CONTENTS

INTRODUCTION

Nucleic acids play an important role in cellular processes including cell division (DNA replication) and protein synthesis (transcription and translation). These processes occur both in healthy cells, and in cancer cells, in which case they are targets for anti-cancer drugs.

Small molecules may interact with or bind with DNA. Such compounds can be classified by their mechanism of action. The main classes of DNA binding molecules are:

- groove binders that sit in the minor groove;
- intercalators that sandwich between base pairs;
- alkylators that can chemically react with DNA, resulting in DNA alkylation; and
- DNA cleavage agents that have the ability to break DNA chains.

Each of these classes of molecules has a different structure and interacts with DNA in a different way.

GROOVE BINDERS

Minor groove binding molecules are usually constructed of a series of heterocyclic or aromatic hydrocarbon rings that possess rotational freedom. This allows the molecule to fit into the minor groove, with displacement of water.

Distamycin and netropsin

Distamycin and netropsin (Figure 1) are natural products possessing amido groups and, respectively, three and two *N*-methylpyrrole rings (distamycin can be denoted PyPyPy, and netropsin PyPy).

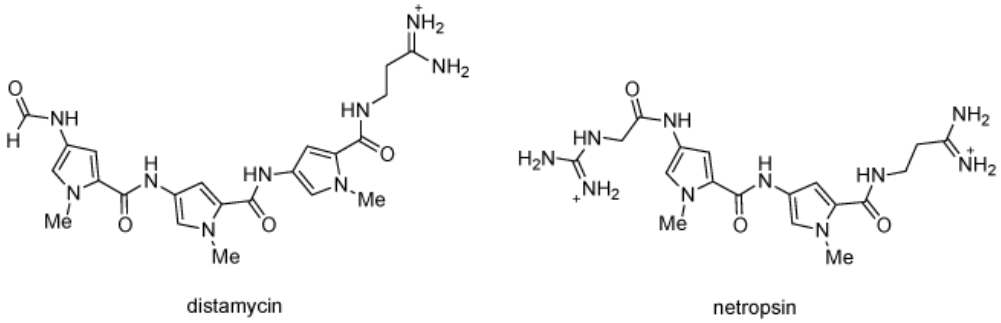

Figure 1 | **Distamycin and netropsin**Structures of the naturally-occurring minor groove binders distamycin and netropsin.

Distamycin and netropsin interact with AT-rich regions of DNA in the minor groove by forming hydrogen bonding and hydrophobic interactions (Figure 2). The terminal amidine group of the small molecule is basic, and serves to attract the drug molecule to the negatively charged DNA phosphodiester backbone. The 2-amino group of guanine prevents distamycin from binding to the minor groove of G·C base pairs by steric hindrance, thus conferring AT-selectivity on the drug molecule.

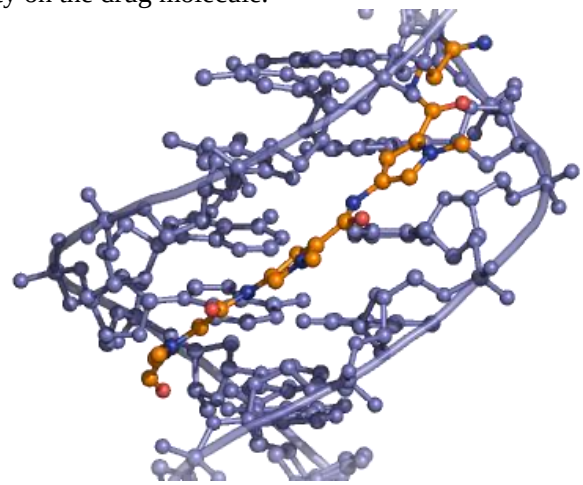

Figure 2 | **Distamycin DNA binding**View from the three-dimensional structure of a complex between distamycin (orange) and a DNA duplex, showing the binding of distamycin in the minor groove.

Lexitropsins

A series of dimers and trimers of distamycin and netropsin have been synthesized and studied in an attempt to increase the DNA binding region from 3 base pairs (monomeric drug) to 10 base pairs or more. These semi-synthetic compounds have been named *Lexitropsins*. As well as pyrrole rings (Py), some lexitropsins also incorporate imidazole (Im) rings. The ability to recognize specific DNA sequences of more than 10 base pairs would give rise to a powerful tool in molecular biology and antisense/antigene therapeutics, as this length of sequence starts to become meaningful in the context of the human genome.

