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Metal-based anticancer drugs: From a past anchored in platinum chemistry to a post-genomic future of diverse chemistry and biology*,**

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Abstract: The field of metal-based anticancer drugs was initiated by cisplatin, one of the leading agents in clinical use. Cisplatin acts by binding to DNA and forming 1,2 intrastrand cross-links. Its importance is reflected by the fact that it is estimated that 50–70 % of cancer patients are treated with a platinum drug [7]. For some time, molecular designs in the metallo-drug field remained obdurately anchored in *cis*-diamine platinum(II) chemistry, but now the field is evolving rapidly with a variety of alternate and very diverse designs being explored. These designs give rise to new spectra of activity and potency and can circumvent cisplatin resistance. This critical review considers the existing clinical platinum drugs, and those currently in commercial development, alongside the new designs including ruthenium anticancer and antimetastatic drugs in clinical trials, polynuclear drugs, organometallic drugs, titanium and gallium drugs, and emerging supramolecular metallo-drugs that act on DNA by noncovalent interactions. The rapid evolution of the field is being informed by post-genomic knowledge and approaches, and further dramatic step-change breakthroughs can be expected as a result; harnessing this knowledge and responding to and taking advantage of this new environment requires integration of chemistry and biology research.

Keywords: metallo-drugs; bioinorganic; DNA; cisplatin; supramolecular.

INTRODUCTION

While many drug molecules are "organic" in nature, other elements in the periodic table, particularly metals, offer a much more diverse chemistry and have important therapeutic applications [1]. In modern medicine, the most striking example is cisplatin (platinol; BristolMyersSquibb), a metal coordination compound containing no organic units and which is currently one of the leading drugs used against cancer [2–6]. The impact of cisplatin can hardly be overstated. Its arrival in the clinic in 1978 suddenly allowed patients to be cured from diseases (such as testicular cancer) which were untreatable. Three other structurally related platinum drugs (carboplatin, nedaplatin, and oxaliplatin) have also entered widespread clinical use and together these have sales in excess of USD 2 billion per annum. Their efficacy and importance is emphasized by the fact that there is hardly any clinical regimen of combination chemotherapy today that does not contain cisplatin or another platinum drug. With the exception of

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**Dedicated to Prof. Einar Sletten, on the occasion of his formal retirement.
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breast and prostate tumors, almost all the most common tumors, plus an increasing number of other less frequent malignancies, are treated with platinum drugs often in combination with other chemotherapeutics: indeed, it is estimated that as many as 50–70 % of cancer patients are treated with a platinum drug [7]. Herein, the use and action of these platinum drugs will be discussed along with an overview of some of the other platinum drugs that are currently in commercial development. The field of metal-based drugs is widening rapidly beyond platinum chemistry, and a variety of quite different and distinctive approaches and biomedical targets will also be discussed together with a critical consideration of the potential and outlook for such agents.

PLATINUM DRUGS

The drug cisplatin (Fig. 1) contains a square-planar platinum(II) center coordinated to two ammonia ligands and two chloride ligands with a *cis*-ligand conformation. Its activity was discovered by chance in an experiment looking at the effect of electric fields on the growth of bacteria and using platinum electrodes. It is a testament to the tenacity of Rosenborg and his coworkers that the active compound was identified and the chance observation led to such a powerful drug [8]. The mode of action is now widely accepted to be through the drug's interaction with DNA. The compound is administered by injection into the bloodstream and is believed to remain in its neutral state until after it crosses the cell membrane where one or both chlorides are displaced by aqua ligands (the chloride concentration being lower inside than outside the cell) affording cationic compounds. These cationic aqua derivatives react with the bases on DNA, most commonly with the N7 of purine bases (with guanine favored over adenine) which displace the aqua/chlorido ligands. A bifunctional adduct is formed between the {*cis*-$Pt(NH_3)_2$} unit and two adjacent bases on the same strand (the 1,2 intrastrand GG adduct accounts for >70 % of all adducts formed: 1,2 intrastrand AG adducts are the next most common at around 20 % of all lesions: 1,3 adducts and monoadducts are much less common). The platinum center is located in the DNA major groove, and the effect of the platinum coordination to two adjacent bases is to bend (kink) the DNA by around 45°, toward the site of platination (Fig. 2a) [9]. This bent DNA structure is then recognized by nuclear high-mobility group (HMG) proteins which bind and are believed to protect the lesion from DNA repair (Fig. 2b). A crystal structure of the DNA-binding domain of an HMG protein bound to platinated DNA reveals a phenylalanine residue from the protein inserting into the cavity created at the kink, forming a face–face π–π interaction with one platinated guanine and a face-edge (CH…π) interaction with the other [10,11].

