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Unusual DNA binding modes for metal anticancer complexes

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Abstract

DNA is believed to be the primary target for many metal-based drugs. For example, platinum-based anticancer drugs can form specific lesions on DNA that induce apoptosis. New platinum drugs can be designed that have novel modes of interaction with DNA, such as the trinuclear platinum complex BBR3464. Also it is possible to design inert platinum(IV) pro-drugs which are non-toxic in the dark, but lethal when irradiated with certain wavelengths of light. This gives rise to novel DNA lesions which are not as readily repaired as those induced by cisplatin, and provides the basis for a new type of photoactivated chemotherapy. Finally, newly emerging ruthenium(II) organometallic complexes not only bind to DNA coordinatively, but also by H-bonding and hydrophobic interactions triggered by the introduction of extended arene rings into their versatile structures. Intriguingly osmium (the heavier congener of ruthenium) reacts differently with DNA but can also give rise to highly cytotoxic organometallic complexes.

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1. Introduction

The interaction of the anticancer drug cisplatin and its ultimate target, DNA, has been the subject of extensive research over the last 40 years.

DNA-binding drugs can be classified according to the type of association with DNA: coordinative binding agents, groove-binders, intercalators, and – most recently classified[1] – phosphodiester backbone-binders. Non-coordinative interactions rely on electrostatic interactions, molecular recognition based on shape and size, and hydrogen bonding. To date, the only DNA-targeted metal-containing drugs in clinical use are platinum-based drugs, and these are believed to interact with the nucleic acid primarily through the formation of coordination bonds [2].

Despite the success of cisplatin and platinum-based drugs, the market is still accessible for new advantageous metal-based drugs that offer better viability, such as oral administration, which might help to diminish severe side-effects and clinic costs. Additionally, research is focussed on drugs with higher efficacy, *i.e.* drugs that interact differently with the target, DNA, can overcome inherent or acquired cisplatin resistance in many tumours, and are active towards tumours which are non-responsive to current chemotherapy.

Some new platinum drugs such as the photoactivatable platinum(IV) complexes developed in our group combine both advantages [3]. These complexes have improved aqueous solubility, are active only when irradiated with certain wavelengths of light and interact with DNA so as to trigger biological processes that eventually lead to apoptosis in a way which is very different from that of cisplatin [4, 5].

Ruthenium drugs have achieved in the last decade the status of very promising candidates for novel cancer therapy, with two drugs already in clinical trials, NAMI-A and KP1019 [6, 7]. Both these ruthenium(III) complexes are believed to be activated through reduction to Ru^{II} , which opens up a new research field focused on organometallic ruthenium(II) complexes. These new complexes, and their heavier congeners, osmium(II) organometallic complexes, offer a more elaborate and intricate interaction with the double helical DNA, not only forming coordination bonds, but also H-bonds and intercalation between DNA base pairs [8]. These complexes demonstrate how transition metals can act as a scaffold for interesting structures that disrupt DNA normal function and deceive the cellular repair strategy leading to cancer cell death.

From this point of view, it is timely to review the interaction of the above metal-based complexes with DNA. Most of the drugs reviewed here show non cross-resistance with cisplatin, an exciting feature which is attributable to the nature of the interactions with the nucleic acid, the configurations of the adducts that are formed and the ways in which these differ from those formed by cisplatin.

2. Historical background for metal-containing anticancer drugs: cisplatin and platinated DNA

In the mid-sixties Rosenberg and coworkers discovered serendipitously the anticancer properties of cisplatin, *cis*-[PtCl₂(NH₃)₂] (Chart 1), during investigations of the effect of electric fields on the growth *Escherichia coli*. They were using platinum electrodes in a solution containing chloride and ammonium salts among other constituents, when they observed an unexpected event. The bacteria become long filaments, and did not replicate. Cell division in the *E. coli* was thus found to be inhibited [9]. Further extensive analysis of this unpredicted observation led them to the conclusion that the bacterial replicative cycle was inhibited by platinum ammine complexes formed by electrolysis at the platinum electrodes and to the subsequent use of *cis*-[PtCl₂(NH₃)₂] as an anticancer drug.

Today, over 30 years after its approval as a chemotherapeutic agent by the North American Food and Drug Administration in the USA (FDA), cisplatin is still one of the world's best selling anticancer drugs. It is responsible for the cure of over 90% of cases of testicular cancer and it plays an important role in some cancer treatments such as ovarian, head and neck cancer, bladder cancer, cervical cancer, melanoma, and lymphomas.

Regardless of the achievements of cisplatin, the drug is efficient in only a limited range of cancers, and for some other tumours cisplatin might induce acquired

immunity during the treatment. Also cisplatin can have severe side-effects: often causing severe nausea and vomiting, bone marrow suppression and kidney toxicity.

Current research on metal complexes is directed partly towards the development of complexes that kill the types of cancer cells that have an inherently low response to current chemotherapy, and partly to drugs whose main aim is diminishing the severe side effects of cisplatin.

Cisplatin mechanism of action

From Rosenberg's early studies with *Escherichia coli* it was suspected that any agent which inhibits cell division should interfere with DNA replication, while the filamentous growth suggested that RNA and proteins were performing normally. Thus, already in the 1970's the implication of DNA in the cytotoxic properties of cisplatin was well established [10-13], and nuclear DNA has since been thought to be strongly implicated if not the ultimate target of cisplatin and related metal-based antineoplastic agents via the process of ligand exchange [2, 14-19].

Prior to DNA attack, cisplatin undergoes aquation. Being a drug able to hydrolyse, cisplatin will be susceptible to aquation as soon as it enters the body. The high concentration of chloride ions in blood plasma (*ca.* 100 mM) preserves its stability towards hydrolysis [20]. However, the significantly lower intracellular chloride concentrations inside cellular compartments (*ca.* 4-23 mM) facilitate rapid ($t_{1/2}$ *ca.* 2 h) hydrolysis of the chloride ligands of cisplatin, leading to activated cationic species

capable of reacting mono- and bifunctionally. The aquation of cisplatin has been extensively investigated and reviewed [21-31]. The two hydrolysis steps, to *cis*-[PtCl(H₂O)(NH₃)₂]⁺ and further to *cis*-[Pt(H₂O)₂(NH₃)₂]²⁺ follow first-order kinetics and have been investigated in the absence and presence of nucleic acid and oligonucleotides.

Although activated (aquated) cisplatin can interact with various biomolecules [32], its antitumour activity derives from its capability eventually to form bifunctional DNA cross-links. The predominant adducts formed by cisplatin with DNA are bifunctional guanine-guanine (GG) intrastrand cross-links, where the chlorido ligands have been replaced first by a water molecule through aquation and then by the nitrogen N7 of adjacent guanine (G) residues [33]. This chelation of DNA triggers a specific distortion or bending (kinking) of the duplex. This particular platinum-induced kink in the DNA is considered to be the critical lesion due to the chain of events that the bimolecular recognition eventually provokes in the cell.

The coordination to N7 of guanines is not surprising as nitrogen N7 atoms of purines are the most electron-dense and accessible sites in DNA for electrophilic attack by platinum. They are exposed in the major groove of the double helix, and not involved in base-pair hydrogen-bonding. The major cisplatin-DNA adducts – comprising around 90% of all the adducts – both *in vitro* and *in vivo* were indeed found to be: 1,2-intrastrand d(GpG) cross-links (between adjacent guanines) in *ca.* 50-65%, and 1,2-intrastrand d(ApG) cross-links in *ca.* 25%, (between adenine and adjacent guanine from 5' to 3'). In addition, 1,3-intrastrand d(GpNpG) cross-links (intrastrand adducts between purines separated by one or more intervening bases) were present in

less than 10%, together with interstrand cross-links, monofunctional adducts to guanine residues, and DNA-Pt-protein cross-links (see Figure 1) [14, 15, 33-36].

[Figure 1]

The significant prevalence of intrastrand GG cross-links over any other adduct has been examined in the context of contributions of H-bonding, the electrostatics in the vicinity of the binding site, and the steric effects of adduct conformation. It has been argued that hydrogen bonding between the NH₃ of the DNA-cisplatin complex and O6 of a nearby guanine – not possible for adenine – has a large impact on the adduct configuration, being essential for stability and eventual cytotoxic activity [37-39]. The small size of the NH₃ group might also be important [40].

Lippard and coworkers published the first crystal structure of a *cis*-{Pt(NH₃)₂}²⁺ adduct of cisplatin with a dinucleotide [41]. The nucleobases are in *anti* conformation, and the two oxygens O6 atoms are on the same side of the platinum coordination plane, *i.e.* head-to-head orientation. The same group published the first crystal structure of the cisplatin fragment coordinated to a double-stranded oligonucleotide (a dodecamer), *cis*-[Pt(NH₃)₂{d(CCTCTG⁶G⁷TCTCC)·d(GGAGACCAGAGG)}] (see Figure 2, left) [42]. They observed rolling of the adjacent guanines towards one another by 49°; however the overall bend in the duplex could not be quantified. Three years after this crystal structure, the same group published the NMR structure of the same adduct in solution [43]. They found similar features, although more pronounced in solution (Figure 2, right). The rolling of the guanines towards one another was 61° leading to an overall helix bend angle of 78°. The NMR study also gave the unwinding of the helix at the site

of platination, which was 25°. Common to both structures is the coordination of the *cis*-{Pt(NH₃)₂}²⁺ fragment to **G**⁶ and **G**⁷ with bending of the duplex significantly towards the major groove without disruption of the Watson-Crick hydrogen bonding. As a result, the minor groove opposite the platinum adduct is widened and flattened, affording a geometry with characteristics of A- and B-type DNA.