Dervan polyamides

Unfortunately, simple oligomeric compounds like distamycin and netropsin do not have the ideal crescent shape to wrap around the minor groove of DNA, and they fail to recognize longer stretches of DNA. Dervan took this approach further in synthesizing a series of oligomeric "hairpin" polyamide molecules containing pyrrole and imidazole ring systems that are able to bind side-by-side in the minor groove of DNA with high affinity and in a sequence-specific manner. These molecules can be prepared by solid-phase methods.

INTERCALATORS

Certain flat aromatic or heteroaromatic molecules can slide between the base pairs of DNA (intercalate) and stabilize the duplex without disrupting base pairing. Intercalation has the effect of lengthening the duplex by around 3 Å per bound drug molecule, causes unwinding of DNA, and prevents replication and transcription by interfering with the action of topoisomerases. The degree of unwinding depends on the structure of the intercalating molecule and the site of intercalation. The tight ternary complex formed between the intercalated

drug, the DNA and the topoisomerase is lethal to proliferating cells, so intercalators are often more toxic to cancer cells than to normal cells.

Acridines

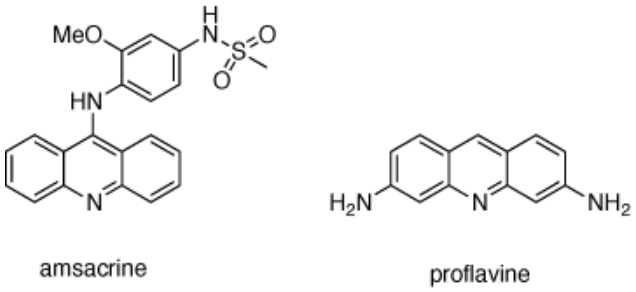

Figure 3 | **Amsacrine and proflavine**Structures of the acridines amsacrine and proflavine, which intercalate between DNA base pairs.

Acridines originated from the aniline dye industry and have been used as anti-malarial and antibacterial drugs. Amsacrine (Figure 3) is used in the treatment of leukemia and proflavine (Figure 3) was used in the Second World War to treat wounds. Proflavine contains amino groups that interact ionically with the negatively charged phosphates groups on DNA, whilst the aromatic ring system intercalates.

Polypeptides

The Actinomycins (Figure 4) are polypeptide antibiotics isolated from *Streptomyces* strains.

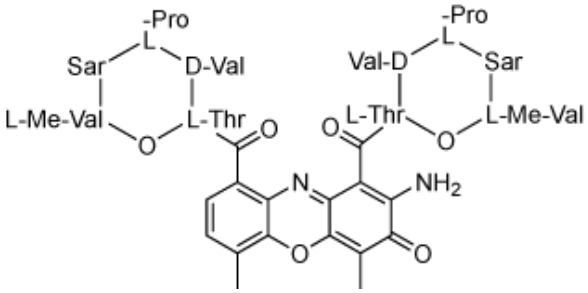

Figure 4 | **Actinomycin D**Structure of actinomyin D (dactinomycin), a polypeptide DNA-binding molecule.

Actinomycins inhibit both DNA synthesis and RNA synthesis by blocking chain elongation. They interact with G·C base pairs as they require the 2-amino group of guanine for binding. The phenoxazone ring slides into the double helix and intercalates, while the pentapeptide side chains interact with the DNA minor groove by forming hydrogen bonding and hydrophobic interactions. The result of these two mechanisms of interaction between small molecule and DNA (intercalation and minor-groove binding) is a very stable complex (Figure 5).

Figure 5 | **Actinomycin DNA binding**View from the three-dimensional structure of a complex between actinomycin D (orange) and a DNA duplex, showing the intercalation of actinomycin D in double-stranded DNA.

Anthracyclines

Doxorubicin (adriamycin) and daunorubicin (daunomycin), isolated from *Streptomyces* strains, are important examples of anthracycline antitumour antibiotics. Both possess an amino group on the sugar which, when protonated, forms an ionic interaction with the negatively charged DNA phosphate backbone. This bond helps to hold the molecule in place, allowing the planar aromatic ring system to slide into the double helix. Although doxorubicin and daunorubicin differ by only one hydroxyl group, they have different activities: daunorubicin is active only against leukaemias, but doxorubicin is active against leukaemias and a wide range of solid tumours.

