9 Nucleic acids as drug targets

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

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

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

9.1 Intercalating drugs acting on DNA

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

Proflavine (Fig. 9.1) is an example of a group of antibacterial compounds called the **aminoacridines,** which were used during the First and Second World Wars to treat deep surface wounds. They proved highly effective in preventing infection and reduced the number of fatalities resulting from wound infections. Proflavine is completely ionized at pH7 and interacts directly with bacterial DNA. The flat tricyclic ring intercalates between the DNA base pairs, and interacts with them by van der Waals forces, while the ammonium cations form ionic bonds with the negatively charged phosphate groups on the sugar phosphate backbone. Once inserted, it deforms the DNA double helix and prevents the normal functions of replication and transcription.

Chloroquine and **quinine** are antimalarial agents that can attack the malarial parasite by blocking DNA transcription as part of its action. A flat heteroaromatic structure is present which can intercalate DNA (Fig. 9.2).

Dactinomycin (Fig. 9.3) (previously called actinomycin D) is a naturally occurring antibiotic that was first isolated from *Streptomyces parvullis* in 1953, and was shown to be an effective anticancer agent in children. It contains two cyclic pentapeptides, but the important feature is a flat, tricyclic, heteroaromatic structure which slides into the double helix via the minor groove. It appears to favour interactions with guanine–cytosine base pairs and, in particular, between two adjacent guanine bases on alternate strands of the helix. The molecule is further held in position by hydrogen bond interactions between the nucleic acid bases of DNA and the cyclic pentapeptides positioned on the outside of the helix. The 2-amino group of guanine plays a particularly important role in this interaction. The resulting bound complex is very stable and prevents the unwinding of the double helix. This in turn prevents DNA-dependent RNA polymerase from catalysing the

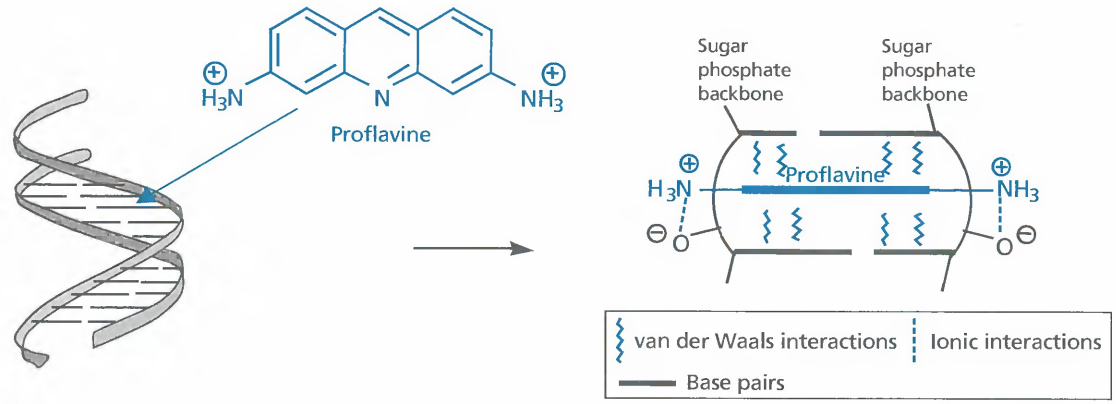


FIGURE 9.1 The intercalation of proflavine with DNA.

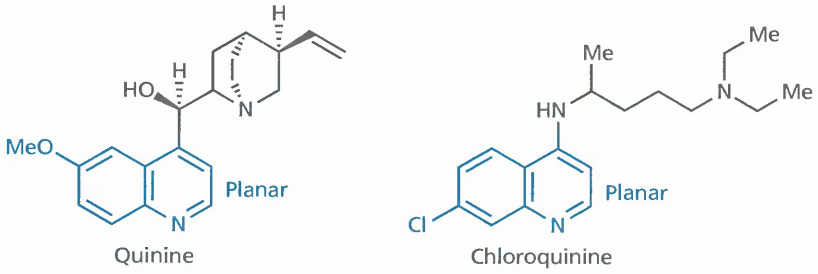


FIGURE 9.2 Intercalating antimalarial drugs.

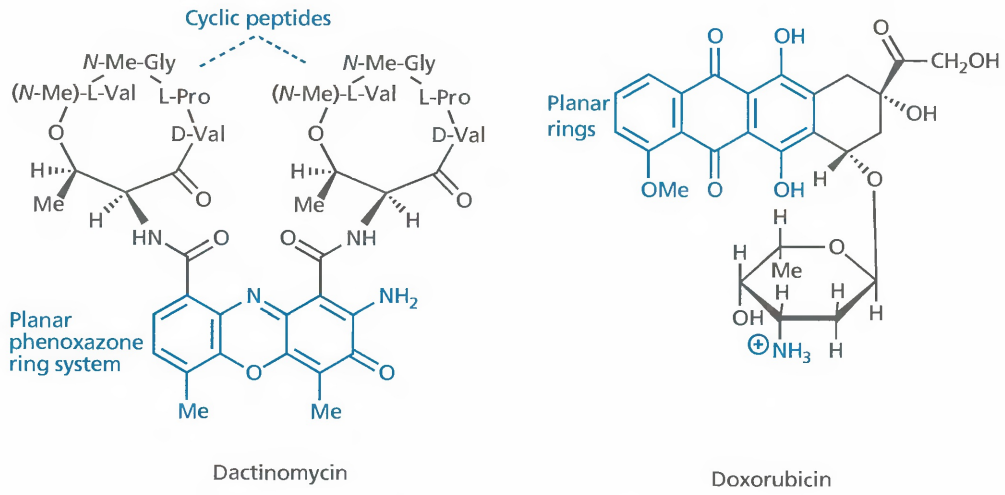


FIGURE 9.3 Dactinomycin and doxorubicin.

synthesis of messenger RNA (mRNA) and thus prevents transcription.

Doxorubicin (Fig. 9.3) is one of the most effective anticancer drugs ever discovered, and belongs to a group of naturally occurring antibiotics called the **anthracyclines**. It was first isolated from *Streptomyces peucetius* in 1967, and contains a tetracyclic system where three of the rings are planar. The drug approaches DNA via the major groove of the double helix and intercalates using the planar tricyclic system. The charged amino group attached to the sugar is also important, as it forms an ionic bond with the negatively charged phosphate groups of the DNA backbone. This is supported by the fact that structures lacking the