[Figure 2]

Once cisplatin has reached and attacked DNA, the response of the cell towards repair of the damage is crucial for the anticancer activity of cisplatin. Cisplatin-DNA adducts are primarily removed by nucleotide excision repair (NER), which consists of recognition, incision, excision, repair synthesis and ligation [19]. If this repair mechanism fails the probability of cancer cell survival decreases.

Why is cisplatin-DNA damage not repaired? Lippard and coworkers have argued that GG cross-links, which help stabilise an opened and flattened minor groove, may be an essential structural feature in the recognition and binding of HMG-domain proteins, leading to “repair shielding” (see Figure 3); the damage persists and ultimately provokes apoptosis [44-46]. A second hypothesis is that cisplatin-damaged DNA hijacks HMG-domain proteins and other nuclear proteins away from their natural binding sites, leading to cellular stress and eventually cell death. These two hypotheses are not mutually exclusive [47].

[Figure 3]

In recent years a great progress has been made in this area and a number of proteins have been implicated in Pt-DNA adduct recognition [48, 49]. The TATA-

binding protein (TBP) binds to cisplatin-modified DNA [50], and appears to have higher specificity for Pt-damaged DNA than for the TATA box, being therefore sequestered and inhibiting *in vitro* transcription [51, 52]. Other proteins, such as p53, can enhance the sensitivity of the cells to the cisplatin [53], and contribute to increase protein binding [54].

DNA adduct formation of cisplatin-like drugs

There are other six platinum-based drugs in clinical use today, *i.e.* cisplatin, carboplatin, and oxaliplatin (*l*-OHP) worldwide, and nedaplatin, (254-S), lobaplatin, (D-19466), and heptaplatin (SKI 2053R) in Japan, China and South Korea, respectively (see Chart 1).

Carboplatin, for example, was developed in the search for a drug less toxic than cisplatin. It was thought that a platinum analogue with the same carrier ligands as cisplatin would form the same DNA lesions, maintaining cisplatin antitumour activity. In carboplatin, the ligands susceptible to nucleophilic substitution are the oxygen carboxylates of chelated cyclobutanedicarboxylate. By having less labile ligands, this analogue reacts with nucleophiles to a lesser extent than cisplatin, and is less toxic. Thus, it was found that carboplatin, is less toxic than cisplatin, with no significant renal or neural toxicity, and at the same time is as effective as, or even more active than cisplatin in some tumours. Due to the presence of the same carrier ligands (NH_3), carboplatin and cisplatin eventually form the same DNA adducts, and the differences between these two drugs is due to the much slower DNA binding kinetics of carboplatin [12, 55, 56]. The same scenario is expected for nedaplatin where the carrier ligands are

also ammonia groups. Thus the principle is established that not only thermodynamic and structural features are important in the design of metal complexes as drugs, but kinetic factors are too.

There appear to be few published studies on DNA adducts formed by lobaplatin or heptaplatin [57, 58]. The case is different for oxaliplatin. Oxaliplatin, (*trans*-1*R*,2*R*-diaminocyclohexane)oxalato platinum(II), first synthesised by Kidani *et al.* in 1978 [59], is the latest worldwide approved platinum-based anticancer agent. It has shown a broad spectrum of antitumour activity [60] with a partial or a non-cross-resistance with cisplatin in a wide range of human tumours *in vitro* and *in vivo* [60-63]. Oxaliplatin also has similar DNA-binding properties to its congener, leading to the same type of DNA-Pt cross-links, although the amount of the different types of adduct varies in comparison to cisplatin. Several studies show that oxaliplatin is less reactive and forms fewer adducts with cellular DNA than cisplatin. Additionally, there are two published structures of oligonucleotide-oxaliplatin adducts for two different dodecamers: a 2.4 Å crystal structure of a 1,2-d(GpG) intrastrand cross-link in a DNA dodecamer duplex formed by reaction with oxaliplatin [64], and a related NMR solution structure [65]. The crystal structure is comparable to that of a cisplatin adduct published by Takahara *et al.* The sequences of the oligonucleotides used were identical, 5'-d(CCTCTGGTCTCC), and the crystal structures of the two adducts are very similar. Therefore, the differences in cytotoxic activity of the two platinum complexes are not readily correlated with these structures. Wu and Chaney have made an attempt to explain these differences based on the solution structure of a similar dodecamer, with the sequence 5'-d(CCTCAGGCCTCC) [65, 66]. In addition, they reported very recently

the solution structures of the above DNA dodecamer duplex with and without a cisplatin 1,2-d(GG) intrastrand cross-link, and the detailed comparison with the 1,2-d(GG) intrastrand cross-link from the reaction of the same oligonucleotide with oxaliplatin. For example, when comparing average solution structures, the platinum-oligonucleotide adduct from reaction of the oligonucleotide with oxaliplatin bends the DNA by 31° (overall bend angle), while the *cis*-{Pt(NH₃)₂(GG)} adduct from cisplatin bends the DNA by 22° [67]. The different response of cellular proteins to these structurally-different DNA adducts may be responsible for the differences in cytotoxicity, and spectrum of activity against different tumours for oxaliplatin and cisplatin.

Therefore, the reason why oxaliplatin adducts lead to fatal lesions in cisplatin-resistant cell lines, and differences in mutagenicity in comparison to cisplatin and carboplatin, might be explained by the different discrimination of cisplatin and oxaliplatin adducts by cellular proteins [65].

In the search for new agents which can circumvent resistance, either inherent or acquired, to cisplatin by many tumours, new drugs are needed that exert their cytotoxicity through a different mechanism of action, *e.g.* via new types of DNA-damage. *Trans*-platinum complexes and multinuclear-platinum complexes are promising candidates as they can give rise to unusual DNA-adducts and hence different activity profiles compared to cisplatin and its analogues.

DNA-damage by platinum complexes with non-cisplatin-like structure

Complexes based on the square-planar geometry *cis*-[PtX₂(amine)₂] complexes produce very similar types of adduct on target DNA – as exemplified by cisplatin, carboplatin, and oxaliplatin. It is therefore not surprising that they induce similar biological responses. This consideration led to the hypothesis that development of platinum compounds structurally-different from cisplatin may lead to different types of DNA-Pt adducts, and to a different spectrum of clinical activity, perhaps complementary to cisplatin.

This is the case for *trans*-platinum complexes [68-73], and is also the case for multinuclear platinum complexes.

The most successful of this type of complex so far is [(*trans*-PtCl(NH₃)₂)₂{ μ -*trans*-Pt(NH₃)₂(NH₂(CH₂)₆NH₂)₂}]⁴⁺, BBR3464 – first reported in 1988 [74] (Chart 2). It has successfully finished [75] phase I after extensive *in vitro* and *in vivo* investigations [76-82]. As is general for polynuclear platinum compounds, BBR3464 shows rapid binding to DNA with a *t*_{1/2} of *ca.* 40 min probably due to its high charge and charge-mediated pre-association. The unwinding angle of 14° for supercoiled plasmid DNA is indicative of bifunctional DNA binding, with *ca.* 20% of the DNA adducts being interstrand cross-links, through binding to guanine bases [76]. BBR3464 forms monofunctional adducts with guanine residues that close to 1,4-interstrand cross-links on the self-complementary DNA 12-mer 5'-{d(ATATGTACATAT)₂} and octamer 5'-{d(ATGTACAT)₂}, with the two platinum atoms coordinated in the major groove at the N7 positions of guanines that are four base pairs apart on opposite DNA strands. The central tetraamine linker unit of [*trans*-H₂N(CH₂)₆NH₂Pt(NH₃)₂NH₂(CH₂)₆NH₂] is located in the minor groove [82, 83].

[Chart 2]

The most relevant feature of this special DNA binding is the lack of severe DNA distortions such as a kink, or significant unwinding of the helices, which are characteristic of DNA adducts of mononuclear platinum complexes. One of the direct consequences of this mild Pt-induced DNA conformational change is that these adducts appear to be poor substrates for recognition by proteins, such as those containing the HMG domain, which bind to rigidly-bent DNA, as found for cisplatin. This might be due to the central platinum unit lying non-covalently on the minor groove in the place in which an HMG phenylalanine intercalates in the case of the ternary adduct formed by HMG and DNA adducts of cisplatin (see Figure 3) [46]. The flexibility of the DNA-Pt adduct might also confer modest attraction for nucleotide-excision repair proteins.

