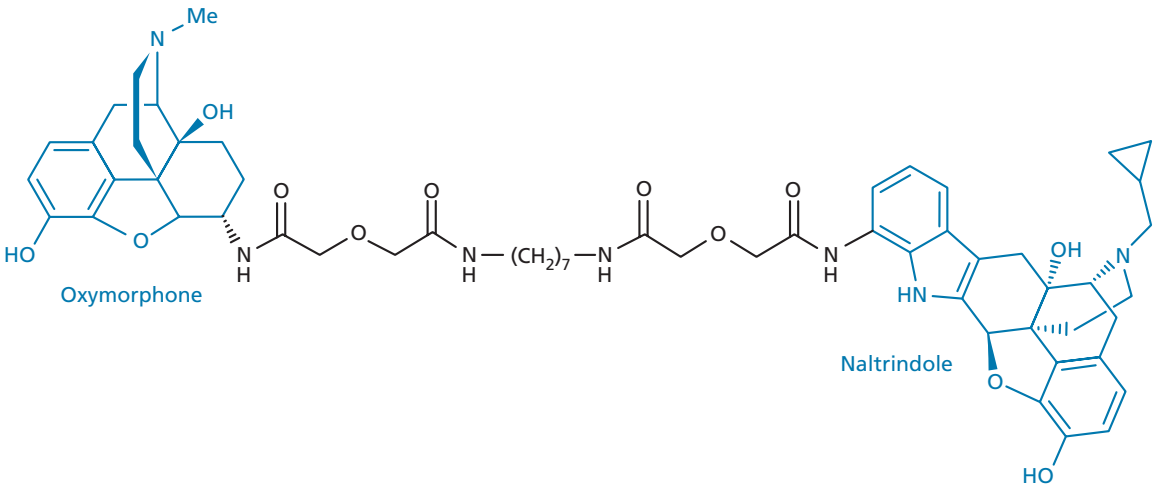


FIGURE 24.37 The bivalent ligand MDAN-21.

tested on different tissues. For example, **norbinaltorphimine** (Fig. 24.38) is a selective κ antagonist when tested on some types of tissue, but a δ antagonist when tested on others. This used to be explained by proposing that different receptor subtypes were present in different tissues, but the same results can be achieved by proposing the presence of κ–δ receptor heterodimers in some tissues, but not others.

Heterodimeric receptors are of current interest because it is believed that the tolerance and dependence effects associated with μ agonists might be caused by their interaction with δ–μ heterodimers, rather than by interaction with unassociated μ receptors. A bivalent ligand (**MDAN-21**) (Fig. 24.37) consisting of the μ-selective agonist **oxymorphone** linked to the δ-selective antagonist **naltrindole** has been found to have 50-fold more potency than morphine, without causing tolerance or dependence. This has exciting potential for the development of a new generation of safer opioid analgesics with fewer side effects (see Web article 12).

24.9.3 Selective opioid agonists versus multi-targeted opioids

The early hopes of finding a highly selective κ agonist with minimal side effects were dashed when it was found that psychotomimetic and dysphoric side effects were associated with activation of the κ receptor. Research into designing δ-selective agonists is still in progress. The main problem is designing structures that are potent and can cross the blood–brain barrier. If this could be achieved, it would allow an understanding of whether the δ receptor has any role in addiction and whether safe δ-selective agonists are feasible. However, it may be more advantageous to design opioids that have controlled activities at a combination of opioid and non-opioid receptors (see Web articles 12 and 15).

Test your understanding and practise your molecular modelling with Exercises 24.12–24.18.

24.9.4 Peripheral-acting opioids

Another approach may be to design opioid analgesics that act on the peripheral nervous system rather than the CNS. **Nalfurafine** (TRK 820) is one such agent that is used in Japan as a κ agonist for the treatment of uremic pruritus (section 24.10).

24.10 Case study: design of nalfurafine

The message–address concept has been a very useful guideline in designing opioid structures showing selectivity for different opioid receptors. In this case study, we will look at how a non-selective antagonist was developed into a κ-selective antagonist and then a κ-selective agonist. The story begins with the design of an opioid dimer that was meant to bind simultaneously to the two κ receptors making up a κ,κ-receptor homodimer (section 24.9.2). There is evidence that the separation between the κ receptors in the κ,κ-homodimer is smaller than for other types of homodimers. Therefore, an opioid dimer with a short linker unit between the two opioid structures should show selectivity. Dimers of the non-selective antagonist **naltrexone** (Fig. 24.11) were synthesized, leading to the discovery of **norbinaltorphimine** (**nor-BNI**) (Fig. 24.38), which is a κ-selective antagonist used frequently in pharmacological studies. Despite the apparent success of the

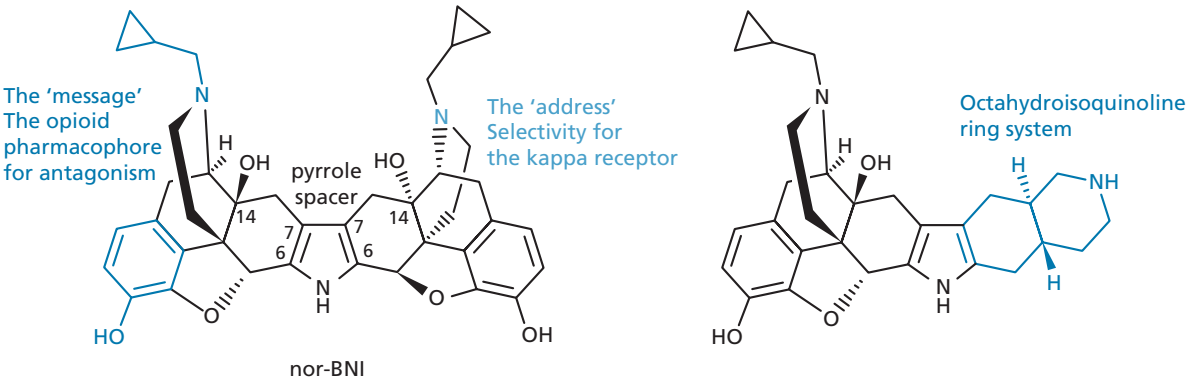


FIGURE 24.38 Norbinaltorphimine and a simplified analogue.

strategy it soon became clear that the separation between the two opioid moieties is too short to allow simultaneous interactions with both components of a receptor dimer. Moreover, SAR studies have demonstrated that only one of the full opioid pharmacophores is required for activity. The second opioid structure certainly plays a role in the high potency and selectivity observed for nor-BNI, but the intact pharmacophore is not required. It was therefore concluded that the dimer was interacting with a single κ receptor, such that one of the opioid components binds to the receptor binding site and acts as the message, while the second opioid component contains a specific feature that serves as the address and interacts with a binding region that is unique to the κ receptor. This extra interaction would explain both the selectivity and the increased potency. Further work demonstrated that the basic amine group is the crucial feature in the address half of the molecule and that it interacts with a unique glutamate residue. Simplification of the structure led to an analogue with an octahydroisoquinoline ring system, which also acts as a κ-selective antagonist.

This antagonist was now used as the lead compound for the design of a κ-selective agonist. There are several examples of projects where an agonist lead compound has been modified to obtain an antagonist, but relatively few where an antagonist has been modified to obtain an agonist. In the former case, the normal strategy is to add an extra functional group in order to form an extra binding interaction with the target receptor such that the resulting induced fit differs from that caused by the binding of an agonist. In order to modify an antagonist to an agonist, the opposite strategy is required—it is necessary to identify an extra interaction that is causing antagonist activity and then remove the group that is involved.

It was thought that the feature responsible for antagonism might be the bulky, hydrophobic octahydroisoquinoline ring system. Therefore, a further simplification was carried out, replacing this ring with a less bulky, flexible, acyclic chain of sufficient length to match the original bicyclic ring (Fig. 24.39). The flexibility of the chain was considered important as this would increase

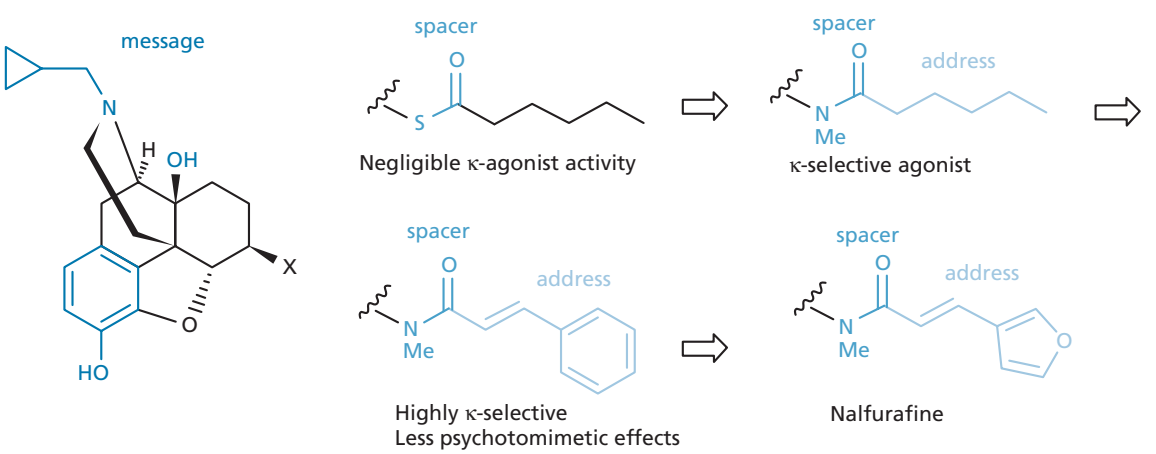


FIGURE 24.39 Design of nalfurafine.

the chances of it adopting the correct active conformation for κ agonist activity. Various chains were studied. For example, a thioester group with a pentyl side chain was essentially inactive. However, replacing the thioester with an amide group resulted in agonist activity with κ selectivity. The side chain was then modified by reintroducing some rigidity in the form of a double bond and an aromatic ring. This resulted in improved selectivity and fewer psychotomimetic side effects. A variety of analogues were prepared containing different heteroaromatic rings instead of the aromatic ring; the best of these was **nalfurafine**.

It is interesting to note that κ selectivity in this structure appears to be related to the presence of the hydrophobic heteroaromatic ring. This appears to contradict the earlier finding with antagonists where an ionized basic group is required for selectivity. However, given the increased flexibility of the modified address segment, it is possible that the heteroaromatic ring is interacting with a different amino acid residue in the binding site.

Nalfurafine is free of the serious side effects of morphine, as well as some of the common side effects of other κ-selective agonists, for example psychotomimetic and dysphoric effects. It was originally proposed as an analgesic in surgery, but sedative effects meant that it was not approved. However, low doses have been found to inhibit the itching associated with the injection of morphine. The compound was subsequently approved as an anti-itching (antipruritic) medication in 2009 for patients undergoing dialysis and was the only κ-selective agent clinically approved at that time.

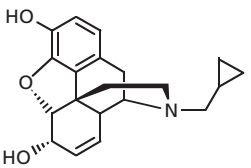
KEY POINTS

- It is proposed that there are two accessory hydrophobic binding regions in the receptor binding site. An agent will act as an agonist or antagonist depending on which of these regions it can access.
- Enkephalins, dynorphins, endomorphins, and endorphins are peptides which act as the body's natural painkillers. The presence of an *N*-terminal tyrosine is crucial to activity.
- Analogues of enkephalins have been designed to be more stable to peptidases by the inclusion of unnatural amino acids, D-amino acids, or *N*-methylated peptide links.
- Enkephalinase inhibitors may have a future role as analgesics by inhibiting the metabolism of enkephalins.
- The existence of homodimeric and heterodimeric opioid receptors has an important role in understanding the activity of opioids and in designing novel opioids.
- The message–address concept has been used to design opioids that are selective for a particular type of opioid receptor.

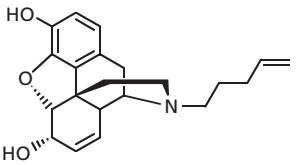
QUESTIONS

1. Morphine is an example of a plant alkaloid. Alkaloids tend to be secondary metabolites that are not crucial to a plant's growth and are produced when the plant is mature. If that is the case, what role do you think these compounds have in plants, if any?
2. The synthesis in Box 24.2 shows that *N*-alkylated analogues can be synthesized by *N*-alkylation directly or by a two-stage process involving *N*-acylation. Why might a two-stage process be preferred to direct *N*-alkylation? What sort of products could not be synthesized by the two-stage process? Is this likely to be a problem?
3. Show how you would synthesize nalorphine (Fig. 24.11).
4. Pethidine has been used in childbirth as it is short-acting and less hazardous than morphine in the newborn baby. Several drugs taken by the mother before giving birth can prove hazardous to a newly born child, but less so after birth. Why is this?
5. Show how you would synthesize diprenorphine and buprenorphine.
6. Why is buprenorphine considered the most lipophilic of the oripavine series of compounds?
7. Identify the potential hydrogen bond donors and acceptors in morphine. Structure–activity relationships reveal that one functional group in morphine is important as a hydrogen bond donor or as a hydrogen bond acceptor. Which group is that?
8. Propose the likely analgesic activity of 3-acetyl morphine relative to morphine, heroin, and 6-acetylmorphine.
9. Describe how you would synthesize the *N*-phenethyl analogue of morphine.
10. The *N*-phenethyl analogue of morphine is a semi-synthetic product. What does this mean?

11. Explain whether you think the following structures would act as agonists or antagonists.



(a)



(b)

12. Thebaine has no analgesic activity. Suggest why this might be so.

13. Morphine is the active principle of opium. What is meant by an active principle?

14. Identify the asymmetric centres in morphine.

15. How could heroin be synthesized from morphine? What problems does this pose for drug regulation authorities?

FURTHER READING

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Titles for general further reading are listed on p. 763.

25 Anti-ulcer agents

25.1 Peptic ulcers

25.1.1 Definition

Peptic ulcers are localized erosions of the mucous membranes of the stomach or duodenum. The pain associated with ulcers is caused by irritation of exposed surfaces by the stomach acids. Before the appearance of effective anti-ulcer drugs in the 1960s, ulcer sufferers often suffered intense pain for many years and, if left untreated, the ulcer could result in severe bleeding and even death. For example, the film star Rudolph Valentino died from a perforated ulcer in 1926 at the age of 31.

25.1.2 Causes

The causes of ulcers have been disputed. Stress, alcohol, and diet have been considered important factors, but there is no clear evidence for this. Scientific evidence indicates that the two main culprits are the use of non-steroidal anti-inflammatories (NSAIDS) or the presence of a bacterium called *Helicobacter pylori*. As far as NSAIDS are concerned, agents such as **aspirin** inhibit the enzyme **cyclooxygenase 1** (**COX-1**). This enzyme is responsible for the synthesis of prostaglandins that inhibit acid secretion and protect the gastric mucosa. Once an ulcer has erupted, the presence of gastric acid aggravates the problem and delays recovery.

25.1.3 Treatment

Anti-ulcer therapy has been a huge money spinner for the pharmaceutical industry with drugs such as **cimetidine**, **ranitidine**, and the **proton pump inhibitors** (**PPIs**). None of these drugs were available until the 1960s, however, and it is perhaps hard for us now to appreciate how dangerous ulcers could be before that. In the early 1960s, the conventional treatment was to try to neutralize gastric acid in the stomach by administering **antacids**. These were bases, such as sodium bicarbonate or calcium carbonate. The dose levels required for neutralization were large and caused unpleasant side effects. Relief was only temporary and patients were often advised to stick to rigid diets, such as strained porridge and steamed fish. Ultimately, the only answer to severe ulcers was a surgical operation to remove part of the stomach.

The first effective anti-ulcer agents were the **H_2 histamine antagonists** which appeared in the 1960s. These were followed in the 1980s by the PPIs. The discovery of *H. pylori* then led to the use of antibacterial agents in anti-ulcer therapy. The current approach for treating ulcers caused by *H. pylori* is to use a combination of drugs, which includes a PPI, such as **omeprazole**, and two antibiotics, such as **amoxicillin** and **metronidazole**.

25.1.4 Gastric acid release

Gastric juices consist of digestive enzymes and hydrochloric acid designed to break down food. Hydrochloric acid is secreted from **parietal cells**, and the stomach secretes a layer of mucus to protect itself from its own gastric juices. Bicarbonate ions are also released and are trapped in the mucus to create a pH gradient within the mucus layer.

The H_2 antagonists and PPIs both work by reducing the amount of gastric acid released into the stomach by the parietal cells lining the stomach wall (Fig. 25.1). These parietal cells are innervated with nerves (not shown on the diagram) from the autonomic nervous system (sections 22.1 and 22.2). When the autonomic nervous system is stimulated, a signal is sent to the parietal cells culminating in the release of the neurotransmitter **acetylcholine** at the nerve termini. Acetylcholine activates the cholinergic receptors of the parietal cells leading to the release of gastric acid into the stomach. The trigger for this process is provided by the sight, smell, or even the thought of food. Thus, gastric acid is released before food has even entered the stomach.