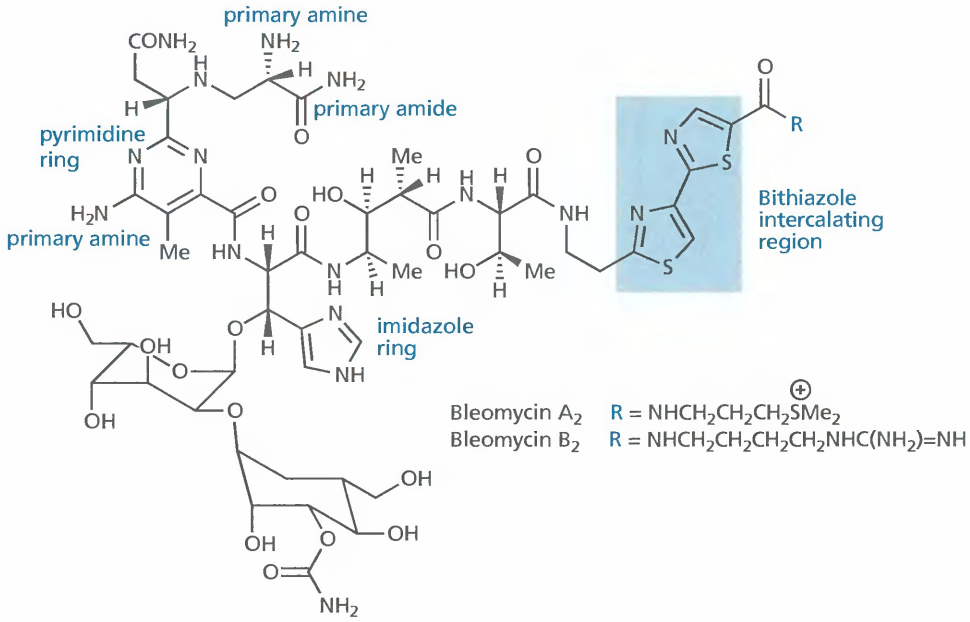


FIGURE 9.4 Bleomycins.

aminosugar have poor activity. Intercalation prevents the normal action of an enzyme called **topoisomerase II**—an enzyme that is crucial to replication and mitosis. The mechanism by which this enzyme works is described in section 6.1.3 and includes the formation of a DNA–enzyme complex, where the enzyme is covalently linked to the DNA. When doxorubicin is intercalated into DNA it stabilizes this DNA–enzyme complex and stalls the process. Agents such as doxorubicin are referred to as topoisomerase II poisons rather than inhibitors since they do not prevent the enzyme functioning directly. Other mechanisms of action for doxorubicin and its analogues have also been proposed—see section 21.2.1.

Bleomycins (Fig. 9.4) are complex natural products that were isolated from *Streptomyces verticillus* in 1962, and are some of the few anticancer drugs not to cause bone marrow depression. Their structure includes a bithiazole ring system as part of their structure. This is the feature that intercalates with DNA. Once the structure has become intercalated, the nitrogen atoms of the primary amines, pyrimidine ring, and imidazole ring chelate a ferrous ion which then interacts with oxygen and is oxidized to a ferric ion, leading to the generation of superoxide or hydroxyl radicals. These highly reactive species abstract hydrogen atoms from DNA, which results in the DNA strands being cut, particularly between purine and pyrimidine nucleotides. Bleomycin also appears to prevent the enzyme **DNA ligase** from repairing the damage caused.

9.2 Topoisomerase poisons: non-intercalating

The following structures are classed as poisons rather than inhibitors, because they stabilize the normally transient cleavable complex that is formed between DNA and topoisomerase enzymes, thus inhibiting the rejoining of the DNA strand or strands (section 6.1.3). We have already mentioned topoisomerase poisons in section 9.1 where we discussed the anthracyclines. In this section, we look at topoisomerase poisons which do not intercalate into the DNA structure. However, since DNA is part of the target complex, we can view these poisons as targeting DNA as well as the topoisomerase enzyme.

The anticancer agents **etoposide** and **teniposide** (Fig. 9.5) belong to a group of compounds called the **podophyllotoxins**, and are semi-synthetic derivatives of **epipodophyllotoxin**—an isomer of a naturally occurring agent called **podophyllotoxin**. Both agents act as topoisomerase poisons. DNA strand breakage is also thought to occur by a free radical process involving oxidation of the 4′-phenolic group, and the production of a semiquinone free radical. Evidence supporting this comes from the fact that the 4′-methoxy structures are inactive. The presence of the glucoside sugar moiety also increases the ability to induce breaks.

Camptothecin (Fig. 9.6) is a natural product which was extracted from a Chinese bush (*Camptotheca acuminata*) in 1966. It stabilizes the cleavable complex formed

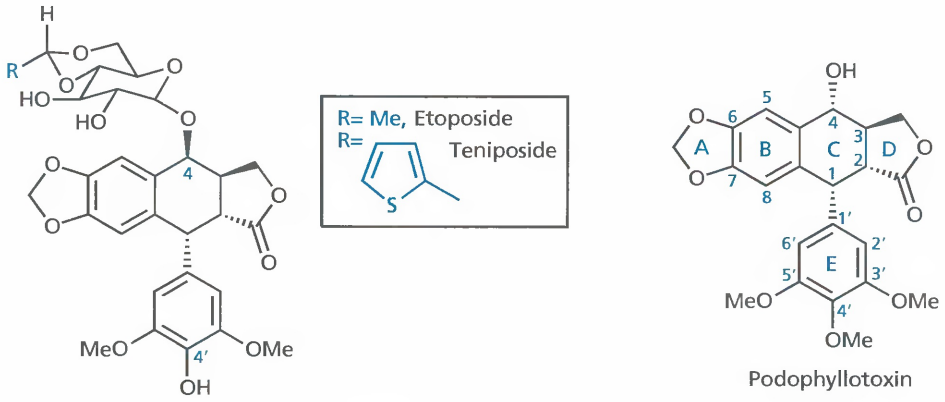


FIGURE 9.5 Podophyllotoxins.

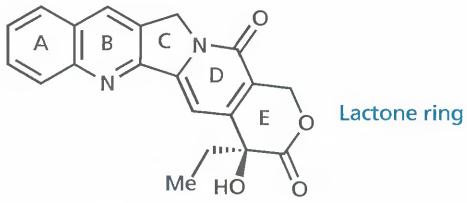


FIGURE 9.6 Camptothecin.

between DNA and the enzyme **topoisomerase I** (section 6.1.3). As a result, single-strand breaks accumulate in the DNA. These can be repaired if the drug departs, but if replication is taking place when the drug–enzyme–DNA complex is present, an irreversible double-strand break takes place which leads to cell death. Semi-synthetic analogues of camptothecin have been developed as clinically useful anticancer agents (section 21.2.2.2).

The antibacterial **quinolones** and **fluoroquinolones** (section 19.8.1) are synthetic agents that inhibit the replication and transcription of bacterial DNA by stabilizing the complex formed between DNA and bacterial topoisomerases. Inhibition arises by the formation of a ternary complex involving the drug, the enzyme, and bound DNA (Fig. 9.7). The binding site for the fluoroquinolones only appears once the enzyme has 'nicked' the DNA strands, and the strands are ready to be crossed over. At that point, four fluoroquinolone molecules are bound in a stacking arrangement such that their aromatic rings are coplanar. The carbonyl and carboxylate groups of the fluoroquinolones interact with DNA by hydrogen bonding, while the fluoro-substituent at position 6, the substituent at C-7, and the carboxylate ion are involved in binding interactions with the enzyme.