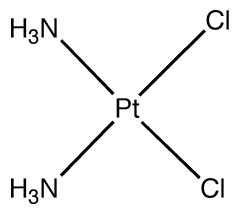


Fig. 1 The anticancer drug cisplatin.

While cisplatin is among the most effective anticancer agents in the armory of drugs available to cancer clinicians, this broad-spectrum cytotoxic is not without its drawbacks [2]. Side effects include nephrotoxicity, nausea, vomiting, and loss of sensation in the extremities (though not the hair loss associated with other chemotherapy agents). These are thought to arise through a combination of the nonspecificity of the drug, and resulting damage in tissues other than the tumor, and platination of the sulfur residues on proteins by the soft platinum(II) center. The effect of the latter can be addressed by coadministration of sulfur-containing "rescue agents" such as diethyldithiocarbamates. Nephrotoxicity

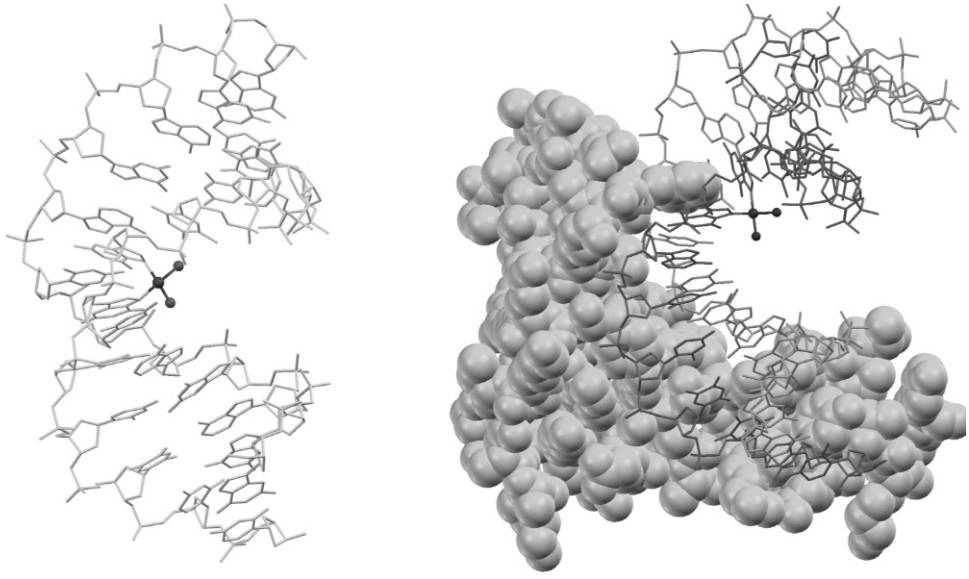


Fig. 2 (a) Structure of DNA with cisplatin bound illustrating the kink caused in the DNA structure (PBD ref. 1AIO) [9]; (b) Structure of HMG (spacefill) recognizing DNA bound to cisplatin (PBD ref. 1CKT) [10].

is addressed by intravenous hydration along with diuretics, and nausea with antiemetic drugs. The intravenous administration and associated hydration therapy necessitates administration in a hospital environment rather than patient self-administration. Still more significantly, many tumors develop resistance to cisplatin after the first course of treatment, preventing readministration, and the agent is not active against all primary tumors (notably breast, the most common cancer among women in Europe, prostate, which is the most common cancer among men in the United States and Europe, and colorectal cancers).