Nerve signals also stimulate a region of the stomach called the **antrum**, which contains hormone-producing

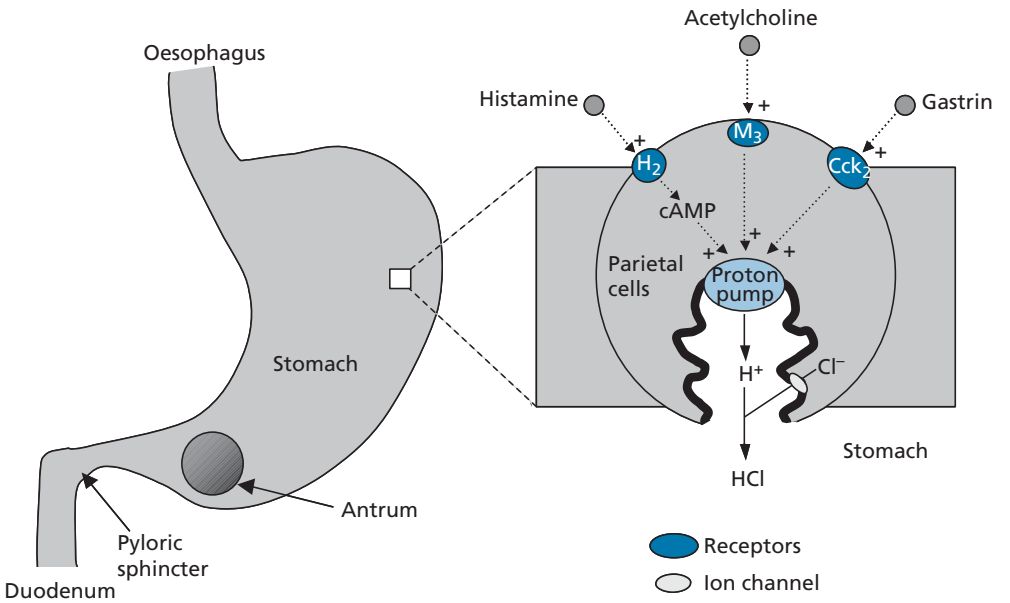


FIGURE 25.1 Factors influencing the release of gastric acid.

cells known as G cells. The hormone released is a peptide called **gastrin** (Fig. 25.2) which is also released when food is present in the stomach. Gastrin moves into the blood supply and travels to the parietal cells, further stimulating the release of gastric acid. Release of gastric acid should, therefore, be inhibited by antagonists blocking either the cholinergic receptor or the receptor for gastrin.

Agents which block the cholinergic receptor are known as **anticholinergic drugs** (section 22.9). These agents certainly block the cholinergic receptor in parietal cells and inhibit release of gastric acid. Unfortunately, they also inhibit cholinergic receptors at other parts of the body and cause unwanted side effects.

The local hormone **histamine** also stimulates the release of gastric acid by interacting with a specific type of histamine receptor called the **H_2 receptor**. Thus, histamine antagonists have proved to be important anti-ulcer drugs, although they have now largely been superseded by the PPIs, which block the mechanism by which hydrochloric acid is released from parietal cells.

25.2 H_2 antagonists

The first breakthrough in anti-ulcer therapy came with the design of the H_2 antagonist **cimetidine** (Tagamet) (Fig. 25.32) produced by the company Smith Kline and French (SKF). The cimetidine programme started in 1964 and was one of the early examples of rational drug design. Up until that time, many of the successes in medicinal chemistry involved the fortuitous discovery of useful pharmaceutical agents from natural sources and the study of analogues often synthesized on a trial-and-error basis. Although this approach yielded a large range of medicinal compounds it was wasteful in terms of the time and effort involved. Nowadays, the emphasis is on rational drug design using the tools of X-ray crystallography, molecular modelling, and genetic engineering (Chapters 13 and 17). Unfortunately, such tools were not available in the 1960s and the story of cimetidine is a good example of how to carry out rational drug design when the target has not been identified or isolated.

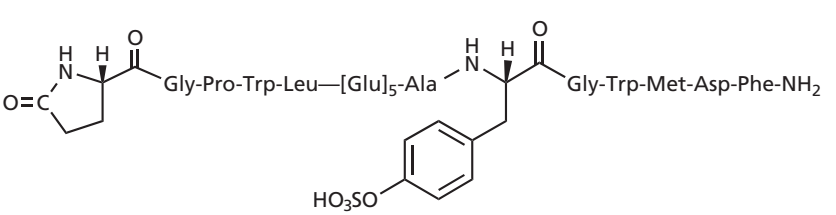


FIGURE 25.2 Gastrin.

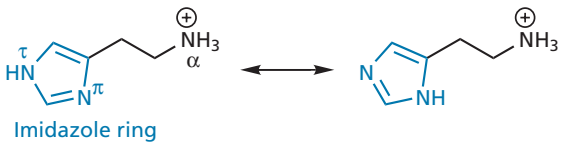


FIGURE 25.3 Histamine.

The remarkable aspect of the cimetidine story is that at the onset of the project there were no lead compounds and it was not even known if the target histamine receptor existed! In 1964, the best hope of achieving an antiulcer agent appeared to be in finding a drug which would block the hormone gastrin. Several research teams were active in this field, but the research team at SKF decided to follow a different tack altogether.

It was known experimentally that histamine (Fig. 25.3) stimulated gastric acid release *in vitro*, so the SKF team proposed that an antihistamine agent might be effective in treating ulcers. At the time, this was a highly speculative proposal as it was by no means certain that histamine played any significant role *in vivo*. Many workers at the time discounted the importance of histamine, especially when it was found that conventional antihistamines failed to inhibit gastric acid release. This suggested the absence of histamine receptors in the parietal cells. The fact that histamine had a stimulatory effect was explained away by suggesting that histamine coincidentally switched on the gastrin or cholinergic receptors. Even if a histamine receptor was present, opponents argued that blocking it would have little effect as the receptors for acetylcholine and gastrin would remain unaffected and could still be activated by their respective messengers. Initiating a project which had no known target and no known lead compound was unprecedented, and represented a massive risk. Indeed, for a long time little progress was made and it is said that company accountants demanded that the project be terminated. It says much for the scientists involved that they stuck to their guns and eventually confounded their critics. Why did the SKF team persevere in their search for an effective antihistamine? What was their reasoning? Before answering that, let us look at histamine itself and the antihistamines available at that time.

25.2.1 Histamine and histamine receptors

Histamine contains an imidazole ring which can exist in two tautomeric forms, as shown in Fig. 25.3. Attached to the imidazole ring is a two-carbon chain with a terminal α-amino group. The pK_a of this amino group is 9.80, which means that at a plasma pH of 7.4, the side chain of histamine is 99.6% ionized. The pK_a of the imidazole ring is 5.74 and so the ring is mostly un-ionized at pH 7.4 (Fig. 25.4). Note that the lower the pK_a value, the more acidic the proton. It is also useful to remember that 50% ionization takes place when the pH is the same value as the pK_a (section 11.3).

Whenever cell damage occurs, histamine is released and stimulates the dilatation and increased permeability of small blood vessels. This allows defensive cells, such as white blood cells, to be released from the blood supply into an area of tissue damage and to combat any potential infection. Unfortunately, the release of histamine can also be a problem. Allergic reactions and irritations are caused by release of histamine when it is not really needed.

The early antihistamine drugs were therefore designed to treat conditions such as hay fever, rashes, insect bites, or asthma. Two examples of these early antihistamines are **mepyramine** and **diphenhydramine** ('Benadryl') (Fig. 25.5), neither of which has any effect on gastric acid release.

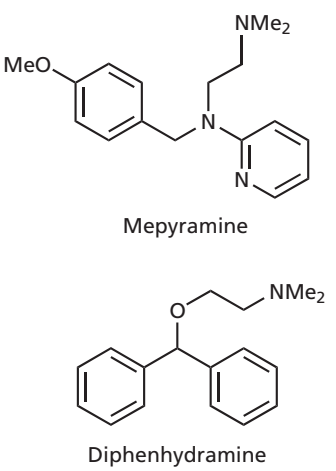


FIGURE 25.5 Early antihistamines.

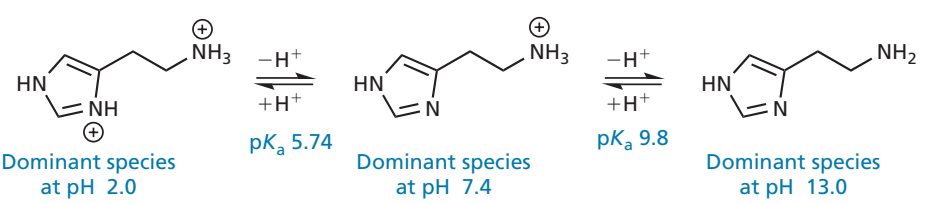


FIGURE 25.4 Ionization of histamine.

Bearing this in mind, why did the SKF team persevere with the antihistamine approach? The main reason was the fact that conventional antihistamines failed to inhibit *all* the then-known actions of histamine. For example, they failed to fully inhibit the dilatation of blood vessels induced by histamine. The SKF scientists therefore proposed that there might be two different types of histamine receptor analogous to the two types of cholinergic receptor mentioned in Chapter 22. Histamine—the natural messenger—would switch both on equally effectively and would not distinguish between them, whereas suitably designed antagonists might be capable of making that distinction. By implication, this meant that the conventional antihistamines known in the early 1960s were already selective in inhibiting the histamine receptors involved in the inflammation process (classified as H_1 receptors), rather than the proposed histamine receptors responsible for gastric acid secretion (classified as H_2 receptors).

It was an interesting theory, but the fact remained that there was no known antagonist for the proposed H_2 receptors. Until such a compound was found, it could not be certain that the H_2 receptors even existed.

Test your understanding and practise your molecular modelling with Exercise 25.1.

25.2.2 Searching for a lead

25.2.2.1 Histamine

The SKF team obviously had a problem. They had a theory but no lead compound. How could they make a start?

Their answer was to start from histamine itself. If the hypothetical H_2 receptor existed, then histamine must bind to it. The task then was to vary the structure of histamine in such a way that it would bind as an antagonist rather than an agonist.

This meant exploring how histamine itself bound to its receptors. Structure–activity relationship (SAR) studies on histamine and histamine analogues revealed that the binding requirements for histamine to the H_1 receptors were as follows:

- the side chain had to have a positively charged nitrogen atom with at least one attached proton. Quaternary ammonium salts which lacked such a proton were extremely weak in activity;
- there had to be a flexible chain between the above cation and a heteroaromatic ring;
- the heteroaromatic ring did not have to be imidazole, but it did have to contain a nitrogen atom with a lone pair of electrons, *ortho* to the side chain.

For the proposed H_2 receptor, SAR studies were carried out experimentally by determining whether histamine analogues could stimulate gastric acid release in stomach tissue. The essential SAR requirements were the same as for the H_1 receptor except that the heteroaromatic ring had to contain an amidine unit (HN – CH = N:).

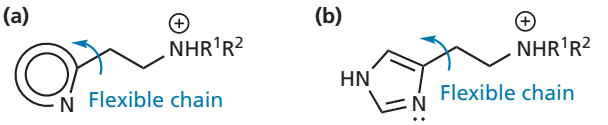


FIGURE 25.6 Summary of structure–activity relationship (SAR) results. (**a**) SAR for agonists at the H_1 receptor; (**b**) SAR for agonists at the proposed H_2 receptor.

The results are summarized in Fig. 25.6 and appear to show that the terminal α-amino group is involved in a binding interaction with both types of receptor via ionic and/or hydrogen bonding, while the nitrogen atom(s) in the heteroaromatic ring interact(s) via hydrogen bonding, as shown in Fig. 25.7.

25.2.2.2 N^{α}-Guanylhistamine

Having gained knowledge of the SAR for histamine, the task was now to design a molecule that would be recognized by the proposed H_2 receptor, but would not activate it. In other words, an agonist had to be converted to an antagonist. This meant altering the way in which the molecule bound to the receptor.

Pictorially, one can imagine histamine fitting into its binding site and stabilizing a change in shape which 'switches on' the receptor (Fig. 25.8). An antagonist can often be found by adding a functional group that binds to an extra binding region in the binding site and prevents the change in shape required for activation.

This was one of several strategies tried out by the SKF workers. To begin with, the structural differences between agonists and antagonists in other areas of medicinal chemistry were identified and similar alterations were tried on histamine. Analogues were tested to see whether they stimulated or blocked gastric acid release—the assumption being that an H_2 receptor would be responsible for such an effect.

Fusing an aromatic ring on to noradrenaline had been a successful tactic used in the design of adrenergic antagonists (see section 23.11.3). This same tactic was tried with histamine to give analogues such as the one shown in Fig. 25.9, but none of these compounds proved to be an antagonist.

Another approach which had been used successfully in the development of anticholinergic agents (section 22.9.2) had been the addition of non-polar, hydrophobic substituents. Similar substituents were attached to various locations of the histamine skeleton, but none proved to be antagonists.

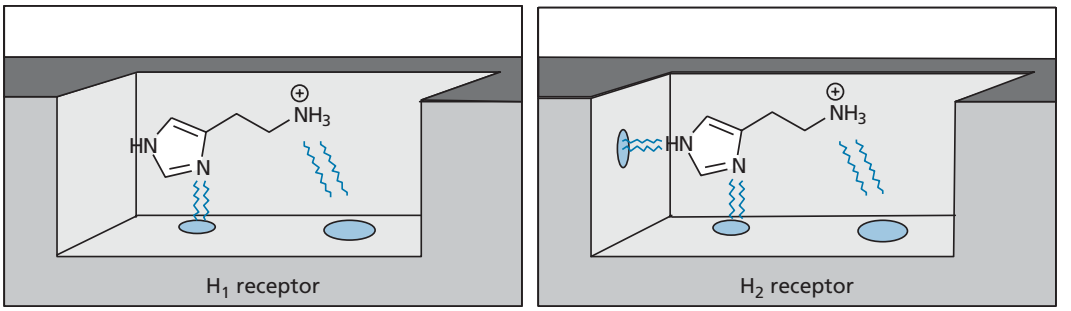


FIGURE 25.7 Binding interactions for the H_1 receptor and the proposed H_2 receptor.

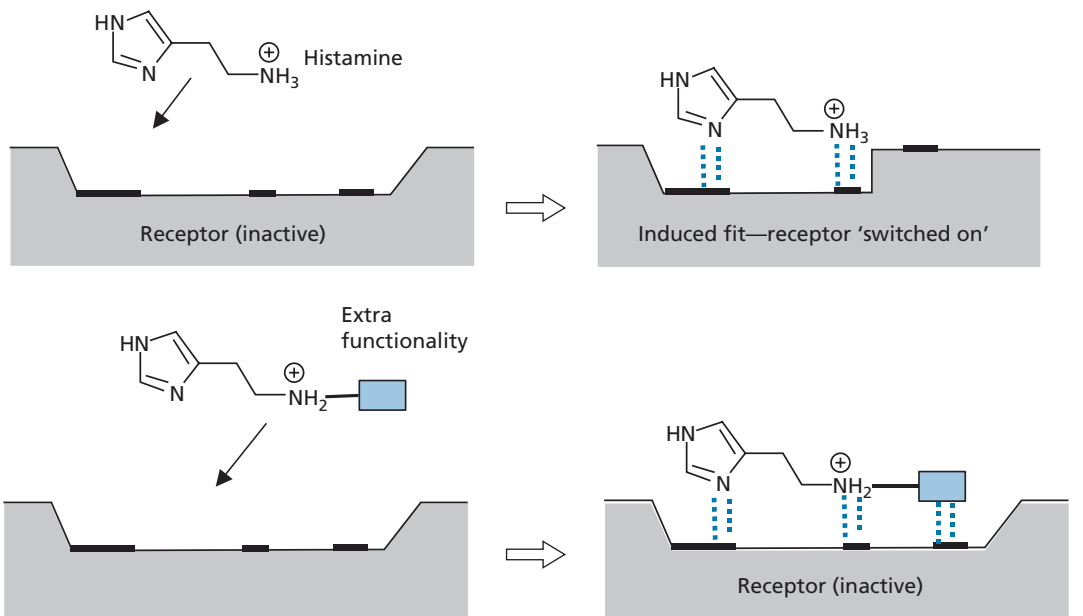


FIGURE 25.8 Possible receptor interactions of histamine and an antagonist.

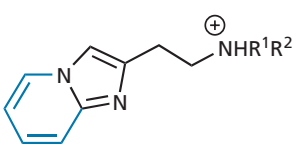


FIGURE 25.9 Histamine analogue with no antagonist activity.

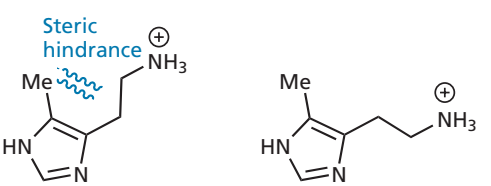


FIGURE 25.10 4-Methylhistamine.

Nevertheless, there was one interesting result which proved relevant to later studies. It was discovered that **4-methylhistamine** (Fig 25.10) was a highly selective H_2 agonist. In other words, it stimulated gastric acid release in the test assay, but had weak activity for all the other actions of histamine. How could this be?

4-Methylhistamine (like histamine) is a highly flexible molecule because of its side chain, but structural studies show that some of its conformations are less stable than others. In particular, conformation I in Fig. 25.10 is not favoured because of a large steric interaction between the 4-methyl group and the side chain. This means that the 4-methyl group is acting as a conformational blocker (section 13.3.10). The selectivity observed suggests that 4-methylhistamine (and by inference histamine) has to adopt two different conformations in order to fit the H_1 and putative H_2 receptor. As 4-methylhistamine is more active at the hypothetical H_2 receptor, it implies that conformation II is required for the H_2 receptor and conformation I is required for the H_1 receptor.

Despite this interesting result, the SKF workers were no closer to an H_2 antagonist. Two hundred compounds had been synthesized and not one had shown a hint of being an antagonist. Research up until this stage had concentrated on adding hydrophobic groups to search for an additional hydrophobic binding region in the proposed receptor binding site. Now the focus switched to study the effect of varying polar groups on the molecule. In particular, the terminal α–NH^{3+} group was replaced by different polar functional groups, the reasoning being that such groups could bond to the same binding region as the NH^{3+} group, but that the geometry of bonding might be altered sufficiently to produce an antagonist. This led to the first crucial breakthrough, with the discovery that N^{α}-**guanylhistamine** (Fig. 25.11) was a weak antagonist of gastric acid release.