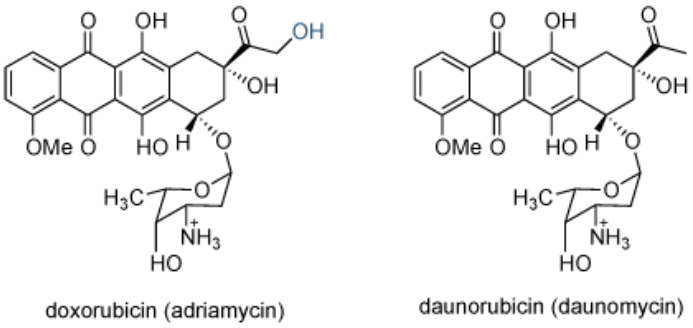

Figure 6 | **Doxorubicin and daunorubicin**Structures of the anthracyclines doxorubicin (adriamycin) and daunorubicin (daunomycin).

ALKYLATORS

Alkylators are strongly electrophilic compounds that react chemically with nucleophilic groups on DNA to form covalent bonds. The resulting DNA adducts are irreversible inhibitors of transcription and translation.

Nucleophilic substitution reactions at the DNA bases occur by both S_N1 and S_N2 mechanisms. The most reactive sites are those that are both nucleophilic and exposed in the grooves of the DNA duplex. The N(7) atom of guanine and the N(3) atom of adenine fulfil both of these criteria. Simple nucleophiles, for example ethyleneimines and methane sulfonates, tend to react via a S_N2 mechanism, whereas the nitrogen mustards can form aziridinium ions that react via an S_N1 mechanism.