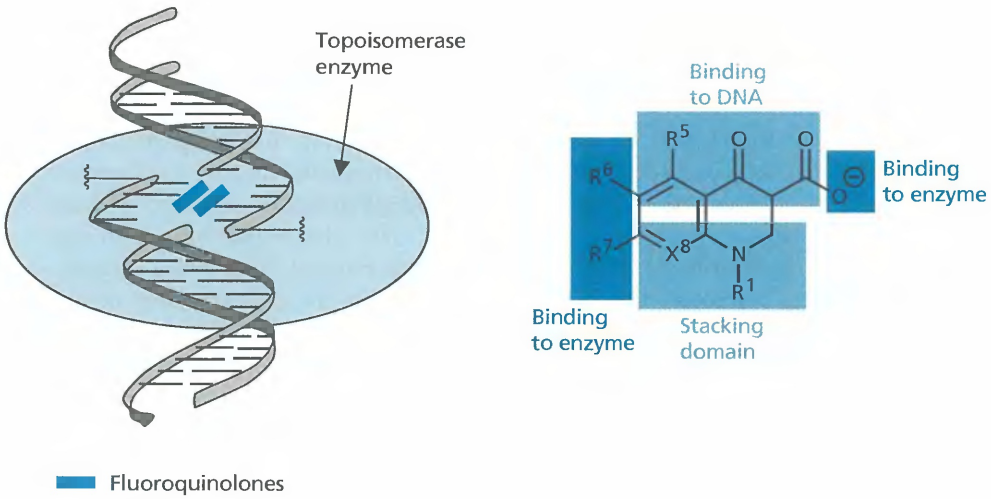


FIGURE 9.7 Complex formed between DNA, the topoisomerase enzyme and fluoroquinolones; R^6 = F for fluoroquinolones.

KEY POINTS

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

9.3 Alkylating and metallating agents

Alkylating agents are highly electrophilic compounds that react with nucleophiles to form strong covalent bonds. There are several nucleophilic groups present on the nucleic acid bases of DNA which can react with electrophiles—in particular the *N*-7 of guanine (Fig. 9.8).

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

Miscoding due to alkylated guanine units is also possible. The guanine base usually exists as the keto tautomer, allowing it to base pair with cytosine. Once alkylated, however, guanine prefers the enol tautomer and is more likely to base pair with thymine (Fig. 9.9). Such miscoding ultimately leads to an alteration in the amino acid sequence of proteins, which in turn can lead to disruption of protein structure and function.

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

9.3.1 Nitrogen mustards

The nitrogen mustards get their name because they are related to the sulfur containing mustard gases used during the First World War. The nitrogen mustard compound **chlormethine** (Fig 9.10) was the first alkylating agent to be used medicinally, in 1942, although full details were

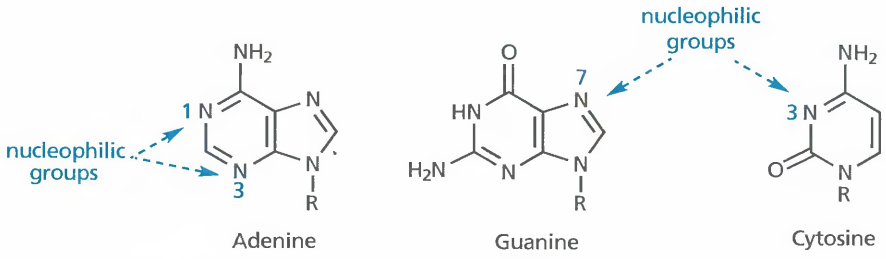


FIGURE 9.8 Nucleophilic groups on adenine, guanine and cytosine.

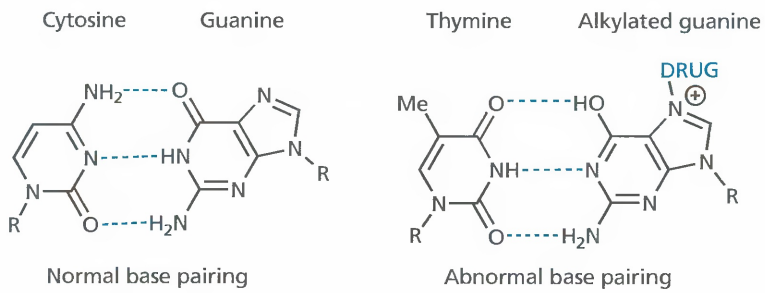


FIGURE 9.9 Normal and abnormal base pairing of guanine.

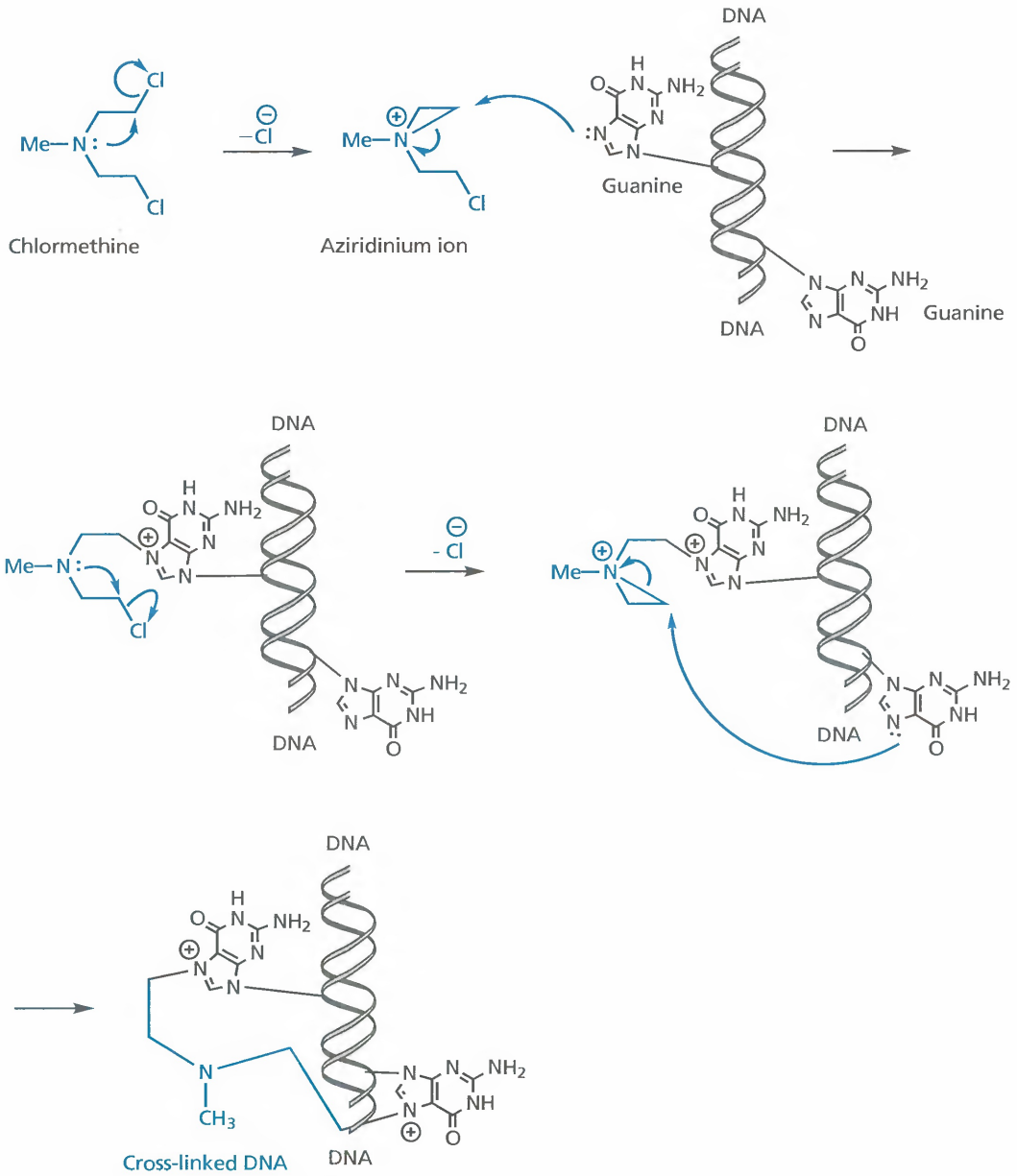


FIGURE 9.10 Alkylation of DNA by chlormethine.

not revealed until after the war due to secrecy surrounding all nitrogen mustards. The nitrogen atom is able to displace a chloride ion intramolecularly to form the highly electrophilic aziridinium ion. This is an example of a **neighbouring group effect**, also called **anchimeric assistance**. Alkylation of DNA can then take place. Since the process can be repeated, cross-linking between chains or within the one chain will occur. Monoalkylation of DNA guanine units is also possible if the second alkyl halide reacts with water, but cross-linking is the major way in which these drugs inhibit replication and act as anticancer agents.