This structure had, in fact, been synthesized very early on in the project, but had not been recognized as an antagonist. This is not too surprising as it acts as an agonist! It was not until later pharmacological studies were carried out that it was realized that N^{α}-guanylhistamine was acting as a partial agonist (section 8.4). This means that N^{α}-guanylhistamine activates the H_2 receptor, but not to the same extent as histamine. As a result, the amount of gastric acid released is lower. More importantly, as long as N^{α}-guanylhistamine is bound to the receptor, it prevents histamine from binding and thus prevents complete receptor activation. This was the first indication of any sort of antagonism to histamine, but still did not prove the existence of the H_2 receptor.

The question now arose as to which parts of the N^{α}-guanylhistamine skeleton were really necessary for this effect. Various guanidine structures were synthesized that lacked the imidazole ring, but none had the desired antagonist activity, demonstrating that both the imidazole ring and the guanidine group were required.

The structures of N^{α}-guanylhistamine and histamine were now compared. Both structures contain an imidazole ring and a positively charged group linked by a two-carbon bridge. The guanidine group is basic and protonated at pH 7.4, so the analogue has a positive charge, similar to histamine. However, the charge on the guanidine group can be spread around a planar arrangement of three nitrogens which means that it can be further away from the imidazole ring (Fig. 25.11). This leads to the possibility that the analogue could be interacting with an extra polar binding region on the receptor which is 'out of reach' of histamine. This is demonstrated in Figs. 25.12 and 25.13. Two alternative binding regions might be available for the cationic group—an agonist region where binding leads to activation of the receptor and an antagonist region where

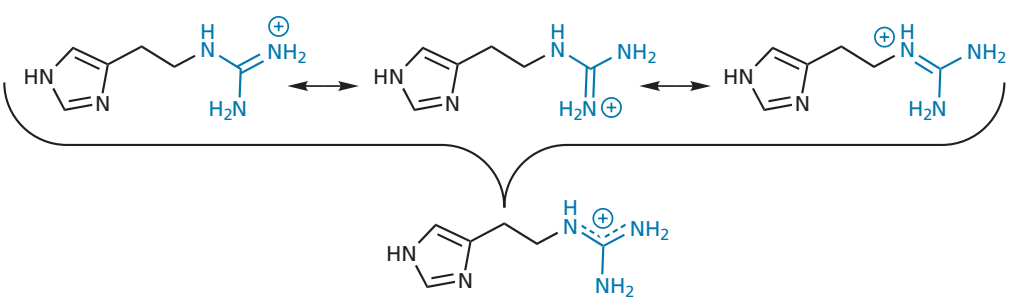


FIGURE 25.11 N^{α}-Guanylhistamine.

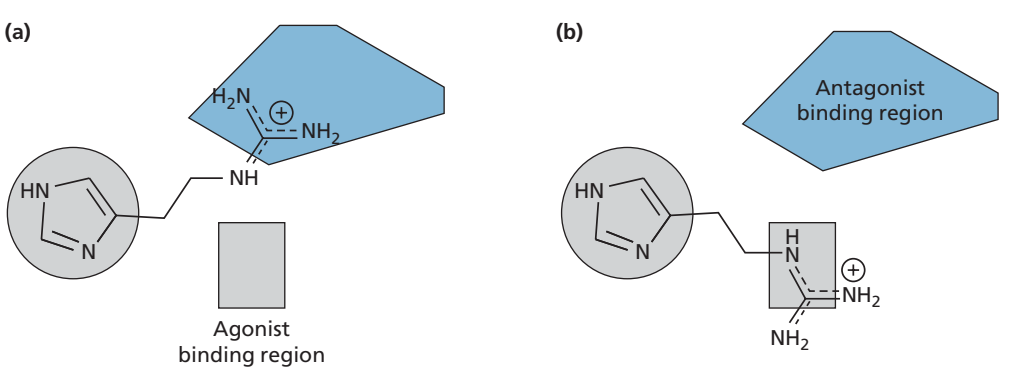


FIGURE 25.12 Possible binding modes for N^{α}-guanylhistamine as (**a**) an antagonist and (**b**) an agonist.

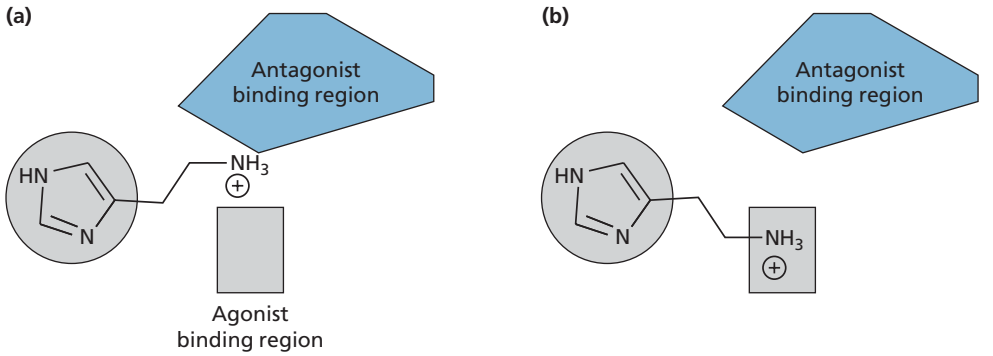


FIGURE 25.13 Binding of histamine: (**a**) no binding to the antagonist binding region; (**b**) binding to the agonist binding region.

binding does not activate the receptor. In Fig. 25.13, histamine is only able to reach the agonist region, whereas the analogue with its extended functionality is capable of reaching either region (Fig. 25.12).

If most of the analogue molecules bind to the agonist region and the remainder bind to the antagonist region, then this could explain the partial agonist activity. Regardless of the mode of binding, histamine would be prevented from binding and an antagonism would be observed owing to the fraction of N^{α}-guanylhistamine bound to the antagonist region.

25.2.3 Developing the lead: a chelation bonding theory

The task was now to find an analogue which would bind to the antagonist region only. The isothiourea (Fig. 25.14a) was synthesized as the positive charge would be restricted to the terminal portion of the chain and should interact more strongly with the more distant antagonist binding region. Antagonist activity did increase, but the compound was still a partial agonist, showing that binding was still possible to the agonist region.

Two other analogues were synthesized where one of the terminal amino groups in the guanidine group was replaced by a methylthio group or a methyl group (b in Fig. 25.14). Both these structures were partial agonists, but with poorer antagonist activity.

From these results, it was concluded that both terminal amino groups were required for binding to the antagonist binding site. It was proposed that the charged guanidine group was interacting with a charged carboxylate residue on the receptor via two hydrogen bonds (Fig. 25.15). If either of these terminal amino groups was absent, then binding would be weaker, resulting in a lower level of antagonism.

The chain was now extended from a two-carbon unit to a three-carbon unit to see what would happen if the guanidine group was moved further away from the imidazole ring. The antagonist activity increased for the

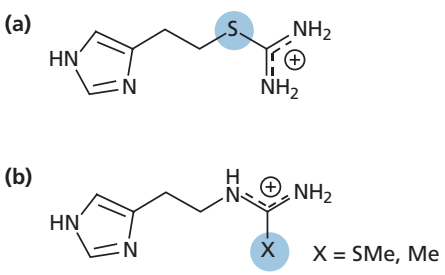


FIGURE 25.14 (**a**) An isothiourea. (**b**) Other analogues.

guanidine structure (Fig. 25.16), but, strangely enough, decreased for the isothiourea structure (Fig. 25.16). Therefore, it was proposed that with a chain length of two carbon units, hydrogen bonding to the receptor involved the terminal NH_2 groups, but with a chain length of three carbon units, hydrogen bonding to the same carboxylate group involved one terminal NH_2 group along with the NH group within the chain (Fig. 25.17). Support for this theory was provided by the fact that replacing one of the terminal NH_2 groups in the guanidine analogue with SMe or Me (Fig. 25.18) did not affect antagonist activity adversely. This was completely different from the results obtained when similar changes were carried out on the C_2 bridged compound. These bonding interactions are represented pictorially in Figs. 25.19 and 25.20.

25.2.4 From partial agonist to antagonist: the development of burimamide

The problem was now to completely remove the agonist activity to get a pure antagonist. This meant designing a structure which would differentiate between the agonist and antagonist binding regions. At first sight this looks impossible, as both regions appear to involve the same type of bonding. Histamine's activity as an agonist depends on the imidazole ring and the charged amino function, with the two groups taking part in hydrogen and ionic bonding respectively. The antagonist activity

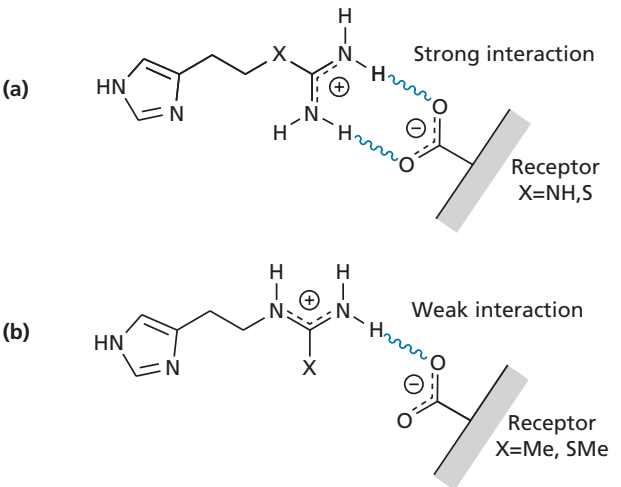


FIGURE 25.15 Proposed hydrogen bonding interactions for (**a**) a structure with two terminal amino groups and (**b**) an analogue with one terminal amino group.

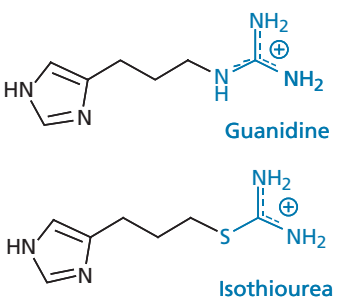


FIGURE 25.16 Guanidine and isothiourea structures with a 3-C linker.

of the partial agonists described so far also appears to depend on a hydrogen bonding imidazole ring and an ionic bonding guanidine group.

Fortunately, a distinction can be made between the charged groups.

The structures which show antagonist activity are all capable of forming a chelated bonding structure, as shown in Fig. 25.17. This interaction involves two hydrogen bonds between two charged species, but is it really necessary for the chelating group to be charged? Could a neutral group also chelate to the antagonist region by hydrogen bonding alone? If so, it might be possible to distinguish between the agonist and antagonist regions, especially as ionic bonding appears mandatory for the agonist region.

It was, therefore, decided to see what would happen if the strongly basic guanidine group was replaced by a neutral group capable of interacting with the receptor by two hydrogen bonds. There are many such groups, but the SKF workers limited the options by adhering to a principle which they followed throughout their research programme. Whenever they wished to alter a specific physical or chemical property, they strove to ensure that other properties were changed as little as possible. Only

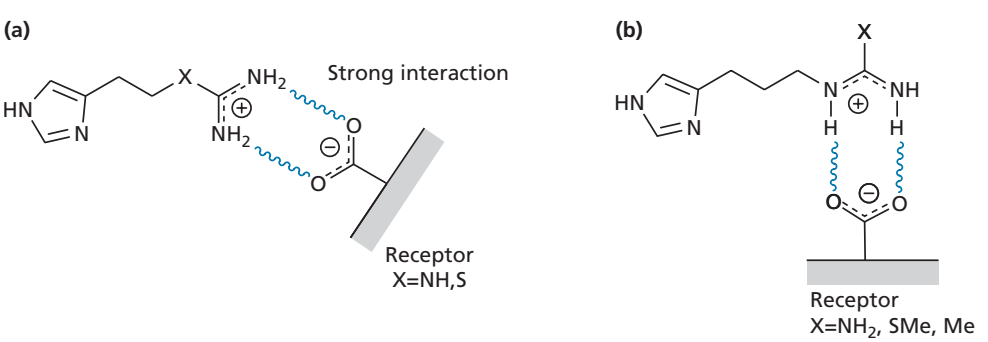


FIGURE 25.17 Proposed binding interactions for analogues of different chain length: (**a**) H-bonding involving two terminal amino groups for the three-atom chain; (**b**) H-bonding involving a terminal and internal amino group for a four-atom chain.

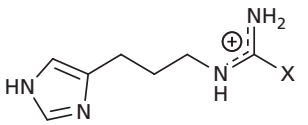


FIGURE 25.18 Guanidine analogue with X = SMe or Me.

in this way could they rationalize any observed improvement in activity. Thus, it was necessary to ensure that the new group was similar to guanidine in terms of size, shape, and hydrophobicity.

Several functional groups were tried, but success was ultimately achieved by using a thiourea group to give **SKF 91581** (Fig. 25.21). The thiourea group is neutral at physiological pH because the C=S group has an electron-withdrawing effect on the neighbouring nitrogens, making them non-basic and more like amide nitrogens. Apart from basicity, the properties of the thiourea group are very similar to the guanidine group. Both groups are planar, similar in size, and can take part in hydrogen bonding. This means that the alteration in biological activity can be reasonably attributed to the differences in basicity between the two groups.

SKF 91581 proved to be a weak antagonist with no agonist activity, establishing that the agonist binding region involves ionic bonding, whereas the antagonist region involves hydrogen bonding.

Further chain extension and the addition of an *N*-methyl group led to **burimamide** (Fig. 25.21), which was found to have enhanced activity, suggesting that the thiourea group has been moved closer to the antagonist binding region. The beneficial addition of the *N*-methyl group is due to an increase in hydrophobicity and a possible explanation for this will be described in section 25.2.8.2 (desolvation).

Burimamide is a highly specific competitive histamine antagonist at H_2 receptors, and is 100 times more potent than N^{α}-guanylhistamine in inhibiting gastric acid release induced by histamine. Its discovery gave the SKF researchers far greater evidence for the existence of H_2 receptors.

Test your understanding and practise your molecular modelling with Exercise 25.2.

25.2.5 Development of metiamide

Despite this success, burimamide was not suitable for clinical trials because its activity was still too low for oral administration. Attention was now directed to the imidazole ring of burimamide and, in particular, to its possible tautomeric and protonated forms. It was argued that if one of these forms was preferred for binding with the H_2 receptor, then activity might be enhanced by modifying the burimamide structure to favour that form.

At pH 7.4, it is possible for the imidazole ring to equilibrate between the two tautomeric forms (I) and (II) via the protonated intermediate (III) (Fig. 25.22). The necessary proton for this process is supplied by water or by an

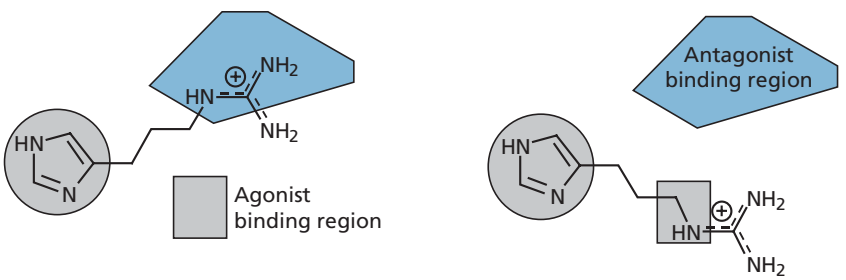


FIGURE 25.19 Proposed binding interactions for the 3-C bridged guanidine analogue.

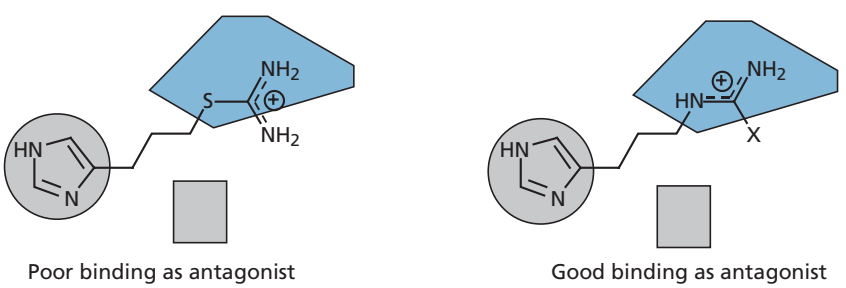


FIGURE 25.20 Proposed binding effect at the antagonist region if the guanidine group is modified.

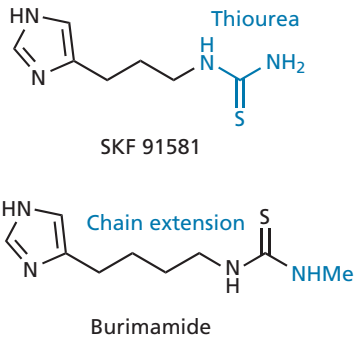


FIGURE 25.21 SKF 91581 and burimamide.

exchangeable proton on a suitable amino acid residue in the binding site. If the exchange is slow, then it is possible that the drug will enter and leave the receptor at a faster rate than the equilibration between the two tautomeric forms. If bonding involves only one of the tautomeric forms or the protonated form, then, clearly, antagonism would be increased if the structure was varied to prefer that form over the other. Our model hypothesis for receptor binding shows that the imidazole ring is important for the binding of both agonists and antagonists. Therefore, it is reasonable to assume that the preferred imidazole form is the same for both agonists and antagonists. If so, then the preferred form for a strong agonist such as histamine should also be the preferred form for a strong antagonist.

