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Mechanism of DNA Binding and Cleavage

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Abstract The necessity for cellular regulation of DNA led to the development of metallonucleases to catalyze and repair DNA strand breaks. Due to cationic character, three-dimensional structural profiles, and propensity for performing hydrolysis, redox, or photoreactions of metal ions and complexes, have a natural ability for interacting with DNA. Since binding and cleavage of DNA is at the heart of cellular transcription and translation, it is an obvious target for therapeutic intervention and the development of diagnostic structural probes. Inorganic constructs such as cisplatin and its analogs exercise antitumor activity by inner-sphere coordination to DNA. During the last decades, the continuous evolution of artificial metallonucleases and metal-based chemotherapeutics such as cisplatin, photo-active octahedral metal complexes have been successfully used as DNA luminescent probes and light-driven reactive agents during the last decades. A recent emerging trend to improve their potential as molecular tools for studies of the genetic material is the design of bifunctional assemblies where the photo-active metal centre is tethered through a flexible linker to a nucleic acid recognition or reactive moiety. In this view, new metal complexes have been designed that utilize or create open coordination positions for DNA binding and hydrolysis, generate reactive oxygen containing species or other radicals for DNA oxidation, or perform direct redox reactions with DNA. This review briefly covers the aspects of drug molecule interaction factors, modes of DNA binding via groove binders, intercalators and alkylators along with the cleavage patterns such as hydrolytic, oxidative and photoinduced DNA cleavage, taking an example of Cisplatin and its mechanism.

Keywords: *DNA, DNA binding, DNA cleavage, DNA drug interaction*

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1. Introduction

Deoxyribonucleic acid (DNA) is of high biological significance [1]. DNA has information stored in it in the form of genes and these are extremely important for various functions. DNA is the primary target molecule for most anticancer and antiviral therapies according to cell biology. Investigations on DNA interactions with transition metal complexes, especially for those containing multidentate aromatic ligands, have aroused considerable interests owing to their potential applications as new therapeutic agents and interesting properties that make them as possible probes of DNA structure and conformation [2,3]. Binding of peptides, small organic and inorganic molecules to DNA will interfere with a number of processes like transcription and replication [4]. By considering this principle various disorders like cancer, cystic fibrosis etc can be cured by using DNA as targets for drugs. And with this emerges a whole new topic if study called DNA drug interaction, which is of great topical importance since 1960 [5]. Ever since then a lot of research has happened in finding a number of metal ions and metal complexes which have been effective in the cancer treatment. Cleavage of DNA can be achieved by targeting its basic constituents like base and/or sugar by an oxidative pathway or by hydrolysis of phosphoester linkages. Among the host of DNA-binding and cleaving agents reported so far, transition metal complexes are of relevance to the present work. Metal complexes have been found to be potential to bind DNA through multitude of interactions and to cleave the duplex by virtue of their intrinsic chemical, electrochemical and photochemical reactivities. Continuous demand for new anti-cancer drugs has stimulated chemotherapeutic research based on the use of metals since potential drugs developed in this way may be less toxic and more prone to exhibit anti-proliferative activity against tumors [6,7]. Transition metal complexes have been extensively studied for their nuclease like activity using the redox properties of the metal and dioxygen to produce reactive oxygen species to promote DNA cleavage by direct strand scission or base modification [8]. Use of metal nanoparticles can be in particular advantageous in generating singlet oxygen [9,10]. A recent report by Geddes and coworkers demonstrated that the presence of metal nanoparticles can enhance singlet oxygen generation [11]. The enhanced electromagnetic fields in proximity to metal nanoparticles are the basis for the increased absorption and various computational methods are available to predict the extent of absorption and the relative increase in singlet oxygen

generation from photosensitizers [12,13]. DNA is preferred to be used as target since its 3D structure makes it easy to locate binding sites for the drug and to detect changes in conformation resulting from the drug binding. It is easy to predict the accessible chemical functional groups for drug binding and it also has the ability to react with a broader range of chemical species that include water, metal ions and their complexes.

2. Mechanism

The different ways in which a drug molecule can interact with the DNA are:

- **Through control of transcription factors:** Here the drug molecule doesn't directly interact with the DNA instead it will interact with the protein that binds to the DNA molecule and hence altering the functions.
- **Forming DNA-RNA hybrids:** By binding to RNA molecule that in turn binds to single stranded DNA forming DNA-RNA hybrids which will interfere with the transcription activity.
- **Direct binding of molecules:** Here the small aromatic ligand molecules directly bind to the DNA double helix and these molecules are of many types like groove binders, intercalators etc.