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

9.3.2 Nitrosoureas

The anticancer agents **lomustine** and **carmustine** (Fig. 9.11) were discovered in the 1960s, and are chloroethylnitrosoureas which decompose spontaneously in

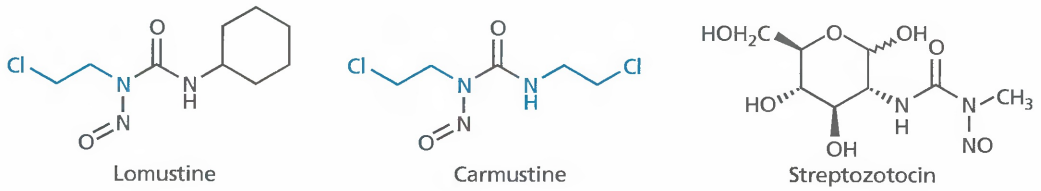


FIGURE 9.11 Nitrosourea alkylating agents.

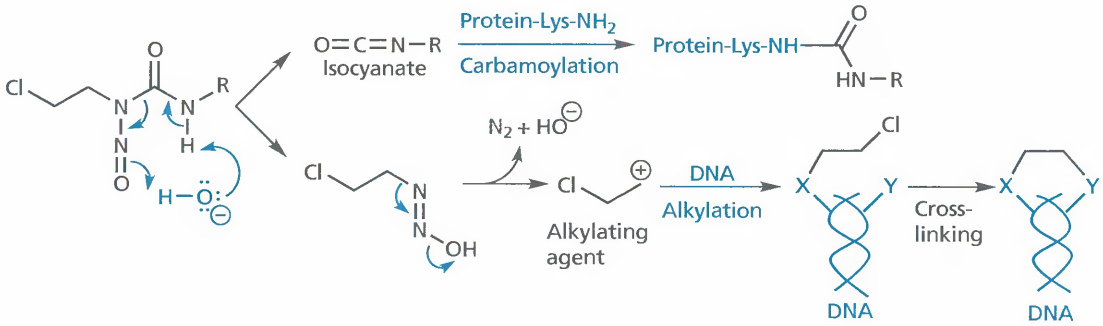


FIGURE 9.12 Mechanisms of action for nitrosoureas.

the body to form two active compounds—an alkylating agent and a carbamoylating agent (Fig. 9.12). The organic isocyanate which is formed carbamoylates lysine residues in proteins and may inactivate DNA repair enzymes. The alkylating agent reacts initially with a guanine moiety on one strand of DNA, then with a guanine or cytosine unit on the other strand to produce interstrand cross-linking (Figs. 9.12 and 9.13). **Streptozotocin** (Fig. 9.11) is a naturally occurring nitrosourea isolated from *Streptomyces achromogenes*.

9.3.3 Busulfan

Busulfan (Fig. 9.14) was synthesized in 1950 as part of a systematic search for novel alkylating agents. It is an anticancer agent which causes interstrand cross-linking between guanine units. The sulfonate groups are good leaving groups and play a similar role to the chlorines in the nitrogen mustards. However, the mechanism involves a direct S_N2 nucleophilic substitution of the sulfonate groups, and does not involve any intermediates similar to the aziridinium ion.

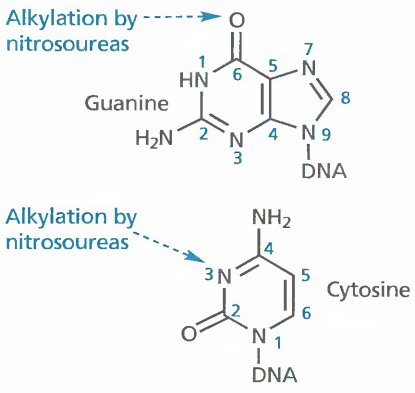


FIGURE 9.13 Alkylation sites on guanine and cytosine for nitrosoureas.

9.3.4 Cisplatin

Cisplatin (Fig. 9.15) is one of the most frequently used anticancer drugs in medicine. Its discovery was fortuitous in the extreme, arising from research carried out in the 1960s to investigate the effects of an electric current on bacterial growth. During these experiments, it was discovered that bacterial cell division was inhibited. Further research led to the discovery that an electrolysis product from the platinum electrodes was responsible for the inhibition and the agent was eventually identified as cis-diammonia dichloroplatinum (II), now known as cisplatin.

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

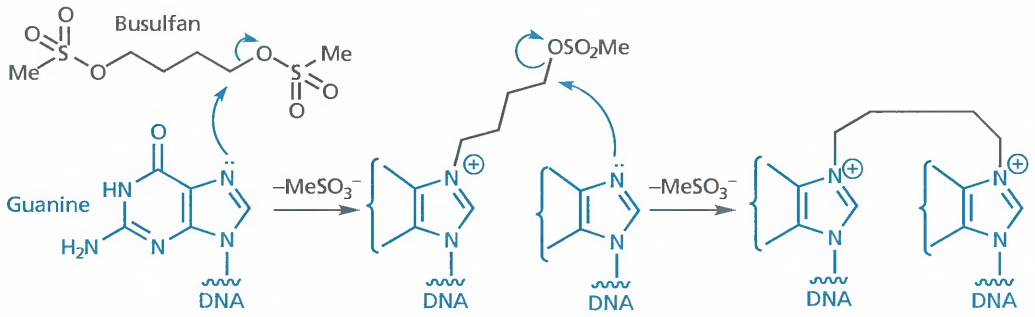


FIGURE 9.14 Cross-linking mechanism involving busulfan.

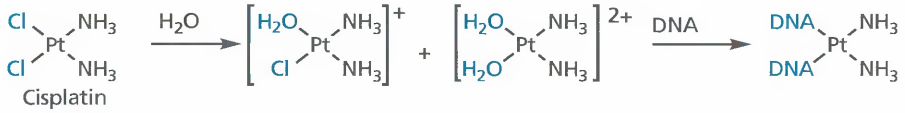


FIGURE 9.15 Activation of cisplatin and intrastrand cross-linking of DNA.