Figure 25.22 shows that the imidazole ring can exist as two un-ionized tautomers and one protonated form. Is the protonated form likely?

We have already seen that the pK_a for the imidazole ring in histamine is 5.74, meaning that the ring is a weak base and mostly un-ionized at physiological pH. The pK_a value for imidazole itself is 6.80 and for the imidazole ring in burimamide it is 7.25, showing that these rings are more basic and more likely to be ionized. Why should this be so?

The explanation lies in the side chains, which have an electronic effect affecting the basicity of the imidazole ring. A measure of the electronic effect of the side chain can be worked out by the Hammett equation (section 18.2.2):

$$pK_{a(R)} = pK_{a(H)} + \rho\sigma_R$$

where p$K_{a(R)}$ is the pK_a of the imidazole ring bearing a side chain R, p$K_{a(H)}$ is the pK_a of the unsubstituted imidazole ring, ρ is a constant, and σ_R is the Hammett substituent constant for the side chain R.

From the pK_a values, the value of the Hammett substituent constant can be calculated to show whether the side chain R is electron-withdrawing or electron-donating. In burimamide, the side chain is slightly electron-donating (of the same order as a methyl group). Therefore, the imidazole ring in burimamide is more likely to be ionized than in histamine, where the side chain is electron-withdrawing. At pH 7.4, 40% of the imidazole ring in burimamide is ionized compared with approximately 3% in histamine. This represents quite a difference between the two structures and, as the binding of the imidazole ring is important for both antagonist and agonist activity, it suggests that a pK_a value closer to that of histamine might lead to better binding and to better antagonist activity.

It was necessary, therefore, to make the side chain electron-withdrawing rather than electron-donating. This can be done by inserting an electronegative atom into the side chain—preferably one which causes minimum disturbance to the rest of the molecule. In other words, an isostere for a methylene group is required—one which has an electronic effect, but which has approximately the same size and properties as the methylene group.

The first isostere to be tried was a sulphur atom. Sulphur is quite a good isostere for the methylene unit, as both groups have similar van der Waals radii and similar bond angles. However, the C–S bond is slightly longer than a C–C bond, leading to a slight extension (15%) of the structure.

The methylene group replaced was next but one to the imidazole ring. This site was chosen, not for any strategic reasons, but because a synthetic route was readily available to carry out that particular transformation. As hoped, the resulting compound—**thiaburimamide** (Fig. 25.23)—had a significantly lower pK_a of 6.25 and was found to have enhanced antagonistic activity, supporting the theory that the un-ionized form is preferred over the protonated, ionized form.

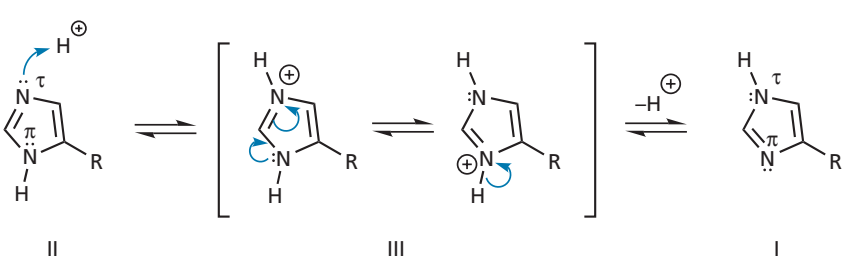


FIGURE 25.22 Imidazole ring can equilibrate between tautomeric forms (I and II) via the protonated intermediate (III).

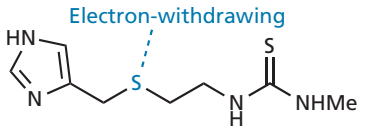


FIGURE 25.23 Thiaburimamide.

Thiaburimamide favours the un-ionized imidazole ring over the ionized ring, but there are two possible un-ionized tautomers. The next question is whether either of these are preferred for receptor binding.

Let us return to histamine. If one of the un-ionized tautomers is preferred over the other, it would be reasonable to assume that the preferred tautomer is the favoured tautomer for receptor binding, as it is more likely to be present. The preferred tautomer for histamine is tautomer I (Fig. 25.22), where Nτ is protonated and Nπ is not. This implies that Nτ in tautomer II is more basic than Nπ in tautomer I. This might not appear obvious, but we can rationalize it as follows. If Nτ in tautomer II is more basic than Nπ in tautomer I, it is more likely to become protonated to form the ionized intermediate (III). Moreover, de-protonation of III is more likely to give the weaker base which would be Nπ in tautomer I. Therefore, the equilibrium should shift to favour tautomer I.

This is all very well, but why should Nτ (tautomer II) be more basic than Nπ (tautomer I)? The answer lies in the side chain R. The side chain on histamine has a positively charged terminal amino group, which means that the side chain has an electron-withdrawing effect on the imidazole ring. As this effect is inductive, the strength of the effect will decrease with distance round the ring, which means that the nitrogen atom closest to the side chain (Nπ) experiences a greater electron-withdrawing effect than the one further away (Nτ). As a result, the closer nitrogen (Nπ) is less basic, and is less likely to bond to hydrogen (Fig. 25.24). As the side chain in thiaburimamide is also electron-withdrawing, then tautomer I will also be favoured here.

It was now argued that tautomer I could be further enhanced if an electron-*donating* group was placed at position 4 of the imidazole ring. At this position, the inductive effect would be felt most strongly at the neighbouring nitrogen (Nτ), further enhancing its basic

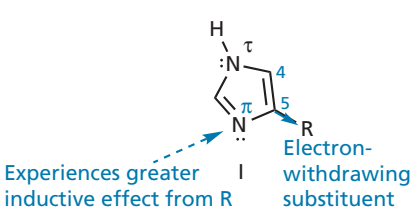


FIGURE 25.24 Inductive effect of the side chain on the imidazole nitrogens.

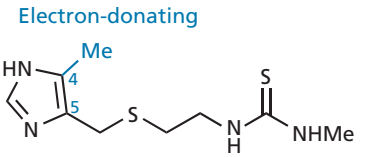


FIGURE 25.25 Metiamide.

character over Nπ. At the same time, it was important to choose a group that would not interfere with the normal receptor binding interactions. For example, a large substituent might be too bulky and prevent the analogue fitting the binding site. A methyl group was chosen because it was known that 4-methylhistamine was an agonist that was highly selective for the H_2 receptor (section 25.2.2.2). This resulted in **metiamide** (Fig. 25.25), which was found to have enhanced antagonist activity, supporting the proposed theory.

It is interesting to note that the percentage increase in tautomer I outweighs an undesirable rise in pK_a. By adding an electron-donating methyl group, the pK_a of the imidazole ring rises to 6.80 compared with 6.25 for thiaburimamide. Coincidentally, this is the same pK_a as for imidazole itself, which shows that the electronic effects of the methyl group and the side chain cancel each other out as far as pK_a is concerned. A pK_a of 6.80 means that 20% of metiamide exists as the protonated form (III), but this is still lower than the corresponding 40% for burimamide. More importantly, the beneficial effect on activity due to the increase in tautomer (I) outweighs the detrimental effect caused by the increase in the protonated form (III).

4-Methylburimamide (Fig. 25.26) was also synthesized for comparison. Here, introduction of the 4-methyl group does not lead to an increase in activity. The pK_a is increased to 7.80, resulting in the population of ionized imidazole ring rising to 72%. This demonstrates the importance of rationalizing structural changes. Adding the 4-methyl group to thiaburimamide is advantageous, but adding it to burimamide is not.

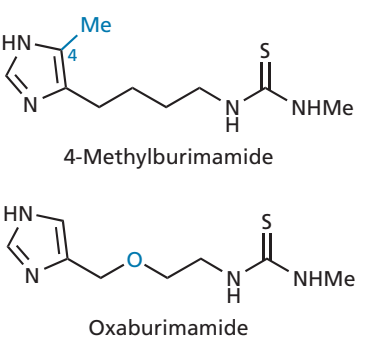


FIGURE 25.26 4-Methylburimamide and oxaburimamide.

The design and synthesis of metiamide followed a rational approach aimed at favouring one specific tautomer. Such a study is known as a **dynamic structure–activity analysis**.

Strangely enough, it has since transpired that the improvement in antagonism may have resulted from conformational effects. X-ray crystallography studies have indicated that the longer thioether linkage in the chain increases the flexibility of the side chain and that the 4-methyl substituent in the imidazole ring may help to orientate the imidazole ring correctly for receptor binding. It is significant that the oxygen analogue **oxaburimamide** (Fig. 25.26) is less potent than burimamide, despite the fact that the electron-withdrawing effect of the oxygen-containing chain on the ring is similar to the sulphur-containing chain. The bond lengths and angles of the ether link are similar to the methylene unit and, in this respect, it is a better isostere than sulphur. This is because the oxygen atom is substantially smaller than sulphur. However, this does not imply that it will be a better bioisostere, as other properties might be detrimental to activity. For example, the oxygen atom is significantly more basic and more hydrophilic than either sulphur or methylene. In fact, oxaburimamide's lower activity might be due to a variety of reasons. The oxygen may not allow the same flexibility permitted by the sulphur atom. Alternatively, the oxygen may be involved in a hydrogen bonding interaction with the binding site that is detrimental to activity. Another possibility is the fact that oxygen is more likely to be solvated than sulphur and there is an energy penalty involved in desolvating the group before binding.

Metiamide is 10 times more active than burimamide and showed promise as an anti-ulcer agent. Unfortunately, a number of patients suffered from kidney damage and granulocytopenia—a condition which results in the reduction of circulating white blood cells and makes patients susceptible to infection. Further developments were now required to find an improved drug without these side effects (see Molecular modelling exercise 25.2).

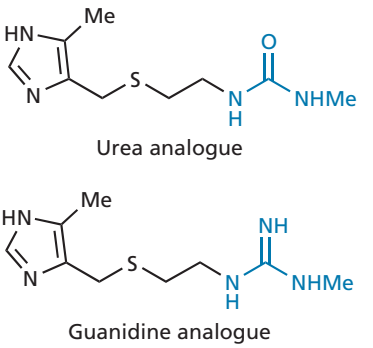


FIGURE 25.27 Urea and guanidine analogues.

25.2.6 Development of cimetidine

It was proposed that metiamide's side effects were associated with the thiourea group—a group which is not particularly common in human biochemistry. Therefore, consideration was given to replacing the thiourea with a group which had similar properties, but which would be more acceptable in a biochemical context. The urea analogue (Fig. 25.27) was found to be less active. The guanidine analogue (Fig. 25.27) was also less active, but it was interesting to note that this compound had no agonist activity. This contrasts with the C_3-bridged guanidine (Fig. 25.16), which is a partial agonist. Therefore, the guanidine analogue (Fig 25.27) was the first example of a guanidine-containing structure having pure antagonist activity.

One possible explanation for this is that the longer four-atom chain extends the guanidine binding group beyond the reach of the agonist binding region (Fig. 25.28), whereas the shorter three-atom chain still allows binding to both agonist and antagonist regions (Fig. 25.29).

The antagonist activity for the guanidine analogue (Fig. 25.27) is weak, but it was decided to look more closely at this compound, as it was thought that the guanidine unit would lack the toxic side effects of the thiourea unit. This is a reasonable assumption as the guanidine unit is present naturally in the amino acid **arginine** (Appendix 1).

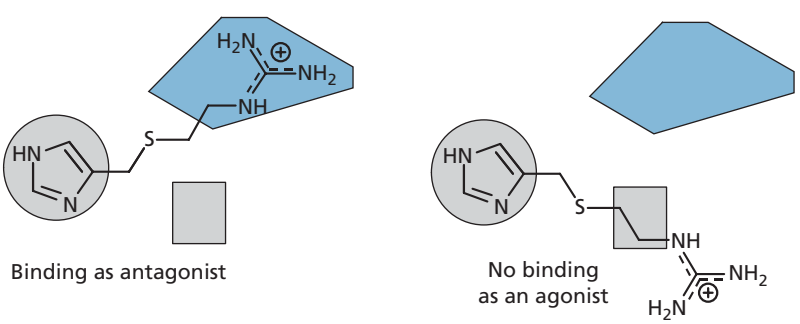


FIGURE 25.28 Binding of the guanidine analogue with a four-atom linker.

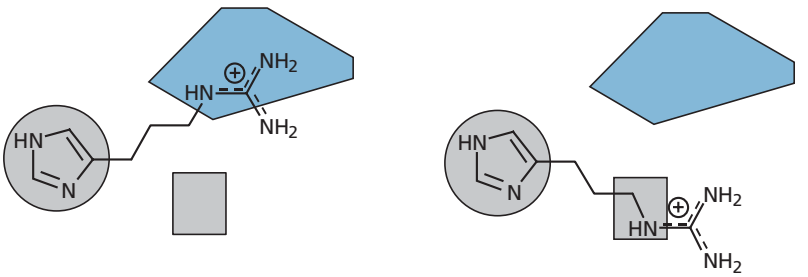


FIGURE 25.29 Binding of the guanidine analogue with a three-atom linker.

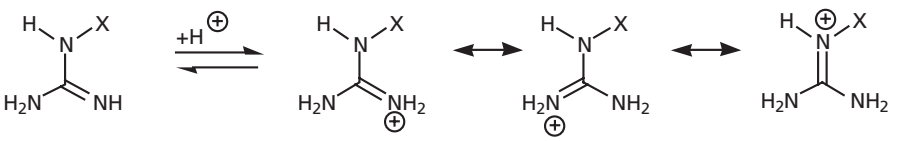


FIGURE 25.30 Ionization of monosubstituted guanidines.

The problem was how to retain the guanidine unit while increasing activity. It seemed likely that the low activity observed was because the basic guanidine group would essentially be fully protonated and ionized at pH 7.4. The challenge was now to make this group non-basic—no easy task as guanidine is one of the strongest neutral organic bases in organic chemistry.

Nevertheless, a search of the literature revealed a useful study on the ionization of monosubstituted guanidines (Fig. 25.30). A comparison of the pK_a values of these compounds with the inductive substituent constants σ_i (section 18.2.2) for the substituents X gave a straight line, as shown in Fig. 25.31, showing that pK_a is inversely proportional to the electron-withdrawing power of the substituent. Thus, strongly electron-withdrawing substituents make the guanidine group less basic and less ionized. The nitro and cyano groups are particularly strong electron-withdrawing groups. The pK_as for cyanoguanidine and nitroguanidine are 0.4 and 0.9, respectively (Fig. 25.31)—similar values to the pK_a for thiourea itself (-1.2).

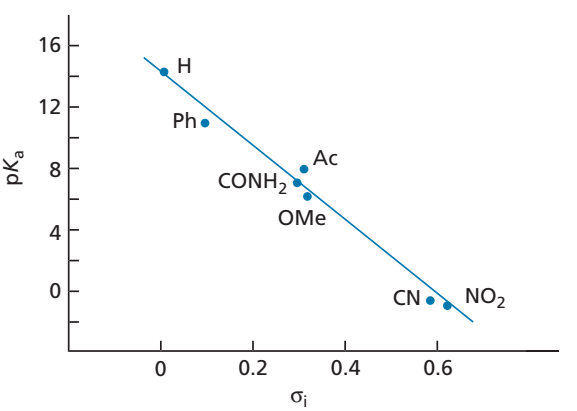


FIGURE 25.31 pK_a versus inductive substituent constants (σ_i).

Both the nitroguanidine and cyanoguanidine analogues of metiamide were synthesized and found to have comparable antagonist activities to metiamide. The cyanoguanidine analogue (**cimetidine**; Fig. 25.32) was the more potent analogue and was chosen for clinical studies. Its synthesis is described in Box 25.1.

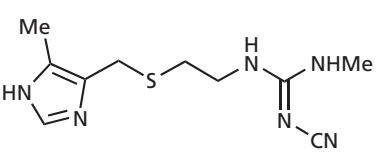


FIGURE 25.32 Cimetidine.

25.2.7 Cimetidine

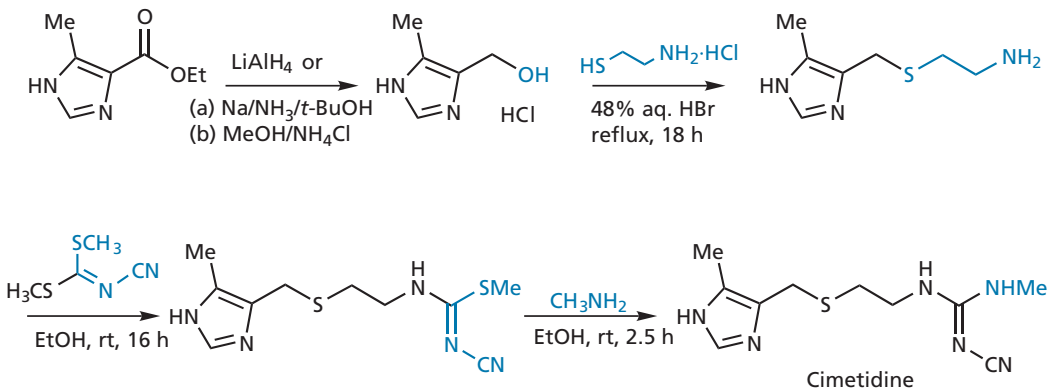
25.2.7.1 Biological activity

Cimetidine inhibits gastric acid release by acting as an antagonist at H_2 receptors. It does not show the toxic side effects observed for metiamide and has been shown to be slightly more active. It has also been found to inhibit **pentagastrin** (Fig. 25.33) from stimulating release of gastric acid. Pentagastrin is an analogue of gastrin and the fact that cimetidine inhibits it suggests some relationship between histamine and gastrin in the release of gastric acid.