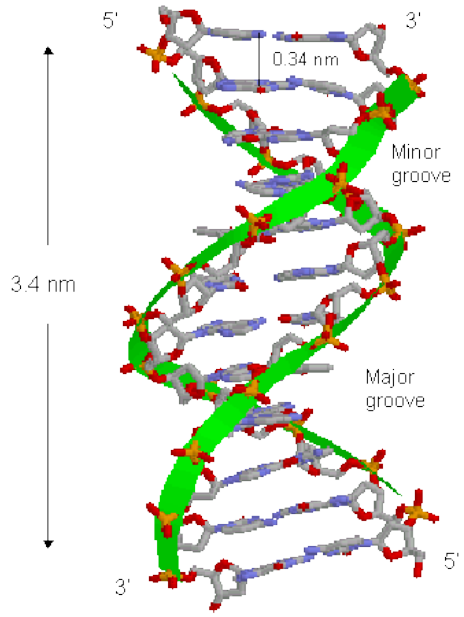


Figure 1. DNA structure and its grooves

Steps in drug development for the target DNA molecule are:

- Based on the knowledge of the DNA of the disease causing micro organism we can isolate specific sequences of DNA that are of functional importance to the organism. For example, in malaria causing parasite P. falciparum 3 such sequences were identified and they are TGCATGCA, GTGTCACAC & GCACGCGCTGC.
- Drugs are then designed to bind to these target sequences.
- The drug should be sufficiently reactive in order to bind to the biological target but not so reactive that it gets deactivated by the other bio molecules in its way [14].
- The frequency with which these sequences occur in humans are determined so that the interaction doesn't tamper with the normal functions of humans.

DNA-drug interaction generally occurs in two steps, i.e. binding and cleavage.

2.1. DNA Binding

The interaction of metal complexes with DNA is a thriving area of research since discovery of cis platin 40 years ago.cis platin when binds to DNA generates intrastrand crosslink's that kinks the DNA structure, inhibits transcription leading to death of cancerous cells. Intercalation was first proposed by Leeman et.al [15] and is defined as insertion between base pairs.

Recently, some transition metal complexes having different ligands such as dipyridoquinoxaline or NSO-donor Schiff base have been reported, which shows efficient DNA binding and cleave DNA on visible light – irradiation [16]. The larger aromatic ring system was proved to account for the higher affinity for DNA and consequently for higher antitumor and photocleaving activities [17]. DNA binding and optical properties have recently been grafted into a single molecular construct toward the development of multifunctional supramolecular complexes. For eg. Ru(II)/ Pt(II) bimetallic polyazine bridging ligand (BL) structures of the form [(tpy)RuCl(BL)PtCl2]PF. It possess optical properties associated with the Ru(II)-polyazine scaffold and labile chloride ligands at the Pt(II) center analogous to cisplatin [6,18].

In this process as the name suggests the drug molecule binds to the DNA at different positions and bind by forming different bonds such as covalent or non covalent bonds. Drug–DNA interactions can be classified into two major categories, intercalation and groove binding.

2.1.1. Groove Binders

These molecules usually place themselves on the minor groove. These bind with direct interaction with the edges of base pairs in either of the major (G-C) or minor (A-T) grooves of nucleic acids. Groove binding does not induce large conformational changes in DNA and may be considered similar to standard lock-and-key models for ligand–macromolecular binding. Groove binders are usually crescent-shaped molecules that bind to the minor groove of DNA [19].

The way they interact can be explained as follows:

- 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 or major groove, with displacement of water.
- The drug molecule first identifies targets which are the specific DNA sequences.
- These targets are in the range of 16 to 18 base pairs.
- Molecules are bonded to the helical structure via non covalent bonds.
- Groove binders interact with base pairs edges in either major or minor grooves.

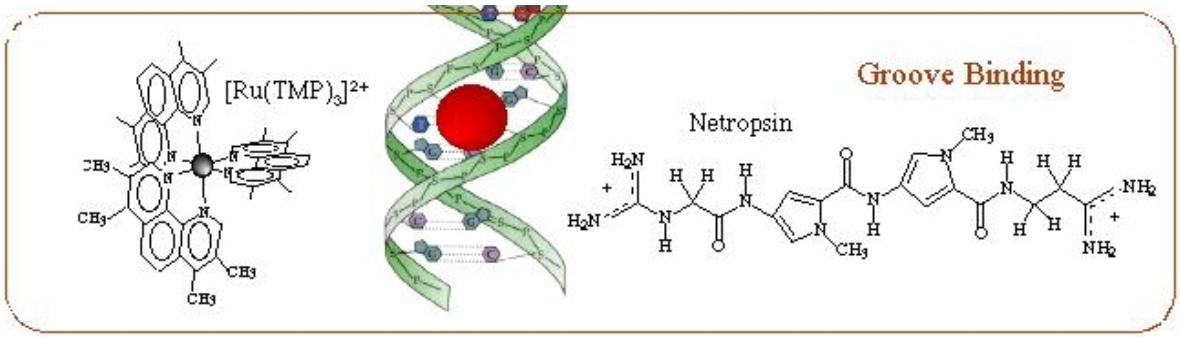


Figure 2. Adsorption of the complex in the DNA grooves [20]