Therefore, it seems that the recognition by HMG domain proteins of DNA-BBR3464 adducts is not a crucial step in the mechanism of cytotoxicity of BBR3464, as claimed for cisplatin. This would support the original hypothesis that polynuclear platinum compounds coordinating and modifying DNA in a different way than the mononuclear complex represent a novel class of platinum anticancer drugs acting by a different mechanism than cisplatin and its analogues.

A comprehensive overview of multi-nuclear platinum drugs has recently been published by Wheate and Collins [84, 85].

3. Photoactivatable platinum(IV) complexes

The oxidation state of platinum in complexes has a major effect on their geometry and reactivity. Octahedral platinum(IV) complexes may be advantageous in

medicinal chemistry because they are less susceptible to substitution reactions and so undergo fewer reactions on route to the tumour. This results in fewer undesired side-effects and reduced drug loss due to deactivation compared to square-planar platinum(II) complexes. In other words they can have reduced toxicity and higher activity, *i.e.* a higher therapeutic index. Another advantage of these complexes is their higher aqueous solubility. Precisely this feature of platinum(IV) complexes has been exploited by Tobe and coworkers, who selected iproplatin (CHIP, JM9, *cis,trans,cis*-[PtCl₂(OH)₂(isopropylamine)₂]) from a range of platinum(IV) complexes for its high aqueous solubility [86]. Iproplatin entered phase I and II clinical trials [87, 88], and even phase III [89], but was ultimately found to be less active than cisplatin and so has not been registered for clinical use [90]. Other complexes such as tetraplatin ([PtCl₄(D,L-cyclohexane-1,2-diamine)]), also called ormaplatin) [91] and orally administrable JM216 (satraplatin, *cis,trans,cis*-[PtCl₂(OAc)₂(NH₃)cyclohexylamine]) [92-94] have also reached clinical trials. More recently, LA-12, an analogue to the latter with an hydrophobic 1-adamantylamine ligand instead of cyclohexylamine (*OC-6-43*)-bis(acetato)adamantylamine(ammine)dichloridoplatinum(IV), has entered clinical phase I after successful pharmacokinetic, *in vitro* and *in vivo* studies [95-100].

Despite studies reporting *in vitro* binding of platinum(IV) species to nucleobase models in the absence of reductants [101-107], evidence that platinum(IV) species can enter cells [108], and that they can bind to DNA more slowly but at similar sites as their platinum(II) counterparts [109-111], it is believed that Pt^{IV} is likely to be reduced before undergoing substitution [112]. Reduction to platinum(II) is probably essential for activation and effective antitumour activity of platinum(IV) complexes

[113-118]. Platinum(IV) complexes would not be expected interact with nucleic acids *in vivo* due to the slow substitution kinetics compared to their reduction rates since substitution is slow. For this reason these complexes are often considered to be pro-drugs.

Controlled activation of platinum(IV) pro-drugs could form the basis of an effective drug design strategy. In the last few years we have been developing a new class of platinum complexes, which are inert until photoactivated by certain wavelengths of light, a procedure which might avoid the severe side-effects of currently-used platinum drugs.

Because anticancer agents stop replication of rapidly-dividing cells, *e.g.* healthy cells of the bone marrow, GI-tract, and skin, these cells are particularly vulnerable to chemotherapy. Additionally, the therapeutic index (*i.e.* correlation between toxic dose and curative dose) of active platinum(II) complexes is relatively narrow, so cancer cells need only to acquire a low level of resistance to a toxic agent to unbalance the ratio, and the treatment will eventually fail. In other words, classical platinum drugs have severe toxicity and the development of resistance can prevent the success of chemotherapy. There is, therefore, a clinical need to develop anticancer drugs with significantly reduced toxicity without diminishing efficiency. The groups of Bednarski and our own are developing new types of anticancer platinum-based drugs that are strong candidates to satisfy this need.

Since reduction to Pt^{II} appears to be an important step in the mechanism of action of Pt^{IV} anticancer complexes, control of when and where this reduction takes place, controls the activation of the pro-drug, and hence its toxicity. In general, many clinically useful antitumour agents are pro-drugs, and indeed their success is due to this fact. The use of photoactivatable Pt^{IV} complexes may allow the activation process to be controlled in an exquisite fashion and provide a new area for drug development.

Photochemical reduction of Pt^{IV} to Pt^{II} occurs by complex mechanisms. With appropriate choice of ligands, hexa-coordinated platinum(IV) photolabile complexes can be prepared. Then, the light-sensitive Pt^{IV} pro-drugs can be photoactivated to antitumour Pt^{II} agents directly at the site of the tumour with laser light.

Desirable features for an ideal photosensitiser include the following. First, stability under physiological conditions is essential with a plasma half-life long enough to allow accumulation, but at the same time the elimination time should be sufficiently rapid to reduce light-toxicity after treatment. Secondly, the longer the wavelength of activation, the greater the depth of penetration of the light, especially red and near infrared light (600-1000 nm, the so-called “phototherapeutic window”). It would also be convenient if the photosensitiser is oxygen-independent as cancer cells are often hypoxic. An ideal compound would also have minimal dark toxicity.

Two types of platinum(IV) photoactivatable anticancer complexes have been reported: diiodo complexes and diazido complexes.

The first photoactivated Pt^{IV} anticancer complexes contained iodide as a reducing ligand with general formula *trans,cis*- $[\text{Pt}(\text{X})_2\text{I}_2(\text{en})]$, where en = ethylenediamine and X = Cl, OH, acetate, or methylsulfonate [119]. Ethylenediamine, a

bidentate chelating ligand, was chosen as the non-leaving ligand to avoid photoisomerizations. The diiodo complexes have intense iodide-to-Pt^{IV} ligand-to-metal charge-transfer (LMCT) bands and are photolabile at *ca.* $\lambda = 400$ nm.

Pt-DNA binding modes range from irreversible platination when X = Cl or SO₂CH₃ (not affected by light), to no platination over 6 h in the case of X = OH, or to 25% platination only after light exposure in the case of X = OAc [119]. The results of the DNA-platination studies were confirmed by NMR studies in which 5'-GMP was used as a model [120]. Moreover, the complexes with X= Cl, OH, or SO₂CH₃ were found to be cytotoxic in a human bladder cancer cell line, but the differences between light and dark toxicities were not as great as had been expected [119].

Thus, the cytotoxicity and DNA binding studies involving irradiation of the diiodo-Pt^{IV} complexes were promising, but these complexes were not suitable as photoactivated drugs because they are too easily reduced by thiols in and outside of cell, causing a facile reduction of the diiodo-Pt^{IV} complexes to cytotoxic Pt^{II} species before light activation. Nevertheless, these studies showed that the development of photoactivatable Pt^{IV} complexes is feasible.

The diazido complexes developed in our laboratory, however, are stable enough to reduction and exhibit the main features required for a photoactivated drug: non-toxicity in the dark and retention of cisplatin-like activity when irradiated.

These azide-Pt^{IV} complexes have the general formula [Pt^{IV}(N₃)₂(OH)₂(am)₂], where only the hydroxo ligands are maintained *trans* to one another, and the am(m)ines can be *cis* and chelated as *e.g.* ethylenediamine (Chart 3) or *trans*. One possible

mechanism involves electron donation from the azido ligands to Pt^{IV} followed by combination of the azidyl radicals ($\bullet\text{N}_3$) (Figure 4) to form molecular nitrogen (N_2), preventing reoxidation of the platinum centre. This fast decomposition may contribute to the efficiency of photoredox reactions involving complexes containing azido ligands.

[Chart 3]

[Figure 4]

The stability in solution and towards potential targets such as the guanine bases of RNA and DNA in the dark has been investigated by NMR. The *cis* complexes, **1** and **2**, are stable towards hydrolysis for 90 days, inert to reaction with nucleobases such as 5'-GMP and d(GpC) in the dark and, most significantly, react only very slowly with GSH over a period of several weeks [101, 121].

Reactions of the ^{15}N -labelled complexes, **1** and **2**, have been followed in aqueous solution in the presence of 5'-GMP and d(GpG), by 1D ^1H , 2D [^1H , ^{15}N] HSQC, and 2D [^1H , ^{15}N] HSQC-TOCSY NMR spectroscopy.

The complexes are cytotoxic toward 5637 human bladder cancer cells under irradiation. Nucleotide cross-linking induced by photoreactions of **1** and **2** was observed. The formation of cisplatin-like bifunctional intrastrand GG adducts in a 212-bp fragment of pSP73KB plasmid and in a 40-mer DNA duplex, has been demonstrated with light of various wavelengths [101, 121, 122].

The all-*trans* complex *trans,trans,trans*- $[\text{Pt}^{\text{IV}}(\text{N}_3)_2(\text{OH})_2(^{15}\text{NH}_3)_2]$, **3**, has comparable cytotoxicity to cisplatin towards human HaCaT keratinocytes, and lacks toxicity to cells in the dark. Additionally, it has a much higher aqueous solubility (>20 mM) than the *cis* isomer **1** and more favourable absorption spectrum, with a LMCT

band more intense and shifted to longer wavelength, *i.e.* more penetrating. Photoreactions and nucleotide interactions have been also investigated in the presence of 5'-GMP by 1D ^1H and 2D [^1H , ^{15}N] HSQC NMR techniques [123], showing stability in the dark and reaction only upon irradiation.