Cimetidine was first marketed in the UK in 1976 under the trade name of **Tagamet** (derived from an**tag**onist and ci**met**idine). It was the first really effective anti-ulcer drug, doing away with the need for surgery. For several

BOX 25.1 Synthesis of cimetidine

The synthesis of cimetidine was originally carried out as a four-step process, where lithium aluminium hydride was used as the reagent for the initial reduction step. Subsequent research revealed that this reduction could be carried out more cheaply and safely using sodium in liquid ammonia, and so this became the method used in the manufacturing process.



Synthesis of cimetidine.

(*t*-Boc)N-β-Ala-Trp-Met-Asp-Phe-NH_2

FIGURE 25.33 Pentagastrin.

years, it was the world's biggest selling prescription product until it was pushed into second place in 1988 by **ranitidine** (section 25.2.9.1).

25.2.7.2 Structure and activity

The finding that metiamide and cimetidine are both good H_2 antagonists of similar activity shows that the cyanoguanidine group is a good bioisostere for the thiourea group. Three tautomeric forms (Fig. 25.34) are possible for the guanidine group with the imino tautomer (II) being the preferred tautomer. This is because the cyano group has a stronger electron-withdrawing effect on the neighbouring nitrogen compared with the two nitrogens further away. As a result, the neighbouring nitrogen is less basic and less likely to be protonated. Moreover, tautomer II has an extra stabilization owing to the conjugation of the double bond and the cyano group.

As tautomer II is favoured, the guanidine group bears a close structural similarity to the thiourea group. Both groups have a planar π electron system with similar geometries (equal C–N distances and angles). They are polar and hydrophilic, with high dipole moments and low partition coefficients. They are weakly basic and also weakly acidic such that they are un-ionized at pH 7.4.

25.2.7.3 Metabolism

It is important to study the metabolism of a new drug in case the metabolites have biological activity in their own right. Any such activity might lead to undesirable side effects. Alternatively, a metabolite might have enhanced activity of the type desired and give clues to further development. Cimetidine itself is metabolically stable and is

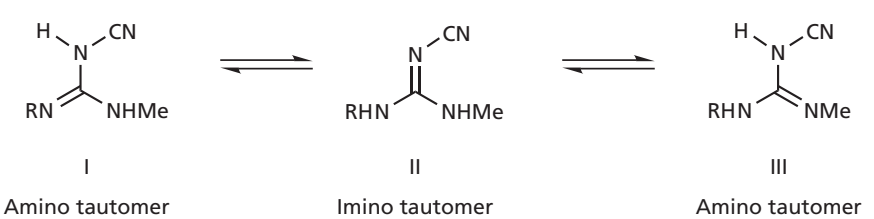


FIGURE 25.34 Three tautomeric forms of guanidine unit.

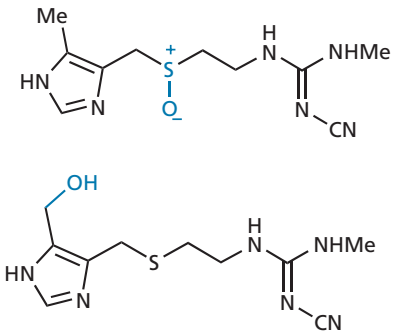


FIGURE 25.35 Metabolites of cimetidine.

excreted largely unchanged. The only metabolites that have been identified are due to oxidation of the sulphur link or oxidation of the ring methyl group (Fig. 25.35).

It has been found that cimetidine inhibits the cytochrome P-450 enzymes in the liver (section 11.5.2). These enzymes are involved in the metabolism of several clinically important drugs, and inhibition by cimetidine may result in toxic side effects as a result of increased blood levels of these drugs. In particular, caution is required when cimetidine is taken with drugs such as **diazepam**, **lidocaine**, **warfarin**, or **theophylline**.

25.2.8 Further studies of cimetidine analogues

25.2.8.1 Conformational isomers

A study of the various stable conformations of the guanidine group in cimetidine led to a rethink of the type of bonding taking place at the antagonist binding region. Up until this point, the favoured theory had been a bidentate hydrogen interaction, as shown in the top diagram of Fig. 25.15, where the two hydrogens involved in hydrogen bonding are pointing in the same direction. In order to achieve this kind of bonding, the guanidine group in cimetidine would have to adopt the *Z,Z* conformation shown in Fig. 25.36. (The *Z* and *E* nomenclature is relevant here, as there is double bond character in the N–C bonds of the guanidine unit.)

However, X-ray and NMR studies have shown that cimetidine exists as an equilibrium mixture of the *E,Z* and *Z,E* conformations. Neither the *Z,Z* nor the *E,E* form is favoured because of steric interactions. If either the *E,Z* or *Z,E* form is the active conformation then it implies that the chelation type of hydrogen bonding described previously is not taking place. An alternative possibility is that the guanidine unit is hydrogen bonding to two distinct hydrogen bonding regions, rather than to a single carboxylate group (Fig. 25.37). Further support for this theory is provided by the weak activity observed for the urea analogue (Fig. 25.27). This compound is known to prefer the *Z,Z* conformation over the *Z,E* or *E,Z* conformations, and would, therefore, be unable to bind to two distinct hydrogen bonding regions.

If this bonding theory is correct and the active conformation is the *E,Z* or *Z,E* form, restricting the group to adopt one or other of these forms may lead to more active

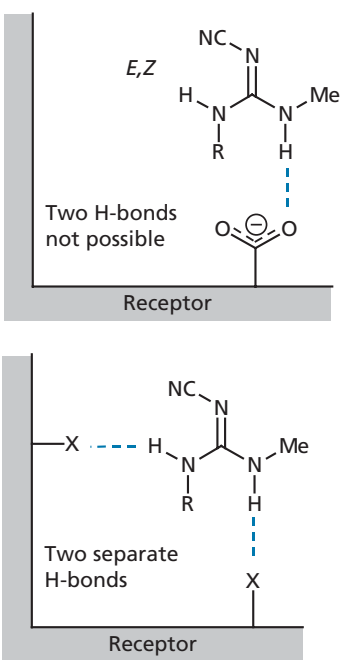


FIGURE 25.37 Alternative theory for cimetidine binding at the agonist region.

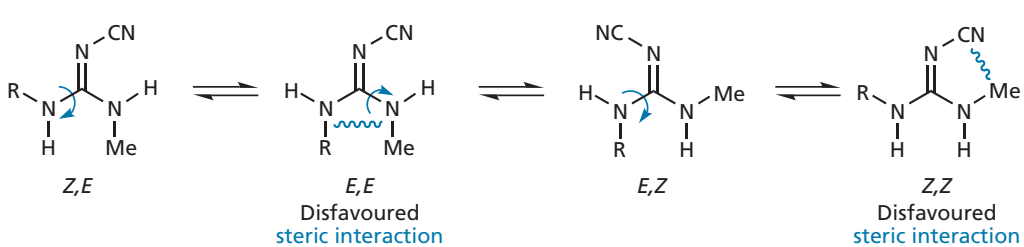


FIGURE 25.36 Conformations of the guanidine group in cimetidine.

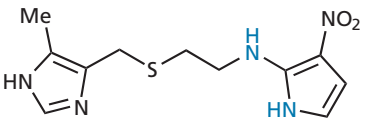


FIGURE 25.38 Nitropyrrole derivative of cimetidine.

compounds and the identification of the active conformation. This can be achieved by incorporating part of the guanidine unit within a ring—a strategy of rigidification (section 13.3.9). For example, the nitropyrrole derivative (Fig. 25.38) has been shown to be the strongest antagonist in the cimetidine series, implying that the *E,Z* conformation is the active conformation.

The isocytosine ring (Fig. 25.39) has also been used to 'lock' the guanidine group, limiting the number of conformations available. The ring allows for further substitution and development, as described in the following sections.

25.2.8.2 Desolvation

The guanidine and thiourea groups, used so successfully in the development of H_2 antagonists, are polar and hydrophilic. This implies that they are likely to be highly solvated and surrounded by a 'water coat'. Before hydrogen bonding can take place to the receptor, this water coat has to be removed. The more solvated the group is, the more difficult that will be.

One possible reason for the low activity of the urea derivative (Fig. 25.27) has already been described above. Another possible reason could be the fact that the urea group is more hydrophilic than the thiourea or cyanoguanidine groups, and is more highly solvated. The energy penalty involved in desolvating the urea group might explain why this analogue has a lower activity than cimetidine, despite having a lower partition coefficient and greater water solubility.

Leading on from this, if the ease of desolvation is a factor in antagonist activity, then reducing the solvation of the polar group should increase activity. One way of achieving this would be to increase the hydrophobic character of the polar binding group.

A study was carried out on a range of cimetidine analogues containing different planar aminal systems (Z) (Fig. 25.40) to see whether there was any relationship between antagonist activity and the hydrophobic character of the aminal system (HZ).

This study showed that antagonist activity was proportional to the hydrophobicity (log *P*) of the aminal unit HZ (Fig. 25.41) and supported the desolvation theory. The relationship could be quantified as follows:

$$\log(\text{activity}) = 2.0\,\log P + 7.4$$

Further studies on hydrophobicity were carried out by adding hydrophobic substituents to the isocytosine analogue (Fig. 25.39). These studies showed that there was an optimum hydrophobicity for activity corresponding to the equivalent of a butyl or pentyl substituent. A benzyl substituent was particularly good for activity, but proved to have toxic side effects. These could be decreased by adding alkoxy substituents to the aromatic ring and this led to the synthesis of **oxmetidine** (Fig. 25.42), which had enhanced activity over cimetidine. Oxmetidine was considered for clinical use, but was eventually withdrawn as it still retained undesirable side effects.

Test your understanding and practise your molecular modelling with Exercise 25.3 and 25.4.

25.2.8.3 Development of the nitroketeneaminal binding group

As we have seen, antagonist activity increases if the hydrophobicity of the polar binding group is increased.

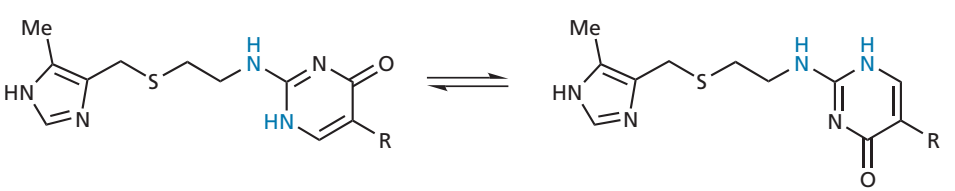


FIGURE 25.39 Isocytosine ring.

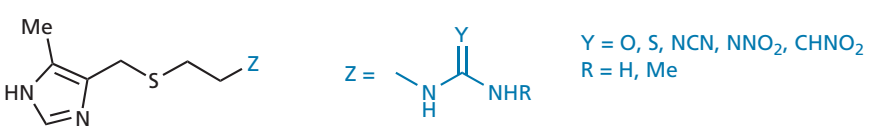


FIGURE 25.40 Cimetidine analogue with planar aminal system (Z).

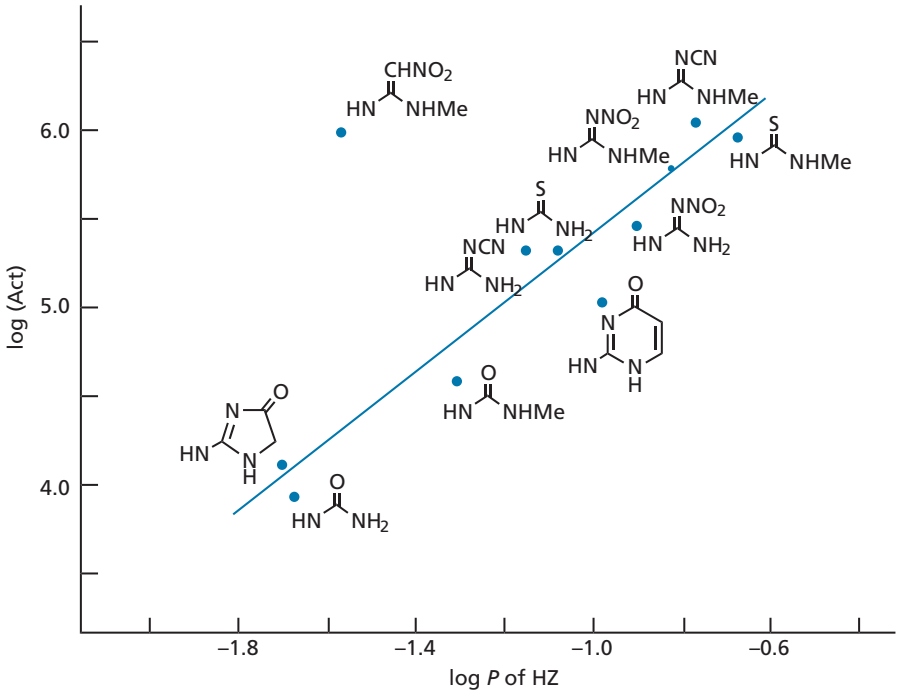


FIGURE 25.41 Antagonist activity is proportional to the hydrophobicity (log *P*) of the aminal unit Z.

It was therefore decided to see what would happen if the polar imino nitrogen of cimetidine was replaced by a non-polar carbon atom. This would result in a keteneaminal group, as shown in Fig. 25.43. Unfortunately, keteneaminals are more likely to exist as their amidine tautomers unless a strongly electronegative group (e.g. NO_2) is attached to the carbon atom.

A nitroketeneaminal group was therefore used to give the structure shown in Fig. 25.44. Surprisingly, there was no great improvement in activity, but, when the structure was studied in detail, it was discovered that it was far more hydrophilic than expected. This explained why the activity had not increased, but it highlighted a different puzzle. The compound was *too* active. Based on its hydrophilicity, it should have been a much weaker antagonist (Fig. 25.41).

It was clear that this compound did not fit the pattern followed by previous compounds as its antagonist activity was 30 times higher than predicted. Nor was the nitroketeneaminal the only analogue to deviate from the expected pattern. The imidazolinone analogue (Fig. 25.44), which is relatively hydrophobic, had a much lower activity than would have been predicted from the equation. Findings like these are particularly exciting, as any deviation from the normal pattern suggests that some other factor is at work, which may give a clue to future development.

In this case, it was concluded that the polarity of the group might be important in some way. In particular, the orientation of the dipole moment appeared to be crucial. In Fig. 25.45, the orientation of the dipole moment is defined by ϕ—the angle between the dipole moment

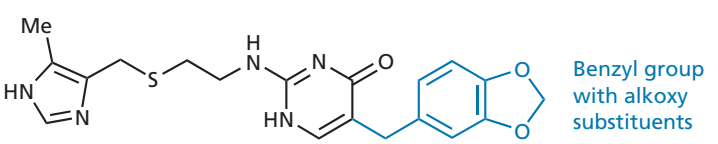


FIGURE 25.42 Oxmetidine.

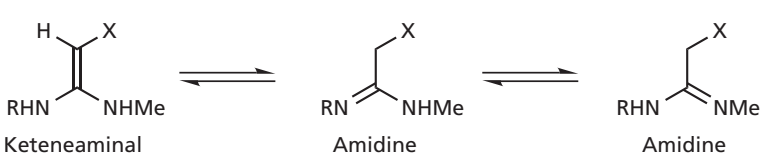


FIGURE 25.43 The keteneaminal group and amidine tautomers.

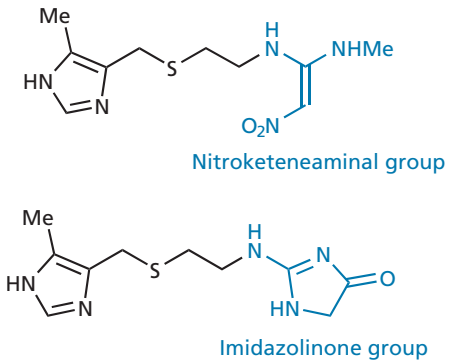


FIGURE 25.44 Cimetidine analogues with unexpected levels of activity.

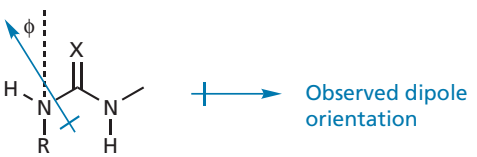


FIGURE 25.45 Orientation of dipole moment.

and an extension of the NR bond. The cyanoguanidine, nitroketeneaminal, and nitropyrrole groups all have high antagonist activity and have dipole moment orientations of 13°, 33°, and 27° respectively (Fig. 25.46). The isocytosine and imidazolinone groups have lower activity and have dipole orientations of 2° and –6° respectively. The strength of the dipole moment (μ) does not appear to be crucial.

Why should the orientation of a dipole moment be important? One possible explanation is as follows. As the drug approaches the receptor, its dipole interacts with a dipole on the receptor surface such that the dipole moments are aligned. This orientates the drug in a specific way before hydrogen bonding takes place and determines how strong the subsequent hydrogen bonding will be (Fig. 25.47). If the dipole moment is correctly orientated as in the keteneaminal analogue, the group is correctly positioned for strong hydrogen bonding and high activity will result. If the orientation is wrong as in the imidazolinone analogue, then the bonding is less efficient and activity is weaker.

Quantitative structure–activity relationship (QSAR) studies (Chapter 18) were carried out to determine what the optimum angle ϕ should be for activity. This resulted in an ideal angle for ϕ of 30°. A correlation was worked out between the dipole moment orientation, partition coefficient, and activity as follows:

$$\log A = 9.12\cos\theta + 0.6\log P - 2.71$$
$$(n = 13,\ r = 0.91,\ s = 0.41)$$

where A is the antagonist activity, P is the partition coefficient, and θ is the deviation in angle of the dipole moment from the ideal orientation of 30° (Fig. 25.48).

