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 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). 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 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. A lifetime's exposure to other damaging mutagens such as tobacco smoke, however, 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. Since 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, since 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. Examples include growth factors called **platelet-derived growth factor** (PDGF) and **transforming growth factor** α (TGF-α). Other cancer cells can produce abnormal receptors which are constantly switched on despite the

lack of growth factors (e.g. Erb-B2 receptors in breast cancer cells). It is also possible for receptors to be over expressed. 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 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 9 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 G_1 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

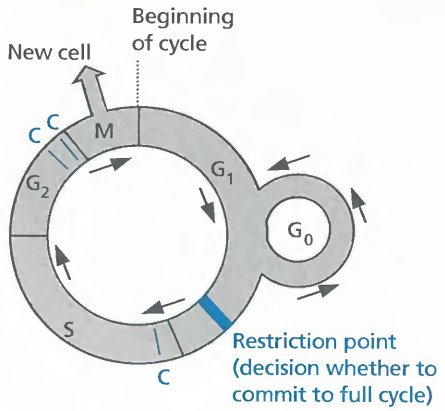


FIGURE 21.1 The cell cycle. G_1, gap 1—cell enlarges and makes new proteins; S, synthesis of DNA; G_2, gap 2—cell prepares to divide; M, mitosis—cell divides; G_0, resting stage—no growth; C, checkpoints.

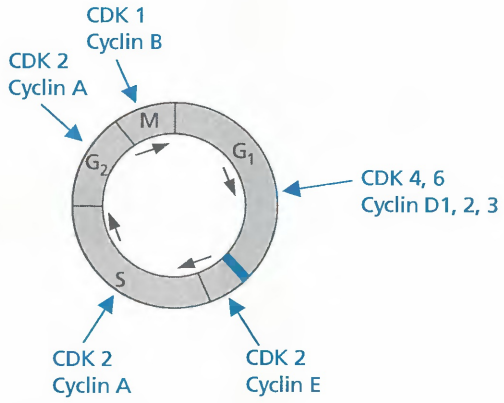


FIGURE 21.2 Control of the cell cycle by cyclins and cyclin dependent kinases.

towards cell growth and division, there is an increase in cyclin D. This binds to CDK4 and CDK6. The resulting 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 lead 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 G_1 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 cyclin 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 lead to 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

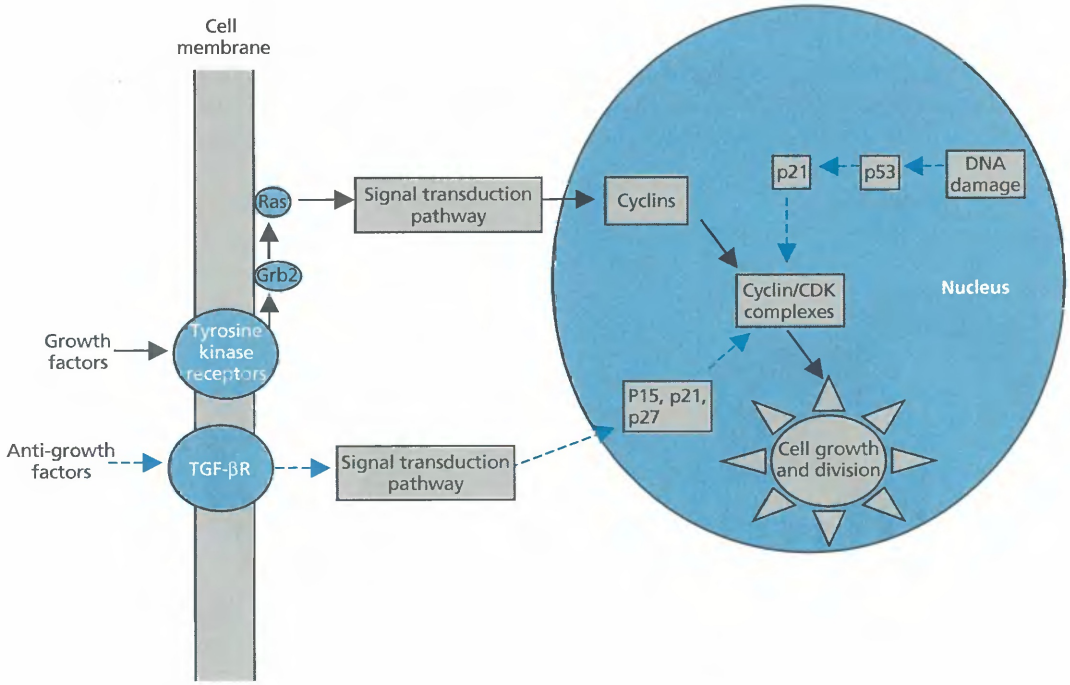


FIGURE 21.3 Signalling pathways that activate and inhibit cell growth and division.

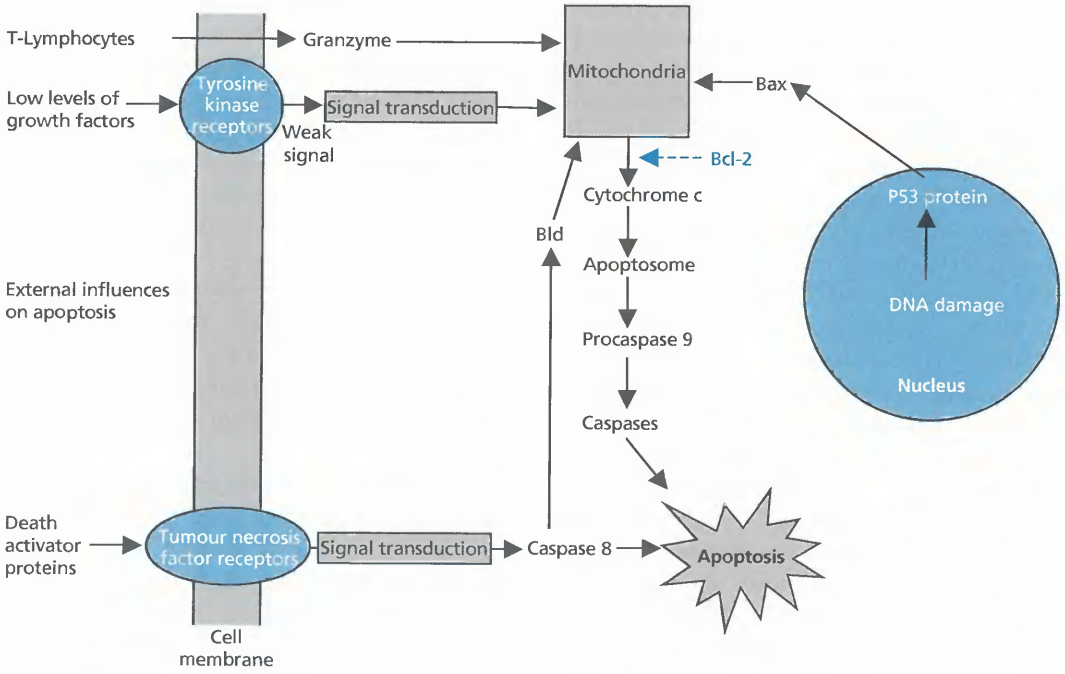


FIGURE 21.4 Signals leading to apoptosis.

protects itself against abnormal or faulty cells. Each cell essentially monitors itself for a series of different chemical signals. 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, which can take three forms. First, there could be a sustained lack of growth factors or hormones. Second, 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 that 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. They are proteases, so they set about destroying the cell's proteins and this 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, some members of the family such as **Bad** and **Bax** promoting it and others such as **Bcl-2** and **Bcl-X** suppressing 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 upregulating 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 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 over-expressed 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, since both these procedures act by triggering apoptosis. For example, many traditional drugs damage DNA. This in itself may not be fatal to the cell, but the cell's monitoring systems detects the damage and goes into self-destruct mode. If the mechanisms involved are defective then apoptosis does not occur, the drugs are not as 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 (6 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 eventually a cancer cell 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 no such drugs have reached the clinic to date.

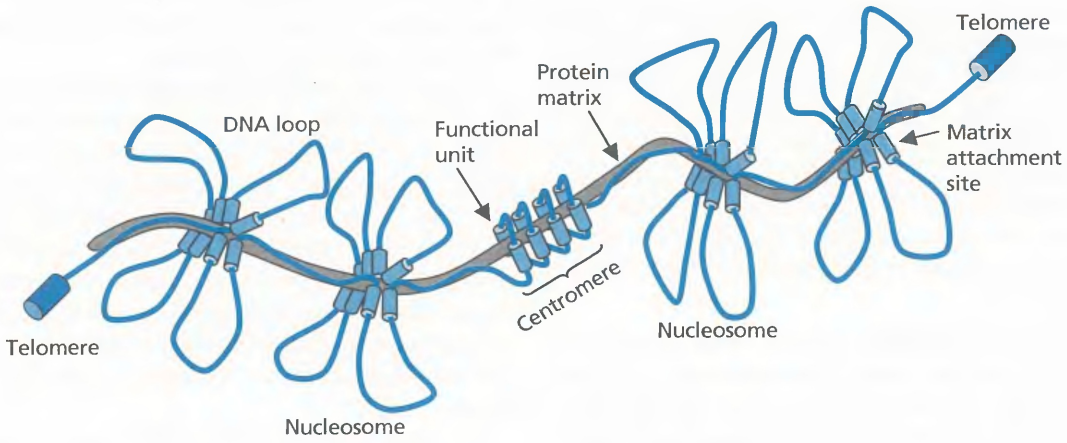


FIGURE 21.5 Chromatin, telomeres and DNA.

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. This is particularly true for the cells in the centre of the tumour. In order to counter this, tumour cells release growth factors 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. 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 be 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. Since tumours generally do not develop an effective lymphatic system, the polymeric drug delivery systems tend to be trapped at the tumour site.

Despite 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. Once the treatment is

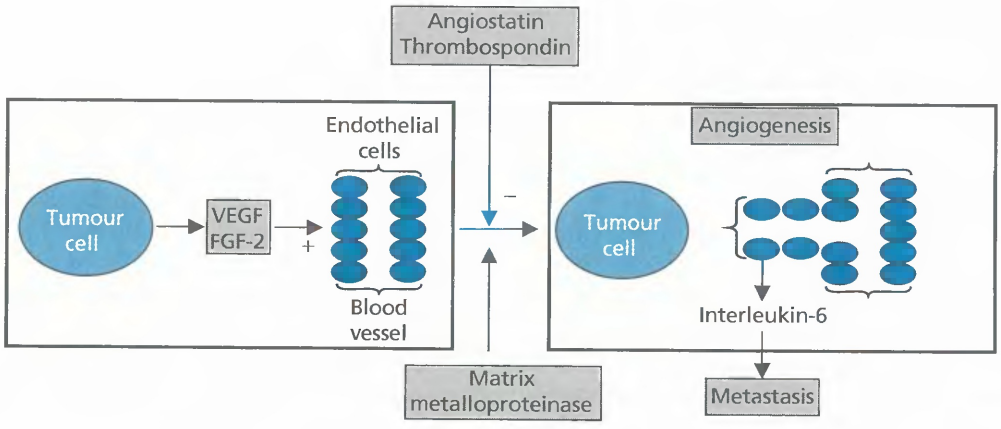


FIGURE 21.6 Angiogenesis.

stopped, though, 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 the tumour are forced to revert to glycolysis in order to produce energy. This 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 that remain localized in a particular part of the body and can grow to the size of a football without a fatal result. Malignant cancers, on the other hand, 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—an anchoring requirement which involves molecules called **integrins**. 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 one 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 usually 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.

Since 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, since 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 rapidly proliferating, since 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 in 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 who are at risk to particular cancers so that they can be regularly screened.

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).

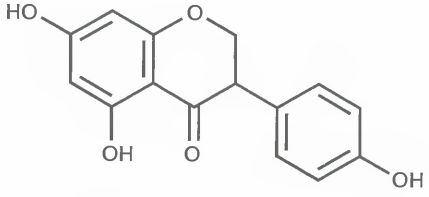


FIGURE 21.7 Genistein.

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 due to 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.
- 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 since 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**, **birocodar** 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.

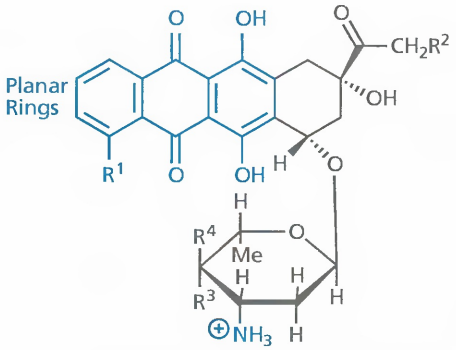
To conclude, regardless of the mechanism involved, it is likely that a drug-resistant cell may be present in a cancer. Therefore, it makes sense to use combinations of anticancer drugs with different targets to increase the chances of finding a weakness in every cell, and not just those susceptible to 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 that 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 that 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 that 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 that fix cells to a specific environment, and which destroy cells that become detached, are overruled.
- 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 that prevent the drug binding to its target, alternative metabolic pathways, or efflux systems that expel drugs from the cell.



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

FIGURE 21.8 The anthracyclines.

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, but 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** since 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. Since these enzymes are most active during active growth and division, the topoisomerase-II poisons are most effective against rapidly proliferating cells. A second mechanism by which doxorubicin 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′, 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 ammonium 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 and this is 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

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 can lead to heart failure, while multi-drug resistance can develop due 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 due 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 11.9) 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 compared to 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 occasionally used for the treatment of acute myeloid leukaemia.

Dactinomycin is mainly given 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 that 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 accumulates here to toxic levels.

pharmacophore involving one of the phenol groups, a 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).

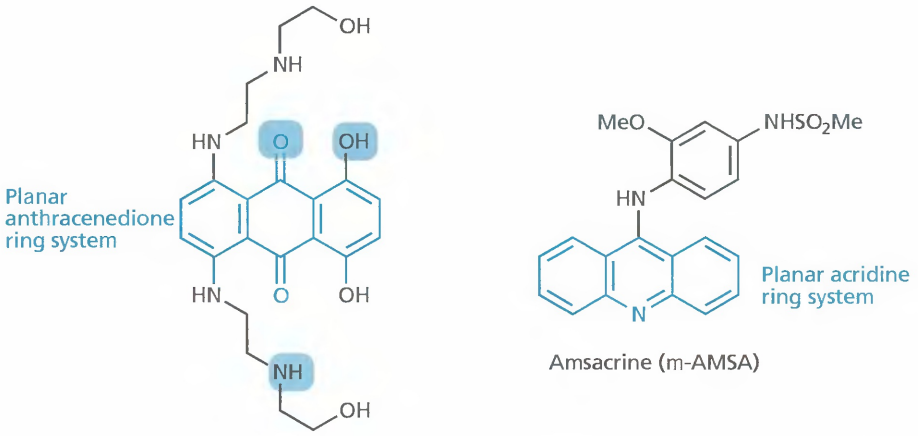


FIGURE 21.9 Mitoxantrone (pharmacophoric groups highlighted in boxes) and amsacrine.

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

21.2.2.1 Podophyllotoxins

Etoposide and **teniposide** 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 due to 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 antitumour 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.

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 due to over-expression 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 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.

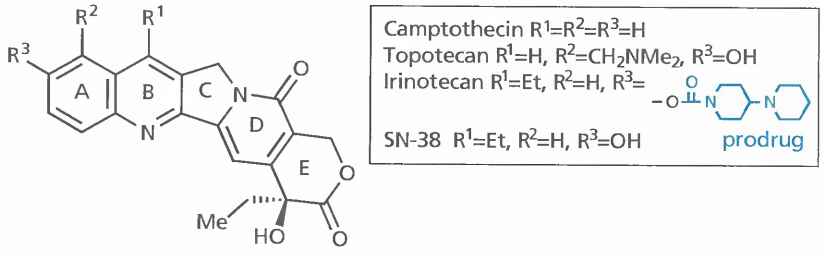


FIGURE 21.10 Camptothecins.

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 alkylate nucleophilic groups on proteins as well, 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. Nevertheless, 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 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, putting an aromatic ring on the nitrogen atom instead of a methyl 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 available to displace the chloride ion. As a result, the intermediate aziridinium ion (Fig. 9.10) 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 be 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. This drug shows a certain amount of selectivity for tumour cells over normal cells. Since 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 **oestradiol**. Since oestradiol normally crosses cell

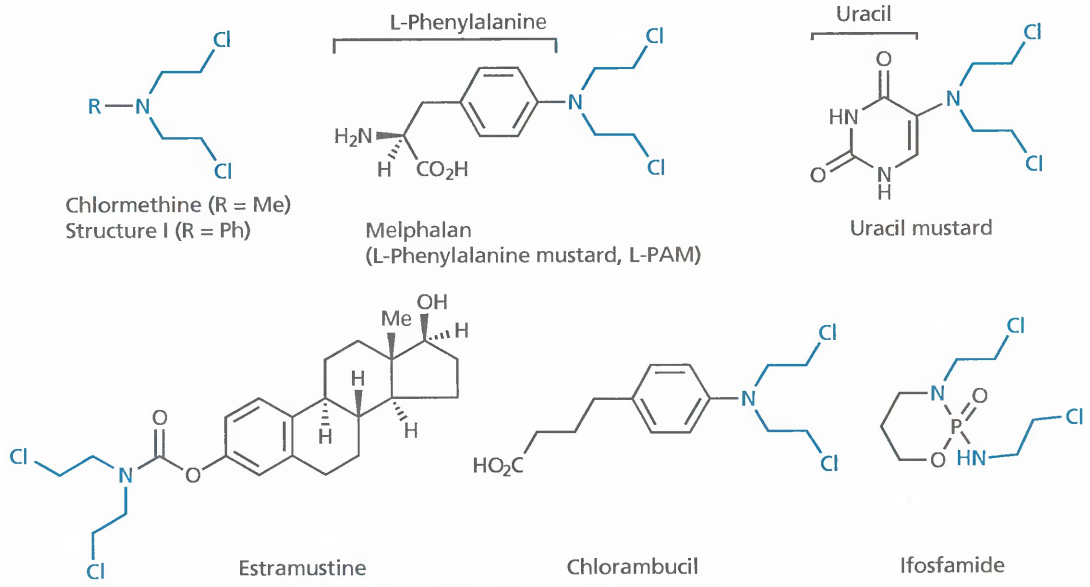


FIGURE 21.11 Mustard-like alkylating agents.