In summary, the Pt^{IV} diazido-complexes **1**, **2**, and **3** are stable under biological conditions, but are reduced and bind to nucleotides upon radiation with light of various wavelengths. They are phototoxic towards several different cell lines, including cisplatin-resistant cells, while remaining inactive in the dark. So far, investigations into the mechanism of action of these compounds indicate that they exert their cytotoxic effect in a different way to cisplatin. This new class of photoactivatable Pt^{IV} -azide complexes has the potential to avoid the side-effects associated with cisplatin, whilst having the advantage of being oxygen-independent.

Recently, a highly potent complex from this class containing pyridine *trans* to ammonia has been synthesised. The complex *trans,trans,trans*- $[\text{Pt}^{\text{IV}}(\text{N}_3)_2(\text{OH})_2(\text{NH}_3)(\text{pyridine})]$, **4**, was found up to 80 times more cytotoxic than cisplatin in the ovarian cancer cell line A2780 whilst inactive and stable toward biological reductants in the dark. It binds to DNA less regularly than cisplatin and mainly to guanines and cytosines, *i.e.* like transplatin. It also binds, but to a less extent, to adenines. Complex **4** was found to form mainly *trans* azido/guanine, and then *trans* diguanine Pt^{II} adducts, which are probably mainly intrastrands between two guanines separated by a third base. It also forms bifunctional interstrand crosslinks with DNA although the percentage is very low (6%) and their relative efficacy is unknown. This may suggest that most of the DNA interactions involve intrastrand crosslinks and/or monofunctional lesions. DNA repair synthesis of these adducts on plasmid DNA was

found to be markedly lower than that induced by either cisplatin or transplatin. Finally, data from ethidium fluorescence assays suggest that the damage to the DNA spans more base pairs around the platinated site than cisplatin [124].

Photoactivation can apparently lead to the formation of platinated DNA lesions which are not as readily repaired as those formed by conventional Pt^{II} anticancer complexes, especially by *trans* diam(m)ine complexes [123]. This may lead to novel mechanisms of action and therefore to clinically useful drugs [3].

4. Ruthenium arene complexes

In the 1970's Clarke *et al.* reported pentaammine(purine)ruthenium(III) complexes capable of inhibiting DNA and protein synthesis in human nasopharyngeal carcinoma cells *in vitro* [125], which triggered interest in ruthenium complexes as potential anticancer pharmaceuticals [126]. During the following decade, Mestroni *et al.* developed hexacoordinated Ru^{II} complexes with dimethylsulfoxide and chloride ligands, particularly, *cis*- and *trans*- $\text{RuCl}_2(\text{dimethylsulfoxide})_4$, which exhibit anticancer activity *in vitro* and *in vivo*. The complexes were shown to interact both *in vitro* and *in vivo* with DNA, their most likely target [127].

Today, there are two Ru-based anticancer drugs in clinical trials: NAMI-A, developed in Trieste by Mestroni, Alessio, and coworkers, and KP1019, developed by Keppler and coworkers in Vienna (Chart 4).

[Chart 4]

NAMI-A has a relatively low toxicity towards cancer cells, but is particularly effective against metastases from solid tumours both in experimental mouse tumours and against human tumours engrafted in the nude mouse [128-131], being scarcely effective in solid tumours. The drug has now completed phase I clinical trials [132].

However, in contrast to the other metal-based anticancer drugs reviewed here, the antimetastatic activity of NAMI-A is thought to be due to the combined effects on the control of angiogenesis (possibly because it interferes with NO metabolism *in vivo*) [131, 133] and anti-invasive properties towards tumour cells and on blood vessels, and not to its interaction with nucleic acids, although it does interact with DNA *in vitro* [129].

There is a number of reviews on the preclinical development of NAMI-A and the different approaches concerning the mechanisms of action of this type of drug by Sava *et al.* [134, 135].

Indazolium *trans*-[(tetrachloride)bis(1H-indazole)ruthenate(III)] (KP1019 or FFC14A) exhibited cytotoxic activity against colon carcinomas in rats in preclinical investigations [136-138]. The preclinical development of KP1019 has been recently reviewed [7, 139, 140].

Octahedral ruthenium(III) complexes are relatively inert towards ligand substitution [141]. The reduction of ruthenium(III) to ruthenium(II) as an activation process prior to DNA binding was first suggested in the late 1970's by Kelman *et al.* [125, 126, 142]. Correlations between the Ru^{III} reduction potential and increased antiproliferative activity for the colon carcinoma cell line SW480 in the series

$[\text{Ru}^{\text{III}}\text{Cl}_4\text{X}_2]^-$, where X is 1H-imidazole, 1H-indazole, or triazole [143-145], confirm this belief. KP1019 is generally considered a pro-drug, with a reduction potential accessible to biological reductants [144, 146]. Although it has been suggested that KP1019 undergoes activation *in vivo* through reduction from Ru^{III} to Ru^{II} , and then promotes interactions with biomolecules after labilization of the $\text{Ru}^{\text{II}}\text{-Cl}$ bonds, aquation of the $\text{Ru}^{\text{III}}\text{-Cl}$ bond has also been investigated [147, 148]. It was found that KP1019 undergoes aquation in aqueous solution in a similar time-scale to that of cisplatin. DNA has been proposed as one of the biological targets of KP1019, and it has also been reported that the drug triggers apoptosis [149]. However, the cellular mechanism of the activation of apoptosis are still under investigation [140, 144, 150].

Recently, we have explored the potential of $\text{Ru}^{\text{II}}(\text{arene})$ complexes $[(\eta^6\text{-arene})\text{Ru}(\text{X})(\text{Y})(\text{Z})]$ as antitumour drugs. They possess characteristic “piano-stool” structures (Chart 5, where X-Y is a neutral chelating ligand, and Z is monoanionic). In these complexes ruthenium is already in its lower (2+) oxidation state which may be important for the cytotoxicity of the drug *in vivo*. The arene ligand, binding as an η^6 electron-donor, and a π -acceptor, confers stability to the +2 oxidation state. The presence of a chelating ligand, X-Y, seems to provide additional stability to the whole structure. The ligand Z is the “leaving group”, such as a halide, and allows activation of the molecule: if labile it can provide a coordination site for biomolecules. Small variations of the arene and the “legs” of the stool afford versatility to the molecule and the possibility of fine-tuning their pharmacological properties.

[Chart 5]

In general Ru-arene complexes show promising cytotoxic activity against human ovarian cancer cell lines, some complexes being as potent as cisplatin and carboplatin. Some structure/activity relationships have been established [151-154]. As an example, when the linker is ethylenediamine and the leaving group is chloride, the cytotoxicity against A2780 human ovarian cancer cells increases with increasing size of the coordinated arene. However, substitution of chloride by other halides has only a small effect on the cytotoxicity of ethylenediamine complexes. Additionally, substitution of the chelating ligand for labile ligands such as other chlorides, acetonitrile or isonicotinamide, led to less active complexes, perhaps due to inactivation of a too-reactive complex before reaching the target. Changing the nature of the donors in the chelating ligand (*e.g.* from a diamine to an azopyridine, or changing an N to an O donor) has also been investigated but the structure-activity relationships become more complicated [153, 155].

Ruthenium(II) complexes often exhibit good solubility in aqueous solution, a feature of great importance for a convenient administration of potential drugs. Such activation through hydrolysis of the Ru-Cl bond may be important for the mechanism of activation of this type of complex, and their chemical behaviour in aqueous media has been extensively investigated [153, 154, 156-158]. In general, arene ruthenium complexes that hydrolyse also exhibit cancer cell cytotoxicity, whereas those that do not undergo little aquation and are not active.

Once the Ru(arene) complex is activated and the aqua species $[(\eta^6\text{-arene})\text{Ru}(\text{X})(\text{Y})(\text{OH}_2)]$ has been formed (Chart 5), the ruthenium centre is a potential

target for nucleophilic attack by biomolecules. The binding of ruthenium(II) arene complexes to nucleobases and DNA in particular is of special interest, as this may be one of the ultimate targets for this type of agent. A number of studies have confirmed this postulate[157, 159] and have investigated in detail such interactions [83, 151, 152, 154, 160-162].

Reactions of ruthenium arene complexes of formula $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}](\text{PF}_6)$, where en = ethylenediamine, arene = biphenyl, dihydroanthracene, tetrahydroanthracene, *p*-cymene, or benzene (complexes **5**, **6**, **7**, **8** and **9** in Chart 6) and aqua adducts, and guanine, thymine, cytosine, and adenine derivatives have been investigated [83]. The reactivity of the various binding sites of nucleobases towards Ru^{II} at neutral pH decreases in the order $\text{G}(\text{N}7) > \text{T}(\text{N}3) > \text{C}(\text{N}3) > \text{A}(\text{N}7), \text{A}(\text{N}1)$ (with binding to cytosine being very weak, and almost none to adenine derivatives). This base selectivity appears to be enhanced by the ethylenediamine NH_2 groups, which form hydrogen bonds with exocyclic oxygens (*e.g.* $\text{C}6\text{O}$ of G) but are non-bonding and repulsive towards exocyclic amino groups of the nucleobases (*e.g.* $\text{C}6\text{NH}_2$ of A) (Chart 7). This pattern of selectivity is found for 5'-mononucleotides [83]. It also appears that the phosphate group might play a role in the binding of these complexes to mono-nucleotides, being the initial binding site before transfer of ruthenium to the base. Reactions with nucleotides appear to proceed *via* aquation of $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}]^+$ followed by rapid binding to the 5'-phosphate group, and then rearrangement to give N7-, N1- or N3-bound products.