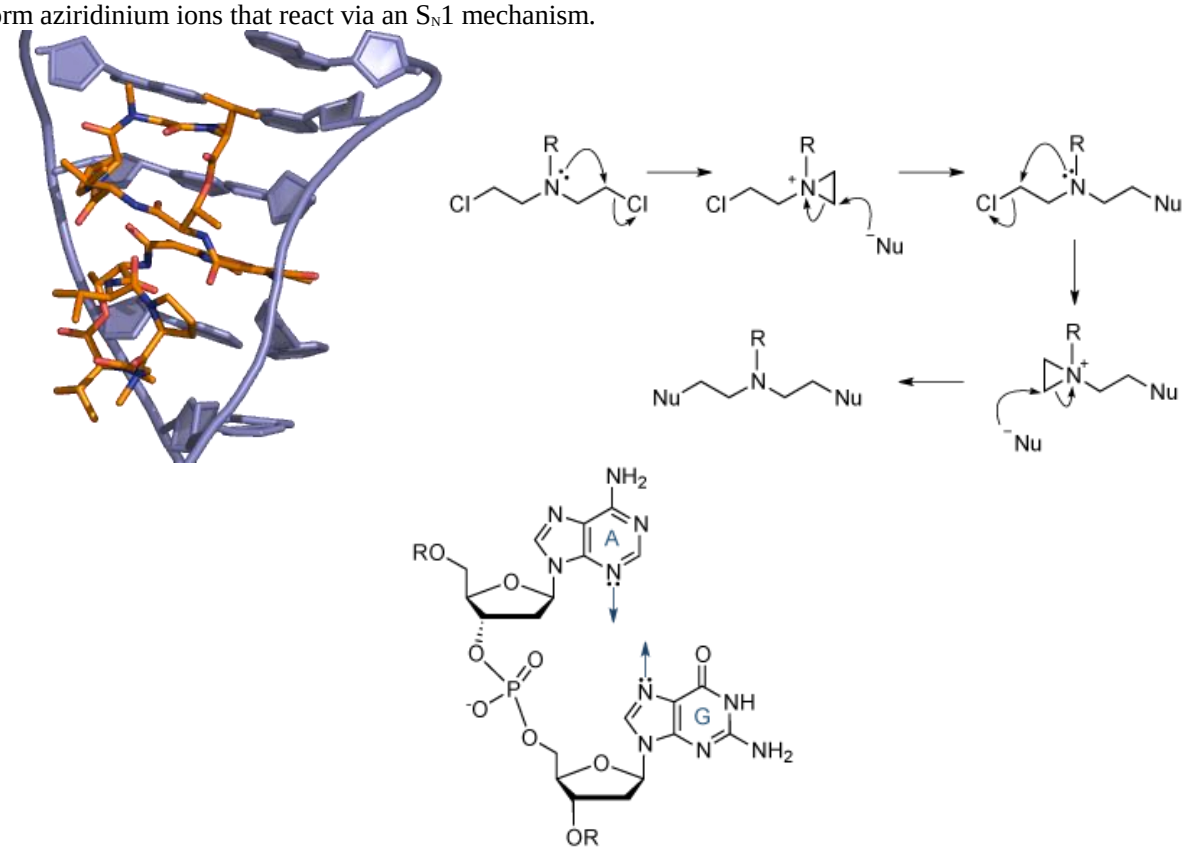

Figure 7 | **Nucleophilicity of adenine and guanine**The N(7) atom of guanine and the N(3) atom of adenine are nucleophilic and are exposed in the grooves of the DNA duplex.
There are several different classes of DNA alkylators, including nitrogen mustards, ethyleneimines, methanesulfonates, nitrosoureas, triazenes and *cis* platinum complexes.

Mustards

Sulfur mustard (Figure 8) is a highly toxic nerve gas that was used during the First World War. Although too toxic for use in cancer therapy, it led to the development of a series of compounds known as nitrogen mustards

(Figure 8) that signalled the beginning of modern cancer chemotherapy. Mechlorethamine (Figure 8) was the first of these compounds to be used in the treatment of advanced Hodgkin's disease.

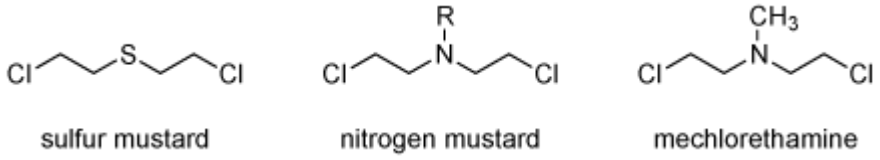

Figure 8 | **Sulfur and nitrogen mustards**General structures of sulfur mustards and nitrogen mustards; and the structure of mechlorethamine, a nitrogen mustard.

The mechanism of action of the mustards begins with the formation of an electrophilic aziridinium ion by displacement of chloride. The aziridinium ion is readily attacked by the nucleophilic DNA bases and the reaction sequence can occur a second time to cross-link two strands of DNA (Figure 9).

Figure 9 | **Mustard mechanism**Mechanism of action of mustards, alkylating agents that cross-link DNA strands.

Mechlorethamine reacts principally with the N(7) atom of guanine, and quaternization of this nitrogen atom leads to scission of the *N*-glycosidic bond (Figure 10).

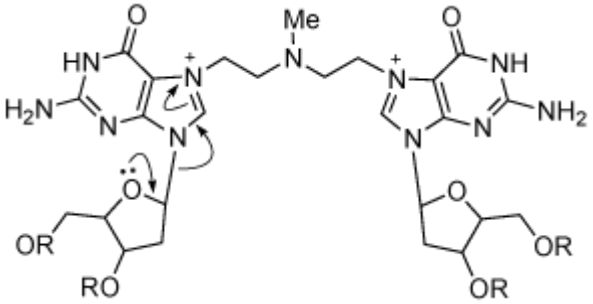

Figure 10 | **Mechlorethamine mechanism**Mechanism of action of mechlorethamine, a nitrogen mustard alkylating agent.

Mechlorethamine is a reactive molecule. It has a lifetime of only minutes in the body and is rapidly hydrolysed. Substitution of the methyl group with an electron-withdrawing aryl group reduces the nucleophilicity of nitrogen, slowing down the rate of aziridinium ion formation and therefore stabilising the compound. However, such compounds are not sufficiently water soluble for intravenous administration. This problem has been solved by the use of carboxylate-containing aryl groups, resulting in the antitumour agent chlorambucil (Figure 11).

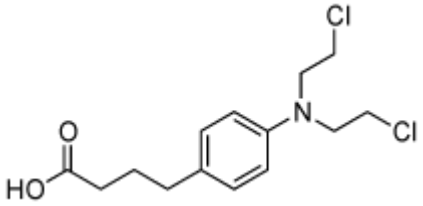

Figure 11 | **Chlorambucil**Structure of chlorambucil, a nitrogen mustard alkylating agent.

Ethyleneimines (aziridines)

Ethyleneimines are pre-formed aziridines and therefore constitute a natural extension of nitrogen mustards (Figure 12). To ensure antitumour activity, at least two ethyleneimine groups must be present in the molecule. To prevent protonation of the ethyleneimine, electron-withdrawing groups are attached (protonated ethyleneimines are too reactive). Lipophilic ethyleneimines are designed to enter the central nervous system.