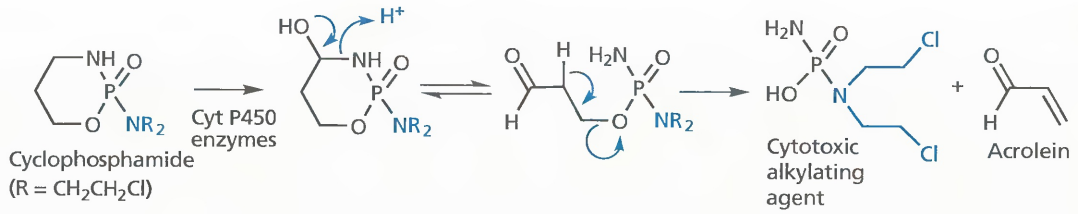


FIGURE 21.12 Phosphoramide mustard from cyclophosphamide.

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.

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 sulfydryl 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.

21.2.3.2 Cisplatin and cisplatin analogues: metallating agents

Cisplatin (Fig. 21.13) 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. 21.13) is a derivative of cisplatin with reduced side effects.

A range of other platinum drugs (Fig. 21.13) (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. **Picoplatin** is another agent nearing the end of clinical trials.

21.2.3.3 CC 1065 analogues

CC 1065 (Fig. 21.14) 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 by which these agents work are described in section 9.3.

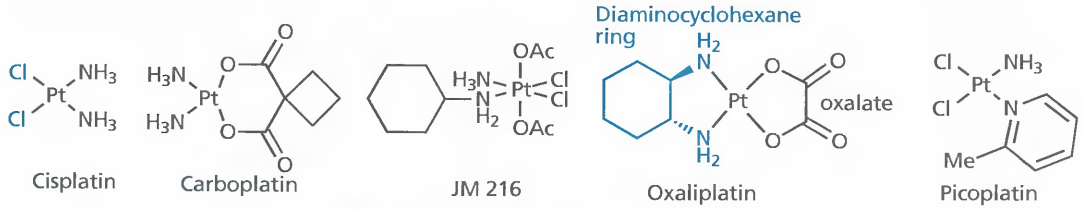


FIGURE 21.13 Platinum-based anticancer drugs.

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 multi-drug regimen. 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 prostrate cancer. Chlorambucil is an orally active drug used primarily in the treatment of chronic lymphocytic leukaemia and Hodgkin's disease.

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 that 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.

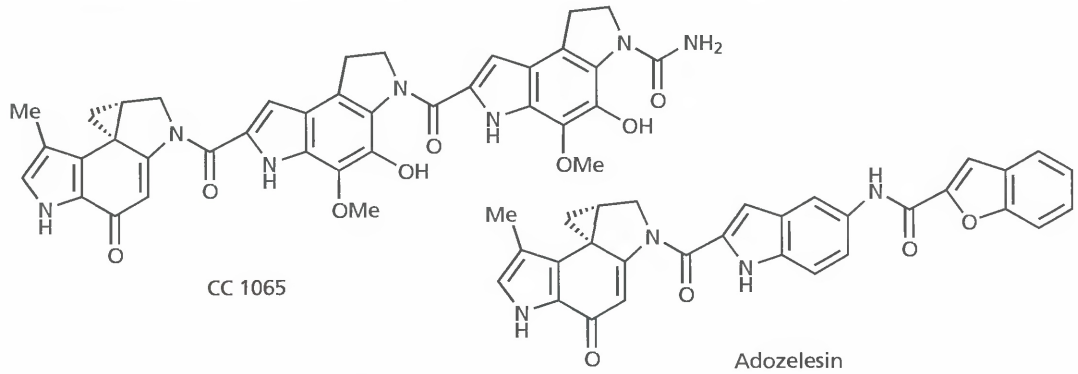


FIGURE 21.14 CC 1065 and adozelesin.

21.2.4 Chain cutters

Calicheamicin γ^1 is an antitumour agent which 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. The mechanism is described in 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 which 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.
- 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 alkylating agents that 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 that 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 proteins which suppress apoptosis.

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. 21.15 and 21.16).

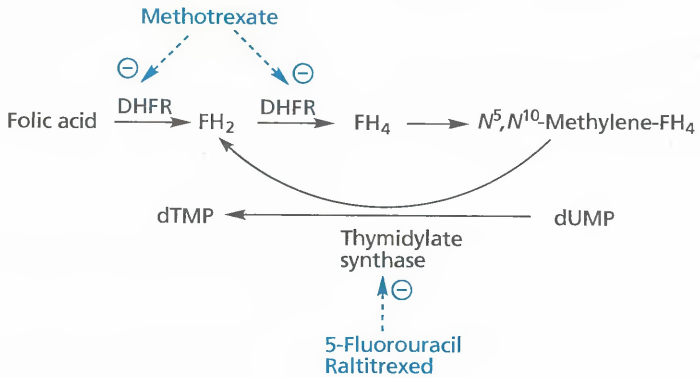


FIGURE 21.15 Reactions catalysed by dihydrofolate reductase and thymidylate synthase.

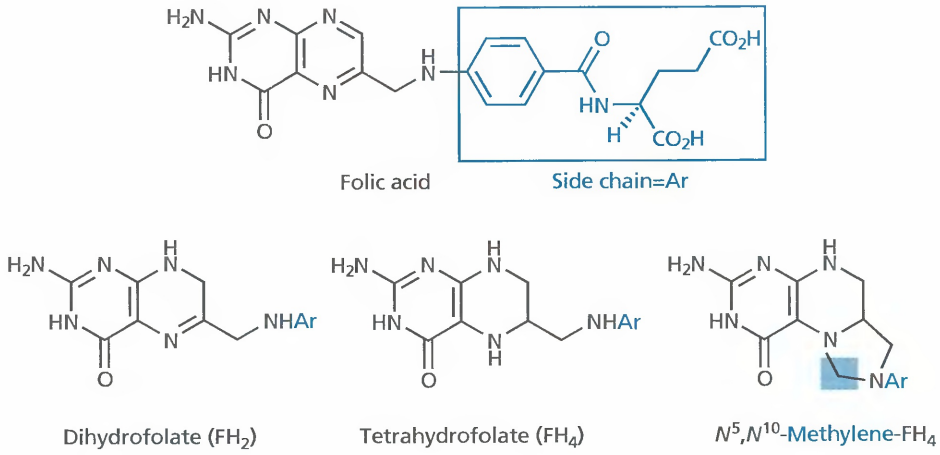


FIGURE 21.16 Structures of folic acid and related cofactors.

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. 21.17) 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, due 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 are trapped within the cell.

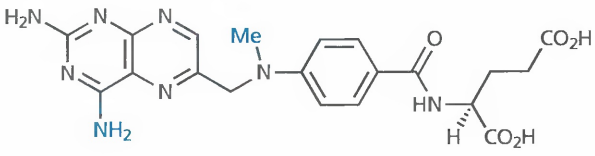


FIGURE 21.17 Methotrexate.

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. 21.18) is an anticancer drug which inhibits this enzyme directly.

It does so by acting as a prodrug for a suicide substrate. 5-Fluorouracil is converted in the body to the fluorinated analogue of 2′-deoxyuridylic acid monophosphate (FdUMP) (Fig. 21.18), which then combines with the enzyme and the cofactor (Fig. 21.19). Up until this point, nothing unusual has happened and the reaction mechanism has been proceeding normally. The tetrahydrofolate has formed a covalent bond to the uracil skeleton via the methylene unit which is usually transferred to uracil. Now things start to go wrong. At this stage, a proton is usually lost from position 5 of uracil. However, 5-fluorouracil has a fluorine atom at that position instead of a hydrogen. Further reaction is impossible, since it would require fluorine to leave as a positive ion. Fluorine is too electronegative for this to occur since 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. 21.20) is the first of a new generation of highly specific folate-based

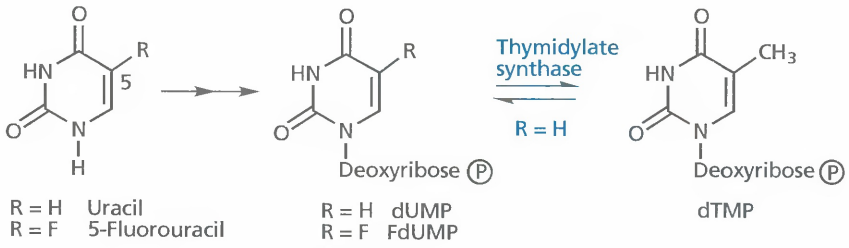


FIGURE 21.18 Biosynthesis of dTMP.

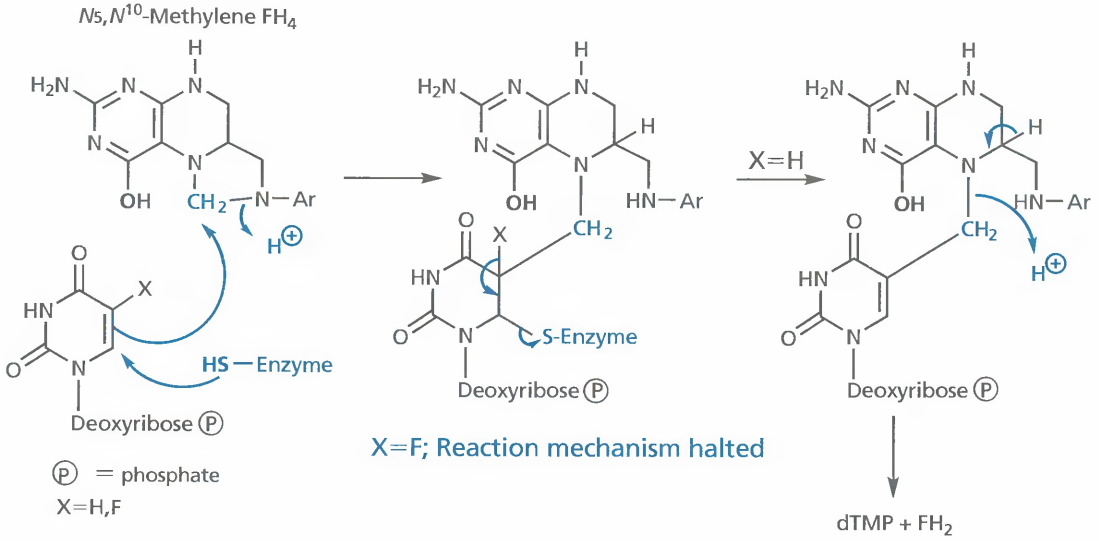


FIGURE 21.19 Use of 5-fluorouracil as a prodrug for a suicide substrate.

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 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.

5-Fluorouracil is usually given intravenously since oral absorption is unpredictable. It is commonly used alongside folinic acid 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** 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.

Nelarabine was given accelerated approval by the US FDA in 2005 for the treatment of T-cell acute lymphoblastic leukaemia and T-cell 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 that is used in the treatment of advanced colorectal cancer. Acquired resistance includes impaired cellular uptake, decreased polyglutamation

(Continued)

BOX 21.4 Clinical aspects of antimetabolites *(Continued)*

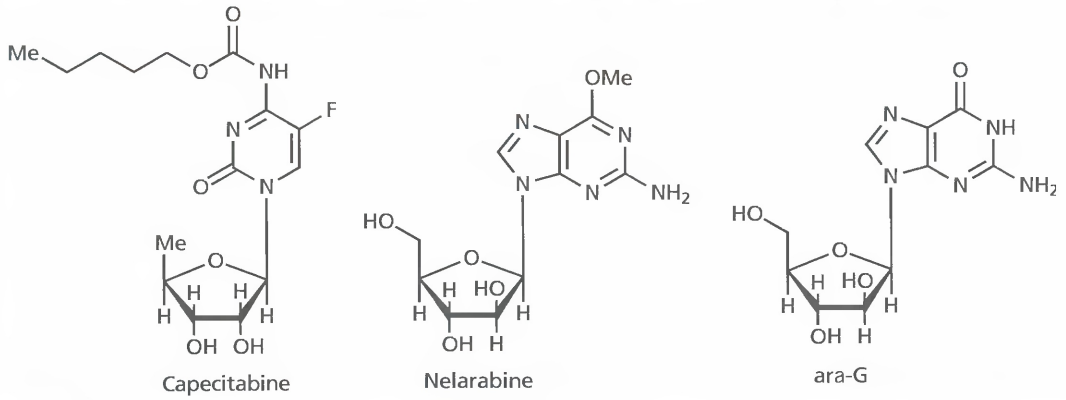


FIGURE 1 Capecitabine.

by folylpolyglutamate synthetase (FPGS), or increased thymidylate synthase expression.

Hydroxycarbamide is orally administered 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 due to increased expression of the enzyme.

Pentostatin is a specialist anticancer drug that 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.

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.

21.3.3 Inhibitors of ribonucleotide reductase

Ribonucleotide reductase is responsible for the conversion of ribonucleotide diphosphates to deoxyribonucleotide diphosphates (Fig. 21.21). The enzyme contains an iron cofactor which is crucial to the reaction mechanism. This involves the iron cofactor reacting with a tyrosine residue to generate and stabilize a tyrosine free radical which then abstracts a proton from the substrate, initiating the mechanism by which reaction takes place. **Hydroxycarbamide** (Fig. 21.36) is a clinically useful agent which inhibits the enzyme by destabilizing the iron centre.

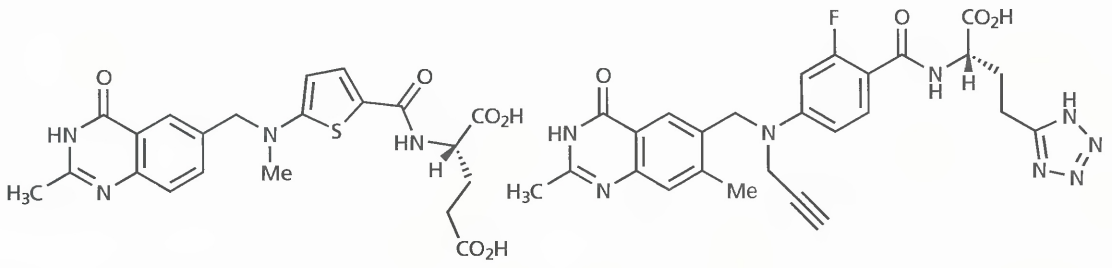


FIGURE 21.20 Raltitrexed and ZD 9331.

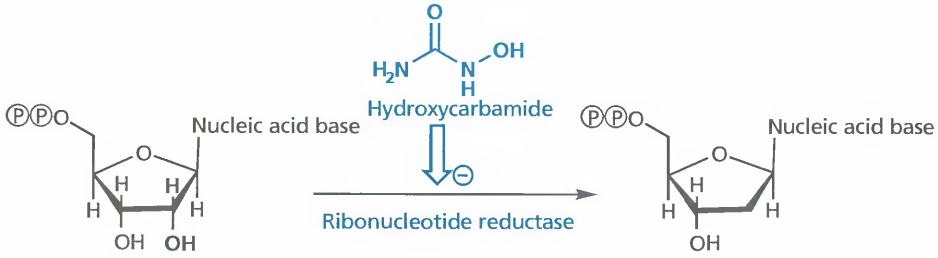


FIGURE 21.21 Reaction catalysed by ribonucleotide reductase (Ⓟ = phosphate).

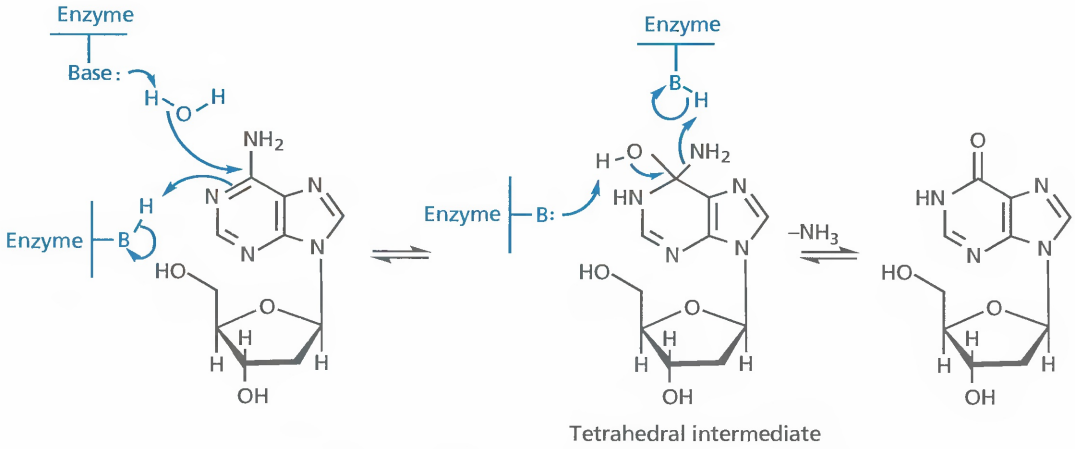


FIGURE 21.22 Mechanism of adenosine deaminase.