The equation shows that activity increases with increasing hydrophobicity (P). The cos θ term shows that activity drops if the orientation of the dipole moment varies from the ideal angle of 30°. At the ideal angle, θ is 0° and cos θ is 1. If the orientation of the dipole moment deviates from 30°, then cos θ will be less than 1 and will lower the calculated activity. The nitroketeneaminal group did not result in a more powerful cimetidine analogue, but we shall see it again in ranitidine (section 25.2.9.1).

Test your understanding and practise your molecular modelling with Exercises 25.5 and 25.6.

25.2.9 Further H_2 antagonists

25.2.9.1 Ranitidine

Further studies on cimetidine analogues showed that the imidazole ring could be replaced by other nitrogen-containing heterocyclic rings. Glaxo moved one step further, however, and replaced the imidazole ring with a furan ring bearing a nitrogen-containing substituent. This led to the introduction of **ranitidine** (Zantac) (Fig. 25.49). Ranitidine has fewer side effects than cimetidine, a longer duration of action, and is 10 times more active. SAR results for ranitidine include the following:

- the nitroketeneaminal group is optimum for activity, but can be replaced by other planar π systems capable of hydrogen bonding;
- replacing the sulphur atom with a methylene atom leads to a drop in activity;
- placing the sulphur next to the ring lowers activity;

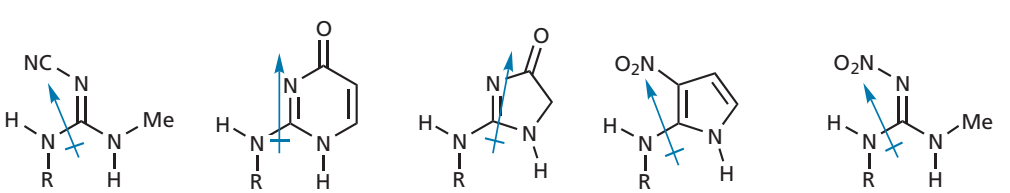


FIGURE 25.46 Dipole moments of various antagonistic groups.

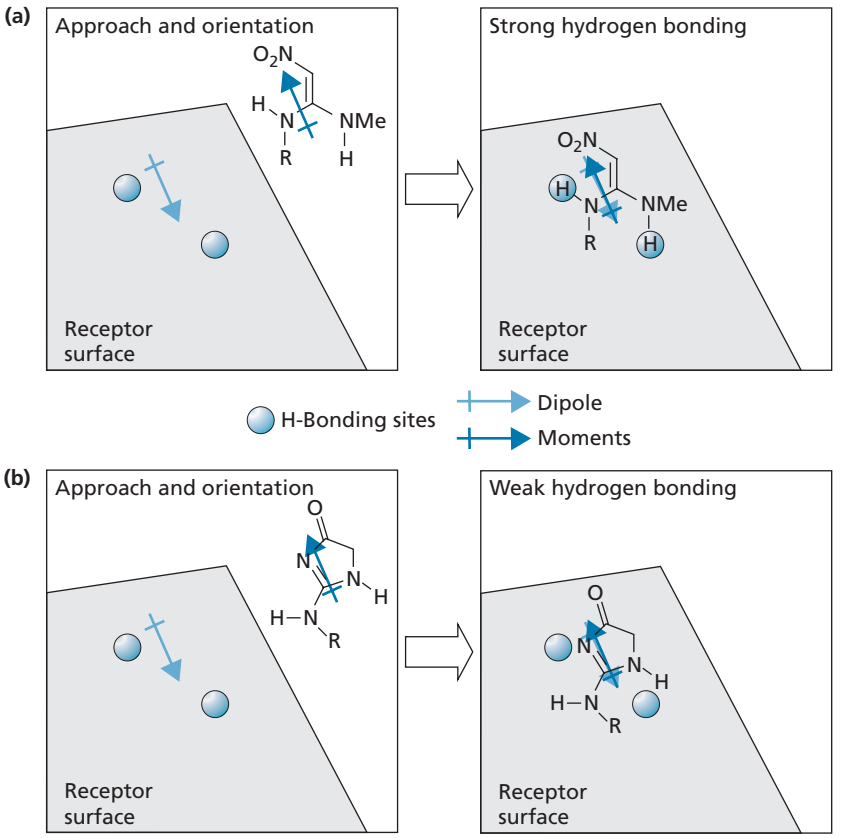


FIGURE 25.47 Dipole–dipole interactions and their effects on orientation and receptor binding: (**a**) strong binding of the nitroketeneaminal group; (**b**) weak binding of the imidazolinone group.

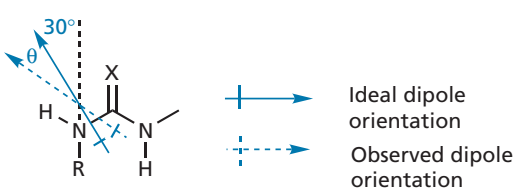


FIGURE 25.48 Definition of the angle θ.

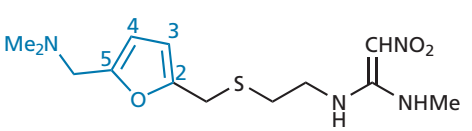


FIGURE 25.49 Ranitidine.

- replacing the furan ring with more hydrophobic rings such as phenyl or thiophene reduces activity;
- 2,5-disubstitution is the best substitution pattern for the furan ring;
- the methyl substituents of the dimethylamino group can be varied, showing that the basicity and hydrophobicity of this group are not crucial to activity;
- methyl substitution at carbon-3 of the furan ring eliminates activity, whereas the equivalent substitution in the imidazole series increases activity;
- methyl substitution at carbon-4 of the furan ring increases activity.

The last two results imply that the heterocyclic rings for cimetidine and ranitidine are not interacting in the same way with the H_2 receptor. This is supported by the fact that a corresponding dimethylaminomethylene group attached to cimetidine leads to a drop in activity. Ranitidine was introduced to the market in 1981 and, by 1988, it had taken over from cimetidine as the world's biggest selling prescription drug. Over a 10-year period, it earned Glaxo profits of around £4 billion ($7 billion) and at one time was earning profits of £4 million ($7 million) per day.

25.2.9.2 Famotidine and nizatidine

Over the period 1985–87, two new anti-ulcer drugs were introduced to the market—famotidine and nizatidine (Fig. 25.50).

Famotidine (Pepcid) is 30 times more active than cimetidine *in vitro*. The side chain contains a sulphonylamidine

group, and the heterocyclic imidazole ring of cimetidine has been replaced by a 2-guanidinothiazole ring. SAR studies gave the following results:

- the sulphonylamidine binding group is not essential and can be replaced by a variety of structures as long as they are planar, have a dipole moment, and are capable of interacting with the receptor by hydrogen bonding. A low pK_a is not essential, which allows a larger variety of planar groups to be used than is possible for cimetidine;
- activity is optimum for a chain length of four or five units;
- replacement of sulphur by a CH_2 group *increases* activity;
- modification of the chain is possible with, for example, inclusion of an aromatic ring;
- a methyl substituent on the heterocyclic ring, *ortho* to the chain leads to a drop in activity (unlike the cimetidine series);
- three of the four hydrogens in the two guanidine NH_2 groups are required for activity.

There are several results here which are markedly different from cimetidine, implying that famotidine and cimetidine are not interacting in the same way with the H_2 receptor. Further evidence for this is the fact that replacing the guanidine of cimetidine analogues with a sulphonylamidine group leads to very low activity.

Nizatidine (Fig. 25.50) was introduced into the UK in 1987 by the Lilly Corporation and is equipotent with ranitidine. The furan ring in ranitidine is replaced by a thiazole ring.

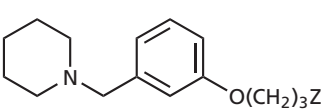
25.2.9.3 H_2 antagonists with prolonged activity

Glaxo carried out further development on ranitidine by placing the oxygen of the furan ring exocyclic to a phenyl ring and replacing the dimethylamino group with a piperidine ring to give a series of novel structures (I in Fig. 25.51). The most promising of these compounds were **lamitidine** and **loxtidine** (Fig. 25.51) which were 5–10 times more potent than ranitidine and three times longer lasting. Unfortunately, these compounds showed toxicity in long-term animal studies with the possibility that they caused gastric cancer, so they were subsequently withdrawn from clinical study. The relevance of these studies has been disputed.

25.2.10 Comparison of H_1 and H_2 antagonists

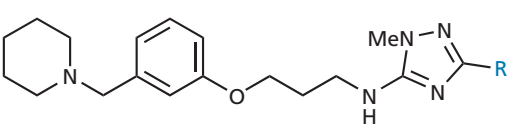
The structures of the H_2 antagonists are markedly different from the classical H_1 antagonists, so it is not surprising that H_1 antagonists failed to antagonize the H_2 receptor. H_1 antagonists, like H_1 agonists, possess an ionic amino group at the end of a flexible chain. Unlike the agonists, they possess two aryl or heteroaryl rings in place of the imidazole ring (Fig. 25.52). Because of the aryl rings, H_1 antagonists are hydrophobic molecules having high partition coefficients.

In contrast, H_2 antagonists are polar, hydrophilic molecules having high dipole moments and low partition coefficients. At the end of the flexible chain they have a polar, π electron system which is amphoteric and un-ionized at pH 7.4. This binding group appears to be the key feature leading to antagonism of H_2 receptors (Fig. 25.52). The heterocycle generally contains a nitrogen atom or, in the case of furan or phenyl, a nitrogen-containing side chain.



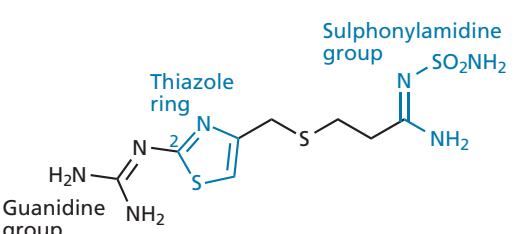
Z = planar and polar H-bonding group

I

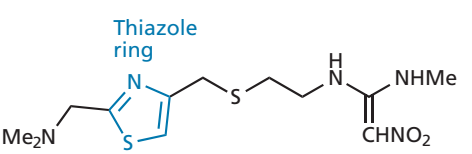


R= NH_2 Lamitidine
R= CH_2OH Loxtidine

FIGURE 25.51 Long-lasting anti-ulcer agents.



Famotidine



Nizatidine

FIGURE 25.50 Famotidine and nizatidine.

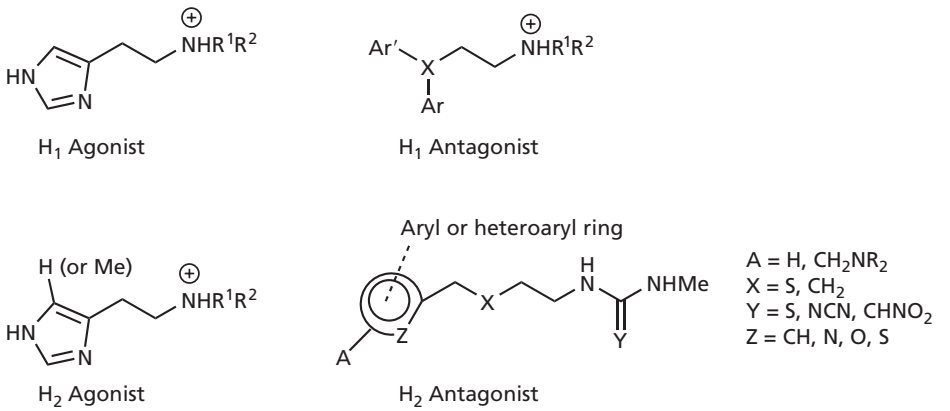


FIGURE 25.52 Comparison of H_1 agonists, H_1 antagonists, H_2 agonists, and H_2 antagonists.

The hydrophilic character of H_2 antagonists helps to explain why H_2 antagonists are less likely to have the CNS side effects often associated with H_1 antagonists.

25.2.11 H_2-receptors and H_2 antagonists

H_2 receptors are present in a variety of organs and tissues, but their main role is in acid secretion. As a result, H_2 antagonists are remarkably safe and mostly free of side effects. The four most used agents on the market are cimetidine, ranitidine, famotidine, and nizatidine. They inhibit all aspects of gastric secretion and are absorbed rapidly from the gastrointestinal tract with half-lives of 1–2 hours. About 80% of ulcers are healed after 4–6 weeks. Attention must be given to possible drug interactions when using cimetidine because of inhibition of drug metabolism (section 25.2.7.3). The other three H_2 antagonists mentioned do not inhibit the P450 cytochrome oxidase system and are less prone to such interactions.

KEY POINTS

- Peptic ulcers are localized erosions of the mucous membranes which occur in the stomach and duodenum. The hydrochloric acid present in gastric juices results in increased irritation and so drugs which inhibit the release of hydrochloric acid act as anti-ulcer agents. Such agents relieve the symptoms rather than the cause.
- The chemical messengers histamine, acetylcholine, and gastrin stimulate the release of hydrochloric acid from stomach parietal cells by acting on their respective receptors.
- H_2 antagonists are anti-ulcer drugs that act on H_2 receptors present on parietal cells and reduce the amount of acid released.
- The design of H_2 antagonists was based on the natural agonist histamine as a lead compound. Chain extension accessed an antagonist binding region, and the replacement of an ionized terminal group with a polar, un-ionized group capable of hydrogen bonding led to pure antagonists.
- The design of improved H_2 antagonists was aided by dynamic structure–activity analysis where changes were made to favour one tautomer over another.
- The orientation of dipole moments between a drug and its binding site plays a role in the binding and activity of H_2 antagonists. Desolvation of polar groups also has an important effect on binding affinity.

25.3 Proton pump inhibitors

Although the H_2 antagonists have been remarkably successful in the treatment of ulcers, they have been largely superseded by the proton pump inhibitors (PPIs). These work by irreversibly inhibiting an enzyme complex called the proton pump and have been found to be superior to the H_2 antagonists. They are used on their own to treat ulcers that are caused by NSAIDs and in combination with antibacterial agents to treat ulcers caused by the bacterium *H. pylori* (section 25.4).

25.3.1 Parietal cells and the proton pump

When the parietal cells are actively secreting hydrochloric acid into the stomach, they form invaginations called **canaliculi** (Fig. 25.53). Each canaliculus can be viewed as a sheltered channel or inlet that flows into the overall 'ocean' of the stomach lumen. Being a channel, it is not part of the cell, but it penetrates 'inland' and increases the amount of 'coastline' (surface area) across which the cell can release its hydrochloric acid. The protons required for the hydrochloric acid are generated from water and carbon dioxide, catalysed by an enzyme called **carbonic anhydrase** (Fig. 25.54). Once the protons have

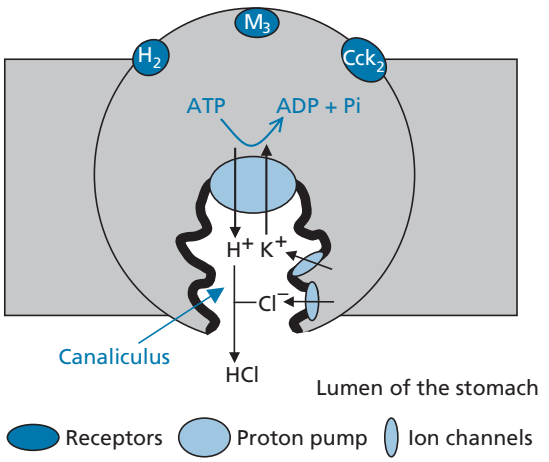


FIGURE 25.53 Role of the proton pump in secreting hydrochloric acid.

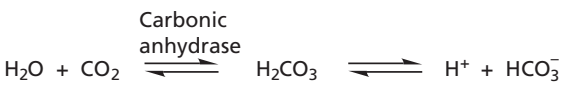


FIGURE 25.54 Enzyme-catalysed generation of protons in the parietal cell.

been generated, they have to be exported out of the cell rather than stored. There are two reasons for this. Firstly, a build-up of acid within the cell would prove harmful to the cell. Secondly, the enzyme-catalysed reaction which generates the protons is reversible, and so a build-up of protons within the cell would encourage the reverse reaction and slow the production down. The export of protons from the parietal cell is achieved by an enzyme complex called the **proton pump** or **H^+/K^+-ATPase**.

The proton pump is only present in the canalicular membranes of parietal cells and is crucial to the mechanism by which hydrochloric acid is released into the stomach. It is called an H^+/K^+-ATPase because it pumps protons out of the cell into the canaliculus at the same time as it pumps potassium ions back in. Energy is required for this process, as both the protons and the potassium ions are being moved against their concentration gradients. In fact, the ratio of protons inside the cell to protons in the canaliculus is 1 to 10^6! The energy required to carry out this exchange is obtained by the hydrolysis of ATP (Fig. 25.55)—hence the term ATPase.

The pump is not responsible for the efflux of chloride ions; these depart the cell through separate chloride ion channels. This outflow closely matches the efflux of protons such that a chloride ion is released for every proton that is pumped out. As a result, hydrochloric acid is formed in the canaliculus, rather than inside the parietal cell.

As each chloride ion departs the cell, it is accompanied by a potassium ion which flows through its own ion channel. No energy is required for this outflow because the potassium ion is flowing down a concentration gradient. The potassium ion acts as a counterion for the chloride ion and, once it is in the canaliculus, it is pumped back into the cell by the proton pump as described previously. Consequently, potassium ions undergo a cyclic movement in and out of the cell.