The success of cisplatin in the clinic led to a period in which many thousands of platinum-containing compounds were screened for their activity, with around 30 entering clinical trials. From these early studies emerged a general rule that activity required a neutral, square-planar platinum(II) center, containing two *cis*-amines and two leaving groups [12]. As will be seen, such a structure does give a high probability of activity but is far from being a prerequisite for anticancer action. The first of these follow-up agents to enter worldwide clinical use was carboplatin (paraplatin; BristolMyersSquibb) [2]. Carboplatin (Fig. 3) contains the {*cis*-$Pt(NH_3)_2$} active fragment of cisplatin, but the two chloride leaving groups are replaced by a bidentate dicarboxylate. In line with this simple change of leaving groups, the biological mechanism of action of carboplatin appears to be entirely analogous to that of cisplatin and the DNA adducts to be the same. The spectrum of cancers that can be treated is also identical. The change of leaving group does reduce the activity of the agent somewhat, however. While it is as effective in ovarian cancer, it is less potent against testicular, head, and neck cancers. Correspondingly, the side effects are less severe. Consequently, cisplatin has tended to remain the agent of choice, with carboplatin used when there is a clinical need to minimize the platinum drug side effects because of other medical conditions. Alongside cisplatin and carboplatin, three other very similar drugs (Fig. 4) have appeared which have been approved for use in specific countries: nedaplatin (Japan; Shionogi & Co. Ltd), heptaplatin (South Korea; SK Pharma), lobaplatin (China). Of these, nedaplatin combines the {*cis*-$Pt(NH_3)_2$} active fragment with a different bidentate leaving group (and thus is a direct analog of cisplatin and carboplatin) while heptaplatin and lobaplatin link the amines into a bidentate ligand structure and use a dicarboxylate leaving group. No dramatic clinical benefits have been described for these drugs over cisplatin.

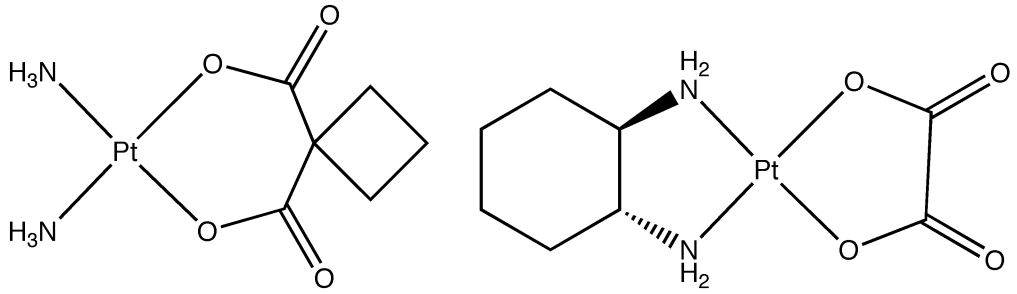


Fig. 3 Other platinum drugs approved for worldwide clinical use: carboplatin and oxaliplatin.