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. 21.22) 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.

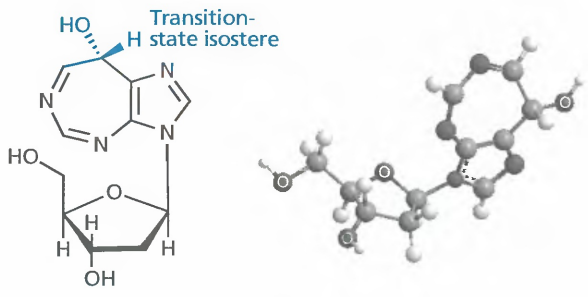


FIGURE 21.23 Pentostatin.

The anti-leukaemia drug **pentostatin** (Fig. 21.23) 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 tetrahedral intermediate in Fig. 21.22.

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. 21.24) 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

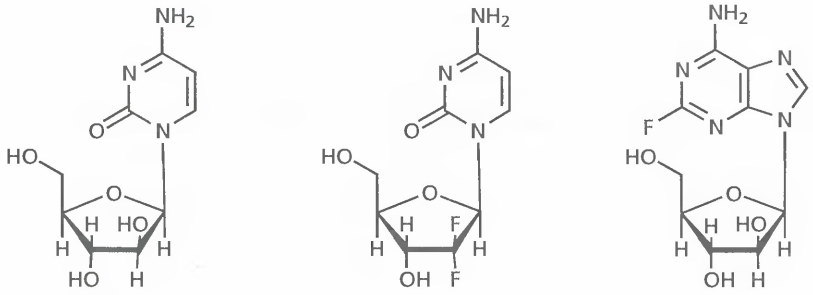


FIGURE 21.24 Inhibitors of DNA polymerase.

a triphosphate and has the same mechanism of action as cytarabine. It too inhibits transcription and can be incorporated into RNA.

21.3.6 Purine antagonists

The thiopurines **6-mercaptopurine** and **6-tioguanine** (Fig. 21.25) 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.

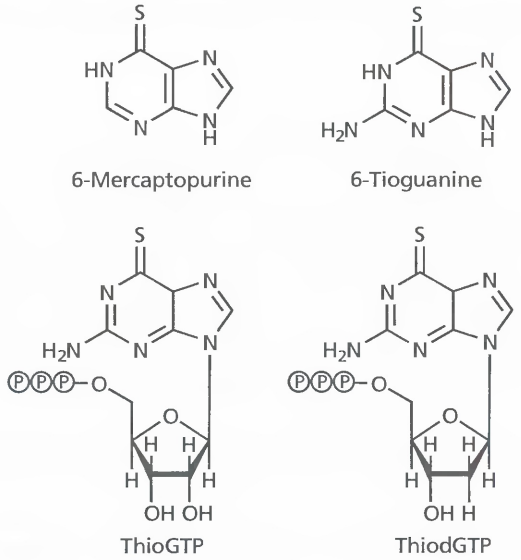


FIGURE 21.25 Purine antagonists, Ⓟ represents a phosphate group.

KEY POINTS

- Antimetabolites are agents that 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 (Box 8.2 and section 4.9).

21.4.1 Glucocorticoids, oestrogens, progestins, and androgens

There are various types of hormones that are used in anticancer therapy such as the glucocorticoids **prednisolone**

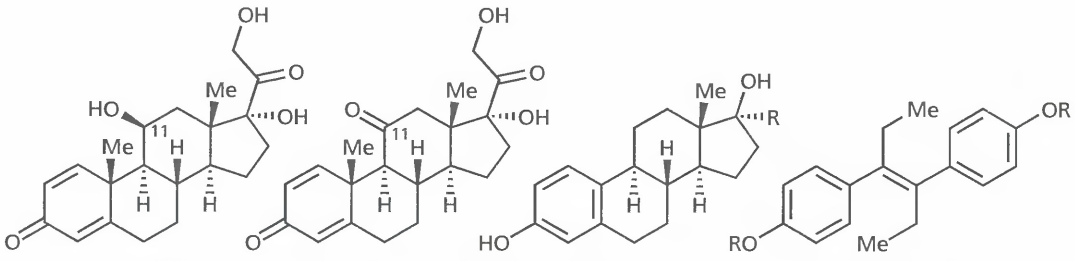


FIGURE 21.26 Glucocorticoids and oestrogens.

and **prednisone** (Fig. 21.26). Prednisone acts as a prodrug and is enzymatically converted to prednisolone in the body.

Oestrogens (spelt estrogens in the USA) 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 **oestradiol**) and **diethylstilbestrol** (a non-steroidal oestrogen) (Fig. 21.26). **Fosfestrol** is the diphosphate prodrug of diethylstilbestrol.

Progestins used as anticancer agents include **medroxyprogesterone acetate** and **megestrol acetate** (Fig. 21.27).

Androgens are thought to suppress production of LH, resulting in a decrease in oestrogen synthesis. The most commonly used agents are **fluoxymesterone** and **testosterone propionate** (Fig. 21.27). The latter is a prodrug which is converted to **dihydrotestosterone**.

21.4.2 LHRH 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. 21.28) which are both decapeptide analogues of LHRH designed to be more resistant to peptidase degradation. This normally takes place next to glycine at 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 Antioestrogens

Tamoxifen and **raloxifene** (Fig. 21.29) are synthetic agents which antagonize oestrogen receptors and prevent oestradiol from binding. The mechanism by which

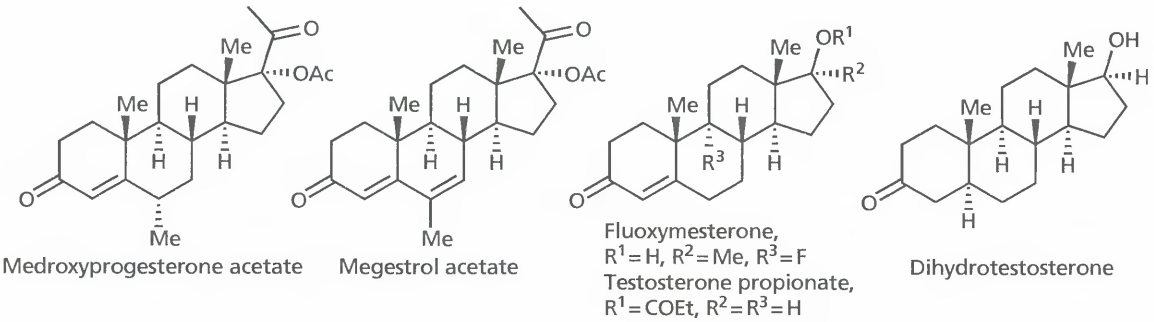
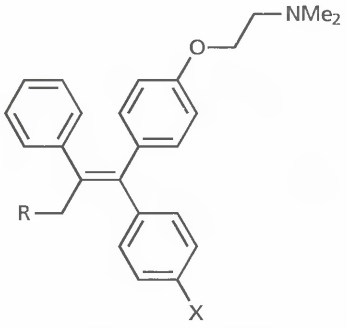


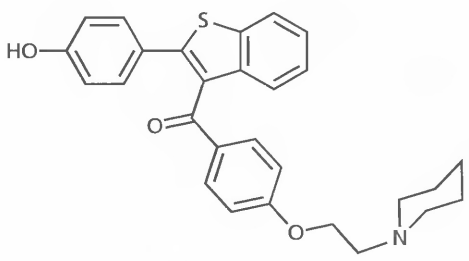
FIGURE 21.27 Progestins and androgens.

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

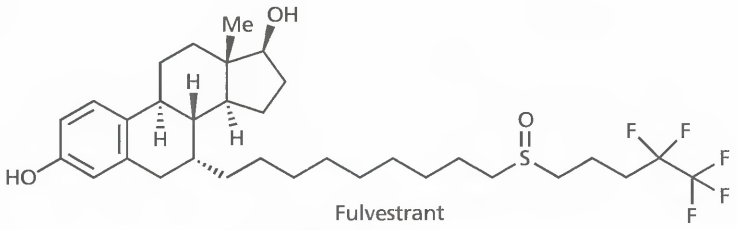
FIGURE 21.28 Luteinizing hormone-releasing hormone (LHRH) agonists.



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

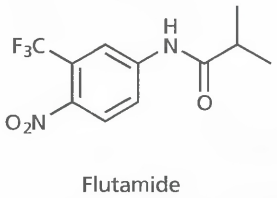


Raloxifene

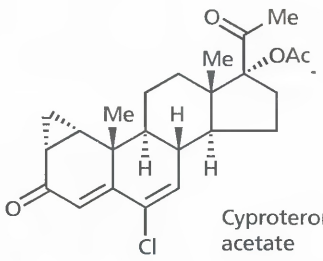


Fulvestrant

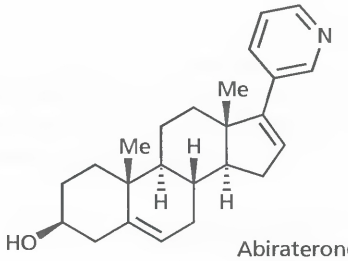
FIGURE 21.29 Antioestrogens.



Flutamide



Cyproterone acetate



Abiraterone

FIGURE 21.30 Antiandrogens.

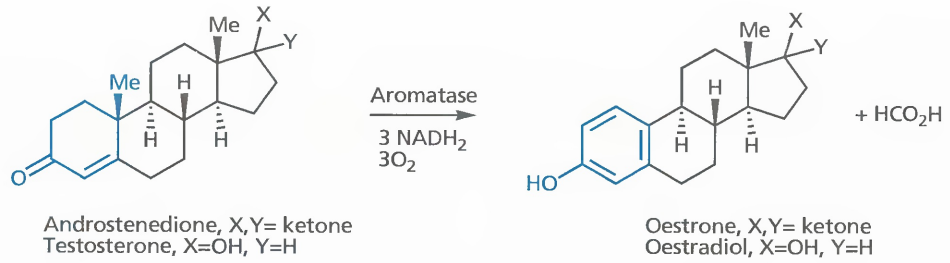
these agents work has been studied extensively and is described in Box 8.2. More recent antioestrogens include **toremifene** and **fulvestrant**, which was approved in 2002.

21.4.4 Antiandrogens

Flutamide and **cyproterone acetate** (Fig. 21.30) are used to treat prostate cancer and work by blocking the action of androgens at their receptors. At present, prostate cancer is treated with a combined therapy of an LHRH agonist and an antiandrogen. It may be possible, however, to use a single agent to inhibit androgen formation by inhibiting a metabolic enzyme called cytochrome $P450_{17a}$, which is involved in the formation of androgens. **Abiraterone** (Fig. 21.30) is a potent inhibitor of this enzyme and has entered clinical trials.

21.4.5 Aromatase inhibitors

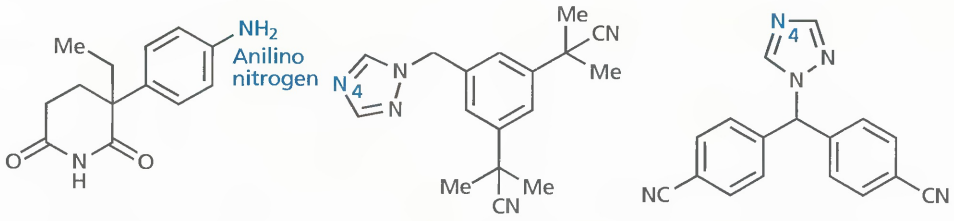
Aromatase inhibitors tend to be used as second line drugs for the treatment of oestrogen-dependent breast cancers that prove resistant to tamoxifen. Aromatase is a membrane-bound enzyme complex consisting of two proteins—one is a cytochrome P450 enzyme containing haem (CYP19), and the other is a reductase enzyme using NADPH as cofactor. Aromatase catalyses the last stage in the biosynthesis of oestrogens from androgens where an aromatic ring is formed (Fig. 21.31). 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 antioestrogenic drugs. There are two types of inhibitor which are used clinically—reversible competitive inhibitors and irreversible inhibitors acting as suicide substrates.



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

Oestrone, X,Y= ketone
Oestradiol, X=OH, Y=H

FIGURE 21.31 Reaction catalysed by aromatase.



Aminoglutethimide

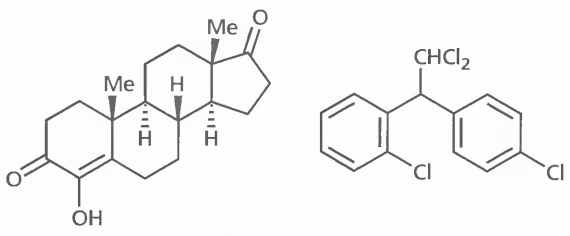
Anastrazole

Letrozole

FIGURE 21.32 Reversible competitive inhibitors of aromatase.

Aminoglutethimide (Fig. 21.32) 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 has resulted in more selective inhibitors such as **anastrazole** 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.

4-Hydroxyandrostenedione (Fig. 21.33) acts as a suicide substrate that permanently inactivates aromatase and is more selective in its action than aminoglutethimide.



4-Hydroxyandrostenedione

Mitotane

FIGURE 21.33 4-Hydroxyandrostenedione 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, anti-hormonal compounds are administered to prevent the offending hormone from binding to its receptor.
- Glucocorticoids, oestrogens, progestins, androgens, and LHRH are used in hormone-based therapy.
- Agents that act as receptor antagonists are used to block oestrogens 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 oestrogens.

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 prevent this process by binding to tubulin to prevent polymerization, or binding to the microtubules to prevent depolymerization.

BOX 21.5 Clinical aspects of hormone-based therapies

Prednisolone is widely used orally for the treatment of leukaemias and lymphomas. **Ethinylestradiol** is the most potent oestrogen 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 most commonly agents 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 as yet unclear 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.

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

Mitotane (Fig. 21.33) interferes with the synthesis of adrenocortical steroids and is used in the treatment of advanced or inoperable adrenocortical tumours. Since 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.

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 that 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*), which bind to tubulin to prevent polymerization. These are discussed in section 10.2.2.

Phyllanthoside (Fig. 21.34) is another natural product which has entered clinical trials, and is thought to bind to tubulin and prevent polymerization. It was obtained from the roots of a Costa Rican tree in the early 1970s. A variety of other naturally occurring agents have been extracted from marine sources and shown to inhibit microtubule formation. For example, **spongistatin 1** (Fig. 21.34) was extracted from a marine sponge in the Maldives and shows potential as an anticancer agent.

Analogues of the naturally occurring compound **podophyllotoxin** (Fig. 9.5) 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 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 plants such as those described above 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 for a while as an anticancer agent. However, its use had to be restricted because of severe side effects. A structural

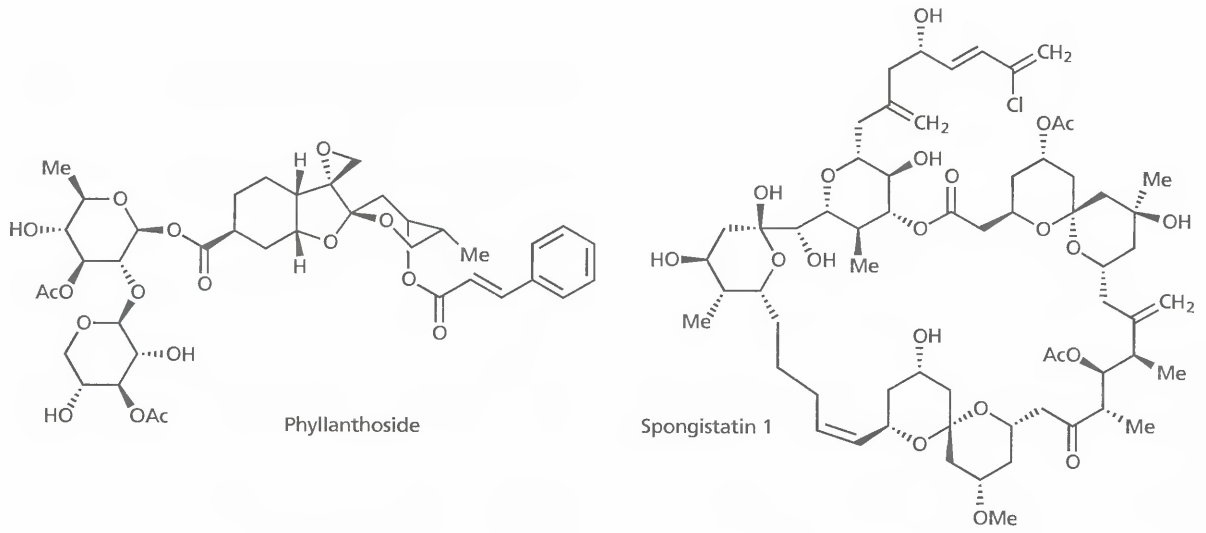


FIGURE 21.34 Natural products inhibiting microtubule formation.

similarity has been noted between podophyllotoxin and **colchicine**—another compound which interacts with tubulin (section 10.2.2).