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

9.3.5 Dacarbazine and procarbazine

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

Dacarbazine (Fig. 9.17) is activated by *N*-demethylation in the liver—a reaction catalysed by cytochrome P450

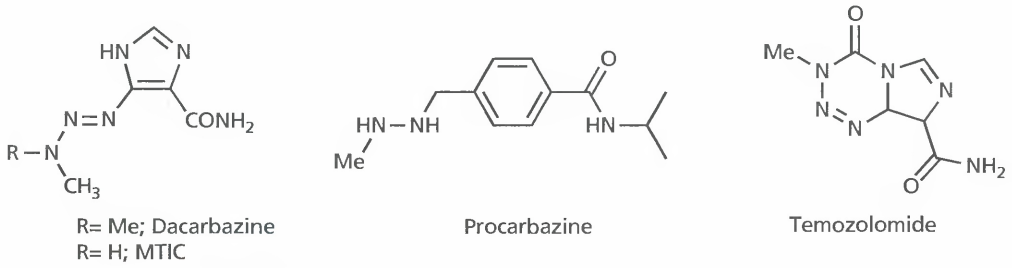


FIGURE 9.16 Dacarbazine, procarbazine and temozolomide.

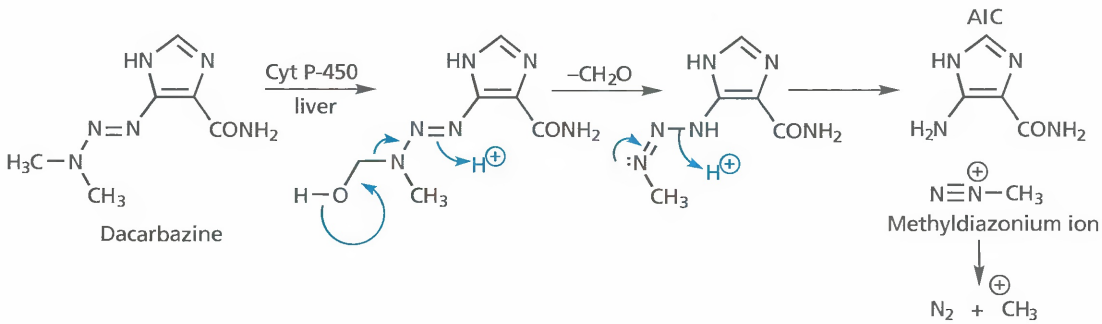


FIGURE 9.17 Mechanism of action of dacarbazine.

enzymes (section 11.4.2). Formaldehyde is then lost to form a product which spontaneously degrades to form 5-aminoimidazole-4-carboxamide (AIC) and the methyldiazonium ion. Reaction of this ion with RNA or DNA results in methylation, mainly at the 7-position of guanine. DNA fragmentation can also occur. AIC has no cytotoxic effect and is present naturally as an intermediate in purine synthesis. **Temozolomide** (Fig. 9.16) also acts as a prodrug and is hydrolysed in the body to form MTIC (Fig. 9.16), which decomposes in a similar fashion to form the methyldiazonium ion.

9.3.6 Mitomycin C

Mitomycin C (Fig. 9.18) was discovered in the 1950s, and is a naturally occurring compound obtained from the microorganism *Streptomyces caespitosus*. It is one of the most toxic anticancer drugs in clinical use, and acts as a prodrug, being converted to an alkylating agent within the body. The process by which this takes place is initiated by an enzyme catalysed reduction of the quinone ring system to a hydroquinone. Loss of methanol, and opening of the three-membered aziridine ring then takes place to generate the alkylating agent. Guanine residues on different DNA strands are then alkylated, leading to interstrand cross-linking, and the inhibition of DNA replication and cell division. Since a reduction step is involved in the mechanism, it has been proposed that this drug should be more effective against tumours in an oxygen-starved (hypoxic) environment such as the centre of solid tumour masses.

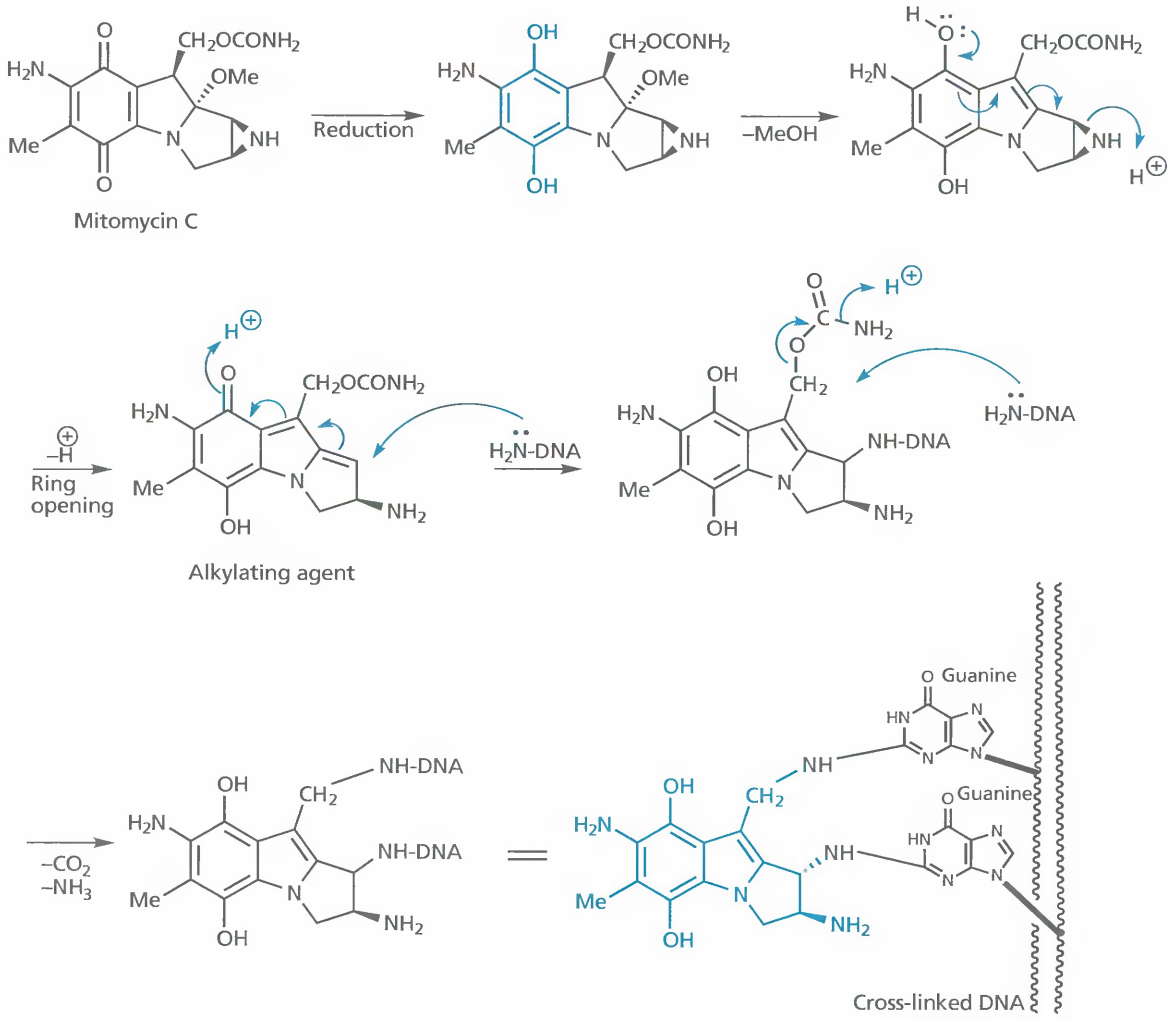


FIGURE 9.18 DNA cross-linking by mitomycin C.