Most of the drugs are groove binders as the minor and major grooves provide a tight fit to the drug which binds to it. As discussed earlier they are of two types, such as minor groove binders and major groove binders [21]. A series of heterocyclic or aromatic hydrocarbon rings get together to form minor groove binding molecules. This allows the displacement of water and the molecule fits into the minor groove. The strand backbones are closer together on one side of the helix than on the other. The minor groove occurs where the backbones are close together while the major groove occurs where the backbones are far apart. It is easier for certain DNA binding proteins to interact with the bases (the internal parts of the DNA molecule) on the major groove side because the backbones are not in the way. On the opposite sides, the grooves twist around the molecule and certain proteins bind to DNA to alter its structure or to regulate transcription or replication [1]. Distamycin and netropsin are natural products possessing amido groups and three and two N-methylpyrrole rings. By means of hydrophobic interactions and hydrogen bonding, distamycin and netropsin interact with AT-rich regions of DNA in the minor groove. The terminal amidine group of the small molecule is basic in nature and attracts the drug molecule to the negatively charged DNA phosphodiester backbone. The 2-amino group of guanine confers AT-selectivity on the drug molecule, preventing distamycin from binding to the minor groove of G·C base pairs by steric hindrance.

Lexitropsins

A series of dimers and trimers of distamycin and netropsin have been synthesized and studied to increase the DNA binding region from 3 base pairs to 10 base pairs or more.

Dervan polyamides

Above discussed molecules do not have the ideal crescent shape to wrap around the minor groove of DNA, and they fail to recognize longer stretches of DNA. So a series of oligomeric "hairpin" polyamide molecules containing pyrrole and imidazole ring systems were synthesized that were able to bind side-by-side in the minor groove of DNA with high affinity and in a sequence-specific manner [22].

2.1.2. Intercalators

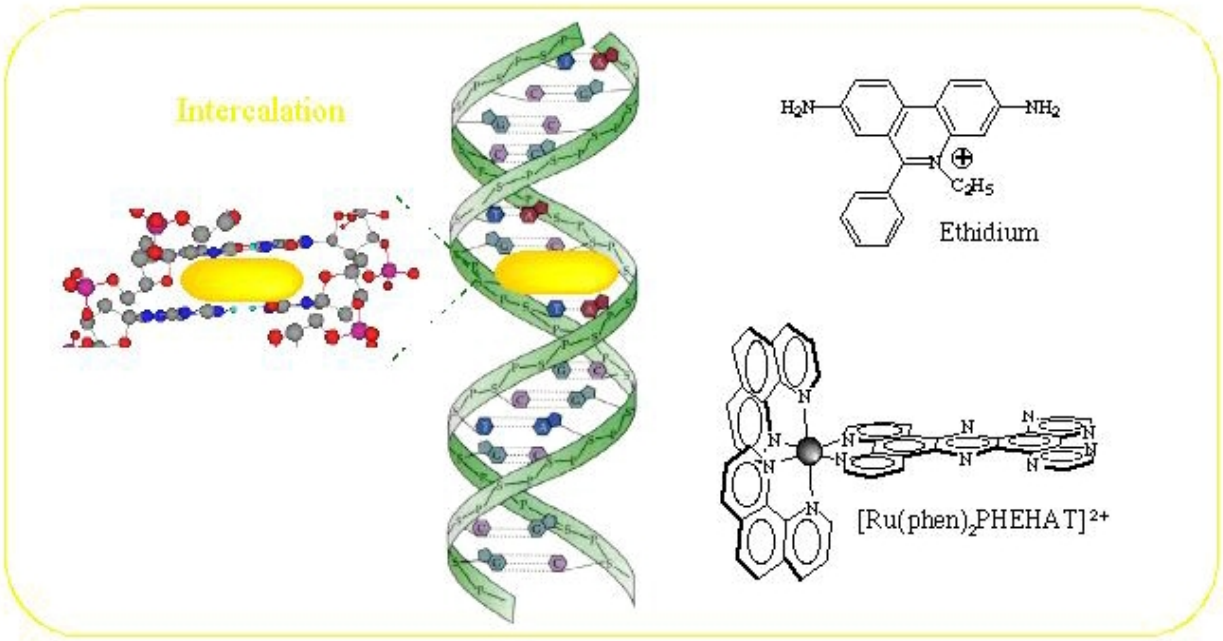


Figure 3. Intercalation of a planar ligand of the complex in the DNA base pairs stack [27]