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), activity is removed altogether. This implies that the sugar moieties in etoposide form a bad steric interaction with tubulin which prevents binding.

Cryptophycins (Fig. 21.35) have been isolated from blue-green algae and shown to have an anticancer mechanism that 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. 21.35) 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 due 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. 21.36) 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

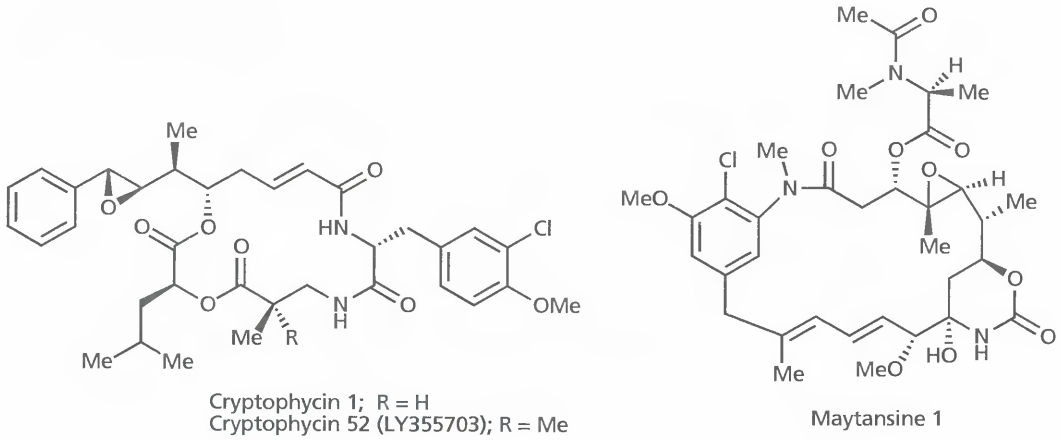
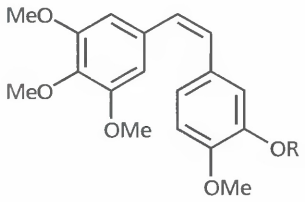


FIGURE 21.35 Cryptophycins and maytansine 1.



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

FIGURE 21.36 Combretastatins.

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 therefore the *cis*-geometry of the double 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 that inhibit tubulin depolymerization

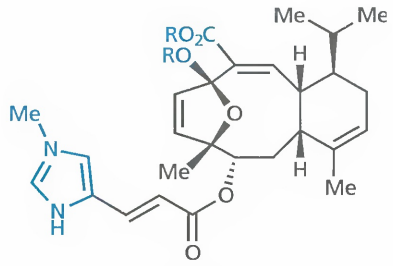
The taxoids are an important group of compounds that inhibit tubulin depolymerization and are discussed in section 10.2.2. The best known example is **paclitaxel** (Taxol). Semi-synthetic taxoids are currently being investigated to find compounds with better oral bioavailability, improved pharmacological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

Since the discovery of paclitaxel a variety of other natural products have been found to have a similar mechanism of action, and are currently being studied as potential anticancer agents (Fig. 21.37). These include bacterial metabolites called **epothilones**, and marine natural products such as **eleutherobin** from coral, and **discodermolide** from a marine sponge. These compounds show several advantages over paclitaxel. First, the epothilones and discodermolide do not appear to be substrates for the P-glycoprotein efflux system, and are therefore potentially effective against drug-resistant cancer cells. Second, it has been found that discodermolide enhances the activity of paclitaxel and it may be possible to use these drugs in combination therapy. Third, 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. 21.37) 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.

These novel agents bind to the same region of tubulin as paclitaxel. A three-dimensional pharmacophore has been developed that 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. 21.38) are similar in structure to eleutherobin, and are also active against drug resistant



Sarcodictyin A; R=Me
Sarcodictyin B; R=Et

FIGURE 21.38 Sarcodictyins.

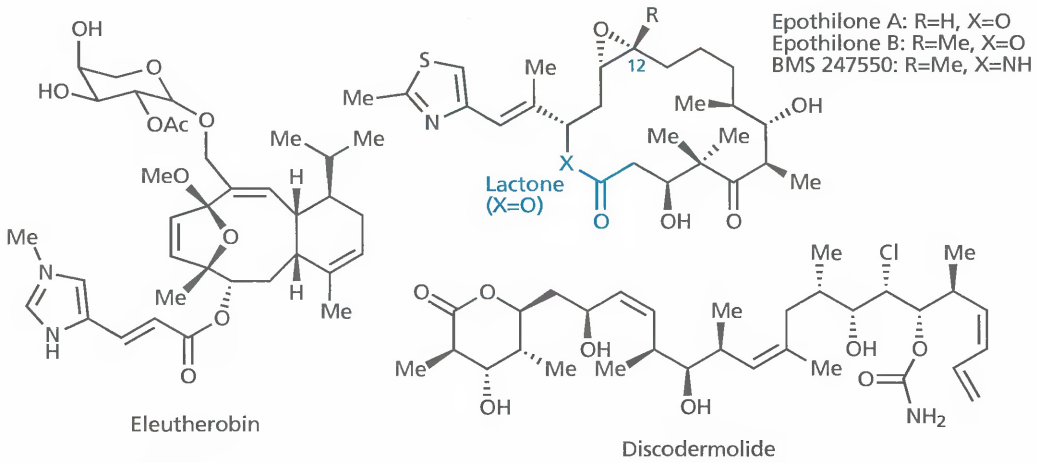


FIGURE 21.37 Recently discovered natural products that inhibit microtubule depolymerization.

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 widely used 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 over-expression 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 G_2/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 over-expression of the P-glycoprotein transport protein, which leads to faster efflux from the cell.

cancers. Structure–activity relationship (SAR) studies of these compounds have demonstrated the importance of the coloured groups in Fig. 21.38.

KEY POINTS

- Agents that 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.
- Paclitaxel and its derivatives bind to tubulin and accelerate polymerization by stabilizing the resulting microtubules. Newer analogues are being investigated that show better oral bioavailability, improved pharmacological properties and activity against drug-resistant cancers.
- A variety of natural products have been discovered that 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. Now, 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 over-expressed 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. Identifying defects in a cell's signalling pathways and identifying suitable targets has 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 5.4.1–5.4.2) 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 bind 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, however, persistently bind GTP and fail to hydrolyse it, such that they are constantly active. Since 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

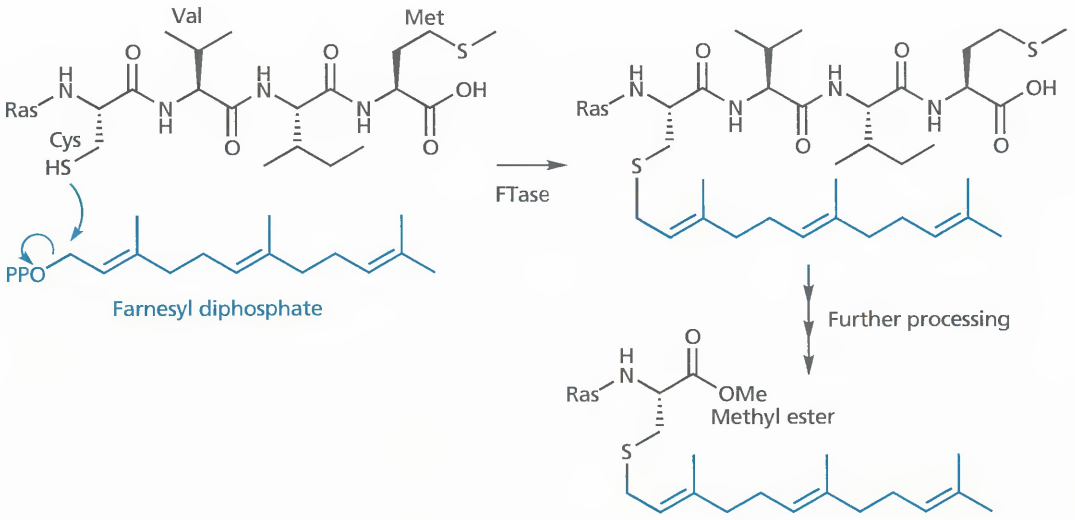


FIGURE 21.39 Mechanism of farnesyl transferase.

cancer. Finding methods of 'neutralizing' Ras, therefore, could be useful in combating cancer.

One of these approaches centres round 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 at 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. 21.39) involves the binding of farnesyl diphosphate (FPP) to the active 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 to the cell membrane by the farnesyl chain.

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

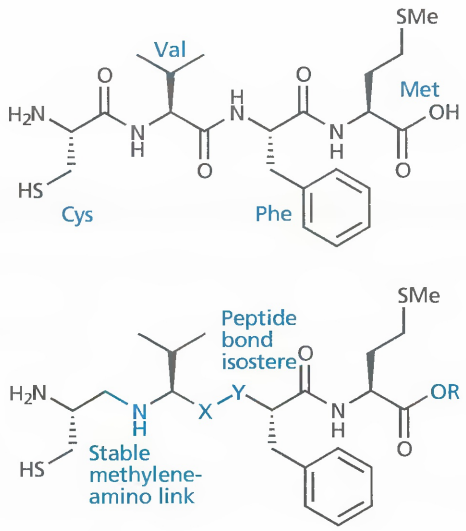


FIGURE 21.40 Design of farnesyl transferase inhibitors.

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. 21.40). 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, be metabolically stable, and have suitable pharmacokinetic properties. However, the lead compound is a tetrapeptide and suffers from various disadvantages.

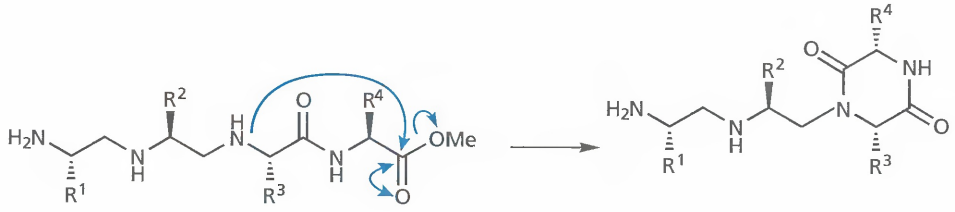


FIGURE 21.41 Diketopiperazine formation.

- First, 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.
- Second, the lead compound has peptide bonds that are susceptible to metabolism, particularly to 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 cyclisation with the terminal ester to form an inactive diketopiperazine structure (Fig. 21.41). 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. 21.42). 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.

Although the thiol group is important as a ligand for zinc, there are problems associated with it, since it is potentially toxic and can be oxidized by metabolic enzymes. The AstraZeneca compound **AZD-3409** (Fig. 21.43) has the thiol group and the 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 FT (K_i < 1 nM)

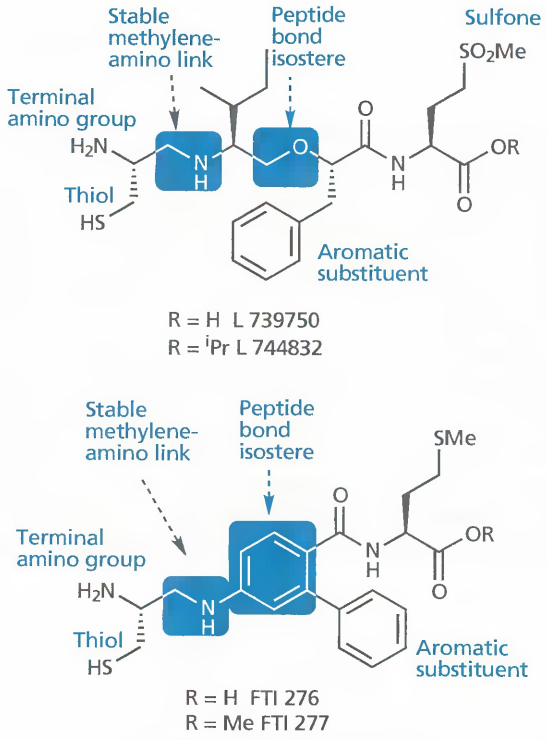


FIGURE 21.42 Farnesyl transferase inhibitors.

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.

[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 a geranylgeranyl group.

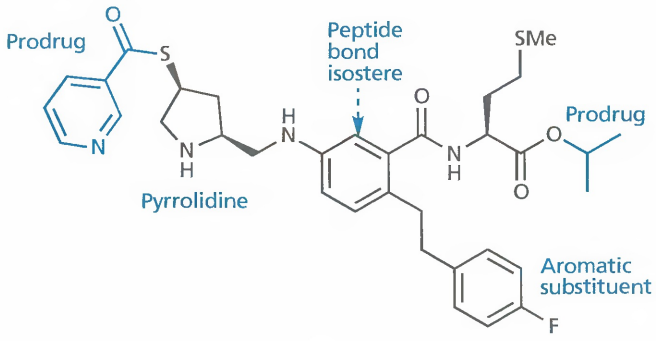


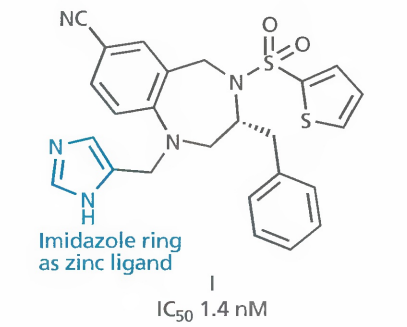
FIGURE 21.43 AZD-3409.

Non-peptide inhibitors have also been designed and are undergoing clinical trials (Box 21.7). Structure I in Fig. 21.44 has an imidazole ring acting as the zinc ligand rather than a thiol group which has been associated with undesirable side effects. The imidazole ring has previously been shown to act as a zinc ligand in other structures.

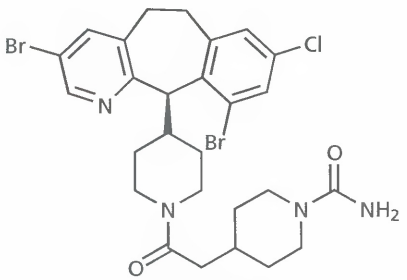
Lonafarnib (Fig. 21.44) was developed from a compound that was discovered by screening compound libraries, and is 10 000 times more active than the original lead compound. Remarkably, it has no ligand for the zinc cofactor and so structure-based drug design was carried out to introduce a suitable group at the correct position, resulting in **Sch 226374** (Fig. 21.45). 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 due solely to 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 inhibit the *cellular* functions of H-Ras only, since 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. Since farnesyl transferase can accept a variety of different protein substrates other than Ras, it is possible that inhibition affects other cellular processes resulting in 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

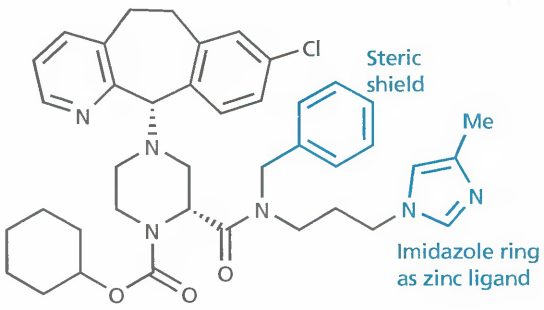


I
IC_{50} 1.4 nM



IC_{50} 1.9 nM

FIGURE 21.44 Non-peptide-like inhibitors of farnesyl transferase undergoing clinical trials.

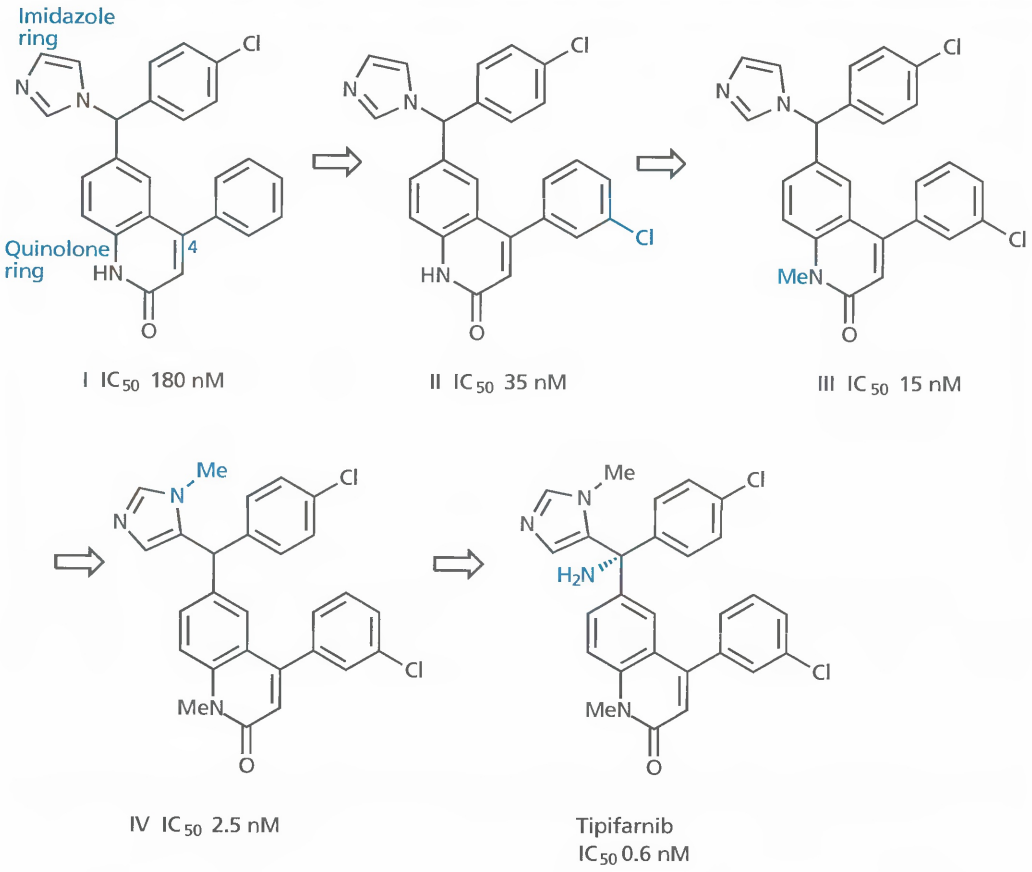


Sch 226374; IC_{50} 0.36 nM

FIGURE 21.45 Development of a lonafarnib analogue.