25.3.2 Proton pump inhibitors

There are four PPIs in clinical use: **omeprazole**, **lansoprazole**, **pantoprazole**, and **rabeprazole** (Fig. 25.56). The *S*-enantiomer of omeprazole has also been approved recently. All the PPIs have a pyridyl methylsulphinyl benzamidazole skeleton and act as prodrugs, since they are activated when they reach the acidic canaliculi of parietal cells. Once activated, they bind irreversibly to exposed cysteine residues of the proton pump and 'block' the pump, preventing further release of hydrochloric acid.

There is a big strategic advantage in inhibiting the proton pump rather than blocking histamine or cholinergic receptors. For example, H_2 antagonists block histamine receptors and block the stimulatory effect of histamine, but this does not block the receptors for acetylcholine or gastrin and so it is still possible for the parietal cell to be activated towards secretion. The proton pump is 'downstream' of all these targets and operates the final stage of hydrochloric acid release. Blocking it prevents the release of hydrochloric acid regardless of what mechanisms are involved in stimulating hydrochloric acid secretion.

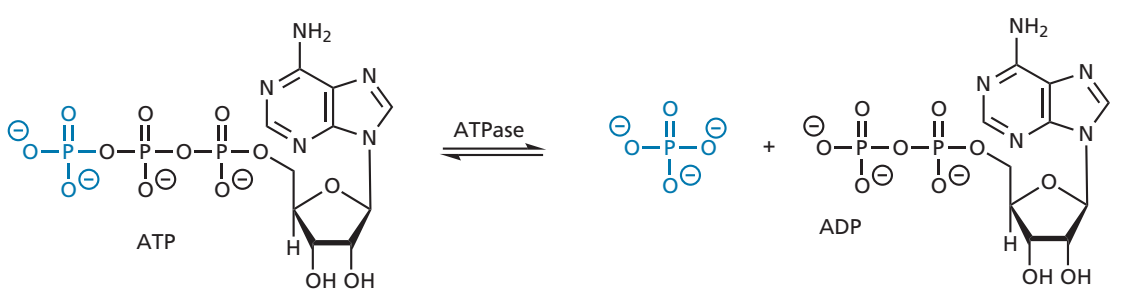


FIGURE 25.55 Enzyme-catalysed hydrolysis of ATP.

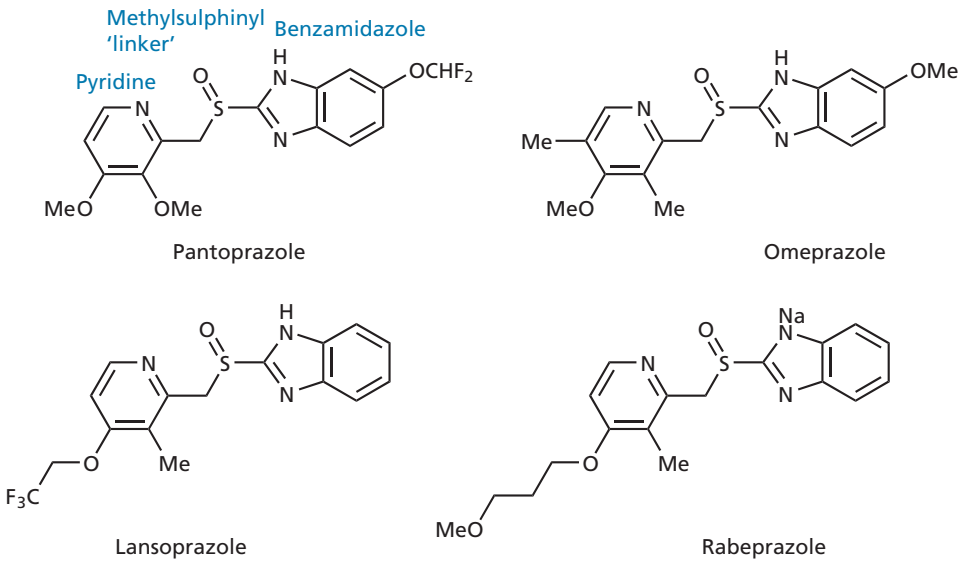


FIGURE 25.56 Proton pump inhibitors (PPIs).

25.3.3 Mechanism of inhibition

The PPIs are weak bases having a pK_a of about 4.0. As a result, they are free bases at blood pH (7.4) and are only ionized in strongly acidic environments where the pH is less than 4. These are conditions found only in the secretory canaliculus of the parietal cell, where the pH is 2 or less. The drugs are taken orally and are absorbed into the blood supply where they are carried round the body as fairly innocuous passengers until they reach the parietal cells. Because they are un-ionized weak bases at this stage, and are also lipophilic in nature, they are able to cross the cell membrane of the parietal cell into the strongly acidic conditions of the canaliculi. Here, the drugs undergo a personality change and become particularly vicious! The canaliculus is highly acidic, so the drug becomes protonated. The consequences of this are twofold:

- the ionized drug is too polar to cross back into the cell through the cell membrane. This leads to a 1000-fold accumulation of the drug in the canaliculi where it is intended to act;
- protonation triggers an acid-catalysed conversion, as shown in Fig. 25.57, which activates the drug.

Protonation takes place on the benzimidazole ring of the drug. The nitrogen of the pyridine ring then acts as a nucleophile and uses its lone pair of electrons to form a bond to the electron-deficient 2-carbon of the benzimidazole ring to form a spiro structure. By doing so, the aromatic character of the imidazole portion of the ring is lost and so there is a high tendency for this ring to re-aromatize. This can be achieved by a lone pair of electrons from nitrogen reforming the double bond and cleaving the S–C bond to form a sulphenic acid. Sulphenic acids are highly reactive to nucleophiles and so a rapid reaction takes place involving an intramolecular attack by the NH group of the benzimidazole on the sulphenic acid to displace the hydroxyl group. A cationic, tetracyclic pyridinium sulphenamide is formed, which acts as an irreversible enzyme inhibitor (Fig. 25.57). It does so by forming a covalent bond to an accessible cysteine residue on the proton pump. There are three such accessible cysteine residues (Cys-813, Cys-892, and Cys-821) and it has been found that the specific cysteine residues attacked depend on which PPI is involved. For example, omeprazole reacts with two of the accessible cysteine residues (Cys-813 and Cys-892), lansoprazole reacts with all three, and pantoprazole only reacts with one (either Cys-813 or Cys-822). Cys-813 is the only cysteine residue which appears to be react with all the PPIs.

As acid conditions are required to activate the PPIs, they are most active when parietal cells are actively secreting hydrochloric acid, and they show little activity when the parietal cells are in a resting state. Since a covalent disulphide bond is formed between the inhibitor and the proton pump, inhibition is irreversible, and so PPIs have a long duration of action. The duration depends on how quickly new pumps are generated by the cell.

PPIs also have very few side effects because of their selectivity of action. This is a result of several factors:

- the target enzyme (H^+/K^+-ATPase) is only present in parietal cells;

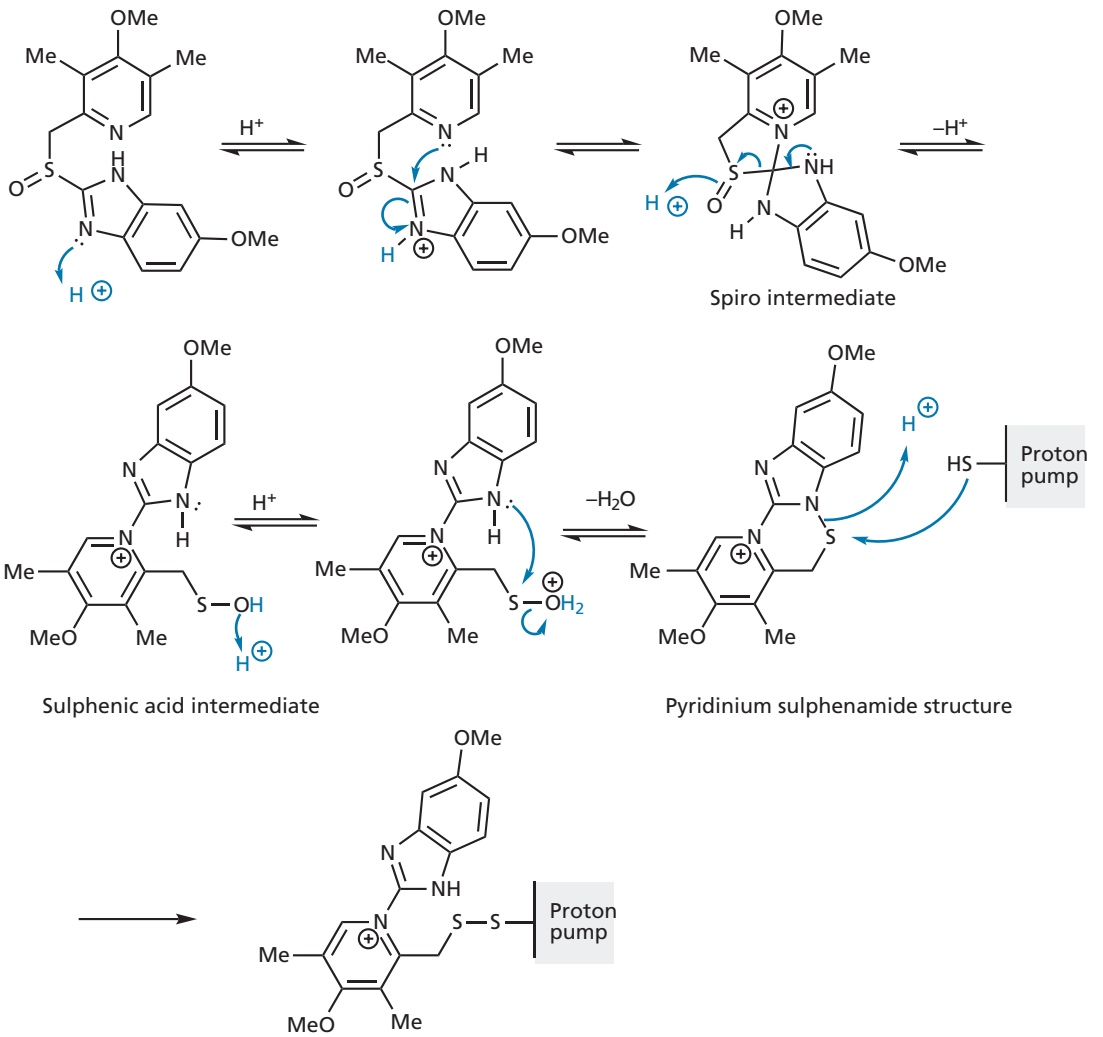


FIGURE 25.57 Mechanism of inhibition by proton pump inhibitors.

- the canaliculi of the parietal cells are the only compartments in the body which have such a low pH (1–2);
- the drug is concentrated at the target site because of protonation and is unable to return to the parietal cell or to the general circulation;
- the drug is rapidly activated close to the target;
- once activated, the drug reacts rapidly with the target;
- the drug is inactive at neutral pH.

25.3.4 Metabolism of proton pump inhibitors

PPIs are metabolized by cytochrome P450 enzymes, particularly *S*-**mephenytoin hydroxylase** (**CYP2C19**) and **nifedipine hydroxylase** (**CYP3A4**). As a result of genetic variations, about 3% of white people of European descent are slow metabolizers of PPIs. Pantoprazole, in contrast to omeprazole and lansoprazole, is also metabolized by the conjugating enzyme **sulphotransferase**.

25.3.5 Design of omeprazole and esomeprazole

Omeprazole was the first PPI to reach the market and was marketed as **Losec** in 1988. In 1996, it became the biggest selling pharmaceutical ever**.** The story of how omeprazole was developed can be traced back to the 1970s. The lead compound for the project was a thiourea structure (CMN 131 in Fig. 25.58). This had originally been investigated as an antiviral drug, but general pharmacological tests showed that it could inhibit acid secretion. Unfortunately, toxicology tests showed that the compound was toxic to the liver, which was attributed to the presence of the thioamide group.

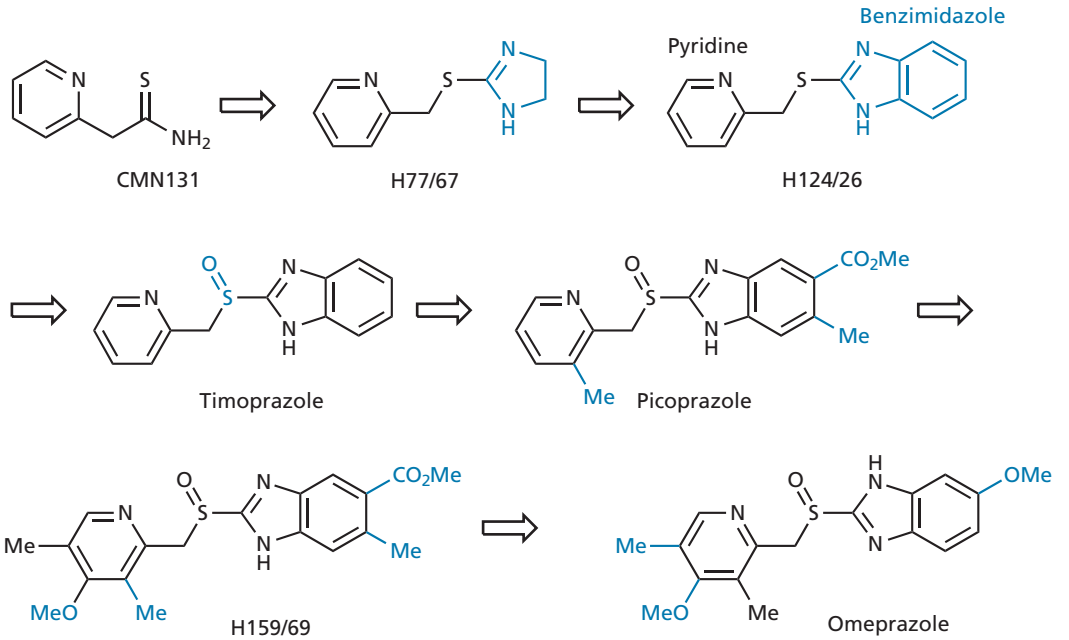


FIGURE 25.58 Development of omeprazole.

Various analogues were made to try to modify or disguise this group, which included incorporating the thiourea group within a ring. This led eventually to the discovery of H 77/67, which was also found to inhibit acid secretion. A variety of analogues having the general structure (heterocycle–X–Y–heterocycle) were synthesized, which demonstrated that the pyridine ring and the bridging CH_2–S group already present in H 77/67 were optimal for activity. However, activity was increased by replacing the imidazole ring of H 77/67 with a benzimidazole group to give H 124/26. At this stage, drug metabolism studies revealed that a sulphoxide metabolite of H 124/26 was formed *in vivo*, which was more active than the original structure. The metabolite was called **timoprazole** and was the first example of a pyridinylmethylsulphinyl benzimidazole structure. It went forward for preclinical trials, but toxicological studies revealed that it inhibited iodine uptake by the thyroid gland and so it could not go on to clinical trials.

Analogues were then synthesized to find a structure which retained the antisecretory properties, but did not inhibit iodine uptake. Eventually, it was found that the two effects could be separated by placing suitable substituents on the two heterocyclic rings. This led to **picoprazole**, which showed potent antisecretory properties over a long period without the toxic side effect on the thyroid. Animal toxicology studies showed no other toxic effects and the drug went forward for clinical trials, where it was found to be the most effective antisecretory compound ever tested in humans. At this point (1977) the proton pump was discovered and identified as the target for picoprazole. Further development was carried out with the aim of getting a more potent drug by varying the substituents on the pyridine ring.

It was discovered that substituents which increased the basicity of the pyridine ring were good for activity. This fits in with the mechanism of activation (Fig. 25.57) where the nitrogen of the pyridine ring acts as a nucleophile. In order to increase the nucleophilicity of the pyridine ring, a methoxy group was placed at the *para* position relative to the nitrogen and two methyl groups were placed at the *meta* positions. The latter have an inductive effect which is electron-donating and increases the electron density of the ring. The methoxy substituent was added at the *para* position to increase electron density on the pyridine nitrogen by the resonance mechanism shown in Fig. 25.59.

It is noticeable that all the PPIs shown in Fig. 25.56 have an alkoxy substituent at the *para* position of the pyridine. The position of the substituent is important. If the substituent was at the *meta* position, none of the possible resonance structures would place the negative charge on the nitrogen atom (Fig. 25.60). Finally, if the methoxy substituent was at the *ortho* position it would be likely to have a bad steric effect and hinder the mechanism.

The introduction of two methyl groups and a methoxy group led to H 159/69 (Fig. 25.58), which was extremely potent but too chemically labile. Further analogues were synthesized where substituents round the benzimidazole ring were varied in order to get the right balance of

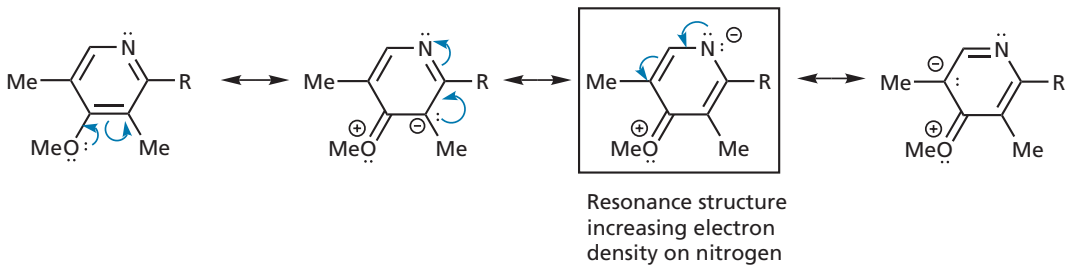


FIGURE 25.59 Influence of the methoxy substituent.