Intercalation involves the insertion of a planar molecule between DNA base pairs, which results in a decrease in the DNA helical twist and lengthening of the DNA [20]. They consist of planar heterocyclic groups that stack between adjacent DNA base pairs. The complex one is stabilized by π-π stacking interactions between the DNA bases and drug. Intercalators show strong structural perturbations in DNA [23]. Certain flat aromatic or

heteroaromatic molecules can fit in between the base pairs of DNA (intercalate) and stabilize the duplex without the disruption of base pairing pattern. Intercalation can lengthen the duplex by around 3 Å per bound drug molecule, causing unwinding of DNA. This prevents DNA replication and transcription by interfering with the action of topoisomerases. The degree of unwinding depends on the arrangement of the intercalating molecule and the site of intercalation. The tight ternary complex created between the intercalated drug, the DNA and the topoisomerase is toxic to proliferating cells, so intercalators are often more lethal to cancer cells than to normal cells. DNA intercalators are used in structural studies and antisense work. Such as in the incorporation of acridine into oligos is striking for antisense applications, since the intercalation of acridine into the DNA-RNA duplex significantly increases the Tm and thereby enhances duplex stability without affecting target specificity [24,25,26].

Acridines

They have its origin from the aniline dye industry. One of its examples is Proflavine which contains amino groups that interact with the negatively charged phosphates groups on DNA due to the presence of ions, whilst the aromatic ring arrangement intercalates.

Polypeptides

Actinomycins (polypeptide antibiotics isolated from Streptomyces strains) by blocking chain elongation, hinder both DNA synthesis and RNA synthesis. They interact with G·C base pairs as they have need of the 2-amino group of guanine for binding. The phenoxazone ring slides into the double helix and intercalates, while the pentapeptide side chains intermingle with the DNA minor groove by hydrogen bonding and hydrophobic interactions. The result of these two mechanisms of interface between small molecule and DNA (intercalation and minor-groove binding) is a very steady complex.

Anthracyclines

They can form antitumour antibiotics such as doxorubicin (adriamycin) and daunorubicin (daunomycin). 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 leukaemia, but doxorubicin is active against leukaemia and also a wide range of solid tumours.

2.1.3. Alkylators

Strong electrophilic compounds that react chemically with nucleophilic groups on DNA to form covalent bonds are known as alkylators. The resultant DNA adducts which are produced are irreversible inhibitors of transcription and translation. Nucleophilic substitution reactions at the DNA bases occur by both SN1 and SN2 mechanisms. The most reactive sites are those that are both nucleophilic and uncovered in the grooves of the DNA duplex. The N(7) atom of guanine and the N(3) atom of adenine complete both criteria's. Simple nucleophiles, for example ethyleneimines and methane sulfonates, tend to react through a SN2 mechanism, whereas the nitrogen mustards can form aziridinium ions that react through an SN1 mechanism.

Ethyleneimines (aziridines)

Ethyleneimines are pre-formed aziridines and as a result constitute a natural addition of nitrogen mustards (mechanism of action of the mustards begins with the formation of an electrophilic aziridinium ion by displacement of chloride). To ensure antitumour activity, at least two ethyleneimine groups must be available in the molecule. To prevent protonation of the ethyleneimine, electron-withdrawing groups are attached (protonated ethyleneimines are too reactive). Lipophilic ethyleneimines are intended to enter the central nervous system.

Platinum complexes

Cisplatin and carboplatin stand for a group of anti-cancer agents used in the treatment of testicular and ovarian tumours. Cisplatin and carboplatin form sturdy platinum-nitrogen bonds with guanine and adenine bases. The cis configuration form intra-strand cross-links which leads to unwinding of the helix, preventing transcription and leading to cell death. The trans-isomer, trans-platin, is not an active anti-cancer agent, perhaps because it cannot eagerly form intra-strand cross-links. It tends to cross-link separate strands and such lesions are repaired easily [22].