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, mainly 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.

the HMGR enzyme (Case Study 1) which is involved in the biosynthetic pathway to both steroids and isoprenoids. Consequently, statins can influence Ras protein farnesylation by lowering the level of farnesyl diphosphate.

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 500–2000 different types of protein kinase. 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 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. Since 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.

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 adenosine triphosphate (ATP) as the phosphorylating agent, and so there is a region within the active site that binds ATP, as well as a region that binds the substrate. Considering the fact that there are so many kinases, and that they all use ATP as the phosphorylating agent, many scientists thought that the protein kinases would be poor targets for drugs. It was believed that the ATP binding region would be very similar for all protein kinases, so that any inhibitor would show poor selectivity and end up inhibiting all protein kinases. This has not turned out to be the case. Crystal structures of protein kinases containing bound ATP have been studied and it has been shown that ATP fits quite loosely to the binding site, and that there are areas of the binding site which remain unoccupied. There are also significant differences in the amino acids present in the ATP binding site from one kinase active site to another. As a result, it is quite possible to design selective inhibitors. Indeed, all the protein kinase inhibitors of clinical interest have been designed to bind to the cofactor binding site, rather than the substrate binding site.

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 epidermal growth factor receptor (EGF-R) are shown in Fig. 21.46 and are representative of all the kinase binding sites. The purine base is buried deep in the binding site and makes two important hydrogen bonding interactions with the protein backbone. 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, two of which are particularly important. One area is known as the specificity surface, while the other consists of a hydrophobic pocket opposite the ribose binding pocket. 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 the pocket and interact with it. The hydrophobic pocket is also lined by different amino acids depending on the kinases involved, and this allows the possibility of designing drugs which can distinguish

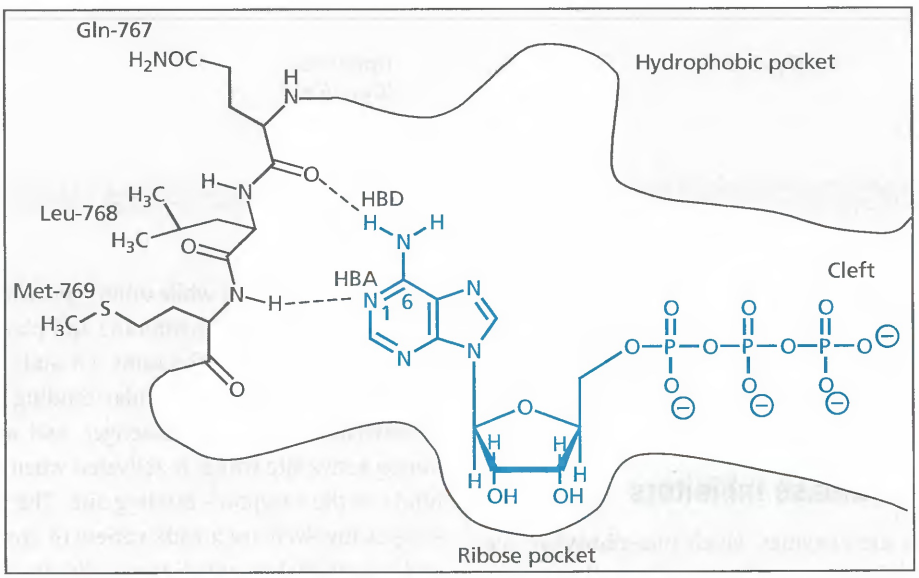


FIGURE 21.46 Binding of ATP to the kinase active site of the epidermal growth factor receptor.

between the hydrophobic pocket of one kinase active site from the hydrophobic pocket of another.

Kinases exist in an inactive or an active conformation. The switch by which a kinase changes from an inactive conformation to an active conformation is controlled by an activation loop. This sits across the active site when the enzyme is in the inactive conformation, blocking access to ATP and/or the protein substrate. Activation usually occurs by phosphorylation of residues on the loop, which causes the loop to move position. In the inactive form of the enzyme, there are hydrophobic regions exposed that are not exposed in the active form of the enzyme and these can provide binding interactions for kinase inhibitors.

Kinase inhibitors are classed as type I or type II inhibitors. Type I inhibitors act on the active conformation of the enzyme. They inhibit the enzyme by binding to the active site, and blocking access to the substrate or cofactor. Type II inhibitors bind to the enzyme when it is in the inactive conformation. They inhibit the enzyme by stabilising the inactive conformation. Protein kinase inhibitors such as gefitinib, erlotinib, SU11248 and seliciclib are type I inhibitors, while agents such as imatinib, lapatinib, sorafenib and vatalanib are type II inhibitors. It has been observed that there are greater differences between the active sites of different kinases when the kinases are in the inactive form than when they are in the active form, suggesting that inhibitors designed to stabilize the inactive conformation may be more selective. To date, all clinically important inhibitors have binding interactions that mimic the adenine interactions of the cofactor ATP.

21.6.2.1 Kinase inhibitors of the epidermal growth factor receptor

EGF-R is a membrane-bound tyrosine kinase receptor having an extracellular binding site for epidermal growth factor (EGF), and an intracellular kinase active site (sections 4.8.2–4.8.3). Several agents have been studied as kinase inhibitors and the first of these to reach the clinic was **gefitinib** (Iressa) (Fig. 21.47 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. 21.48) which had various important features previously identified by SAR studies, mainly 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 rapidly metabolized 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 11.4). Therefore, it was decided to modify the structure such that both metabolic routes were blocked. In structure IV in Fig. 21.48, the methyl group was replaced by a chloro substituent. This can be viewed as a bioisostere for the methyl group since 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 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. a 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

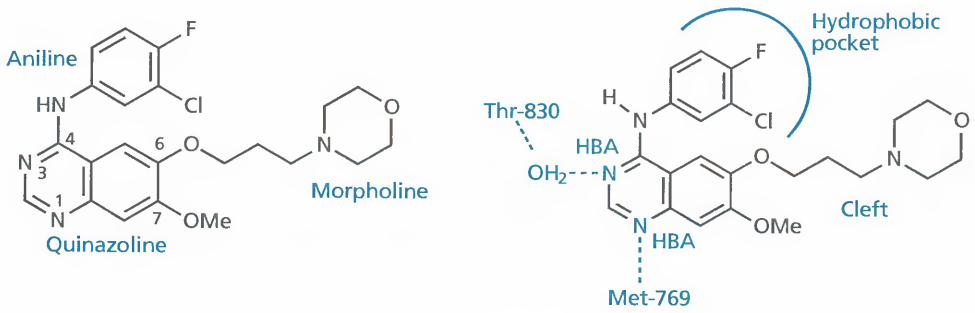
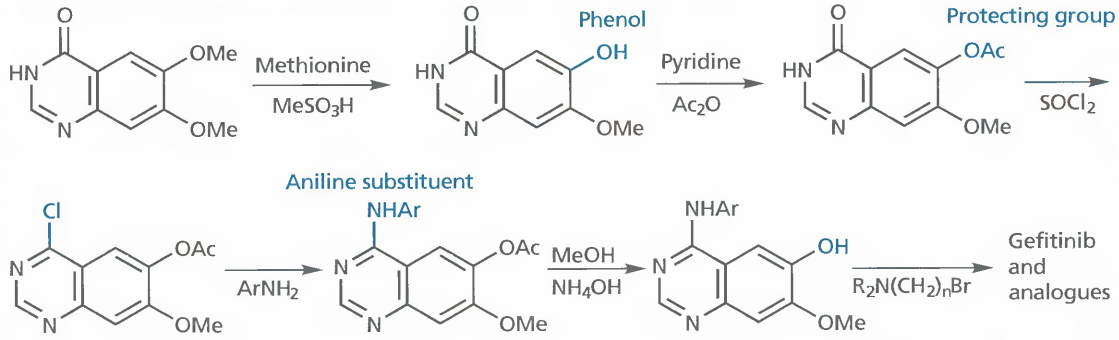


FIGURE 21.47 Structure and binding interactions of gefitinib (Iressa).

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 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.

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 (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.

A molecular modelling study was carried out to investigate how gefitinib might bind to a model of the kinase binding site. The model was constructed from a knowledge of the structures of similar active sites. Gefitinib was found to mimic ATP and bind to the ATP-binding region of the kinase active site. The binding of ATP itself involves two important hydrogen bonding interactions between the purine base of ATP and the protein backbone between amino acids Gln-767 and Met-769 (Fig. 21.46). One of these interactions involves the purine group acting as a hydrogen bond donor, while the other involves purine acting as a hydrogen bond acceptor. Gefitinib has two nitrogen atoms in the quinazoline ring which both act as hydrogen bond acceptors (Fig. 21.47). One of these interacts directly with Met-769, while the other interacts with a bridging water molecule which interacts in turn with the hydroxyl group of Thr-830. The aniline ring occupies the normally vacant hydrophobic pocket opposite the ribose binding pocket (Figs. 21.46 and 21.47).

Other EGF-R kinase inhibitors include **PKI-166** which is undergoing clinical trials and **erlotinib** (Fig. 21.49), 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

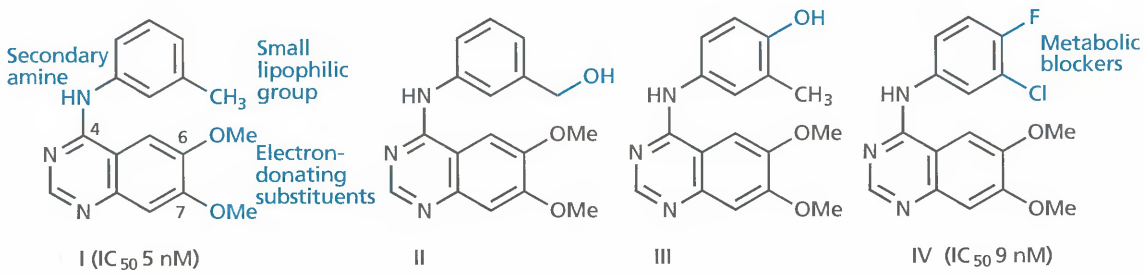


FIGURE 21.48 Design of a metabolically stable analogue of structure (I).

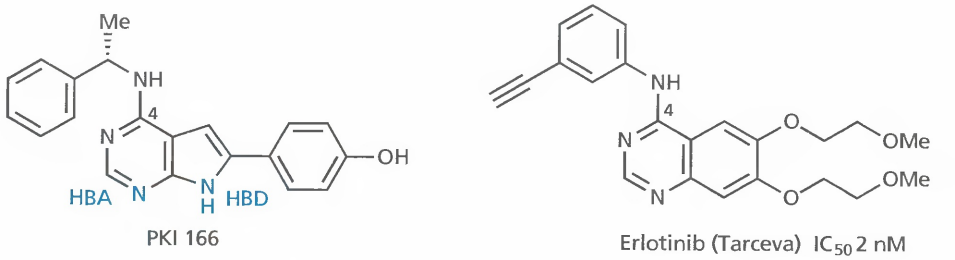


FIGURE 21.49 Inhibitors of the epidermal growth factor receptor kinase.

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. 21.46), 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. 21.50) has the same quinazoline 'core' as erlotinib and gefitinib and was approved in March 2007.

21.6.2.2 Inhibitors of the Abelson tyrosine kinase

As the first protein kinase inhibitor to reach the market, **imatinib** (**Glivec** or **Gleevec;** Fig. 21.51 and Box 21.9) represents a milestone in anticancer therapy. It was also the first drug 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 kinase active site resides on the Abl portion of the hybrid protein.

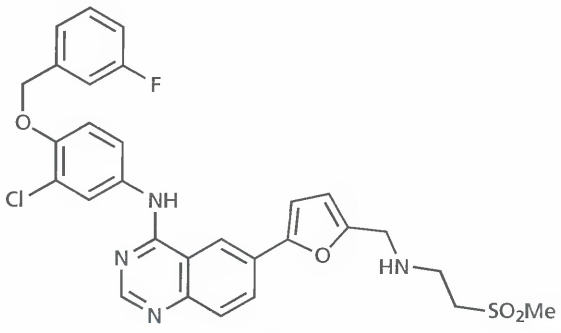


FIGURE 21.50 Lapatinib.

The lead compound (I in Fig. 21.68) 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 showed inhibitory activity against tyrosine kinases as well. 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. 21.51), 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 Abl kinase has been determined. This demonstrates the importance of the amide group within imatinib which serves as an 'anchoring group' (Fig. 21.52). The amide forms hydrogen bonds to glutamate and aspartate residues which are both essential to the catalytic mechanism of the enzyme. These interactions orientate the molecule allowing either half of the structure to access binding

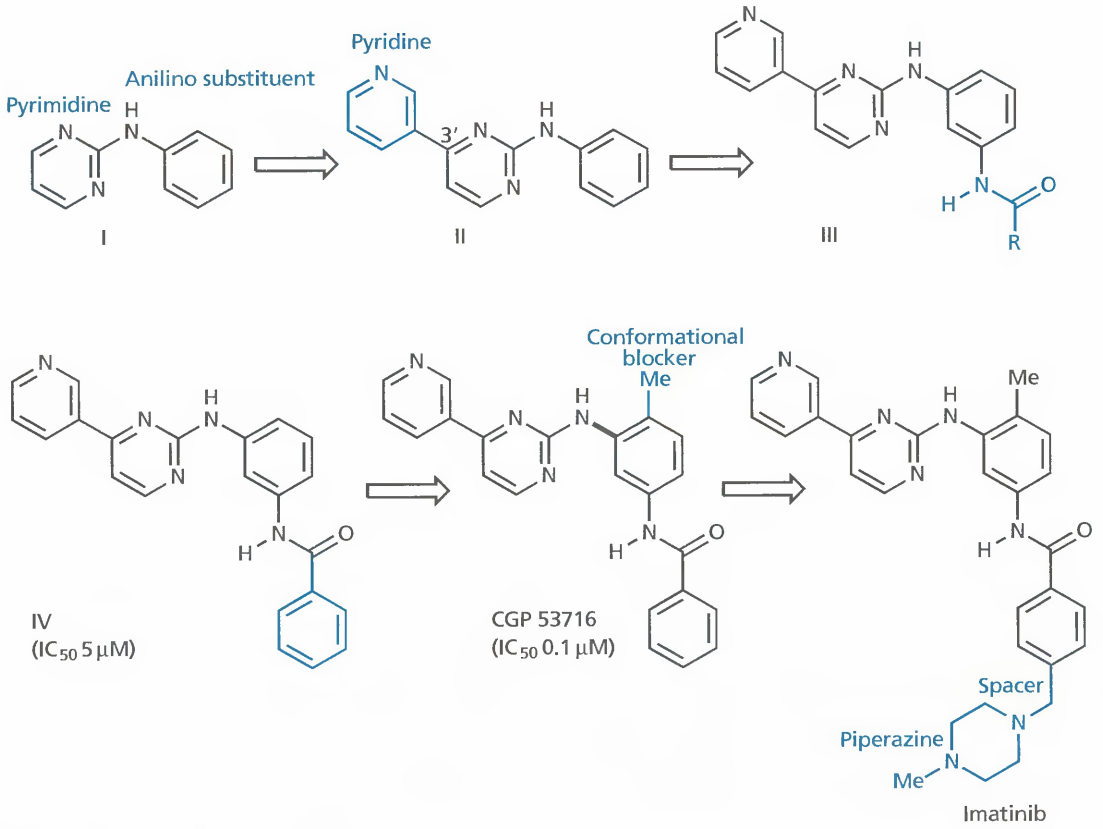
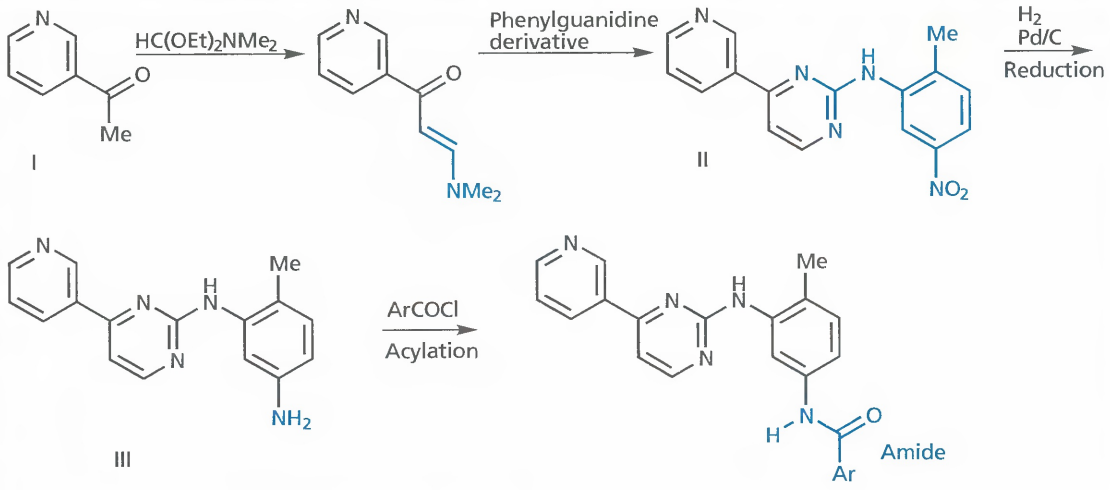


FIGURE 21.51 Development of imatinib.

