Chimica Farmaceutica

Nucleic Acids as Drug Targets
Nucleic Acids: Primary Structure

Deoxyadenosine  Deoxyguanosine  Deoxythymidine  Deoxycytidine

Adenine  Guanine  Cytosine  Thymine

Purines  Pyrimidines
Nucleic Acids: Secondary Structure

---

**FIGURE 6.4** The secondary structure of DNA.
Nucleic Acids: Minor and Major Grooves
Nucleic Acids: Minor and Major Grooves
Nucleic Acids: Tertiary Structure

The tertiary structure of DNA is often neglected or ignored, but it is important to the action of the quinolone group of antibacterial agents and to several anticancer agents. DNA is an extremely long molecule, so long in fact that it would not fit into the nucleus of the cell if it existed as a linear molecule. It has to be coiled into a more compact three-dimensional shape which can fit into the nucleus: a process known as **supercoiling**. This process requires the action of a family of enzymes called **topoisomerases**, which can catalyse the seemingly impossible act of passing one stretch of DNA helix across another stretch.

Supercoiling allows the efficient storage of DNA, but the DNA has to be uncoiled again if replication and transcription are to take place. If uncoiling did not take place, the unwinding process (catalysed by helicase enzymes) that takes place during replication and transcription would lead to increased tension due to increased supercoiling of the remaining DNA double helix.

The same topoisomerase enzymes are responsible for catalysing the uncoiling process, so inhibition of these enzymes would effectively block transcription and replication.
Topoisomerase II is a mammalian enzyme that is crucial to the effective replication of DNA. The enzyme binds to parts of DNA where two regions of the double helix are in near proximity.
Nucleic Acids: Tertiary Structure

The enzyme binds to one of these DNA double helices and a tyrosine residue is used to nick both strands of the DNA. This results in a temporary covalent bond between the enzyme and the resulting 5' end of each strand, thus stabilizing the DNA. The strands are now pulled in opposite directions to form a gap through which the intact DNA region can be passed. The enzyme then reseals the strands and departs.
Nucleic Acids: Tertiary Structure

**Topoisomerase I** is similar to **topoisomerase II** in that it relieves the torsional stress of supercoiled DNA during replication, transcription, and the repair of DNA. The difference is that it cleaves only one strand of DNA, whereas topoisomerase II cleaves both strands. The enzyme catalyses a reversible transesterification reaction, but where the tyrosine residue of the enzyme is linked to the 3' phosphate end of the DNA strand rather than the 5' end. This creates a 'cleavable complex' with a single-strand break. Relaxation of torsional strain takes place either by allowing the intact strand to pass through the nick or by free rotation of the DNA about the uncleaved strand. Once the torsional strain has been relieved, the enzyme rejoins the cleaved strand of DNA and departs.

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

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
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.
Intercalating drugs acting on DNA

Proflavine is an example of a group of anti bacterial 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 pH 7 and interacts directly with bacterial DNA. The flat tricyclic ring intercalates between the DNA base pairs, and interacts with them by van der Waals forces, while the 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 tran:
Intercalating drugs acting on DNA

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

Doxorubicin is one of the most effective anticancer drugs ever discovered, and belongs to a group of naturally occurring antibiotics called the anthracyclines. It was first isolated from Streptomyces peucetius in 1967, and contains a tetracyclic system where three of the rings are planar. The drug approaches DNA via the major groove of the double helix and intercalates using the planar tricyclic system. The charged amino group attached to the sugar is also important, as it forms an ionic bond with the negatively charged phosphate groups of the DNA backbone. This is supported by the fact that structures lacking the aminosugar have poor activity. Intercalation prevents the normal action of an enzyme called topoisomerase II — an enzyme that is crucial to replication and mitosis. The mechanism by which this enzyme works 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.
Intercalating drugs acting on DNA

*Bleomycins* 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.
Topoisomerase poisons: non-intercalating

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. 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 belong to a group of compounds called the podophyllotoxins, and are semisynthetic 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.
Topoisomerase poisons: non-intercalating

**Camptothecin** is a natural product which was extracted from a Chinese bush (Camptotheca acuminata) in 1966. It stabilizes the cleavable complex formed between DNA and the enzyme topoisomerase I. 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.
Topoisomerase poisons: non-intercalating

The antibacterial quinolones and fluoroquinolones are synthetic agents that inhibit the replication and transcription of bacterial DNA by stabilizing the complex formed between DNA and bacterial topoisomerasers. Inhibition arises by the formation of a ternary complex involving the drug, the enzyme, and bound DNA. 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 fluorosubstituent at position 6, the substituent at C-7, and the carboxylate ion are involved in binding interactions with the enzyme.
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.

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.

![Diagram of nucleophilic groups in adenine, guanine, and cytosine](image)
Alkylating and metallating agents

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. 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.
Alkylation and metallating agents: 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 was the first alkylating agent to be used medicinally, in 1942, although full details were 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. 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.
Alkylating and metallating agents: Nitrogen mustards
Alkylating and metallating agents: Nitrosoureas

**FIGURE 9.11** Nitrosourea alkylating agents.
Alkylating and metallating agents: Busulfan and Cisplatin

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.
Alkylating and metallating agents: Dacarbazine and procarbazine

**FIGURE 9.16** Dacarbazine, procarbazine and temozolomide.
Alkylating and metallating agents: Mitomycin C
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 and the podophyllotoxins 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. Another example is the antitumour agent calicheamicin γ which was isolated from a bacterium.

This compound binds to the minor groove of DNA and cuts the DNA chain by the mechanism shown. 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 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 UNA becomes a diradical. Reaction with oxygen then leads to chain cutting.
Chain cutters
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 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.
Chain terminators
Aciclovir 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'. Therefore the nucleic acid chain cannot be extended any further. Several other structures acting in a similar fashion are used in antiviral therapies.
Agents that act on RNA

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.

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. 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.
Agents that act on RNA: Antisense therapy
Agents that act on RNA

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. The first antisense oligonucleotide to be approved for the market was the antiviral agent fomivirsen (Vitravene) in 1998.
Agents that act on RNA

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. 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.

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). 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.
Agents that act on RNA

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 IDS. One of the advantages of these mechanisms over conventional anti-sense 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.
Agents that act on RNA

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