Table 1. DNA alkylating agents [28]

Alkylating agents	Examples
DNA -targeted mustards	• Oilgopyrrole and oligoimidazole carriers • Bis-(benzimidazole) carriers • Polybenzamide carriers • 9-Anilinoacridine-4-carboxamide carriers
Guanine-specific alkylating agents	• Mitomycins • Carmethizole analogues • Pyrrolobenzodiazepines • Ecteinascidin analogues
Adenine-specific alkylating agents	• Duocarmycin and analogues • Benz[e]indolones • Analogues of KW-2189 • Amino analogues • Bizelesin and other bis analogues

2.2. DNA Cleavage

Cleavage of DNA is a vital process in all living systems. For example, topoisomerase enzymes resolve topological problems of DNA in replication, transcription and other cellular transactions by cleaving one or both strands of the DNA [25]. Another example are restriction enzymes (or restriction endonucleases), which protect the cell against virus infection by cleavage of the foreign DNA [29], or by degrading cellular DNA during apoptosis of the affected cell [30]. Finally, the activity of many anticancer drugs rely on their ability to introduce extended damage to the DNA in the (affected) cells (*e.g.* bleomycin) [31], which can trigger apoptosis [32], leading to the cell death [33]. In general, three different types of DNA cleavage can be distinguished, namely i) DNA hydrolysis, ii) photochemical cleavage, and iii) oxidative cleavage, although the last two categories are quite closely related.

2.2.1. Hydrolytic Cleavage

It can be defined as a method of DNA cleavage by the cleavage of phosphor diester bonds to generate fragments in the presence of water. The fragments produced here can be relegated. The half life of a typical phosphate diester bond of DNA in neutral water under ambient conditions

(25°C) is estimated to be in the order of tens to hundred billions of years. This means that a catalyst has to accelerate this reaction 1017-fold to achieve an effective hydrolysis of the phosphate backbone of DNA within an acceptable timeframe (*i.e.* a couple of min). General mechanism of this method is the hydrolysis reaction is facilitated by the presence of metal ions, acting as Lewis Acids. These Lewis acids can activate the phosphate group towards nucleophilic attack, activate water or hydroxide as nucleophile or increase the leaving group ability of the departing alcohol. The general accepted mechanism of the DNA hydrolysis reaction is a nucleophilic attack at the DNA phosphate backbone, to form a five coordinate intermediate, which can be stabilized by the catalyst. Subsequent cleavage of either the 3'-PO (as seen is most often in enzymatic systems) or the 5'-PO results in a strand scission. After this nucleophillic attack one group leaves as an alcohol.

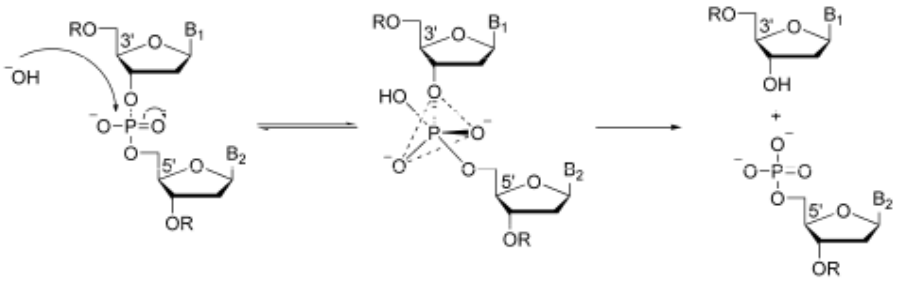


Figure 4. Proposed reaction mechanism for the hydrolysis of DNA

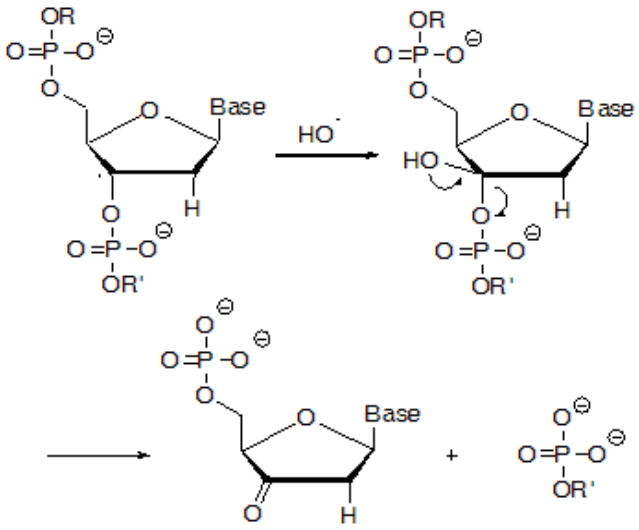
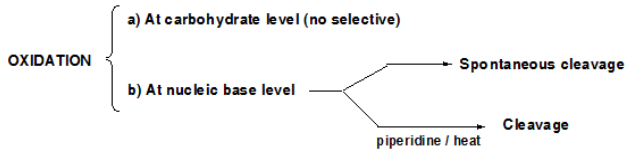


Figure 5. Cleavage at nucleobases

2.2.2. Oxidative Cleavage

This method of cleavage involves the oxidation of deoxy ribose by abstraction of sugar hydrogen or oxidation of nucleobases. Oxidative cleavage is usually mediated by the presence of additives and photo induced DNA cleaving agents i.e. an external agent like light or H_2O_2 is required to initiate cleavage. Like in hydrolytic cleavage in this method the DNA fragments cannot be religated. Oxidative cleavage can occur both at the carbohydrate level and at the nucleic base level. Oxidative cleavage of DNA can result in the damage of all four nucleobases or the deoxy ribose sugar. Generally Hydroxyl radical species of O_2 (OH) are involved in this oxidative cleavage. The mechanism of oxidative cleavage occurs in 3 ways: hydrogen abstraction, addition and electron transfer.