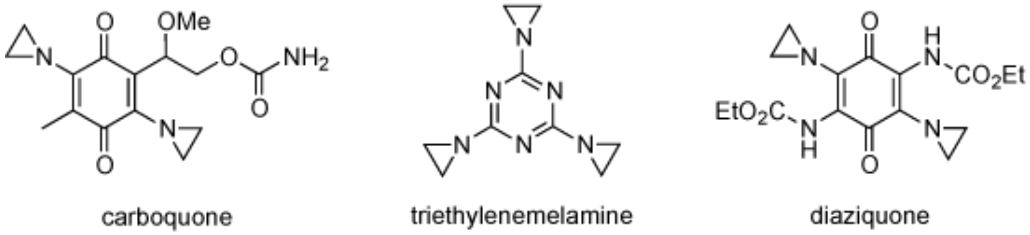

Figure 12 | **Ethyleneimines**Structure of ethyleneimines (aziridines), alkylating agents derived from nitrogen mustards.

Methanesulfonates

Methanesulfonates alkylate guanine at the N(7) position. Unlike nitrogen mustards, which tend to form inter-strand bridges, the methanesulfonates form intra-strand cross-links. An example is the bifunctional anti-cancer drug busulfan (Figure 13).

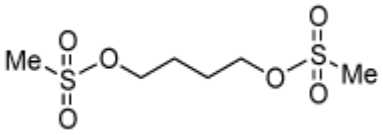

Figure 13 | **Busulfan**Structure of busulfan (or busulphan), an anti-cancer drug that forms intra-strand DNA cross-links.

Platinum complexes

Cisplatin and carboplatin (Figure 14) represent a group of anti-cancer agents used in the treatment of testicular and ovarian tumours. Cisplatin and carboplatin form strong platinum—nitrogen bonds with guanine and adenine bases. The *cis* configuration leads to intra-strand cross-links, causing unwinding of the helix, preventing transcription and leading to cell death.

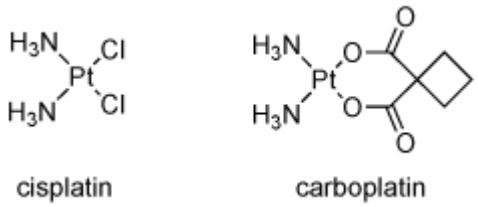

Figure 14 | **Cisplatin and carboplatin**Structures of cisplatin and carboplatin, alkylating agents used in the treatment of testicular and ovarian cancer.

The *trans* isomer, *trans*-platin, is not an active anti-cancer agent, probably because it cannot readily form intra-strand cross-links. It tends to cross-link separate strands and such lesions are more easily repaired (Figure 15).

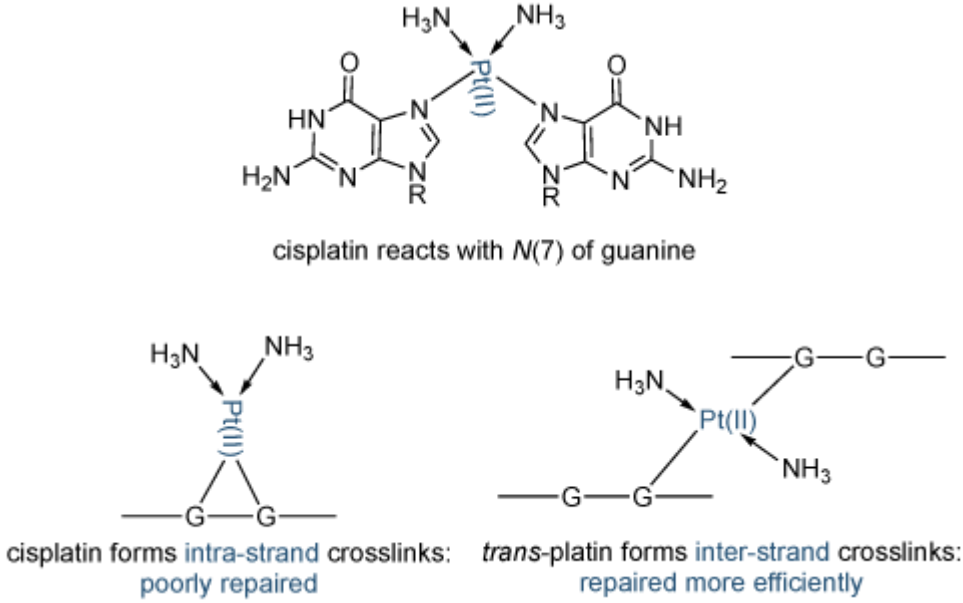

Figure 15 | **The difference between cisplatin and *trans*-platin**Cisplatin forms intra-strand cross-links, while *trans*-platin forms inter-strand cross-links, which are more readily repaired.