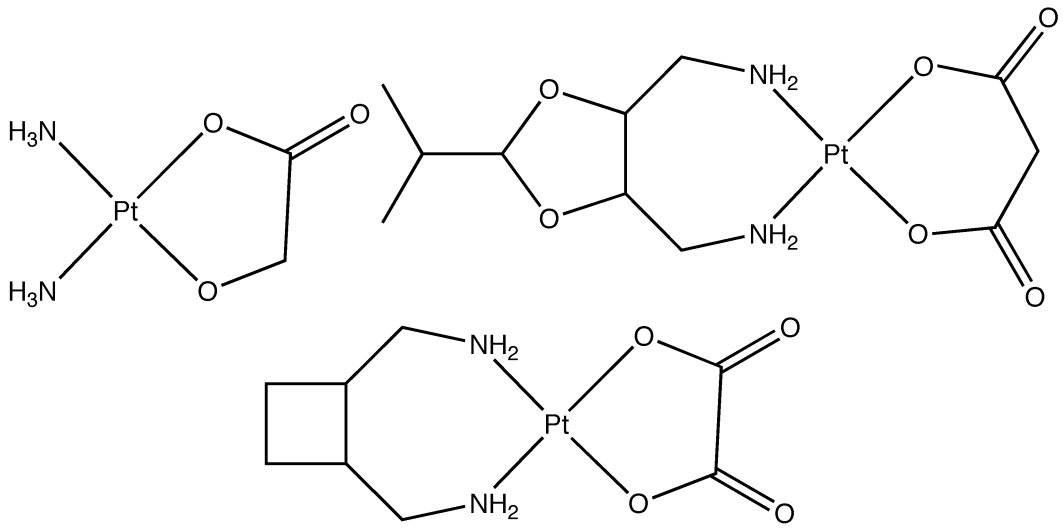
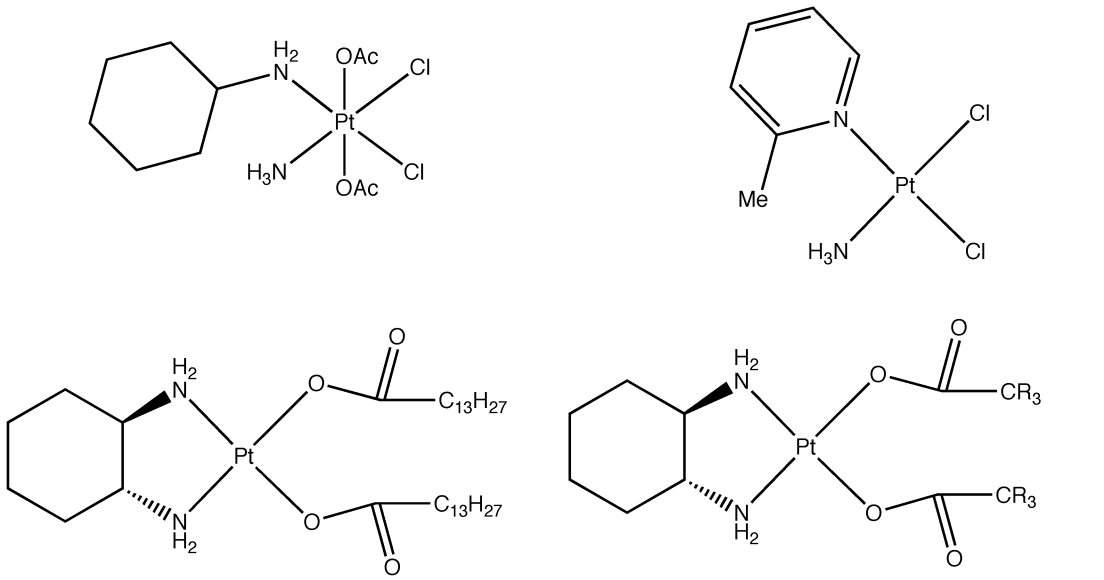


Fig. 4 Other platinum drugs approved for regional clinical use: (top left) nedaplatin (Japan), (top right) heptaplatin (South Korea), (bottom) lobaplatin (China).

Very recently (2004), a further platinum drug, oxaliplatin (eloxatin; Sanofi-Aventis) has become the third to achieve worldwide clinical acceptance. The clinical advantage of oxaliplatin is that it has a different spectrum of activity: in particular, it is effective against colorectal cancer, a disease not treatable using cisplatin or carboplatin [2]. Moreover, oxaliplatin is active against some cisplatin-resistant cancers. The predominant DNA adducts formed by oxaliplatin are 1,2 intrastrand GG adducts analogous to those formed by cisplatin. However, in oxaliplatin the amines are incorporated into a 1,2-diaminocyclohexane (dach) framework and the adducts are thus not {*cis*-Pt(NH_3)$_2$} adducts but {Pt(dach)} DNA adducts. The precise biological reasons for the difference in spectrum of activity and the ability of this agent to circumvent some cisplatin resistance mechanisms remain to be fully elucidated, but seems to hinge on the dach ligand: The alternate diamine ligands used in heptaplatin and lobaplatin do not confer these same effects.

Although these platinum(II) cytotoxics are fairly non-specific in their action and have been in the clinic for 30 years, their continuing importance (and the size of the market) is illustrated by the fact that at least seven further platinum drugs are currently in commercial development: satraplatin (GPC Biotech & Pharmion); miriplatin (Dainippon Sumitomo Pharma and Bristol-Myers K.K.); prolindac (Access Pharmaceuticals); BP-C1 (Meabco); cisplatin Lipid Complex (Transave); aroplatin (Antigenics); picoplatin (Poinard) [13].