FIGURE 25.60 Possible resonance structures for methoxy substitution at the *meta* position.

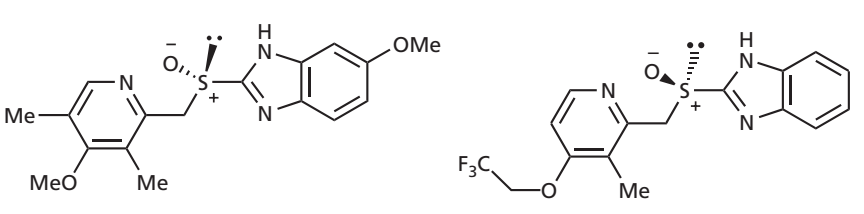
potency, chemical stability, and synthetic accessibility. Finally, omeprazole was identified as the structure having the best balance of these properties.

Omeprazole was launched in 1988 and became the world's biggest-selling drug, earning its makers vast profits. For example, worldwide sales in 2000 were $6.2 billion (£3.6 billion). The patents on omeprazole ran out in Europe in 1999 and in the USA in 2001, but its makers (Astra) had already started a programme to find an even better compound. In particular, they were looking for a compound with better bioavailability.

Substitution was varied on both the pyridine and benzimidazole rings, but the best compound was eventually found to be the *S*-enantiomer of omeprazole—**esomeprazole** (**Nexium**; Fig. 25.61). At first sight, it may not be evident that omeprazole has an asymmetric centre. In fact, the sulphur atom is an asymmetric centre as it has a lone pair of electrons and is tetrahedral. Unlike the nitrogen atoms of amines, sulphur atoms do not undergo pyramidal inversion and so it is possible to isolate both enantiomers. The *S*-enantiomer of omeprazole was found to be superior to the *R*-enantiomer in terms of its pharmacokinetic profile, and was launched in Europe in 2000 and in the USA in 2001. The story of esomeprazole is an example of **chiral switching** (section 15.2.1) where a racemic drug is replaced on the market with a single enantiomer. There is no difference between the two enantiomers of omeprazole as far as the mechanism of action is concerned, but it is possible to use double the dose levels of esomeprazole compared to omeprazole, resulting in greater activity. Esomeprazole is metabolized mainly by CYP2C19 in the liver, to form the hydroxy and desmethyl metabolites shown in Fig. 25.62. However, it undergoes less hydroxylation than the *R*-isomer and has a lower clearance rate. Owing to these differences in metabolism and excretion, higher plasma levels of the *S*-enantiomer are achieved compared with the *R*-enantiomer. The synthesis of omeprazole and esomeprazole is described in Box 25.2. **Dexlansoprazole**, the *R*-enantiomer of lansoprazole, was also approved by the US Food and Drug Administration (FDA) in 2009.

25.3.6 Other proton pump inhibitors

The other PPIs shown in Fig. 25.56 retain the pyridinylmethylsulphinyl benzimidazole structure of omeprazole. They also share the alkoxy substituent at the *para* position of the pyridine ring. Variation has been limited to the other substituents present on the heterocyclic rings. These play a role in determining the lipophilic character



Esomeprazole Dexlansoprazole

FIGURE 25.61 Esomeprazole and dexlansoprazole.

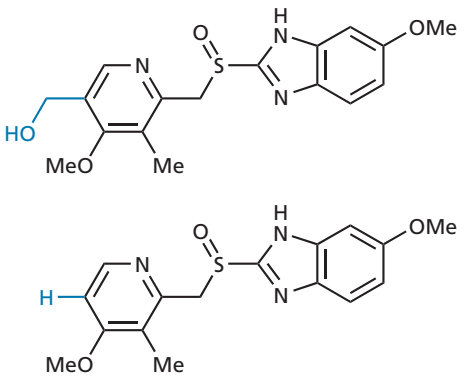


FIGURE 25.62 Metabolites of esomeprazole.

of the drug, as well as its stability. As far as the latter is concerned, there has to be a balance between the drug being sufficiently stable and un-ionized at neutral pH to reach its target unchanged, and its ability to undergo rapid acid-induced conversion into the active sulphenamide when it reaches the target. Stability to mild acid is important to avoid activation in other cellular compartments, such as lysosomes and chromaffin granules. Drugs which undergo the acid-induced conversion extremely easily are more active, but they are less stable and are more likely to undergo transformation in the blood supply before they reach their target. Drugs which are too stable are less reactive under acid conditions and react slower with the target.

The various PPIs all work by the same mechanism, but have slightly different properties. For example, pantoprazole is chemically more stable than omeprazole or lansoprazole under neutral to mildly acidic conditions (3.5–7.4), but it is a weaker, irreversible inhibitor under strong acid conditions. Rabeprazole is the least stable at neutral pH and is the most active inhibitor.

KEY POINTS

- The proton pump is responsible for pumping protons out of the parietal cell in exchange for potassium ions which are pumped in. The process involves the movement of protons against a concentration gradient, which requires energy provided by the hydrolysis of ATP.
- PPIs prevent the proton pump from functioning. They offer a strategic advantage over H_2 antagonists because they act on the final stage of hydrochloric acid release.
- PPIs are prodrugs that are activated by the acidic conditions found in the canaliculi of parietal cells. They undergo an acid-catalysed rearrangement to form a reactive tetracyclic pyridinium sulphenamide which acts as an irreversible inhibitor. Reaction takes place with accessible cysteine residues on the proton pump to form a covalent disulphide bond between cysteine and the drug.
- PPIs need to be reactive enough to undergo acid-catalysed interconversion in the canaliculi of parietal cells, but stable enough to survive their journey through the bloodstream.

25.4 *Helicobacter pylori* and the use of antibacterial agents

25.4.1 Discovery of *Helicobacter pylori*

One of the problems relating to anti-ulcer therapy, both with the H_2 antagonists and the PPIs, is the high rate of ulcer recurrence once the therapy is finished. The reappearance of ulcers has been attributed to the presence of a microorganism called *Helicobacter pylori*, which is naturally present in the stomachs of many people, and can cause inflammation of the stomach wall. As a result, patients who are found to have *H. pylori* are currently given a combination of three drugs—a PPI to reduce gastric acid secretion and two antibacterial agents (such as **nitroimidazole**, **clarithromycin**, **amoxicillin**, or **tetracycline**) to eradicate the organism.

It was once considered unthinkable that a bacterium could survive the acid conditions of the stomach. However, in 1979, it was shown that *H. pylori* can do just that. The organism is able to attach to a sugar molecule on the surface of the cells that line the stomach wall and use the mucus layer which protects the stomach wall from gastric juices as its own protection. As there is a pH gradient across the mucus layer, the organisms can survive at the surface of the mucus cells where the pH is closer to neutral (Fig. 25.63). *Helicobacter pylori* is a spiral, curved bacterium which is highly motile and grows best in oxygen concentrations of 5%, matching those of the mucus layer. The bacterium also produces large amounts of the enzyme **urease** which catalyses the hydrolysis of urea to ammonia and carbon dioxide, thus neutralizing any acid in the local environment (Fig. 25.64). The bacterial cells can contribute to the formation of stomach ulcers, because they secrete proteins and toxins that interact with the stomach's epithelial cells, leading to inflammation and cell damage. It is also thought that the microorganism increases the risk of gastric cancers.

25.4.2 Treatment

As mentioned earlier, *H. pylori* is treated with a triple therapy of a PPI and at least two antibacterial agents. A PPI is administered because the antibiotics used work best at higher pH levels than those normally present in the stomach. The combination of **omeprazole**,

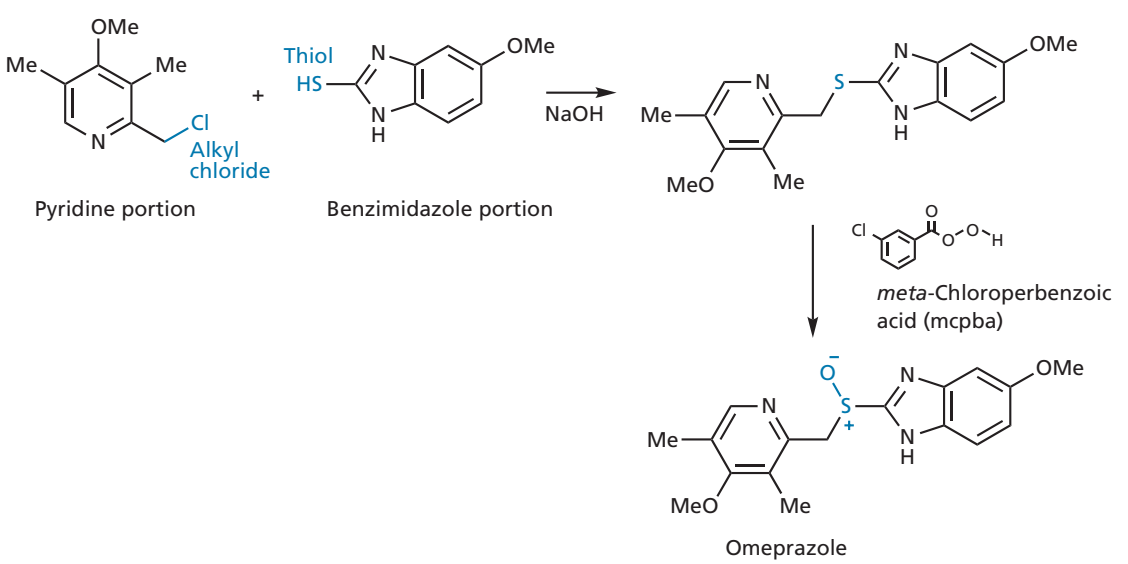
BOX 25.2 Synthesis of omeprazole and esomeprazole

The synthesis of omeprazole appears relatively simple, involving the linkage of the two halves of the molecule through a nucleophilic substitution reaction. The benzimidazole half of the molecule has a thiol substituent which is treated with sodium hydroxide to give a thiolate. On reaction with the chloromethylpyridine, the thiolate group displaces the chloride ion to link up the two halves of the molecule. Subsequent oxidation of the sulphur atom with *meta*-chloroperbenzoic acid gives omeprazole. What is not obvious from the scheme is the effort required to synthesize the required chloromethylpyridine starting material. This is not the sort of molecule that is easily bought off the shelf and its synthesis involves six steps.

The same route can be used for the synthesis of esomeprazole (the *S*-enantiomer of omeprazole) by employing asymmetric conditions for the final sulphoxidation step. Early attempts to carry out this reaction involved the Sharpless reagent formed from $Ti(O\text{-}iPr)_4$, the oxidizing agent cumene hydroperoxide ($Ph(CH_3)_2OOH$), and the chiral auxiliary (*S,S*)-diethyl tartrate. Although sulphoxidation took place, it required almost stoichiometric quantities of the titanium reagent and there was little enantioselectivity. The reaction conditions were modified in three ways to improve enantioselectivity to over 94% enantiomeric excess and which required less of the titanium reagent (4–30 mol%).

- Formation of the titanium complex was carried out in the presence of the sulphide starting material.
- The solution of the titanium complex was equilibrated at an elevated temperature for a prolonged time period.
- The oxidation was carried out in the presence of an amine such as *N,N*-diisopropylethylamine. The role of the amine is not fully understood, but it may participate in the titanium complex.

The enantiomeric excess can be enhanced further by preparing a metal salt of the crude product and carrying out a crystallization which boosts the enantiomeric excess to more than 99.5%.



Synthesis of omeprazole.

amoxicillin, and **metronidazole** is frequently used, but combinations involving other antibacterial agents, such as **clarithromycin** and **tetracycline**, are also possible. **Bismuth chelate** (bismuth subcitrate and tripotassium dicitratobismuthate) is present in some combination therapies. This preparation has a toxic effect on *H. pylori* and may help to prevent adherence to the mucosa. Other protective properties include an enhancement of local prostaglandin synthesis, a coating of the ulcer base, and an adsorption of pepsin.

Combination therapy has been shown to eradicate *H. pylori* in over 90% of duodenal ulcers and significantly

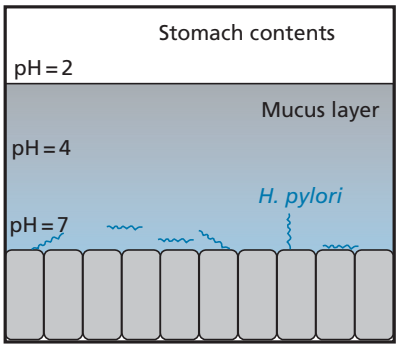


FIGURE 25.63 *Helicobacter pylori* attached to stomach cells.

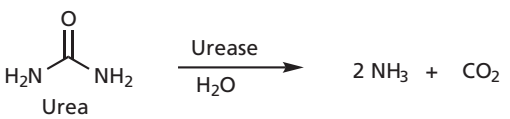


FIGURE 25.64 Action of urease.

reduce ulcer recurrence. Similar treatment is recommended for *H. pylori*-related stomach ulcers.

Eradication of *H. pylori* can be difficult because of the emergence of resistant strains and the difficulty in delivering the antibacterial agents at the required therapeutic concentration. *Helicobacter pylori* can also assume a resting coccoid form that is more resistant to therapy.

It has been found that PPIs have an inherent anti-*H. pylori* action and it has been suggested that they inhibit urease, possibly by linking to exposed cysteine residues. However, the PPIs also inhibit strains of *H. pylori* which do not have urease, so this is not the full story. This antibacterial activity is sufficient to suppress the organism but not eradicate it, so traditional antibacterial agents are still required.

Research has been carried out into the design of drugs which act as sugar decoys to prevent *H. pylori* binding with stomach cells in the first place.

25.5 Traditional and herbal medicines

Several herbal remedies have been used for the treatment of ulcers.

Liquorice has been reported to have a variety of medicinal properties and has been used as a medicine for several thousand years. It is reported to have anti-ulcer properties and this has been attributed to a component called **glycrrhetinic acid**—the aglycone of **glycyrrhizin**. **Carbenoxolone** is a derivative of glycrrhetinic acid and has been used in ulcer therapy. It is thought to have a mucosal protective role by increasing mucus production and has some antibacterial action against *H. pylori*.

Silymarin is a mixture of compounds (mainly **silibinin**, **silichristin**, and **siliianin**) obtained from the fruit of the milk thistle (*Silybum marianum*) and has anti-ulcer activity. It has been shown to reduce histamine concentrations in rats.

Extracts from the **neem tree** (*Azadirachta indica*) have been used extensively in India as a medicine for a variety of ailments. The aqueous extract of the neem bark has been reported to have anti-ulcer effects. Possible mechanisms include proton pump inhibition or anti-oxidant effects in the scavenging of OH radicals.

Other herbal medicines include **comfrey** and **marshmallow**.

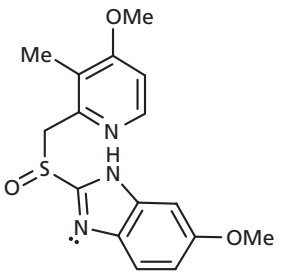
KEY POINTS

- *Helicobacter pylori* is a bacterium which is responsible for many ulcers. It can survive at the surface of mucus cells and produce proteins and toxins which damage epithelial cells.
- Ulcers which are caused by *H. pylori* are treated with a combination of drugs which includes a PPI and at least two antibiotics.
- Several traditional and herbal remedies are used in the treatment of ulcers.

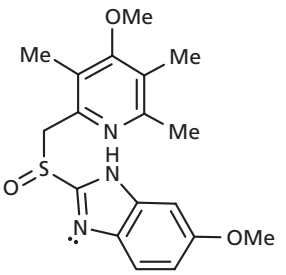
QUESTIONS

1. Omeprazole is administered orally as a galenic formulation to protect it from being activated during its journey through the acidic contents of the stomach. Once it is released in the intestines, it is absorbed into the blood supply and carried to the parietal cells where it crosses the cell membrane into the canaliculi and is activated. As the canaliculi lead directly into the lumen of the stomach, why is omeprazole not orally administered directly to the stomach?
2. In the development of omeprazole, the methoxy and methyl groups were added to the pyridine ring to increase the pK_a. Subsequently, it was found that analogue (I) with only one of the methyl groups had a higher pK_a than omeprazole. Suggest why this might be the case.
3. Suggest whether you think structure (I) would be a better PPI than omeprazole.
4. The acid-catalysed activation of PPIs requires pyridine to be nucleophilic, which is why two methyl groups and a methoxy group are present in omeprazole. Suggest whether the addition of an extra methyl group (structure II) would lead to a more potent PPI.

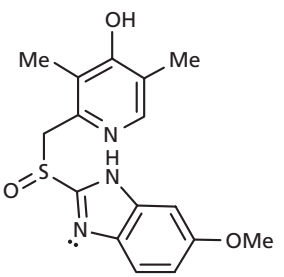
5. The phenol (III) is a very difficult compound to synthesize and is unstable at neutral pH. Suggest why this might be the case.



I



II



III

6. Suggest what types of metabolite might be possible from omeprazole.

7. One of the metabolic reactions that takes place on cimetidine is oxidation of the methyl substituent on the imidazole ring (Fig. 25.35). A common strategy to prevent such a metabolic reaction occurring is to replace a susceptible methyl group with a chloro substituent. Why is a chloro substituent used commonly for this purpose? Do you think an analogue of cimetidine with a 4-chloro substituent would be an improvement over cimetidine itself?

8. The acidic contents of the stomach encourage the digestion of food and the destruction of cells. Why are the cells lining the stomach not digested in that case?

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Titles for general further reading are listed on p. 763.