[Chart 7]

Ruthenium arene complexes **5**, **6**, **7**, **8** and **9** have been found to interact with natural DNA in a cell-free medium [163]. The kinetics of the binding for those with extended arenes (**5**, **6** and **7**) to natural double-helical CT DNA is about an order of magnitude faster than that for cisplatin. The *p*-cymene complex has slower kinetics. The DNA binding is quantitative in every case and the Ru-DNA adducts are stable. Transcription mapping experiments have shown that all the complexes bind preferentially to guanine residues in double helical DNA. For complexes **5**, **6** and **7**, with extended π -electron systems, in addition to coordination to guanine, non-coordinative, hydrophobic interactions between the arene ligand and DNA can occur. These may include arene-base stacking, and arene intercalation, and minor groove binding, and are thought to contribute to the driving force for the binding of chloro Ru^{II} arene complexes to double-helical DNA [157]. The data obtained from CD and polarographic analyses of Ru^{II}-modified DNA with complexes **5**, **6** and **7** demonstrate that monofunctional adducts of these compounds distort the DNA conformation and that the distortion has a nondenaturational character as it is for cisplatin [164]. Complex **4** distorts the DNA conformation and thermally destabilises DNA more severely, and even denaturational changes can occur. The unwinding angles of supercoiled pSP73KB DNA induced by Ru^{II} arene complexes are very similar for **5**, **6** and **7**, but markedly smaller for **8**. The linear dichroism (LD) data suggest that there might be insertion into the minor groove, and the displacement of the intercalator ethidium bromide is more noticeable by **5**, **6** and **7** than for the *para*-cymene-Ru complex [163]. When comparing complexes **7** and **8**, the complexes with tetrahydroanthracene and *p*-cymene, the lesser destabilisation of the DNA in the case of complex **7** is attributable to additional hydrophobic interactions. As an intercalator, the complex can thermodynamically

stabilise DNA by lengthening and unwinding DNA, increasing the phosphate spacing along the helix axis. The nature of the arene can also influence the level of repair synthesis of ruthenium-damaged DNA. When the arene is tetrahydroanthracene, the level of repair by nuclear excision repair proteins (NER) is lower than for *p*-cymene as arene, or for cisplatin [159]. This observation provides additional support for a mechanism underlying antitumour activity of Ru^{II} arene compounds different from that of cisplatin.

Additional investigations into new ruthenium organometallic complexes with extended arenes such as $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}]^+$, where the arene is *ortho*-, *meta*- or *para*-terphenyl also sustain these findings [165].

In order to investigate nucleoside selectivity we synthesised Ru-arene complexes with an H-bond acceptor *O,O*-chelating ligand such as acetylacetonate (acac) in the place of the H-bond donor *N,N*-chelating ligand such as ethylenediamine (en). If our hypothesis was correct these complexes should show inverted selectivity. We observed that the affinity of acac-type complexes for adenosine (N1- and N7-bound) can be greater than for guanosine, and there is little binding to cytidine or thymidine [153, 158].

Due to the success of these complexes as anticancer drug candidates, and taking into account that they coordinate to DNA only monofunctionally as opposed to lethal bifunctional cisplatin-DNA adducts, bifunctional Ru-arene analogues were synthesised [166]. Surprisingly, for the dinuclear complexes $[\eta^6:\eta^1\text{-C}_6\text{H}_5(\text{CH}_2)_n\text{NH}_2]\text{RuCl}_2$ 1 ($n = 3$) and 2 ($n = 2$), both single hydrolysis and binding to one molecule of the model base 9-ethylguanine is relatively fast, whilst the second

hydrolysis step and further reactions with nucleobases is slow and the binding weak. The unwinding angle induced in negatively supercoiled DNA (9°) is less than that induced by bifunctional cisplatin binding (13°). It was found that only monofunctional DNA adducts formed (by interstrand assays and by the Ru-DNA adducts failing to produce stopsites for pSP73KB plasmid DNA transcription by RNA polymerase). Perhaps less surprising was finding the complexes exhibit little cytotoxicity toward the ovarian cancer cell line A2780 ($IC_{50} > 100 \mu M$) [166].

Since the intracellular tripeptide glutathione (γ -L-glutamyl-L-cysteinylglycine, GSH) is present at millimolar concentrations in cells and can inactivate cisplatin, reactions of Ru^{II} -arene complexes with guanine derivatives have been studied in the presence of excess GSH. These studies have revealed a new mechanism for DNA ruthenation involving oxidation of coordinated S-bound glutathione to the sulfenate which weakens the Ru-S bond and allows guanine to coordinate [154].

Recently, Liu *et al.* have studied the interaction of the monofunctional fragment $\{(\eta^6\text{-biphenyl})Ru(en)\}^{2+}$ with the 14-mer d(ATACATGGTACATA)-d(TATG¹⁸TACCATGTAT) by HPLC-ESI-MS and 2D NOESY NMR. Single strands can be ruthenated at specific guanines and purified by HPLC, but on heating and annealing with the complementary strand, ruthenium migrates and ruthenation is found at N7 of every guanine residue of the 14-mer duplex. At one site (G¹⁸), two different conformers were identified by NMR. In one conformer, intercalation of the arene between G¹⁸ and adjacent T is observed. In the second

conformer, the arene is non-intercalated but stacked on a tilted adjacent thymine, lying on the surface of the major groove (Figure 5) [161].

[Figure 5]

Some appealing DNA interactions have been found for the dinuclear Ru-arene complex $[\{(\eta^6\text{-biphenyl})\text{RuCl(en)}\}_2(\text{CH}_2)_6]^{2+}$ (**10** in Chart 8, en = ethylenediamine) [157]. Both this binuclear complex and its half unit $[(\eta^6\text{-biphenyl})\text{RuCl(Et-en)}]^+$ (**11** in Chart 8, where Et-en is $\text{CH}_3\text{CH}_2(\text{H})\text{NCH}_2\text{CH}_2\text{NH}_2$) show a very surprising and interesting feature. These organometallic complexes have dynamic stereogenic centres in aqueous solution. Dynamic chiral recognition can play a role in the interaction of the dinuclear complex with chiral DNA allowing selection of the most favourable diastereomer for binding. The dinuclear complex **10** induced a large unwinding (31°) of plasmid DNA, twice that of mononuclear **11** (14°). This value is very high, even compared to multinuclear platinum complexes [167]. Can it be due to intercalation of the two pendant phenyl rings? Complex **10** effectively inhibited DNA-directed RNA synthesis *in vitro*. This dinuclear complex gave rise to interstrand cross-links on a 213-bp plasmid fragment with efficiency similar to bifunctional cisplatin, and to 1,3-GG interstrand and 1,2-GG and 1,3-GTG intrastrand cross-links on site-specifically ruthenated 20-mers. Complex **10** blocked intercalation of ethidium considerably more than mononuclear **11**, where both the free arene rings and the linker are believed to contribute. The ruthenium dimer, **10**, introduces the concept of induced-fit recognition of DNA by organometallic ruthenium complexes containing dynamic stereogenic centres via dynamic epimerization, intercalation, and cross-linking.

5. Osmium arene complexes

Recently, we have investigated the chemical and biological activity of analogous half-sandwich osmium arene complexes [168-170]. Osmium, the heavier congener of ruthenium and a third row transition metal, commonly exhibits slower kinetics than ruthenium, and is often considered to be relatively inert. However, it is apparent that it is possible to tune the biochemical reactivity of the Os^{II} -arene complexes through understanding their aqueous solution chemistry. Thus, active Os^{II} -arene complexes have been designed [171, 172]. Peacock *et al.* found that the chelating ligand plays a major role in the stability, nucleobase binding, and cancer cell toxicity. They synthesised complexes containing a series of *N,O*-chelates, and found that the choice of the type of N- and O-donors is crucial. Stability toward formation of inactive hydroxo-bridge dimers (which can form at micromolar concentrations under similar conditions to those in cell culture media) and therefore activity of the osmium complexes is provided by the more acidic chelating oxygen donors and the introduction of a π -acceptor nitrogen such as pyridine, which reduces the electron density on the metal lowering the pK_{a} of the coordinated water of the aquo- Os^{II} adduct [171].