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.

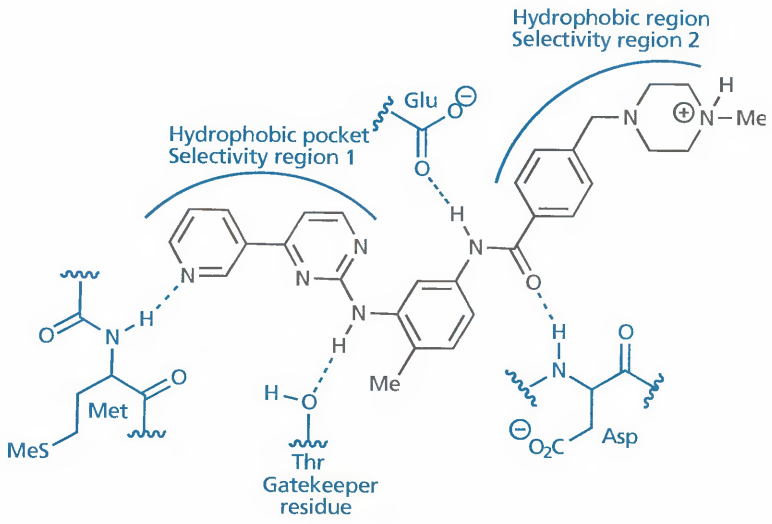


FIGURE 21.52 Binding interactions of imatinib in the active site of Abl kinase.

regions 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 emphasised by the loss of activity which is observed when the amino group is alkylated. The pyridine and pyrimidine rings are located within a hydrophobic region representing selectivity region 1. 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.

As mentioned above, imatinib inhibits three kinds of protein kinase target and clinical applications have been found for two of these (Bcr-Abl and cKit). Investigations are also in progress relating to platelet-derived growth factor receptor (PDGF-R), which is responsive to the platelet-derived growth factor (PDGF). It has been suggested that excessive levels of PGDF contribute to the appearance of certain types of cancer, so imatinib may be useful in this respect as well.

The fact that imatinib is not totally selective and inhibits various 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 due 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 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 been observed as well. 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. 21.53) represent a second generation of Bcr-Abl inhibitors that are active against some imatinib-resistant tumours, but not against the T315I mutant. The *N*-methylpiperazine ring in imatinib has been replaced in nilotinib, increasing affinity for Bcr-Abl by 20–30 fold, while retaining activity for c-Kit and PDGF-R.

Dasatinib binds to the kinase target with greater affinity than either nilotinib or imatinib, and can bind when the enzyme is in different states. This has the effect of limiting resistant strains to those that have mutations which affect amino acid residues directly involved in binding interactions.

There are several projects underway which are attempting to design kinase inhibitors that are less likely to fall prey to the problems of drug-resistance. One way of tackling the problem of resistance is to design drugs which have a dual target. For example, structures are being developed that inhibit the kinase active sites of Abl and

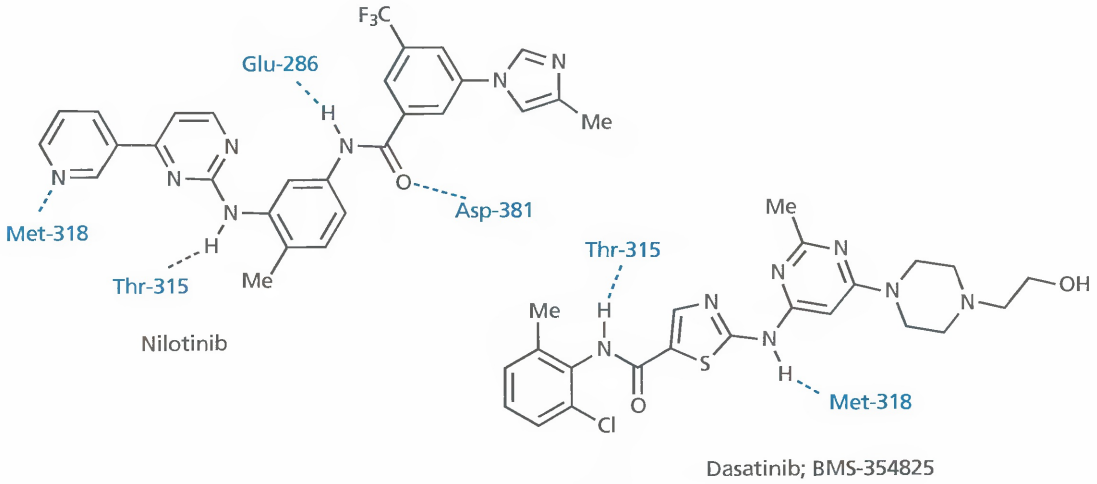


FIGURE 21.53 Nilotinib and dasatinib.

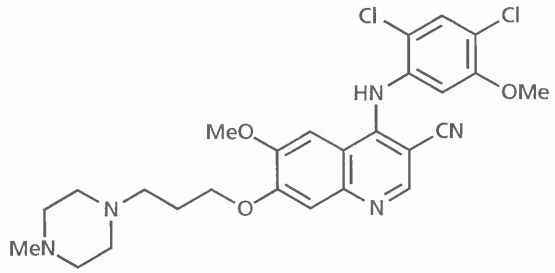


FIGURE 21.54 Bosutinib.

another kinase called Src. The latter plays a crucial role in cell movement and proliferation. **Bosutinib** (Fig. 21.54) is one such structure currently in clinical trials.

Another strategy is to develop kinase inhibitors which bind to Bcr-Abl at sites other than the ATP binding site. 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. 21.55) is one such compound in 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. 21.55) which inhibit the kinase enzyme by binding to the substrate binding site rather than the ATP cofactor binding site.

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

21.6.2.3 Inhibitors of cyclin-dependent kinases

CDKs are involved in the control of the cell cycle (section 21.1.6), but are over-expressed or overactive in many cancer cells. Since 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

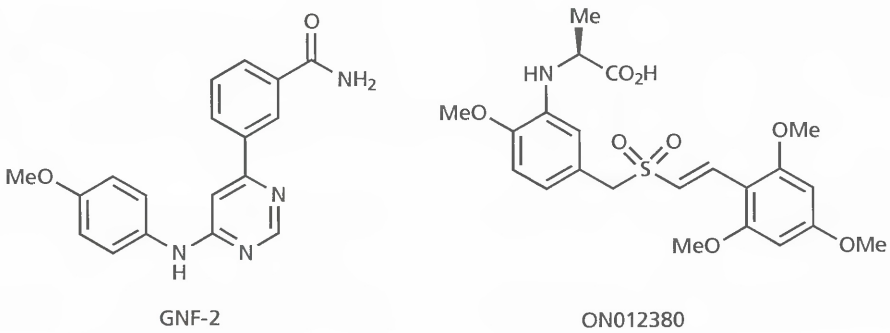


FIGURE 21.55 Structures of GNF-2 and ON012380.

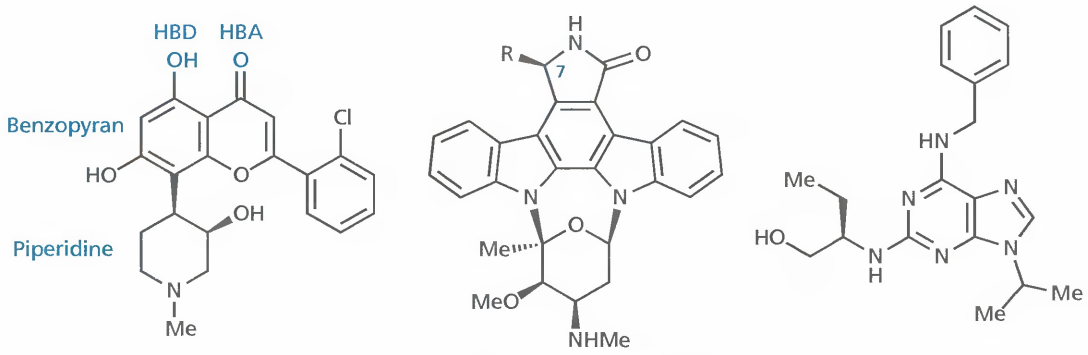


FIGURE 21.56 Inhibitors of cyclin dependent kinases.

cyclin-dependent kinase inhibitors (CKIs). There are at least nine CDKs, and typically they are 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. 21.56) 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 show such 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, and 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. 21.56). 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. 21.56) 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 Kinase inhibitors of FGF-R and VEGF-R

Elevated expression levels of fibroblast growth factor receptor (FGF-R) and the vascular endothelial growth factor receptor (VEGF-R), along with their corresponding ligands have been associated with angiogenesis. Consequently, kinase inhibitors of these receptors may disrupt the angiogenesis process. This will starve tumours of nutrients in the long term while improving access of conventional drugs in the short term. The receptor for the vascular endothelial growth factor (VEGF) is a protein kinase receptor which dimerizes when it binds VEGF and triggers the start of a signal transduction pathway leading to angiogenesis. Inhibitors designed to bind to the ATP binding region of the kinase active site have entered clinical trials as potential anticancer agents (Fig. 21.57). For example, **SU-5416** is an oxindole structure that reached phase III clinical trials for the treatment of colorectal cancer. The oxindole ring can provide a hydrogen bond acceptor and a hydrogen bond donor to bind to the same region of the peptide backbone as the adenine ring of ATP. There is also an intramolecular hydrogen bond between the NH of the pyrrole ring and the carbonyl oxygen which helps to stabilize the active conformation. **PTK 787** is a phthalazine structure which was also in phase III clinical trials during 2006.

Despite the potential of these agents, inhibition of the VEGF receptor kinase is not guaranteed to inhibit angiogenesis completely since there are other growth factors which can promote angiogenesis; for example PDGF, TGF-β, TNF-α and interleukins IL-1 and IL-8. It is therefore likely that a variety of agents targeting angiogenesis in different ways may be the best approach.

Another approach is to use multi-kinase inhibitors which include FGF-R and/or VEGF-R in their list of targets. Examples include the clinically useful agents **sorafenib** and **sunitinib** (section 21.6.2.6).

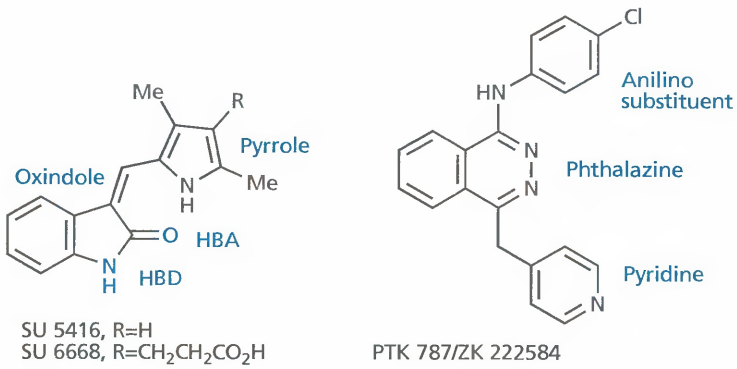


FIGURE 21.57 Kinase inhibitors of VEGF-R and FGF-R.

21.6.2.5 Other kinase targets

There are other types of protein kinases and protein kinase receptors which have been found to be over-expressed in certain types of cancer cells, and research is being carried out to find selective inhibitors for these. The main targets other than those already described are the platelet-derived growth factor receptor (PDGF-R), protein kinase B (PKB), Protein kinase C (PKC), mitogen-activated protein kinases (MAPK), insulin growth factor 1 receptor (IGF-1R), the Met receptor, Raf-1 kinase and c-Src tyrosine kinase. IP_3 Kinase inhibitors are being studied as anti-angiogenesis agents.

It is worth noting that most research has been carried out on ATP mimics, but there is potential in designing inhibitors that bind to the substrate binding region. Many researchers feel that such inhibitors could be more selective and safer to use.

21.6.2.6 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. 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 within the one drug. Such drugs are sometimes referred to as '**dirty drugs**' since they affect a variety of different targets. mTRKIs are likely to be particularly promising agents for the treatment of cancers which are driven by several abnormalities.

Sorafenib is one example of a mTRKI. It was developed from a urea lead compound using a mixture of traditional medicinal chemistry strategies and multiple point variations (Box 21.10). It has been approved as a VEGF-R inhibitor, and is undergoing clinical trials against a variety of other kinase targets.

Sunitinib (Fig. 21.58) was approved in 2006, and inhibits multiple kinase targets including VEGF-R, PDGF-R and the KIT receptor. **Vatalanib** (Fig. 21.58) is another multi-kinase inhibitor undergoing clinical trials.

The idea of targeting several targets with the one drug is described as a '**poly-pharmacology**' approach. Drugs acting in this way may have potentially enhanced antitumour activity relative to more selective kinase inhibitors.

An alternative poly-pharmacology approach is to use a 'cocktail' of different selective kinase inhibitors.

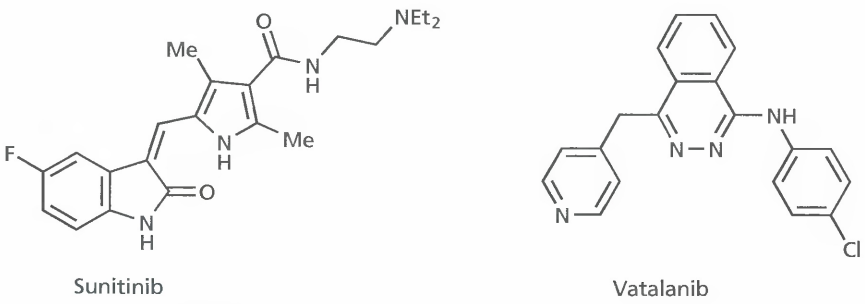


FIGURE 21.58 Structures of sunitinib and vatalanib.

BOX 21.10 Design of sorafenib

In order to find the lead compound for sorafenib, high throughput screening of 200000 compounds was carried out against recombinant Raf-1 kinase (also called c-Raf). This led to the identification of a urea lead compound (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 tenfold increase in activity. However, despite the synthesis of many more analogues, no further improvement in activity could be obtained. Up to this point, conventional medicinal chemistry strategies were followed which involved altering one group at a time. This allows one to rationalise any alterations in activity that result from the change of any ring or substituent. It was now 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 but has low activity compared to the lead compound. Similarly, structure VI has the isoxazole ring but has poor activity. 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.

Structure IV was now adopted as the new lead compound. Replacing the phenyl ring with a pyridine ring led to structure (V) and a five fold 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.

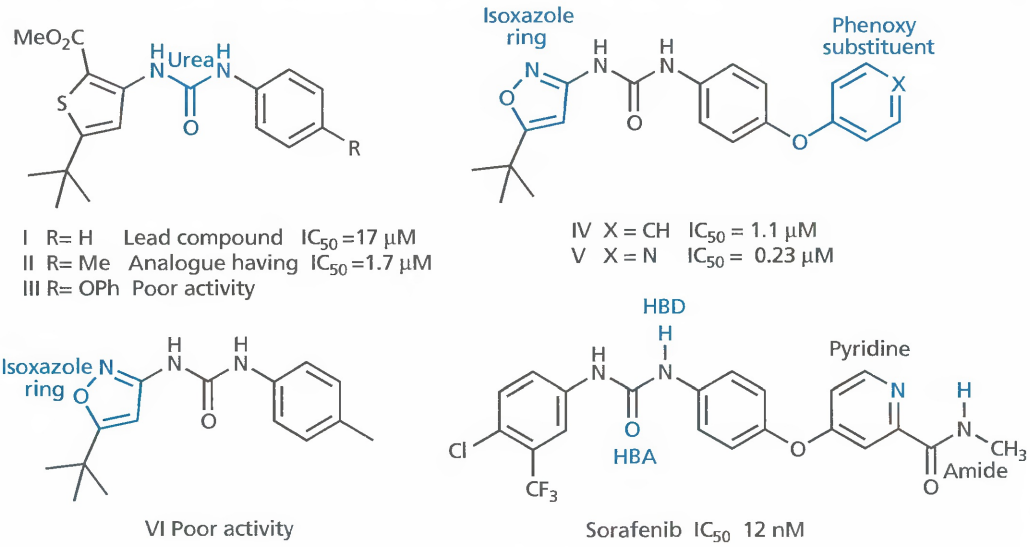


FIGURE 1 From lead compound to sorafenib.