Many of these agents are aimed at addressing the issue of administering a cisplatin-like or oxaliplatin-like drug into patients. The closest to market appears to be satraplatin (Fig. 5), a platinum(IV) agent which can be taken orally [13]. It is believed to be reduced in the body (with loss of the acetate ligands) to afford a cisplatin-like complex, which then creates DNA adducts containing $\{Pt(NH_3)(NH_2C_6H_{11})\}$ fragments. Aside from the clear patient benefits of oral delivery, there may be additional benefits in the spectrum of activity, with approval currently being sought for the use of this agent in conjunction with prednisone (an anti-immune/anti-inflamatory agent) against hormone refractory prostate cancer.



CR3 = C9H19; usually branched; a mixture of alkanes

Fig. 5 Some platinum metallo-drugs currently in commercial development: satraplatin (top left), picoplatin (top right), miriplatin (bottom left), aroplatin (bottom right).

Picoplatin is similar in structure to cisplatin but with one of the amines replaced with a 2-methylpyridine unit. Like oxalipatin, picoplatin is active against some cisplatin-resistant cancers [14]. It is clear from the activity of oxaliplatin, satraplatin, and picoplatin that modifying those ligands on platinum that are retained in the DNA adduct can affect the way the compound acts in the biological system. Yet, a deep understanding that would permit deliberate design remains elusive. Clinical trials on a formulation of picoplatin contained in an oral capsule have also been initiated (2007).

Aroplatin and miriplatin are liposomal formulations of a Pt(dach) agent similar to oxaliplatin. Their rationale is that liposomal delivery can increase a drug's bioavailability and liposomes can also in some cases accumulate at tumor sites by enhanced vascular permeability and subsequent retention. The key to the success of this approach appears to lie in formulation issues rather than in the design of the platinum agent [15]. A liposomal formulation of cisplatin (lipoplatin; Regulon) is being explored with a similar rationale [16] and an inhaled liposomal formulation (cisplatin lipid complex; Transave) is being explored as a means to localize delivery to the lung for the site-specific treatment of lung cancer. Like aroplatin, prolindac is also based on Pt(dach) fragments. In this case, the leaving groups on the platinum, rather than discrete individual ligands, are residues on a polymer. The drug thus contains multiple Pt(dach) units attached onto a polymer scaffold. Release (of the same active component as

oxaliplatin) from the polymer is favored at acidic pH, which may provide a level of targeting toward hypoxic tumors [13].

All these drugs, in the clinic or in development, are "classical" Pt-drug structures containing a square-planar platinum(II) center, two *cis*-amines, and two leaving groups or, in the case of the platinum(IV) agent satraplatin, are converted into classical Pt agents in the body. None contain tissue-specific targeting vectors. Oxaliplatin has undoubtedly pushed the field forward, widening a little the spectrum of cancers that can be treated and circumventing some cisplatin resistance. Some of the others such as satraplatin and picoplatin may in the future widen the scope of these platinum agents still further. Yet these agents are all structurally similar and rely on essentially the same molecular-level action (formation of a 1,2 intrastrand GG cross-links on DNA) and the steps forward are small incremental progressions of the design, resembling a lead-optimization process. Obtaining truly novel spectrums of activity requires agents that act through different molecular-level interactions. This can only be achieved by breaking the design rules [12] that originally guided the platinum metallo-drug field.