Cleavage at deoxyribose sugar: If the oxidative cleavage occurs at the carbohydrate, abstraction of one hydrogen of deoxyribose can initiate the oxidative cleavage process. In the next figure the process following the C-3' abstraction of deoxyribose is shown in Figure 5.

The oxidation at the nucleic base level occurs preferably at guanine because it's lower oxidation potential. Hydroxyl radical reacts with the heterocyclic bases in DNA by addition. In pyramidines OH adds to the C5 or C6 double bond leading to cleavage. In purines the hydroxyl ion binds to the C4, C5 & C8 [34].

2.2.3. Photoinduced DNA Cleavage

Photocleavage of nucleic acids allows the use of light to trigger nuclease activity. Nucleases that are activated by visible or near-UV light can be used for examination of processes such as transcription and to probe nucleic acid structure as photofootprinting and photo-sequencing agents.On the other hand, photosensitization of DNA by drugs may be useful as a potential anti-tumor therapy. DNA photocleavage can occur by a wide variety of mechanisms such as [35] hydrogen atom abstraction from the sugar ring by photochemically generated radicals [36], direct electron transfer from the base (usually guanine) to the photoexcited cleaver [37], singlet oxygen production by transfer of energy from the excited photocleaver, and [38] formation of base adducts.

DNA damage initiated by photosensitization can be divided in two major types; Type I process a one electron process and Type II process a pathway involving singlet oxygen [39,40].

In the first type (Type I process), the cleaving agent is excited and generates sequentially a superoxide radical from molecular oxygen via an electron transfer step. Superoxide itself is a rather poor oxidant [41], and it can be further reduced (leading to H_2O_2 and OH) or it can function as a reductant. The DNA damage observed via this pathway is mainly guanine oxidation, formed via guanine radical cations [39,40]. This results in the formation of base labile sites in the DNA.

In a Type II process, the photo excited compound generates singlet oxygen, which only modifies guanine

residues, in contrast to superoxide. Two pathways can be distinguished [39,40,42,44] A Diels-Alder reaction with singlet oxygen results in the formation 4,8-dihydro-4-hydroxy-8-oxo-dG, and after further reduction in 8-oxo-dG. A [2+2] cycloaddition with singlet oxygen results after a cascade of reactions in the formation of cyanuric acid. The modified residues are base labile positions in the DNA and alkaline.