KEY POINTS

- Alkylating agents contain electrophilic groups that react with nucleophilic centres on DNA. If two electrophilic groups are present, interstrand and/or intrastrand cross-linking of the DNA is possible.
- Nitrogen mustards react with guanine groups on DNA to produce cross-linking.
- Nitrosoureas have a dual mechanism of action whereby they alkylate DNA and carbamoylate proteins.
- Cisplatin is an alkylating agent that causes intrastrand cross-linking.
- Dacarbazine and procarbazine are prodrugs that are activated by enzymes to produce a methyldiazonium ion, which acts as an alkylating agent.
- Mitomycin C is a natural product that is converted to an alkylating agent by enzymatic reduction. Interstrand cross-linking takes place between guanine groups.

9.4 Chain cutters

'Chain cutters' cut the strands of DNA and prevent the enzyme **DNA ligase** from repairing the damage. They appear to act by creating radicals on the DNA structure. These radicals react with oxygen to form peroxy species, and the DNA chain fragments. The bleomycins (section 9.1) and the podophyllotoxins (section 9.2) are examples of drugs that can act in this way, as are the **nitroimidazoles** and **nitrofurantoin**, which target bacterial DNA and are used as antibacterial agents (section 19.8.4). Another example is the antitumour agent **calicheamicin** γ^1 (Fig. 9.19) which was isolated from a bacterium. This compound binds to the minor groove of DNA and cuts the DNA chain by the mechanism shown in Fig. 9.20. The driving force behind the reaction mechanism is the formation of an aromatic ring from the unusual enediyne system. The reaction starts with a nucleophile attacking the trisulfide

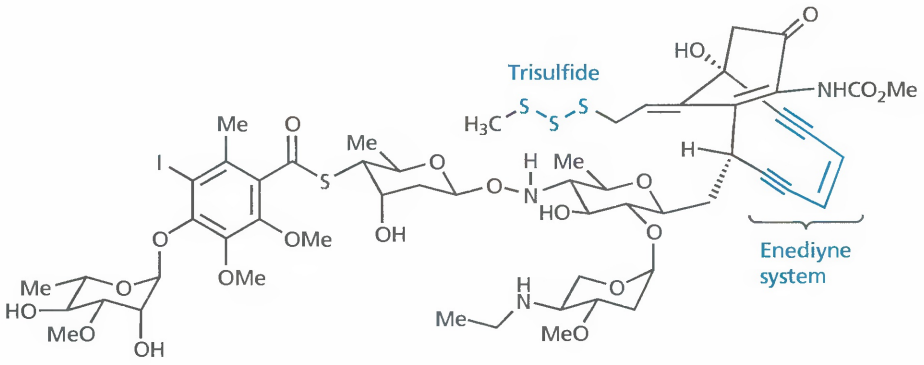


FIGURE 9.19 Calicheamicin γ^1.

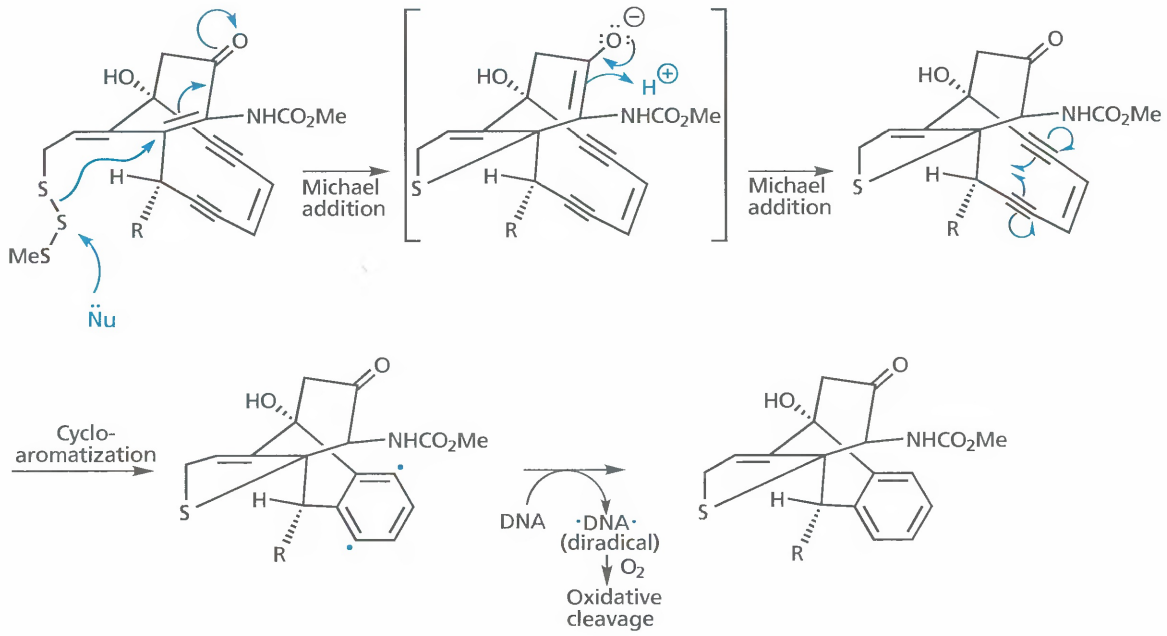


FIGURE 9.20 Mechanism of action of calicheamicin γ^1.

group. The thiol which is freed then undergoes an intramolecular Michael addition with a reactive α, β-unsaturated ketone. The resulting intermediate then cycloaromatizes (a reaction known as the Bergman cyclization) to produce an aromatic diradical species which snatches two hydrogens from DNA. As a result, the DNA becomes a diradical. Reaction with oxygen then leads to chain cutting.

9.5 Chain terminators

Chain terminators are drugs which act as 'false substrates' and are incorporated into the growing DNA chain during replication. Once they have been added, the chain can no longer be extended and chain growth is terminated. The drugs which act in this way are 'mistaken' for the nucleotide triphosphates that are the authentic building blocks for DNA synthesis. The mechanism by which these nucleotides are added to the end of the growing DNA chain is shown in Fig. 9.21 and involves the loss of a diphosphate group—a process catalysed by the enzyme **DNA polymerase**. Before each building block is linked to the chain, it has to be 'recognized' by the complementary nucleic acid base on the template chain. This involves base pairing between a nucleic acid base on the template and the nucleic acid base on the nucleotide.