21.6.2.7 Inhibitors of heat shock protein 90

There is intense interest in developing drugs that inhibit the heat shock protein 90 (HSP 90), a protein which acts as a molecular chaperone (section 2.7.4). Chaperones are important to the survival of normal cells, but they are even more important to the survival of cancer cells which are under greater stress. Consequently, the inhibition of chaperones should have a greater effect on cancer cells than normal cells. HSP90 is a chaperone that has been identified as a potential target for anticancer agents since

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. Over-expression 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. **Lapatinib** was approved in 2007 for the treatment of patients with advanced or metastatic breast tumours which over-express 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 due to tumour resistance. Tumours having resistance to trastuzumab are unlikely to have resistance to lapatinib since the former drug binds to the extracellular region of the receptor and the latter binds intracellulularly to the 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 to trastuzumab. The drug is currently undergoing clinical trials for a range of other cancer treatments such as those involving the head, neck and kidney.

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. This in turn leads to excessive quantities of white blood cells (leukocytes). Imatinib has been successful in 90% of patients. 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 over expressed. 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.

Nilotinib and dasatinib have been approved for the treatment of CML when imatinib therapy is unsuccessful due to drug resistance. Dasatinib is also being considered for metastatic melanoma.

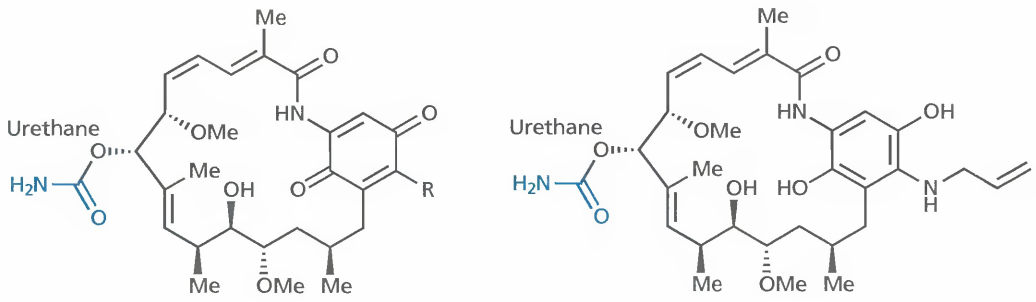
Other kinase inhibitors

Sorafenib has been approved as a VEGF inhibitor for the treatment of renal cell carcinoma. **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.

Temsirolimus is an analogue of the antibiotic rapamycin and was approved in 2007 for the treatment of advanced renal cell carcinoma. It binds to the FK506-binding protein, and the resulting drug-protein complex then inhibits a kinase called mTOR.

it is quite selective in the number of proteins with which it interacts (ca 300). Moreover, many of the proteins with which HSP90 interacts are overexpressed in different cancer cells, and are anticancer targets in their own right (e.g. Bcr-Abl, c-Kit, Her2, EGFR, VEGFR). Targeting HSP90 may well prove highly effective in treating cancers which have gained resistance against anticancer drugs targeting these proteins. Resistance to such drugs is often associated with mutations that have led to changes in the target protein such that they fail to bind the anticancer drug. Since these proteins are more unnatural, they have a greater reliance on HSP90 during the folding process. Therefore, the most mutated protein is the one that is most likely to be vulnerable if HSP90 is inhibited.

HSP90 is a kinase protein, and hydrolyses ATP in order to carry out its role as a chaperone. Several companies are investigating inhibitors that bind to the ATP binding site. The natural product **geldanamycin** (Fig. 21.59) binds to the site and is a potent inhibitor, but it suffers from a reactive quinone moiety and poor solubility. **Tanespimycin, alvespimycin** and **IPI-504** (Fig. 21.59) are analogues with improved properties and are undergoing clinical



R=OMe, Geldanamycin
R=NHCH$_2$CH=CH$_2$, 17-AAG, Tanespimycin
R=NHCH$_2$CH$_2$NMe$_2$, 17-DMAG, Alvespimycin

IPI-504

FIGURE 21.59 Structures that inhibit HSP90.

trials. All these structures contain a urethane group that is crucial to activity and which forms hydrogen bonds to the region of the binding site that normally interacts with the adenine moiety of ATP.

Other research is looking at drugs which will block the protein-protein interactions that are involved when HSP interacts with different kinds of protein. Since the protein-protein interactions differ, it should be possible to design drugs that block HSP interacting with some proteins but not with others, leading to greater selectivity.

KEY POINTS

- Many cancers produce abnormal or over-expressed proteins that are involved in the signalling pathways that stimulate cell growth and division. Agents that act selectively against these targets are less likely to have the serious side effects associated with traditional cytotoxic agents.
- An abnormal form of the Ras protein that is permanently active 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 the 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 that 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 over-expressed or abnormal in nature. Anticancer agents have been designed to bind to the ATP binding region of the kinase active site, and to act as inhibitors. Since there is a variation in the active site from kinase to kinase, it is possible to design agents that are selective between 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

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 the 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

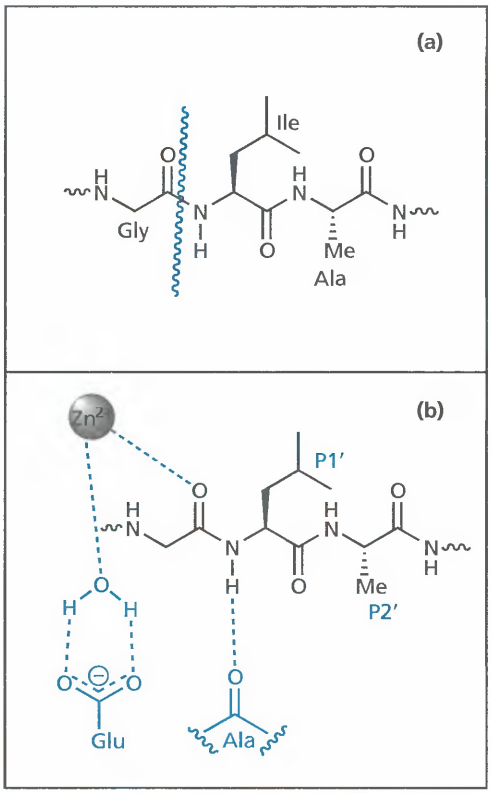


FIGURE 21.60 Peptide bond cleaved by matrix metalloproteinases.

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. 21.60). 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 that is stable to hydrolysis.
- 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. 21.61) which is an orally active, synthetic compound in 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.

The nature of the P1′ group can be varied, and determines the activity and selectivity of the inhibitors against

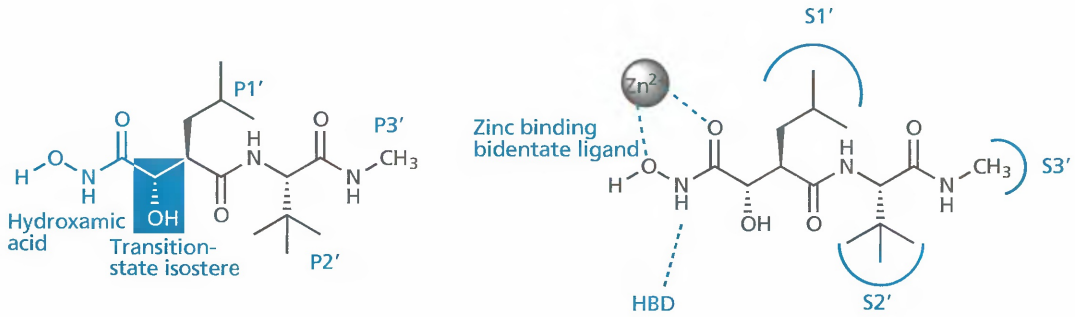


FIGURE 21.61 Structure and binding interactions of marimastat.

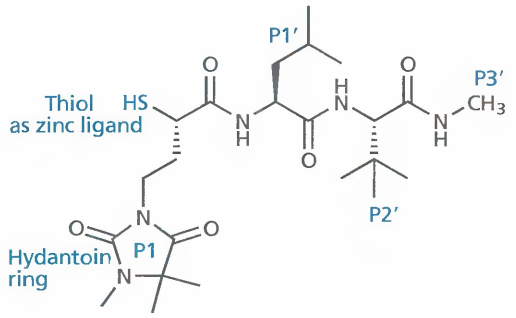


FIGURE 21.62 BMS 275291 (D 2163).

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. 21.62), which has a thiol group as the ligand for zinc. The hydantoin ring is thought to access the S1 subsite. The right-hand 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, due 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. 21.63). Examples include **CGS 27023A** and **prinomastat** which are both in clinical trials. These structures have decreased peptide character, but still suffer from a lack of selectivity. 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.

21.7.2 Cyclooxygenase 2 inhibitors

Cyclooxygenase (COX; see section 7.6) is an enzyme involved in prostaglandin synthesis and is over-expressed in several human cancers. It has two isozymes: COX-1, which is present in normal cells and plays an important role in many processes, and COX-2 which is not present in normal tissues, but is induced by mitogenic and inflammatory stimuli resulting in enhanced prostaglandin synthesis in cancerous or inflamed tissue respectively. COX-2 is thought to be important to various important mechanisms of carcinogenesis including angiogenesis, apoptosis, tumour invasiveness and multidrug resistance via efflux pumps.

Therefore, it has been proposed that selective inhibitors of COX-2 could help prevent further growth of tumours and/or augment the effect of currently used anticancer drugs. It is possible to design COX-2 selective inhibitors, that distinguish between an isoleucine group in the binding site of COX-1 and a valine group at the equivalent position in COX-2. A clinical trial involving the COX-2 inhibitor **celecoxib** has shown that there may be potential in using COX-2 inhibitors for both the prevention and treatment of cancers, especially of the colon and prostate.

21.7.3 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

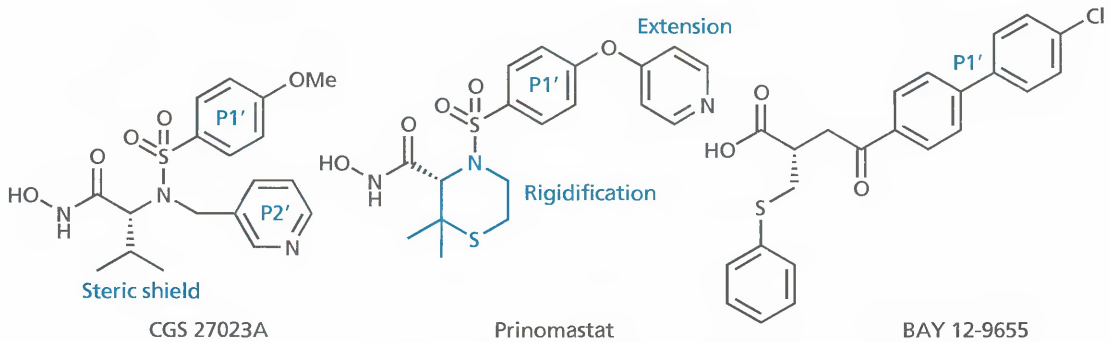


FIGURE 21.63 Second-generation MMIs.

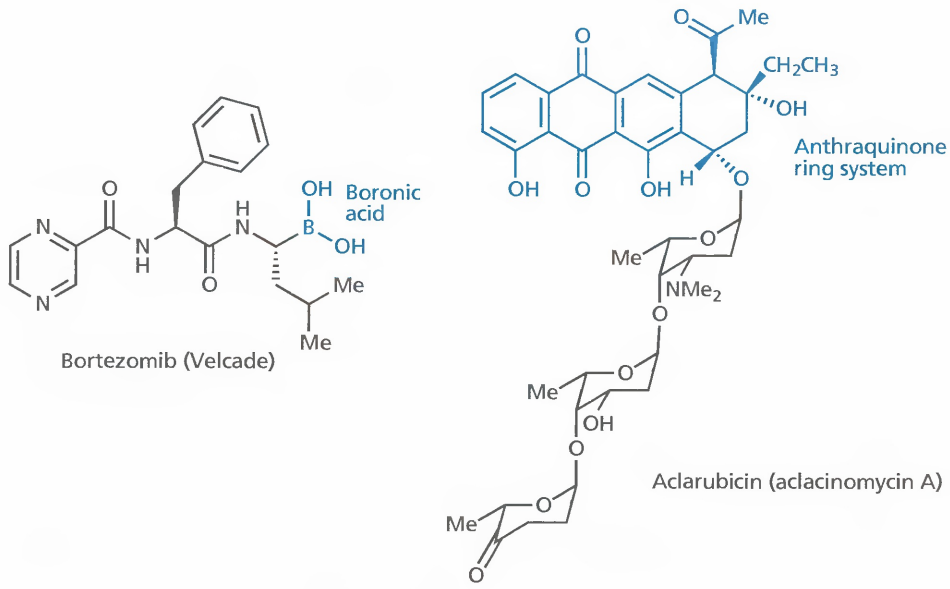


FIGURE 21.64 Proteasome inhibitors.

ones. Therefore, cells mark their defective proteins with a molecular label so that they can be recognized by the protein killing machine. This molecular label is a protein called **ubiquitin**.

Since 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. 21.64) 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 multi-drug resistance. **Aclarubicin** (**aclacinomycin A**) (Fig. 21.64) 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.

21.7.4 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 eight 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 neutralises 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.

Inhibitors for these enzymes are currently being studied, and several inhibitors of histone deacetylase are in clinical trials (Fig. 21.65). **Depsipeptide** is a natural product derived from a bacterial strain. It is reduced inside cells to an active compound which promotes apoptosis and inhibits cell proliferation and angiogenesis. Synthetic agents such as **MS-275** are also being investigated. Since the enzyme contains a zinc cofactor, many of these agents contain a hydroxamate group to act as a ligand; for example **vorinostat**, which was approved in 2006 by the US FDA for the treatment of cutaneous T-cell lymphoma.

21.7.5 Other enzyme targets

There are many other enzymes which are being studied as potential targets for anticancer agents. For example,

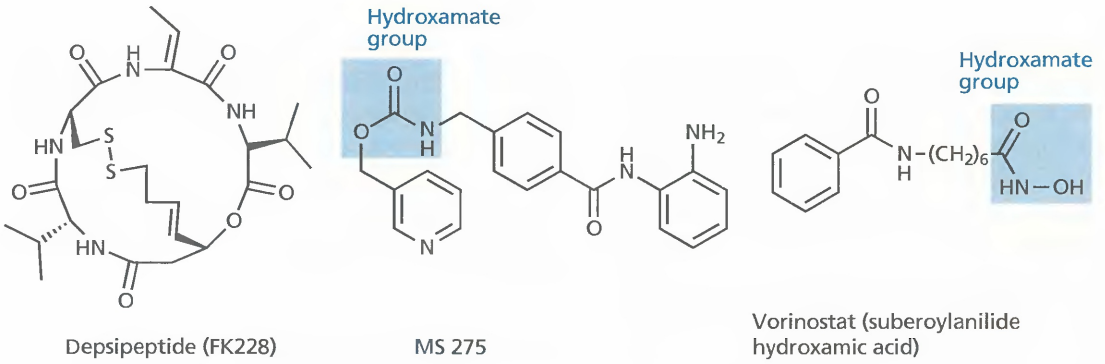


FIGURE 21.65 Histone deacetylase inhibitors.

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. 21.66) 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.

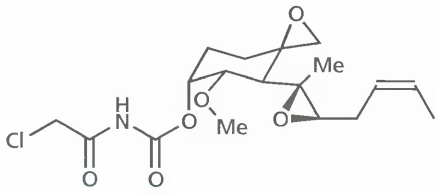


FIGURE 21.66 TNP 470.

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.

21.8.1 Synthetic agents

Thalidomide (Fig. 21.67) 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'. Since 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 had been 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

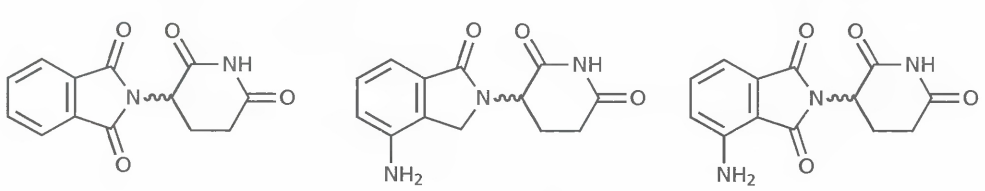


FIGURE 21.67 Thalidomide and thalidomide analogues.

thalidomide since 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. Since thalidomide can suppress or boost the immune system depending on individual circumstances, it is known as an **immunomodulator.** Thalidomide also appears to directly arrest the growth of cells and promote apoptosis.

Analogues of thalidomide have been synthesized with the aim of removing its teratogenic properties. **Lenalidomide (Revlimid)** and **actimid** (Fig. 21.67) are two such examples that are showing great promise and are in clinical trials. 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 currently 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.