Trans and polynuclear platinum drugs

Early on in the studies of cisplatin it was recognized that the *trans*-isomer (transplatin) was inactive [17], and thereafter the need for a *cis* geometry at platinum rapidly became a dogma. However, this dogma (as the other design rules) has more recently been shown to be invalid and three distinct classes of *trans* compounds shown to possess anticancer activity [18]: *Trans* compounds containing pyridine ligands (developed by Farrell) [19], *trans* compounds containing an alkylamine and an isopropylamine (developed by Navarro-Ranninger) [20], and *trans* compounds containing iminoether ligands (developed by Natile and Coluccia) (Fig. 6) [21]. These three classes of agents show potencies similar to that of cisplatin and, perhaps more importantly, are active against cisplatin-resistant cell lines. The DNA lesions formed by a *trans*-platinum agent will be inherently different from those formed by a *cis* agent. Indeed, these *trans* agents preferentially form monofunctional adducts with the DNA or interstrand cross-links, rather than the 1,2 intrastrand cross-link preferred by cisplatin. Thus, their molecular-level interactions with DNA are different and hence their activity profiles differ. Despite their promising activity, representatives of these *trans*-platinum(II) classes have not been evaluated in the clinic.

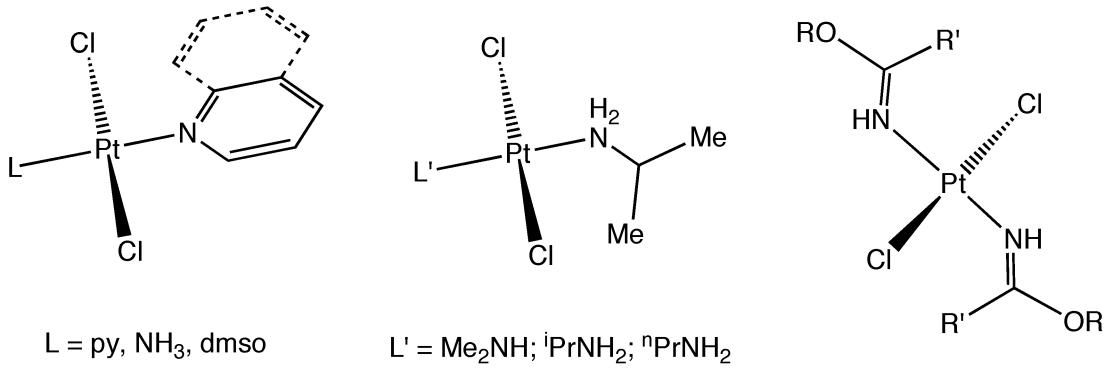


Fig. 6 Active *trans*-platinum(II) compounds: (left) pyridine-containing (center), isopropylamine-containing (right), and iminoether-containing.

The most dramatic example of platinum(II) agents that break the traditional design rules are Farrell's di- and tri-nuclear platinum compounds (Fig. 7) in which the metal centers are linked by flexible diamine chains [22]. These compounds are not only multinuclear but also polycationic. Moreover, they do not contain platinum centers with *cis* leaving groups. The reward for creating a design which

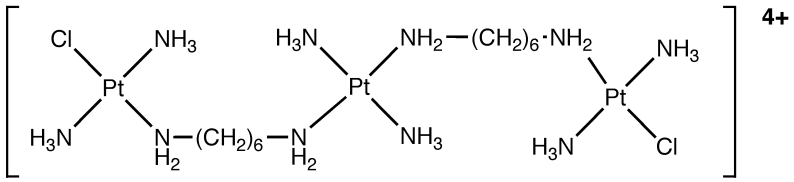


Fig. 7 BBR3464; an example of Farrell's active multinuclear cationic *trans*-platinum(II) compounds.

breaks all the rules is that these agents are much more potent anticancer drugs than cisplatin itself and exhibit activity against a broad spectrum of tumors, including cisplatin-resistant tumors. Bifunctional long-range DNA adducts are formed (both inter- and intrastrand cross-links). In addition, a DNA conformational change is induced from a right-handed (B) to a left-handed (Z) DNA double helix. This induction of Z-DNA is irreversible and is associated with the cross-linking [23]. The lead agent of this class, a trinuclear compound BBR3464, has undergone phase II clinical trials [24].

DRUGS BASED ON OTHER METALS

Ruthenium drugs

An alternative way to break the cisplatin design rules is to explore other metal centers. Ruthenium is particularly attractive as the ligand exchange kinetics in its complexes can be similar to those of platinum complexes. Two ruthenium-based anticancer agents (Fig. 8) are currently in clinical trials.