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

Aciclovir (Fig. 9.22) is an important antiviral drug that was discovered in the 1970s, and acts as a chain terminator, satisfying all three requirements. It contains a guanine base, which means that it can base pair to cytosine moieties on the template chain. Second, although it does not contain a triphosphate group, this is added to the molecule in virally infected cells. Third, the sugar unit is incomplete and lacks the required OH group normally present at position 3′—compare the structure of deoxyguanosine in Fig 9.22. Therefore the nucleic acid chain

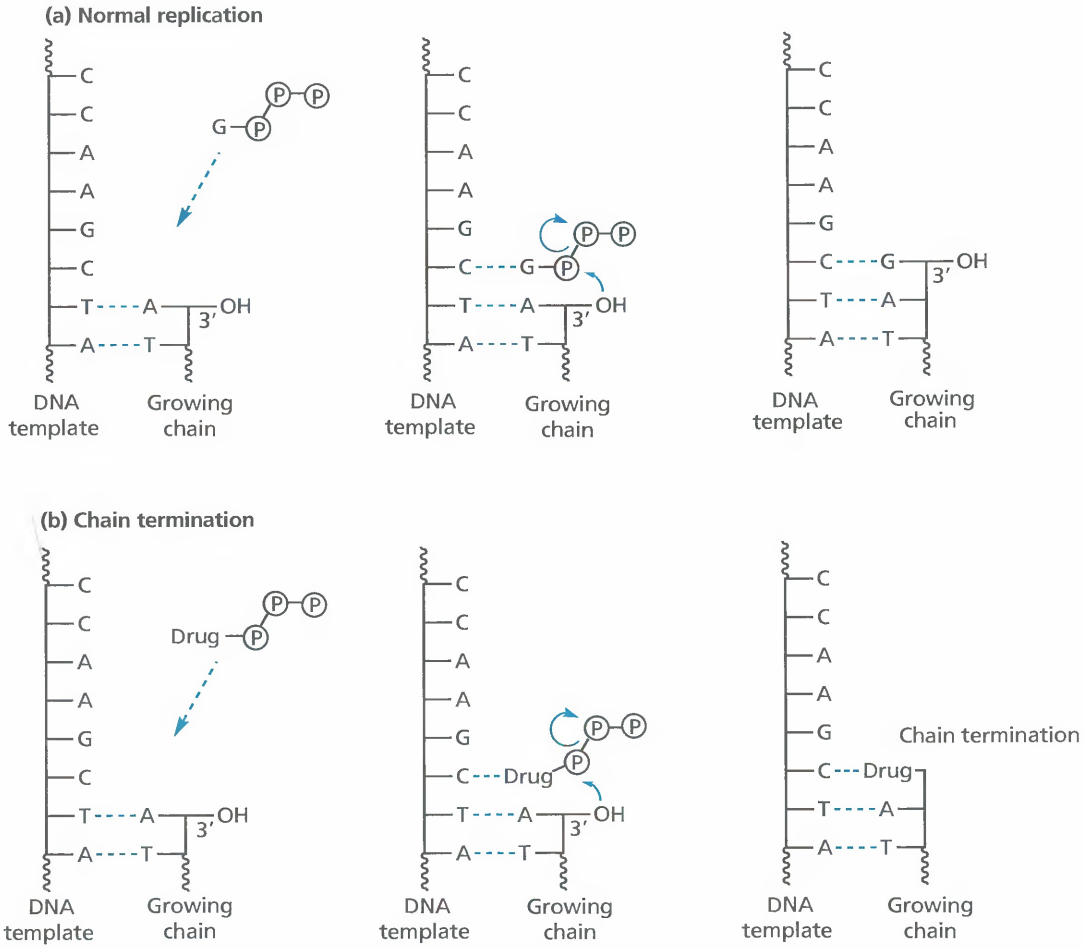


FIGURE 9.21 (a) The normal replication mechanism. (b) A drug acting as a chain terminator.

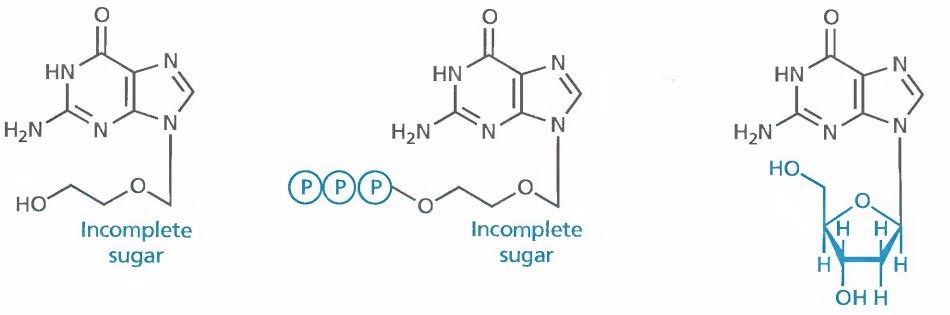


FIGURE 9.22 Structure of aciclovir, aciclovir triphosphate and deoxyguanosine.

cannot be extended any further. Several other structures acting in a similar fashion are used in antiviral therapies and are described in sections 20.6.1 and 20.7.3.1.

9.6 Control of gene transcription

Various research groups are looking into the design of synthetic molecules that can bind to DNA by recognizing nucleic acid base pairs, and by doing so control gene transcription. It has been found that 'hairpin' polyamide structures containing heterocyclic rings have this capacity and bind in the minor groove of DNA (Fig. 9.23). The molecule is made up of two arms connected by means of a linker unit. The molecule attaches itself to DNA like a clamp with each arm binding to one of the DNA strands. The binding interactions are through hydrogen bonding to the base pairs of DNA, and involve both the heterocyclic rings and the amide bonds. Polyamides containing eight heterocyclic rings bind with an affinity

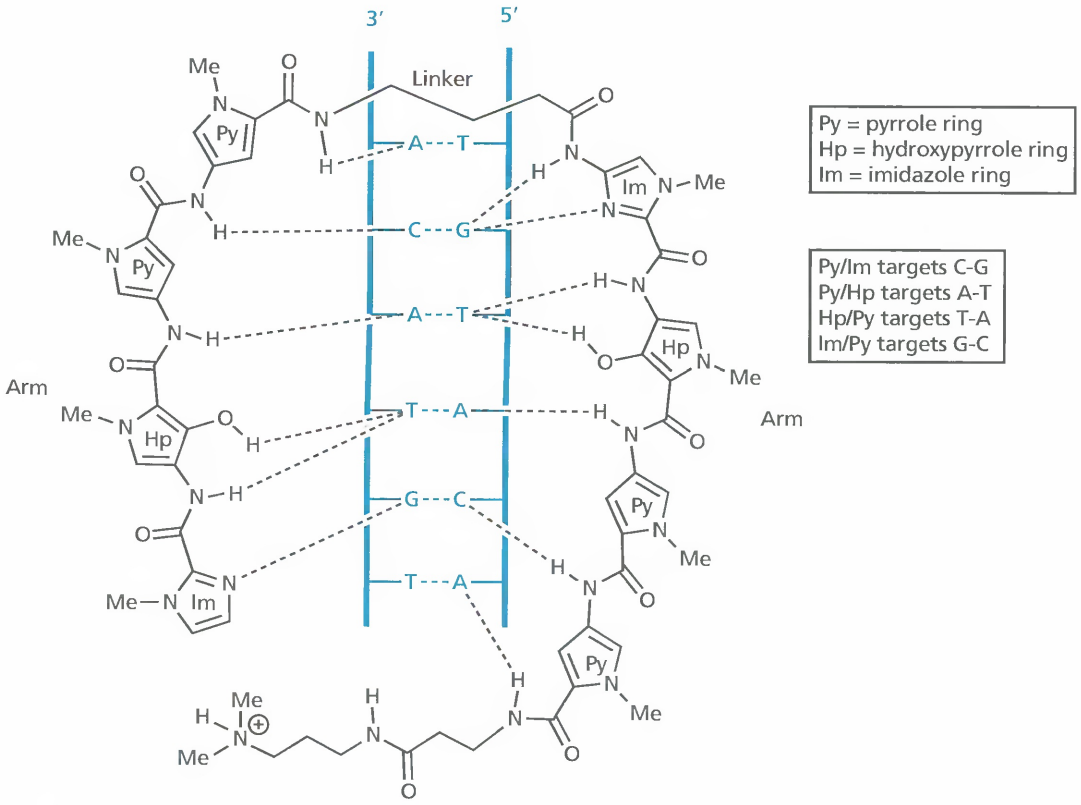


FIGURE 9.23 Synthetic polyamides capable of recognizing and binding to a particular sequence of nucleic acid base pairs.