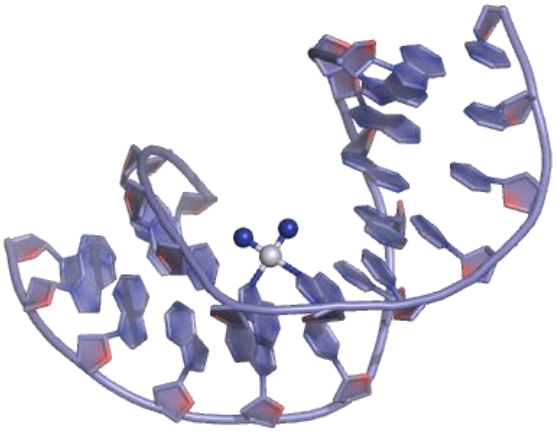

Figure 16 | **Cisplatin DNA binding**View from the three-dimensional structure of a cisplatin intra-strand adduct. The platinum atom is shown as a white sphere; the NH_3 ligands are shown as blue spheres.

DNA CLEAVAGE AGENTS

Bleomycin

The classic DNA-cleaving anti-cancer antibiotic is bleomycin, a mixture of related antibiotics isolated from *Streptomyces verticillus*. Bleomycin is a glycopeptide antibiotic, the major component of which is bleomycin A_2 (Figure 17).

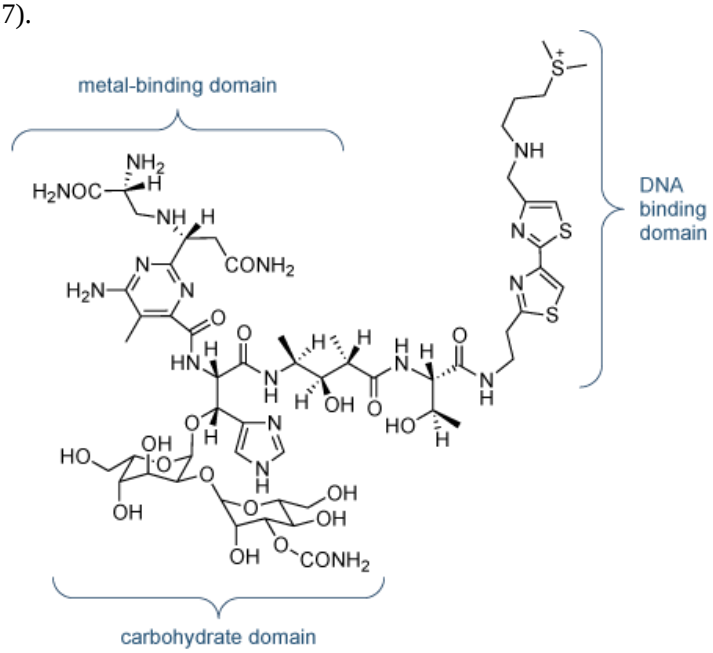

Figure 17 | **Bleomycin**Structure of Bleomycin A_2, an anti-cancer antibiotic.

There are three domains in bleomycin A_2:

- **Metal-binding domain**: the pyrimidine, β-aminoalanine and β-hydroxyimidazole are involved in the formation of a stable complex with Fe(II). Reaction with O_2 gives a ternary complex believed to be responsible for the DNA cleavage activity.
- **DNA-binding domain**: the bithiazole moiety intercalates into the double helix and the attached side chain containing a sulfonium ion is attracted to the phosphodiester backbone.
- **Carbohydrate domain**: the gluose and carbamoylated mannose disaccharide are thought to be responsible for selected accumulation of bleomycin in some cancer cells. This domain does not appear to be involved directly in DNA cleavage.

Bleomycin binds tightly to guanine bases in DNA, particularly in G-T and G-C-rich sequences. When the ternary complex of Fe(II), bleomycin and oxygen attacks DNA, it abstracts hydrogen atoms. The resultant radicals react with oxygen to form peroxy species which then fall apart, resulting in chain cleavage.