DNA binding studies on a series of complexes of the type $[(\eta^6\text{-arene})\text{Os}(\text{LL})\text{Cl}]^{\text{n}+}$, where arene = biphenyl or *p*-cymene and LL = ethylenediamine, picolinate, or oxinate (see Chart 9, complexes **12-15**) [173], show that these complexes all bind and distort polymeric DNA with a rate of binding comparable to that of cisplatin. These complexes vary in cytotoxicity and the toxicity does not appear to correlate with the extent of cell uptake. The extent of the interaction with DNA correlates well with the cytotoxicity of these osmium complexes. They coordinate mainly to guanine with additional non-coordinative interactions, and only complex **13**

undergoes quantitative reaction with DNA. Osmiated-DNA inhibits RNA synthesis like cisplatin and the ruthenium analogue of **12** (complex **5** in this review). Surprisingly, ligation experiments suggest that no DNA bending occurs, which differs from the behaviour of cisplatin. The unwinding angle induced in supercoiled plasmid DNA by osmium(II) arene complexes is large (21-27°) in comparison to the ruthenium analogues (7-14°) or cisplatin (6° for monofunctional and 13° for bifunctional adducts). For complexes **12**, **13** and **14** this may arise from intercalation of the arene interacts into the duplex. Complexes **12** and **13**, with extended electron-rich π -systems, can displace the intercalator ethidium bromide from DNA, so supporting this hypothesis.

5. Conclusions and perspectives

The introduction of platinum drugs into the clinic for cancer treatment has been very successful and their mechanism of action is known to involve attack on DNA. Structural distortions of DNA induced by platination are important to protein recognition and the downstream events which lead to apoptosis and cell death. Hence there is current focus on the design of new agents which can induce different DNA structural distortions compared to cisplatin. These new agents may then be effective in cases where cisplatin is not and also circumvent cisplatin acquired resistance. Recent developments include di-nuclear and tri-nuclear platinum complexes which can cross-link distant sites on DNA, and *trans* complexes. Photoactivatable diazido platinum(IV) complexes appear to produce lesions of DNA which are more difficult to repair than those produced by cisplatin and provide the basis for a novel form of photochemotherapy.

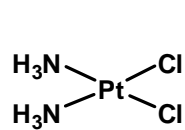
Organometallic ruthenium(II) monoarene complexes appear to be non-cross-resistant with cisplatin and can also distort DNA despite being only monofunctional. DNA base-selectivity can be achieved by coordination of ruthenium to N7 of G, H-bonding between an amine such as ethylenediamine chelated to Ru^{II} and the C6O of G, as well as intercalation of the arene if it has an extended structure (*e.g.* biphenyl or an anthracene derivative). Intriguingly cytotoxic osmium(II) arene complexes appear to induce significant unwinding of double-helical DNA but cause little bending, in contrast to ruthenium analogues and to cisplatin. Further work is necessary to understand the subtleties of such DNA interactions and to optimise the design of osmium arene complexes for activity.

Acknowledgements

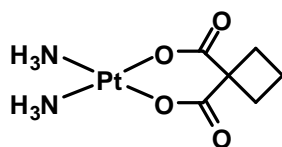
We thank the MRC, EPSRC, EC and Körber Foundation for their support for our work, our co-workers for collaboration, and EC COST Action D39 for stimulating discussions.

**STRUCTURAL FORMULAE, CHARTS, CAPTION TO FIGURES AND
FIGURES**

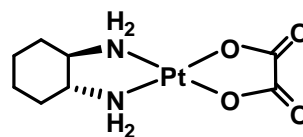
Chart 1



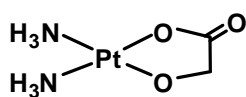
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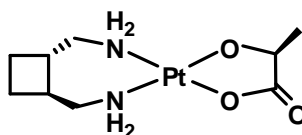
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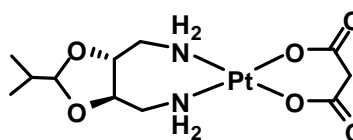
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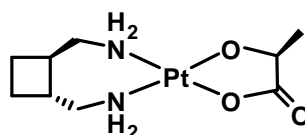
nedaplatin



lobaplatin, LP-D1



heptaplatin



lobaplatin, LP-D2

Chart 2

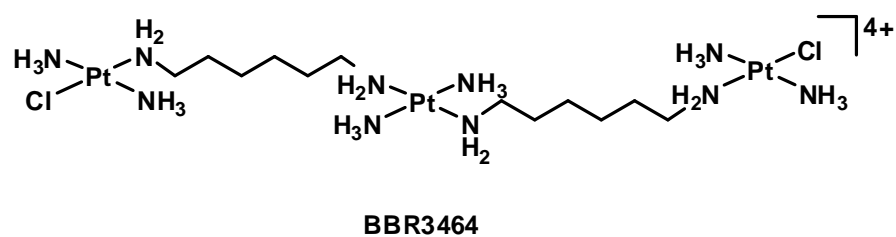


Chart 3

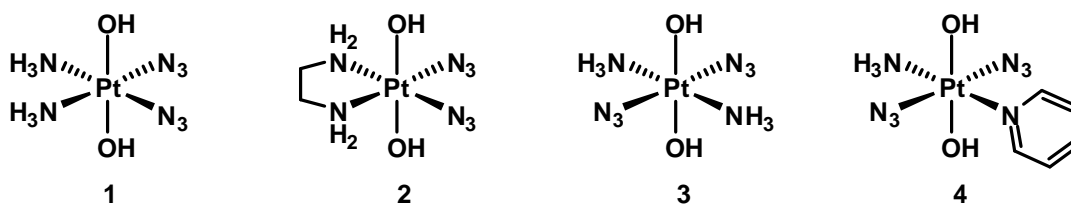
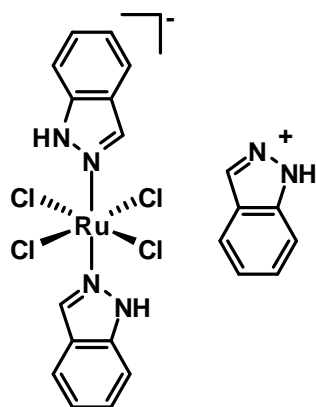
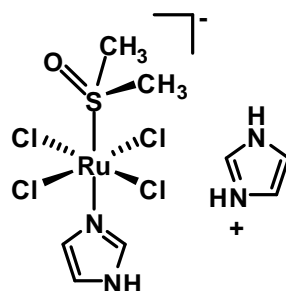