and specificity that is comparable to naturally occurring DNA-binding proteins. Experiments have shown that it is feasible for these drugs to cross the cell membrane and to inhibit transcription by binding to the regulatory element of a gene—in other words where a transcription factor would normally bind. Binding at this specific region is achieved by designing the drug to recognize the base pair sequences in that region. This is possible by using particular patterns of pyrrole, hydroxypyrrole and imidazole rings on each arm of the molecule. Binding to the regulatory element of the gene is crucial, since polyamides that bind to the coding region of the gene do not appear to prevent transcription. Presumably, they are displaced during the transcription process. However, it may be possible to attach an alkylating agent to the molecule such that a covalent bond is formed and the gene is 'knocked out'.

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

9.7 Agents that act on RNA

9.7.1 Agents that bind to ribosomes

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

9.7.2 Antisense therapy

A great deal of research has been carried out into the possibility of using **oligonucleotides** to block the coded messages carried by mRNA. This is an approach known as **antisense therapy** and has great potential. The rationale is as follows (Fig. 9.24). Assuming that the primary sequence of a mRNA molecule is known, an oligonucleotide can be synthesised containing nucleic acid bases that are complementary to a specific stretch of the mRNA molecule. Since the oligonucleotide has a complementary base sequence, it is called an **antisense oligonucleotide**. When mixed with mRNA, the antisense oligonucleotide recognizes its complementary section in mRNA, interacts with it and forms a duplex structure such that the bases pair up by hydrogen bonding. This section now acts as a barrier to the translation process and blocks protein synthesis.

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

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

Nevertheless, antisense oligonucleotides are potential antiviral and anticancer agents, as they should be capable of preventing the biosynthesis of 'rogue' proteins, and have fewer side effects than currently used drugs. Design strategies aimed at solving many of the pharmacokinetic problems of oligonucleotides are described in section 14.10, and the first antisense oligonucleotide to

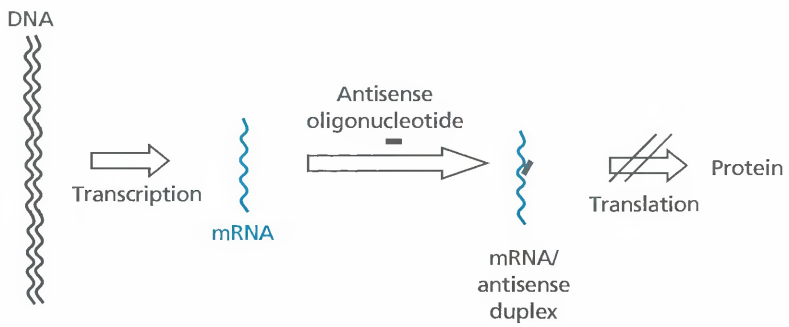


FIG. 9.24 The principles of antisense therapy.

be approved for the market was the antiviral agent **fomivirsen (Vitravene)** in 1998 (section 20.6.3).

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

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

In the nucleus, an endonuclease enzyme excises segments of base paired RNA from normal RNA. These segments exit the nucleus into the cytoplasm and are further cleaved by an endonuclease enzyme called **Dicer** to produce short segments of double stranded molecules called micro-RNAs (**miRNA**) which are typically 21 nucleotides in length (Fig. 9.25).

Each miRNA is recognized and bound by a complex of enzymes called **RISC** (RNA induced silencing complex) which catalyses the unravelling of the strands to produce single stranded segments of RNA called small interfering or small inhibitory RNAs (**siRNA**) (Fig. 9.26). One of the strands is discarded while the other remains bound to the protein and base pairs to any mRNA molecule that contains a complementary sequence of nucleic acid bases. This brings mRNA and RISC together and the enzyme complex then cleaves the mRNA.

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

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

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

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

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

FIGURE 9.25 Cleavage of RNA to produce micro-RNAs (miRNA).

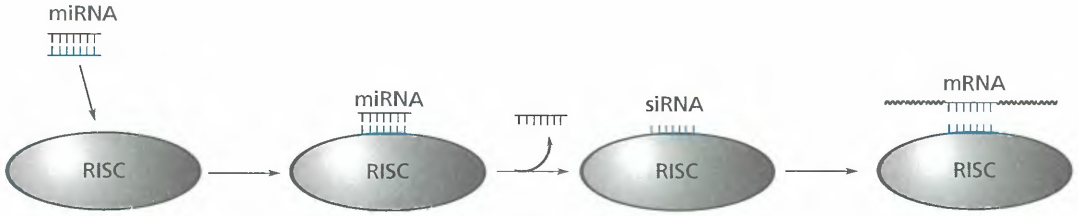


FIGURE 9.26 Interaction of micro-RNA (miRNA) with RNA induced silencing complex (RISC).

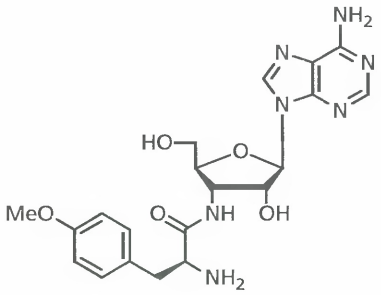
KEY POINTS

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

QUESTIONS

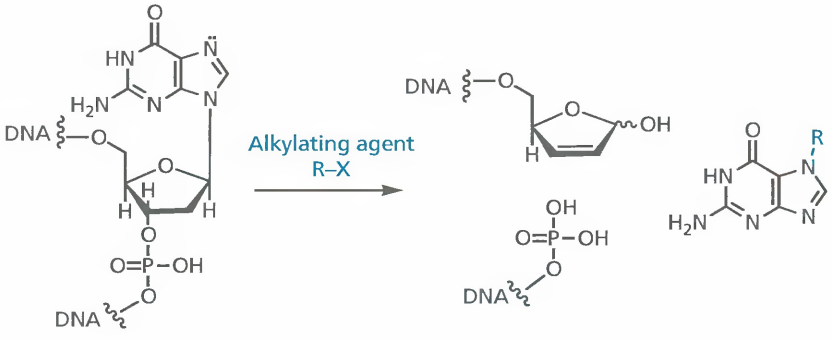
1. Puromycin is an antibiotic that inhibits the translation of proteins. When inhibition is taking place, partially constructed proteins are present in the cytoplasm, and are covalently linked to the drug.

Suggest a mechanism by which this drug causes inhibition.

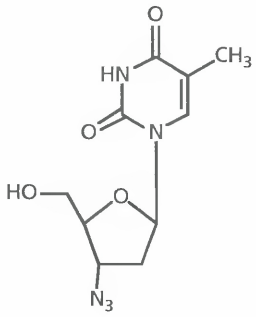


Puromycin

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



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



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