Enediyne antitumour antibiotics

The enediyne antitumour antibiotics are anti-cancer compounds that cause the oxygen-dependent cleavage of the DNA phosphate backbone. They interact with the minor groove of DNA and then either a thiol or NADPH triggers a reaction that produces radicals. These radicals cleave the DNA chain. The major characteristic of these enediynes is the presence of a macrocyclic ring with at least one double bond and two triple bonds.

The two steps in antitumour activity are therefore

- activation of the enediyne
- action of the activated antitumour agent on DNA.

Neocarzinostatin

The first of the enediyne antitumour agents, neocarzinostatin (Figure 18), was isolated in 1965. Further enediynes, principally esperamicins and calichemicins, were not isolated until many years later.

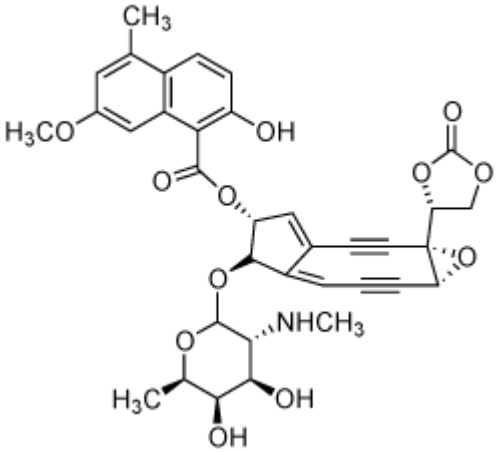

Figure 18 | **Neocarzinostatin**Structure of neocarzinostatin, the first enediyne antitumour agent. Neocarzinostatin contains an active naphthoate ester which intercalates in DNA, positioning the diyne in the minor groove. Activation with a thiol followed, by a Bergman rearrangement, produces a diradical that is somewhat different from those produced by other enediynes (it is not a 1,4-dehydrobenzene diradical, but it is thought to behave similarly). The reactive diradical effects DNA strand scission by reaction with the C4′- and the C5′-atoms of the deoxyribose sugar, usually at deoxyadenosine and thymidine residues, consuming one equivalent of O_2 per strand break (Figure 19). As neocarzinostatin is the oldest member of the enediyne class of antitumour agents, it is the most thoroughly studied.

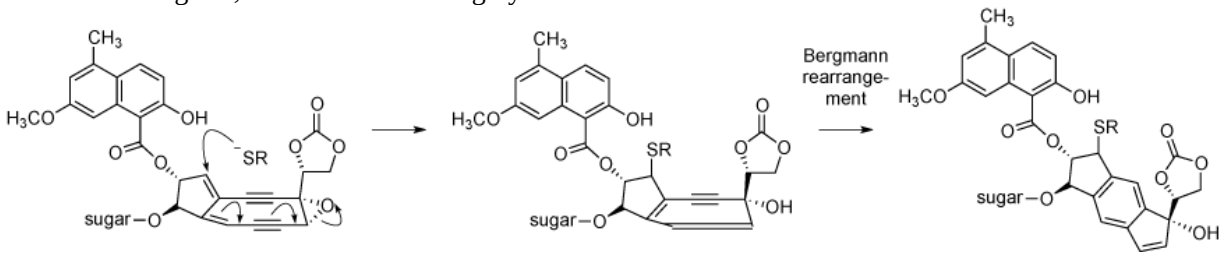

Figure 19 | **Neocarzinostatin mechanism**Mechanism of activation of neocarzinostatin by thiols.

Esperamicins and calichemicins

The esperamicins and calichemicins are similar to neocarzinostatin, having a bicyclo [7.3.1] ring and an allylic trisulfide attached to the bridgehead carbon, a 3-ene-1,5-diyne as part of the macrocycle and an α,β-unsaturated ketone in which the double bond is at the bridgehead of the bicyclic system (Figure 20).

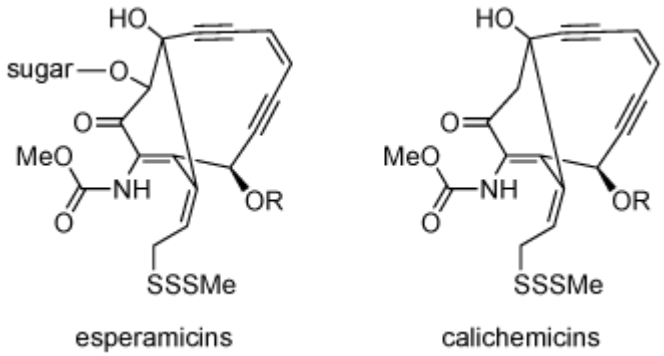

Figure 20 | **Esperamicins and calichemicins**Structures of esperamicins and calichemicins, enediyne antitumour agents.