Chart 4



KP1019



NAMI-A

Chart 5

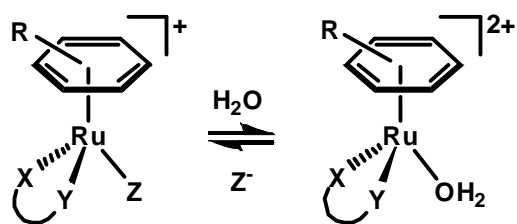


Chart 6

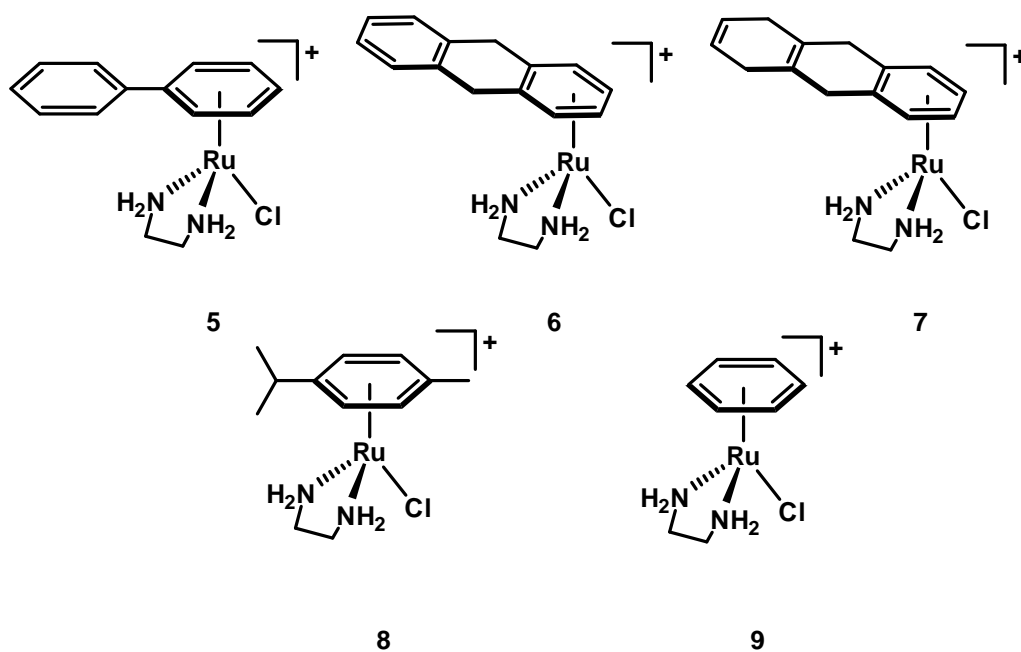


Chart 7

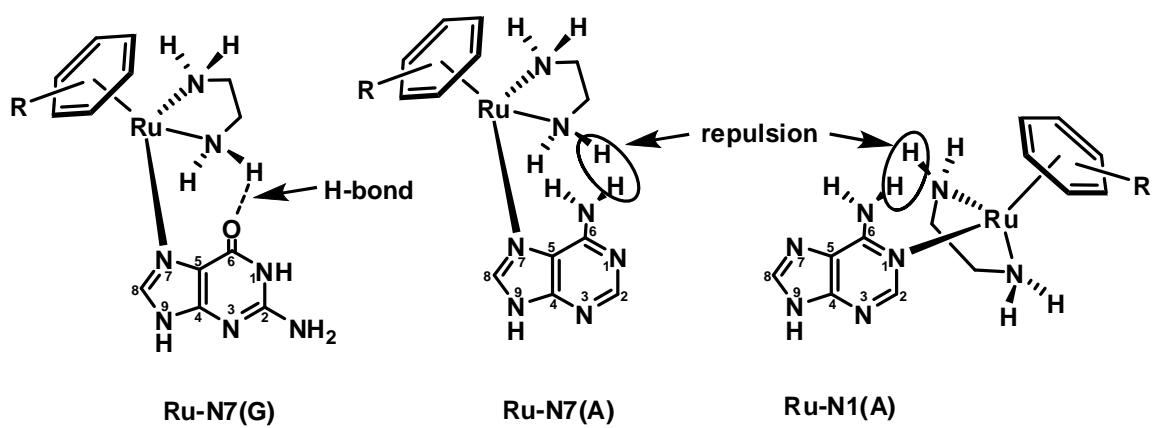


Chart 8

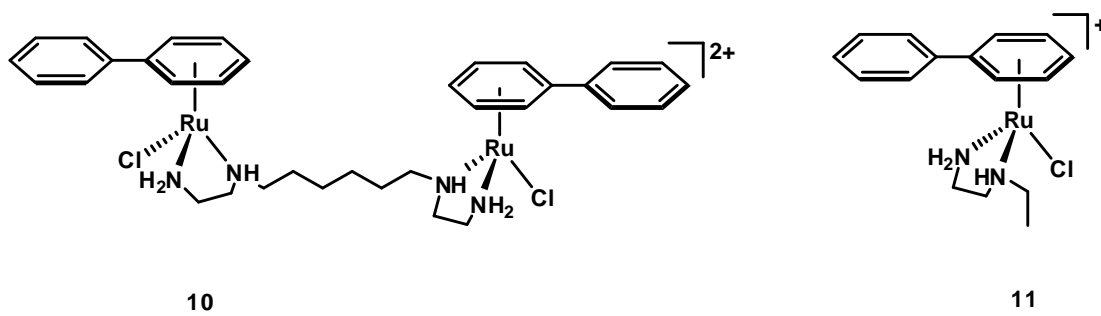
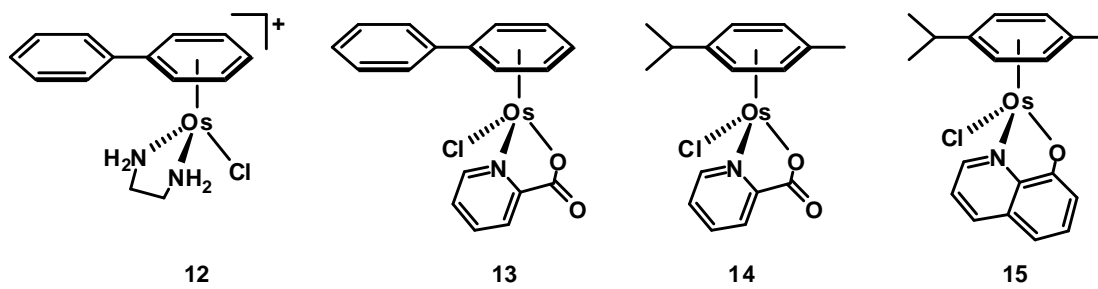


Chart 9



CAPTIONS TO FIGURES

Figure 1. Different types of DNA-cisplatin adducts for a given random sequence. The 1,2-intrastrand GG (top left) is believed to be the critical lesion. Y = H₂O/OH, Cl, protein.

Figure 2. X-ray structure [42] (left) and NMR solution structure [43] (right) of the adduct formed by interaction of cisplatin and the 12-mer d(CCTCTGGTCTCC)-d(GGAGACCAGAGG). Pt, pink; N, deep blue; O, red; P, orange; C, grey. (PDB: 1AIO and 1A84, respectively).

Figure 3. Adduct formed between the nonsequence-specific domain A of HMG1 and cisplatin-modified DNA. Pt, pink; N, deep blue; O, red; P, orange; C, grey. HMG is a purple ribbon with a intercalating phenylalanine highlighted in yellow. (Coordinates taken from the Protein Data Bank, 1CKT) [46].

Figure 4. Possible mechanism for the photoreduction of a Pt^{IV}-diazido complex.

Figure 5. Molecular models of two conformers of duplex dodecamer d(ATACATGGTACATA)-d(TATG¹⁸TACCATGTAT) ruthenated at N7 of G¹⁸ with monofunctional fragment {(η⁶-biphenyl)Ru(en)}²⁺. (A) showing the intercalation of the arene between G¹⁸ and T¹⁷. (B) in which the arene is non-intercalated but stacked on a tilted T¹⁷. Color code: en, dark blue; biphenyl, green; ruthenium, purple (Adapted from ref. 161).