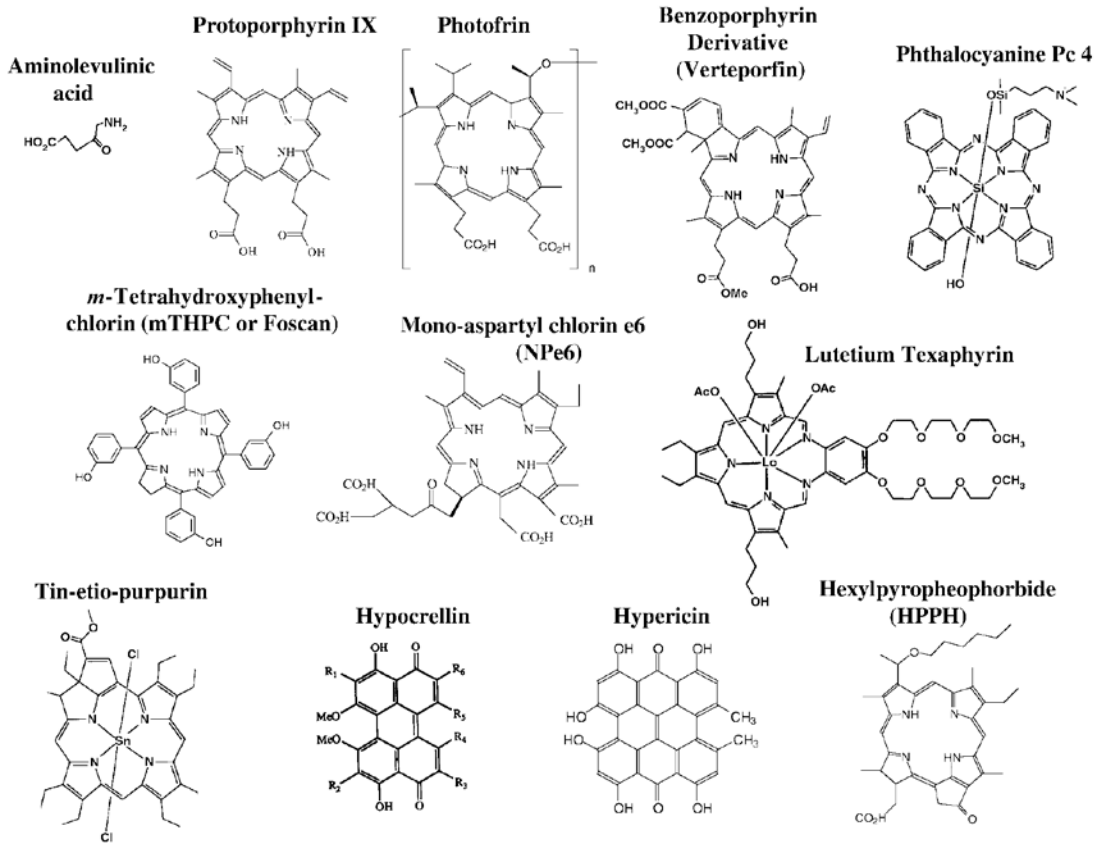


Figure 6. Names and structures of selected photosensitizers in clinical or pre-clinical studies

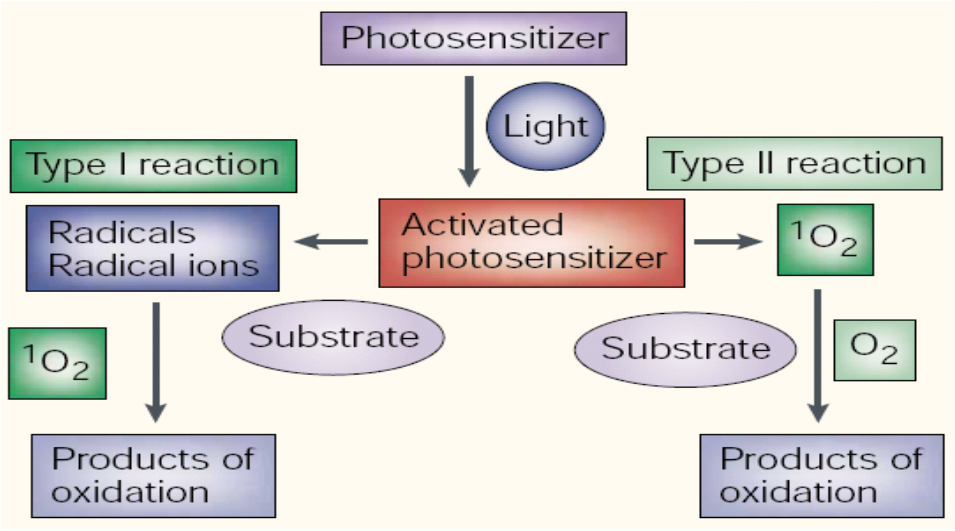


Figure 7. Schematic representation of PDT mechanism

2.3. Cisplatin a Classical Example of DNA Binding and Cleavage Agent

The inorganic compound cis-diamminedichloroplatinum (II) ***cis*-[Pt(NH$_3$)$_2$(Cl)$_2$]** commonly referred to as cisplatin, also called as Peyrone's salt was named after Michel Peyrone who first synthesized it in 1845. It was the first member of a class of platinum-containing anti-cancer drugs, which now also includes carboplatin and oxaliplatin. Cisplatin and its analogs are heavy metal complexes containing a central atom of platinum surrounded by two chloride atoms and two ammonia molecules in the cis position. Cisplatin is a white lyophilized powder soluble in water or saline at 1mg/ml and in diethylformamide at 24 mg/ml with a melting point of 270°C. Cisplatin has biochemical properties similar to that of bifunctional alkylating agents, producing interstrand, intrastrand and monofunctional adduct cross-linking in DNA.

2.3.1. Mechanism of Cisplatin

One chloride ligand is slowly displaced by water resulting in the formation of [PtCl $(H_2O)(NH_3)_2$] [16]. Because of this the complex can bind to bases easily especially to guanine [PtCl (guanine-DNA) $(NH_3)_2]^+$. On displacement of another Chlorine molecule by water the cisplatin molecule can bind to another guanine in the same DNA molecule forming a cross link between the two strands. After complete binding of the Cisplatin the DNA molecule will bend at a 30 deg. Angle. This leads to DNA damage. The damaged DNA elicits DNA repair mechanism which leads to apoptosis.