The proposed mechanism of activation of the esperamicins and calichemicins begins with reduction of the trisulfide to a thiolate by NADPH or thiols in the cell. This results in an intramolecular Michael addition, which followed by a Bergman rearrangement gives the 1,4-dehydrobenzene diradical, the activated form of the esperamicin or calichemicin (Figure 21).

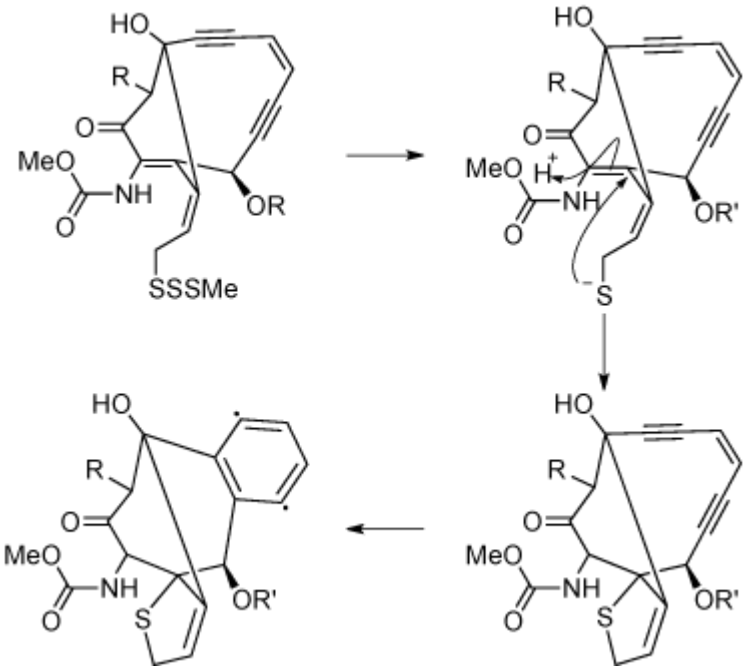

Figure 21 | **Esperamicin mechanism**Mechanism of activation of esperamicins, enediyne antitumour agents.

Enediynes interact with the DNA minor groove, so that the pro-radical centers of the enediyne moiety are positioned close to the proton abstraction sites (Figure 22).

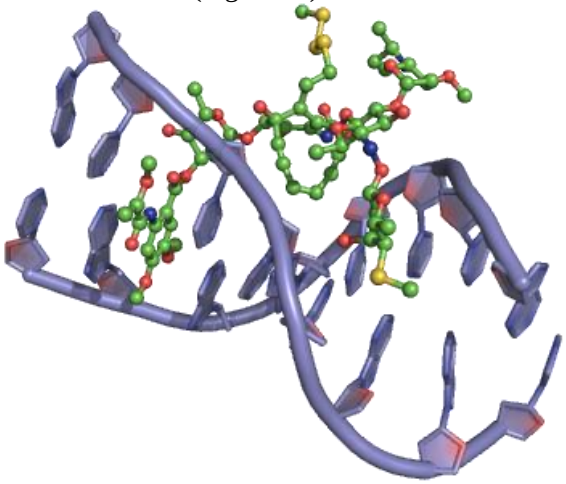

Figure 22 | **Esperamicin DNA binding**View from the three-dimensional structure of a complex between esperamicin A1 (green) and a DNA duplex, showing the binding of esperamicin A1 in the minor groove of double-stranded DNA.

Dynemicin A

Dynemicin A (Figure 23) combines structural features of both anthracyclines and enediynes. Dynemicin A binds to DNA by a combination of intercalation and minor groove binding. Dynemicin A can be activated by NADPH, thiols or light, and, once bound to DNA, can cause cleavage of one or both strands of the DNA helix.

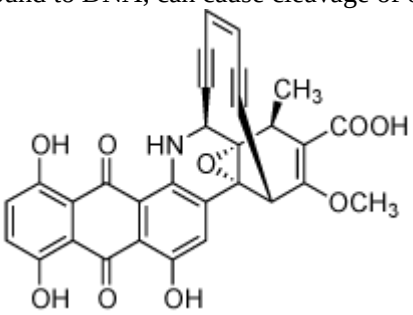

Figure 23 | **Dynemicin**Structure of Dynemicin A, a small molecule that binds to DNA by intercalation and minor groove binding, and effects DNA strand cleavage.