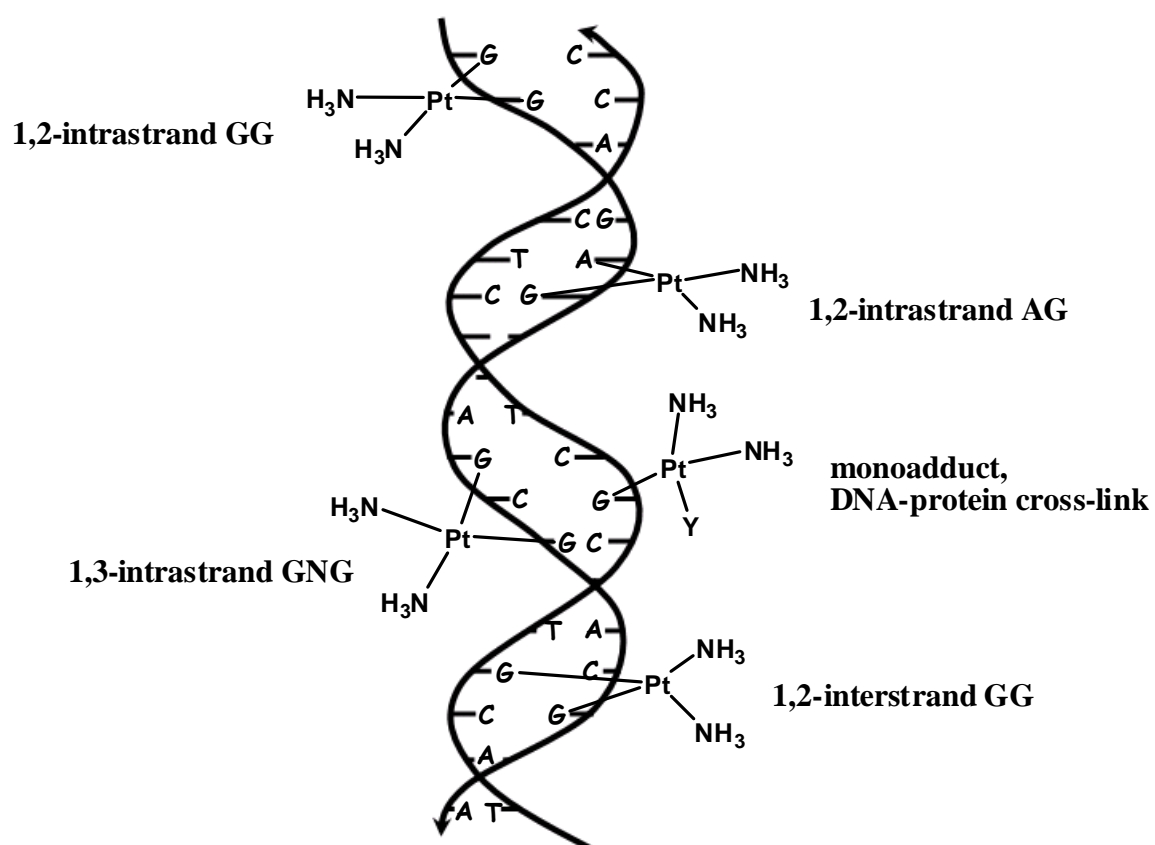


Figure 1

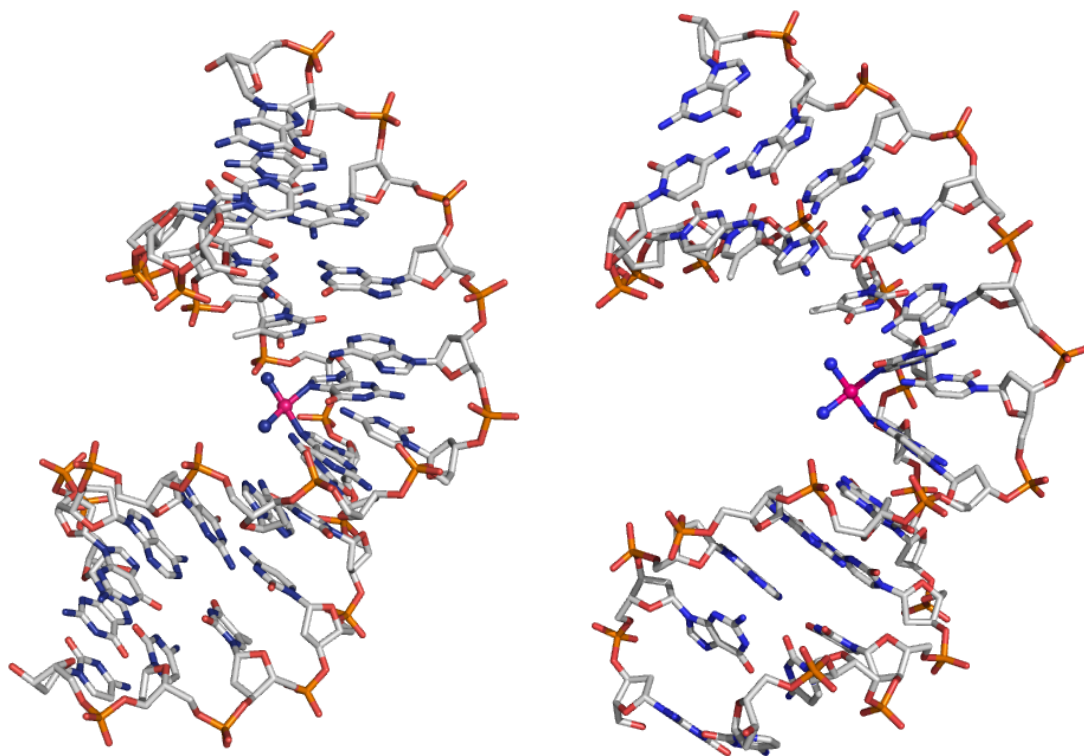


Figure 2

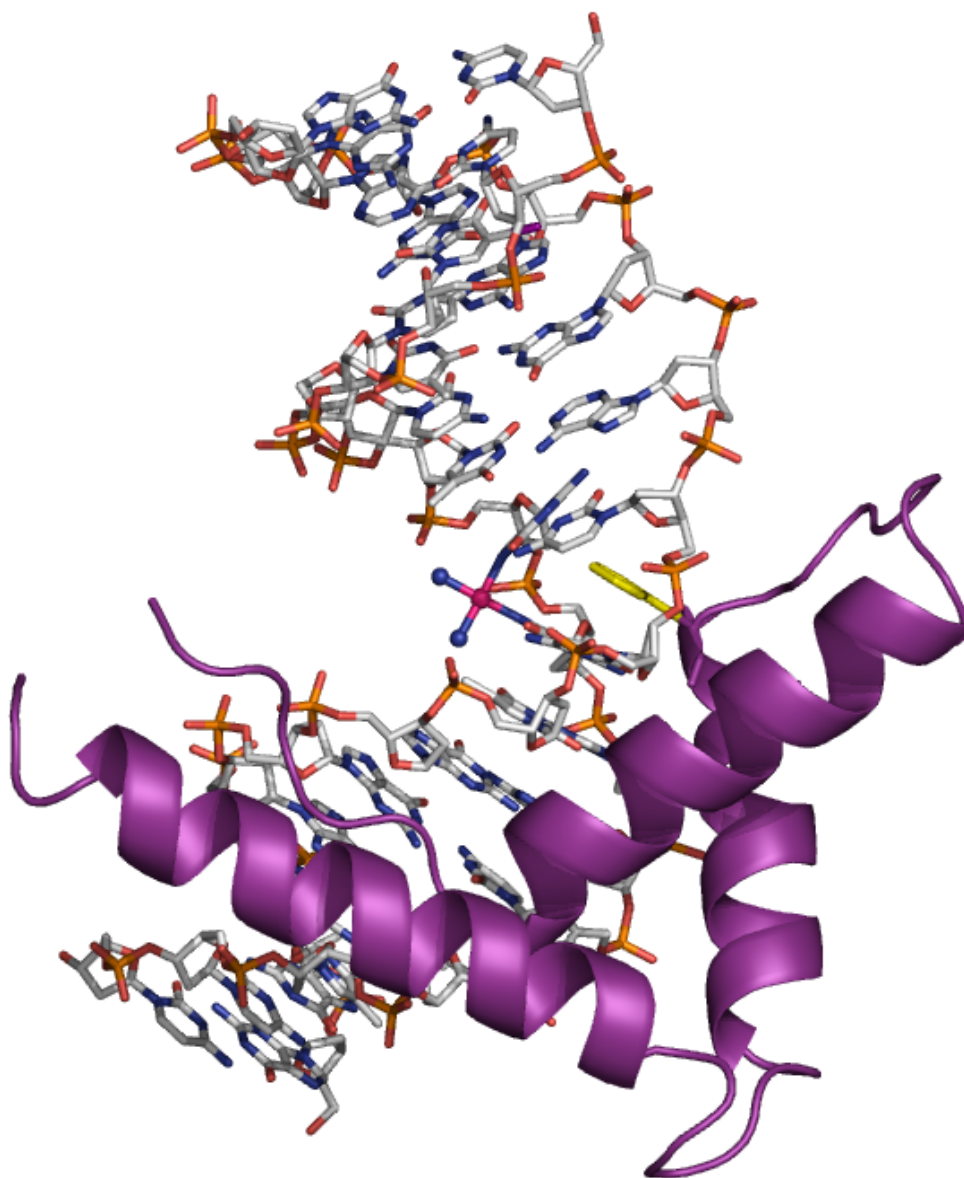


Figure 3

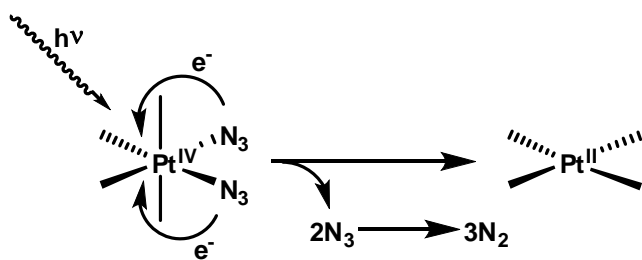


Figure 4

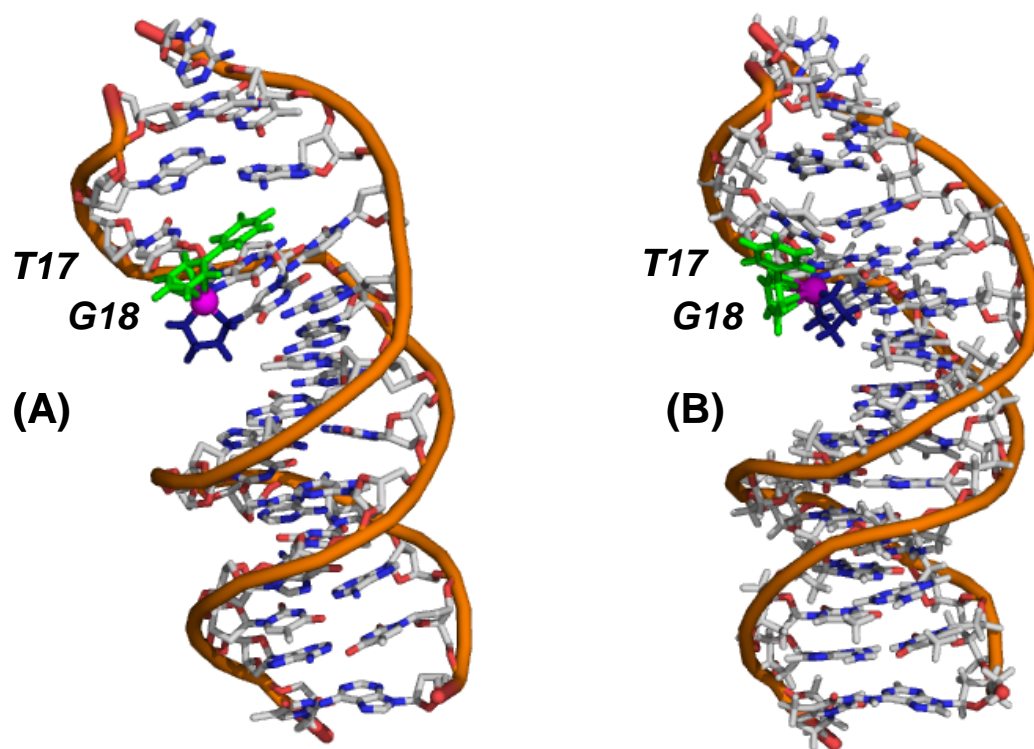


Figure 5

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