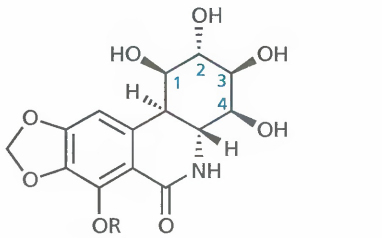
Arsenic trioxide is an orphan drug used for a variety of leukaemias. It is thought to promote cell suicide by targeting the cell's mitochondria.

21.8.2 Natural products

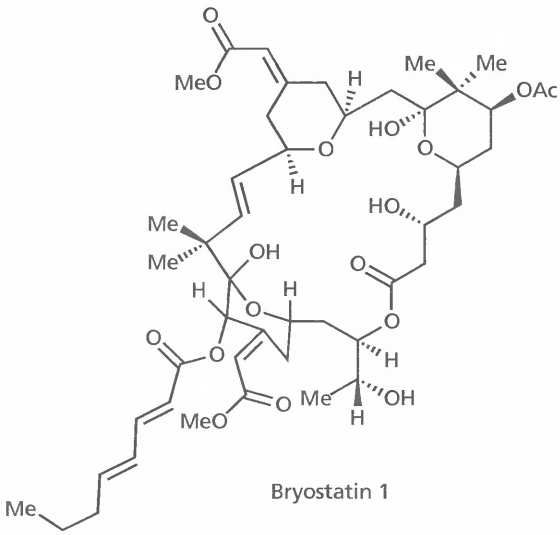
Pancratistatin (Fig. 21.68) is a natural product isolated from a plant called *Pancratium littoralis* which belongs to the genus *Narcissus*. Records show that extracts from plants in this genus were used by Hippocrates in 200 BC to treat breast cancer. The drug also inhibits angiogenesis, and its phosphate 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. 21.68) 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.

Dolostatins 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 **dolostatin 10**, **auristatin PE** and **dolostatin 15** (Fig. 21.69), which are all undergoing clinical trials.



Pancratistatin; R=H
Pancratistatin prodrug; R=phosphate



Bryostatin 1

FIGURE 21.68 Miscellaneous natural products having anticancer activity.

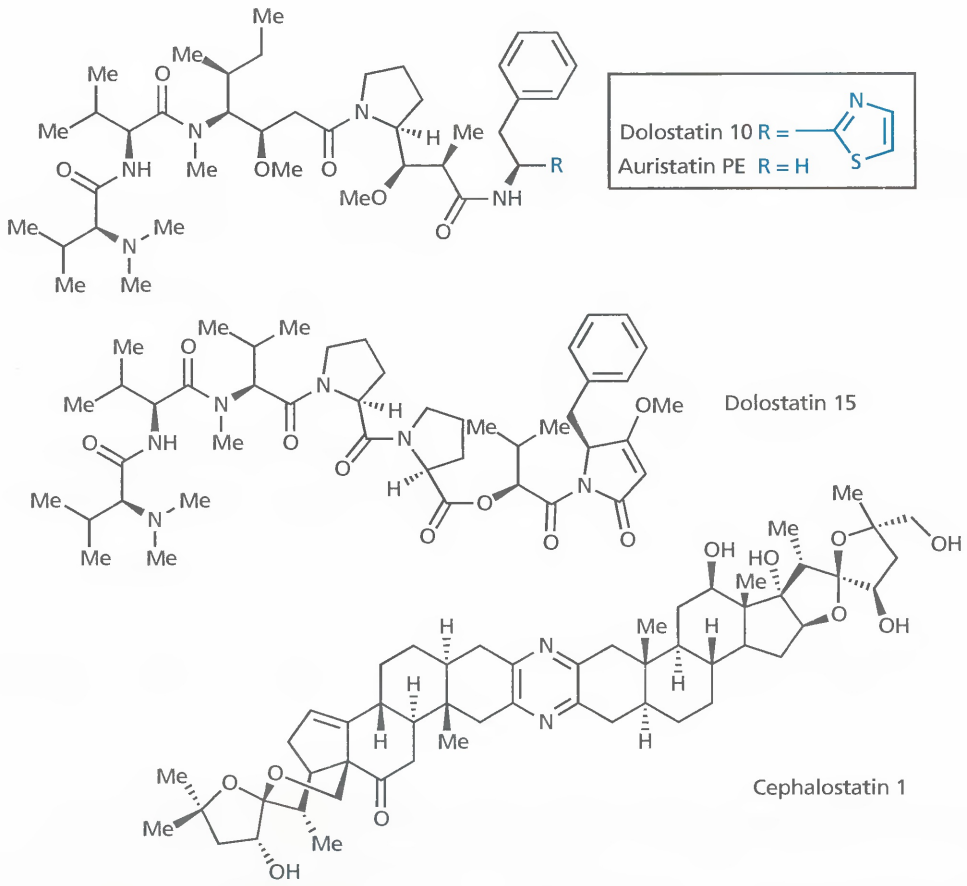


FIGURE 21.69 Natural products with anticancer activity.

Cephalostatin 1 (Fig. 21.69) 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**, **aldesleukin** (a preparation of interleukin-2) and **tretinoin**.

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–coactivator 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 coactivator protein, and thus prevent the formation of the complex required to signal the start of transcription (Box 10.2).

KEY POINTS

- Matrix metalloproteinases are zinc-dependent enzymes that 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.
- Inhibitors of COX-2 are being studied as potential anticancer agents.
- 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

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 treatment of metastatic breast cancer in combination with paclitaxel. HER-2 is a member of the EGF-R family of tyrosine kinase receptors, which is over-expressed 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, and is used alongside paclitaxel as the standard first line treatment for HER2-positive metastatic breast cancer. Unfortunately, the drug cannot cross the blood brain barrier and is ineffective against any tumour cells that have metastasised to the brain. Drug resistance and cardiac toxicity are further problems that 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 since there are reported fatalities relating to the release of cytokines.

Cetuximab is a chimeric monoclonal antibody that targets the extracellular domain of the EGF-receptor and blocks epidermal growth factor 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. It also used in the first-line treatment of metastatic breast cancer alongside paclitaxel.

Antibody-drug conjugates

Gemtuzumab (Box 21.13) has been approved for the treatment of acute myeloid leukaemia (AML) in patents older than 60 years.

Ibritumomab was the first approved drug involving radioimmunotherapy 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.

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. These serve to activate the body's immune response to direct killer cells against the tumour. Alternatively, if the antigen is an over-expressed receptor, the antibody may bind to it, such that the chemical messenger can no longer bind. In this case, the antibody acts as a receptor antagonist.

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 the tumour was limited due 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. Since 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 that is over expressed 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.

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, since 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 disulfide links at the hinge region of the antibody (Fig. 21.70) to produce eight thiol groups, and to attach drugs to these by alkylation or via a disulfide 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. 21.71). 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 linkers have been tried such as acid-labile linkers, peptidase-labile linkers and disulfide linkers. The disulfide linker can be cleaved by disulfide exchange with an intracellular thiol such as glutathione which has a higher concentration within cells than in plasma.

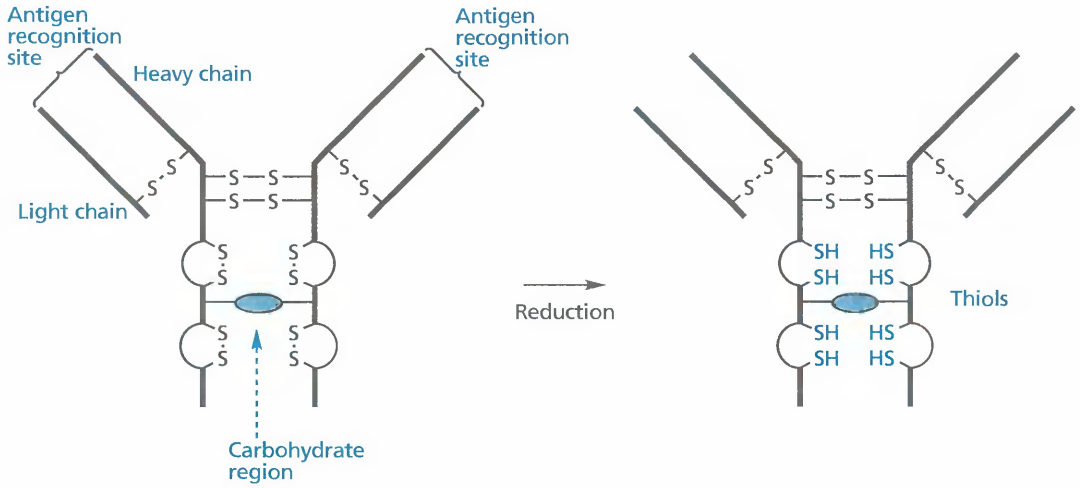


FIGURE 21.70 Reduction of disulfide links.

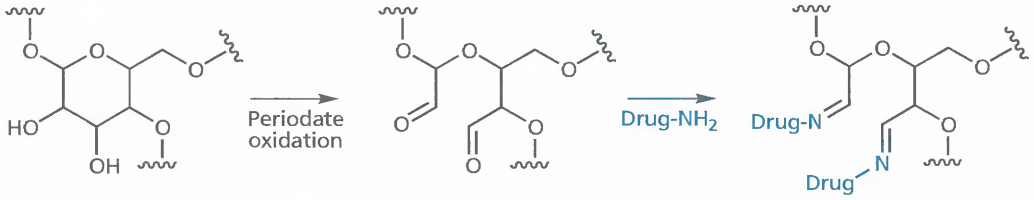


FIGURE 21.71 Linking drugs to carbohydrates.

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, 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 90Y and tositumomab carries 131I.

21.9.3 Antibody-directed enzyme prodrug therapy (ADEPT)

Antibody-directed enzyme prodrug therapy (ADEPT) 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. 21.72). 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 mammalian enzymes during its circulation round the body. It is also possible to use foreign enzymes even if a counterpart exists 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 selectively react with the foreign enzyme rather than the mammalian version. Examples of enzymes in this category include β-glucuronidase and nitroreductase.

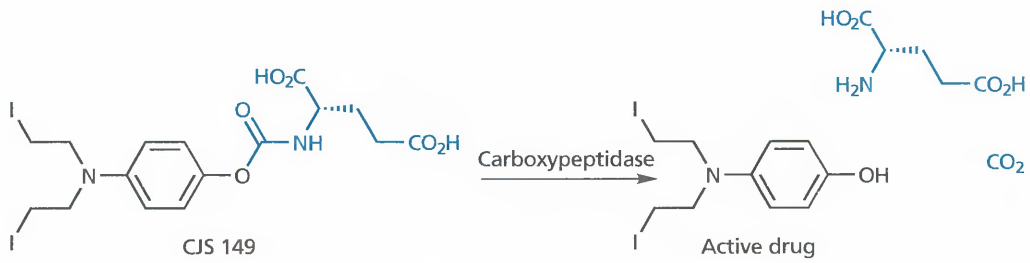


FIGURE 21.72 Activation of a prodrug by carboxypeptidase.

Box 21.13 Gemtuzumab: an antibody–drug conjugate

A humanized antibody has been linked to the highly potent anticancer drug calicheamicin. The trisulfide group normally present in calicheamicin was first modified to a disulfide 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 and has been approved for the treatment of acute myeloid leukaemia (AML) in patients older than 60 years.

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 disulfide group occurs later in the cell nucleus to produce the active thiol (Fig. 2).

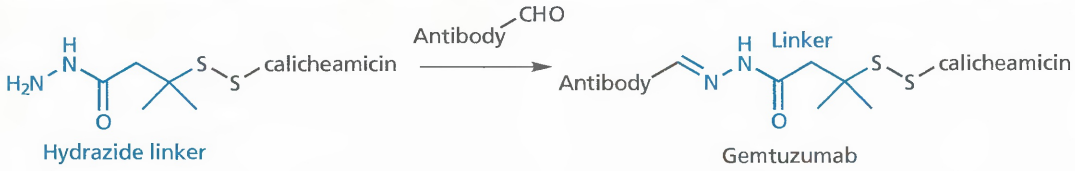


FIGURE 1 Linking calicheamicin to an antibody.

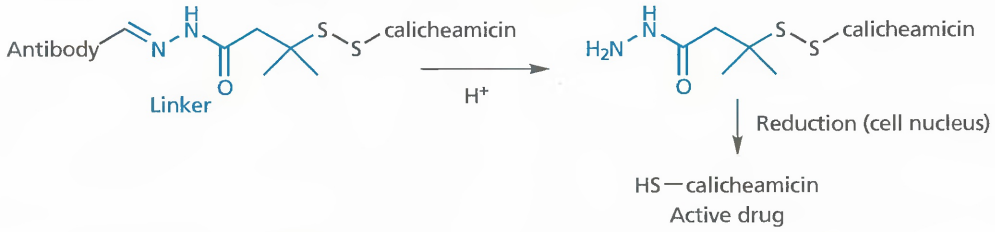


FIGURE 2 Release and activation of calicheamicin.

Many studies have been carried out on ADEPT. One example is an antibody—β-lactamase complex capable of reacting with the cephalosporin prodrug of an alkylating agent (Fig. 21.73) 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

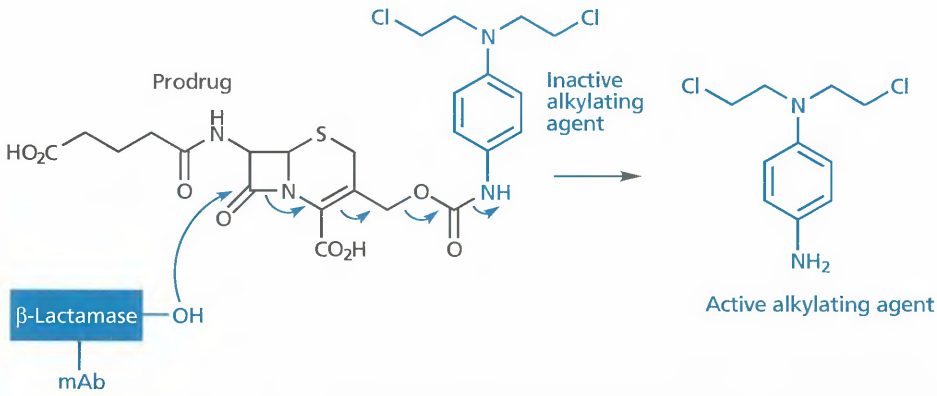


FIGURE 21.73 ADEPT strategy to release an alkylating agent.

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 catalytic properties of the antibody. 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. Since 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 (GDEPT) involves the delivery of a gene to the cancer cell. Once delivered, the gene codes for an enzyme capable of transforming 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 genetically modified 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). Since 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 that 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), and in this form they are non-toxic. 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. 21.74) 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.

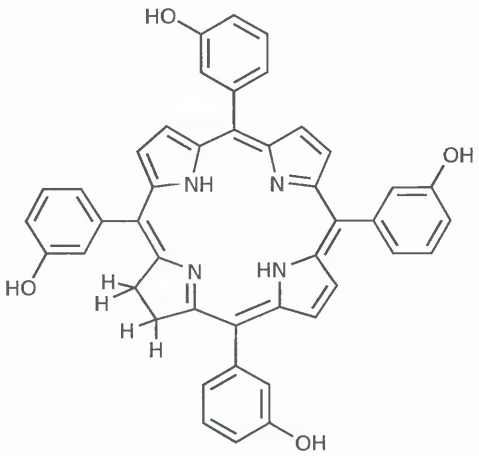


FIGURE 21.74 Temoporfin.

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 (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 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 revealing red, fanglike teeth. It is likely that sufferers from 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.

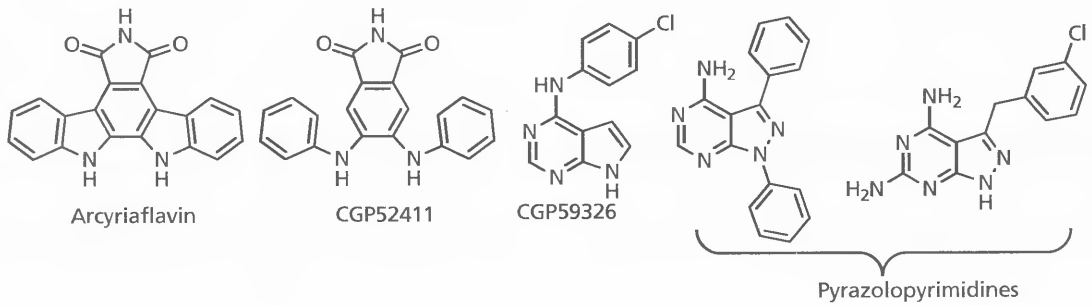
Problems such as photosensitivity have limited the application of PDT, but research is underway to find improved methods of delivering the agent.

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. 21.45) 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 sulfonamides would be suitable antibacterial agents to treat opportunistic infections in cancer patients?
3. Since 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 21.56) is a kinase inhibitor that shows no selectivity, but is a useful lead compound for potential antitumour agents. A simplification strategy resulted in arcyriaflavin (shown below), 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 (shown below) is a further simplification of arcyriaflavin A. Remarkably, this compound is inactive against PKC and is selective for the kinase active site of the epidermal growth factor receptor. 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. 21.46). Suggest how this structure might bind, showing the binding interactions that are possible. 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 epidermal growth factor receptor. Suggest how they might be bound to the active site.

8. In the development of imatinib, a conformational blocker was introduced (Fig. 21.51). Suggest a conformation that 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.

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Titles for general further reading are listed on p. 725.