Table 2. DNA Cleavage agents

Types of cleavage	Examples	References
Hydrolytic cleavage	Cu(II)TACH complex	[45]
	copper-ATCUN complexes	[46]
	copper(II)−l-histidine complex	[47]
	[CoII(CysGly)(HisSer)]	
	[CoII(CysGly)(HisPhe)]	[48]
Oxidative cleavage	Zn(F-BDPA)(NO3)2	[49]
	[Cu(mbpzbpy)Br2](H2O)2.5	[50]
	[Cu(mpzbpya)Cl](CH3OH)	[50]
	[Cu2(mTPXA)Cl4]3 H2O	
	[Cu2(pTPXA)Cl4]3 H2O	
	[Cu3(HPTAB)Cl5]Cl3 H2O	[51]
Photo induced cleavage (photosensitizers)	Photofrin® (HpD)	
	Levulan® (ALA)	
	Metvix® (M-ALA)	
	Visudyne® (Vertiporfin)	
	Antrin® (Lutexaphyrin)	
	Foscan® (Temoporfin) LS11 (Talaporfin)	
	Photosens® (Phthalocyanine)	[52]

Cisplatin is administered intravenously as short-term infusion in normal saline for treatment of solid malignancies. It is used to treat various types of cancers like ovarian, bladder, cervical, lungs etc.

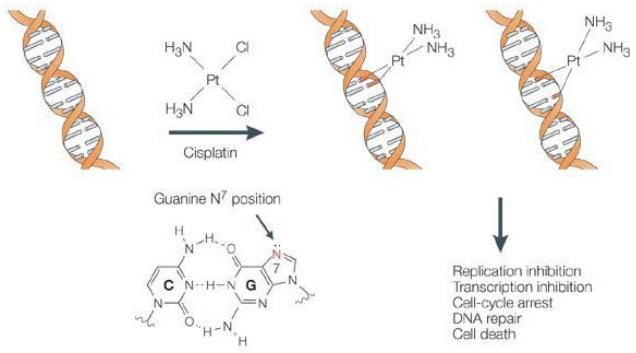


Figure 8. Mechanism of Cisplatin [53]

3. Conclusion

Recent advances in medicinal inorganic chemistry demonstrate significant prospects for the utilization of metal complexes as drugs, presenting a flourishing arena for inorganic chemistry. Compounds containing metal ions have and will continue to play an important role in biomedical technology. Whether as imaging agents, therapeutics or probes for chemical genetics, inorganic compounds will continue to provide the research community with tools that cannot be achieved with organic chemistry alone. Significant progress in platinum based anticancer agents has been achieved, based in part on a mechanistic understanding of the DNA-binding and pharmacological effects of cisplatin. Several new compounds with reduced toxicity and high specificity have been developed. Ruthenium complexes with antitumor activity are also emerging rapidly. The future development of medicinal inorganic chemistry requires an understanding of the physiological processing of metal complexes or drugs with DNA, to provide a rational basis for the design of new metal-based drugs. In this direction of designing new drugs understanding the mechanism of DNA drug interaction is vital. Application of new methodologies such as combinatorial chemistry, extensively used in organic drug discovery, will be beneficial for the development of inorganic compounds as therapeutics.

Abbreviations

TACH:	1,3,5-triaminocyclohexane
ATCUN:	amino terminal copper nickel
CysGly:	cysteinylglycine
HisSer:	histidylserine
HisSer:	histidylphenylalanine
BDPA:	*N*,*N*′-bis(benzyl)-*N*,*N*′-bis(2-pyridylmethyl)-6,6′-bis(aminomethyl)-2,2′-bipyridine)
mbpzbpy:	6,6-bis(3,5-dimethyl-N-pyrazolmethyl)-2,2-bipyridine)
Hmpzbpya:	6-(3,5-dimethyl-N–pyrazolmethyl)-2,2-bipyridine-6-carboxylic acid)
mTPXA:	N,N,N',N'-tetra-(2-pyridylmethyl)-m-xylylene diamine
pTPXA:	N,N, N',N'-tetra-(2-pyridylmethyl)-p-xylylenediamine
HPTAB:	N,N,N',N',N'',N''-hexakis(2-pyridylmethyl)-1,3,5-tris-(aminomethyl)benzene
HpD:	Hematoporphyrin derivative
ALA:	5-Aminolevulinic acid
M-ALA:	Methylated 5-Aminolevulinic acid
BPD:	Benzoporphyrin derivative
LS11:	talaporfin Sodium.

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