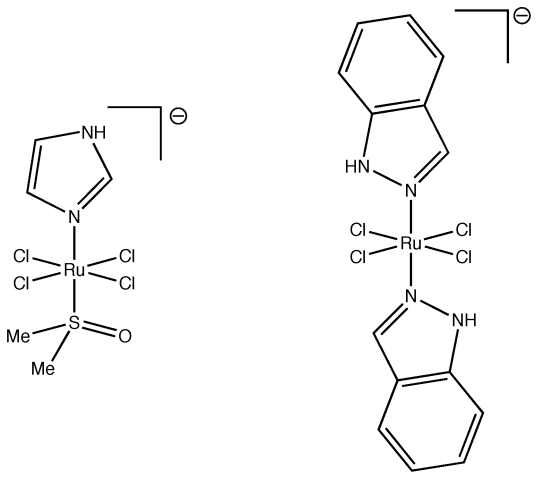


Fig. 8 The metal-containing anions in the ruthenium drugs NAMI-A (left) and KP1019 (right).

The first ruthenium agent to enter clinical trials was NAMI-A [1,25], imadozolium *trans*-[tetrachloro(dimethylsulfoxide)(imidazole)ruthenate(III)]. The drug, developed by Alessio and Sava, is an anionic complex which contains an octahedral ruthenium(III) center bound to one imidazole ligand, with a S-coordinated dmso ligand *trans* to the imidazole and four chlorides completing the coordination sphere. That this drug has made it to clinical trials is quite remarkable because it is not very active against cancer cell lines, which are the usual first screen for activity (indeed, the compound failed the NCI cell line panel screen). Despite its lack of activity against primary tumors, the drug is, however, a potent agent against metastasis tumors. This is potentially very important because although great leaps have been made in treating primary cancers (including surgery, chemotherapy, and radiotherapy) sec-

ondary metastases represent a major clinical challenge. While NAMI-A can bind to DNA, this is not believed to be the source of its biological action. Rather, NAMI-A seems to act as an anti-angiogenic and anti-invasive agent. Its anti-angiogenic properties may relate to the ability of NAMI-A (and other ruthenium compounds) to scavenge NO produced by epithelial cells. The anti-invasive properties appear to be a result of its interaction with extracellular or external cell membrane receptor proteins.

The second of the ruthenium agents currently in clinical trials is indazolium *trans*-[tetrachlorobis(1H-indazole)ruthenate(III)] (KP1019 or FFC14A) developed by Keppler [26]. Despite its structural similarity to NAMI-A, this agent is a cytotoxin which is active against primary tumors, and is being investigated for activity against colorectal cancers. The agent causes apoptosis via the mitochondrial pathway and is believed to act as a pro-drug with the actual active species as yet not identified. Under physiological conditions, at least one of the chlorides is exchanged for aqua (or hydroxy) ligands. The complex can react with serum albumin in the blood and also transferrin. Human serum albumin can bind up to four of these ruthenium complexes and may act as a "reservoir" of agent. Transferrin is normally used to transport iron centers into the cell and is over-expressed in cancer cells. It is believed that the ruthenium complex is transported into cells by transferrin, which can bind two ruthenium centers. There is crystallographic evidence that the ruthenium can bind to residues in the two iron binding pockets, and that the two *trans*-indazole ligands can remain attached to the ruthenium in the transferrin complex. The complex is released from transferrin at acidic pHs such as those found in endosomes, which is also used to release iron from transferrin inside the cell. Thus, it appears that KP1019 can very effectively use the natural iron transport systems to locate itself inside the cell. Once inside the cell, the agent can bind to DNA with a preference shown for G and A residues: indeed, it is observed to bind to DNA in tumor cells and causes some (though not many) strand breaks. However, the DNA adducts are not very effective at terminating transcription. Although DNA might be the biomolecular target through which the drug's cytotoxic activity is revealed, the demonstrated and effective binding to protein histidine residues means that action via other biomolecules cannot be excluded. A substantial body of information has already been assembled regarding the effect of this compound on cells and their components and to drug resistance pathways. The continuing studies into its action seem likely to provide valuable information to guide others in the metallo-drug field and may reveal new potential intracellular targets.

More recently, two further classes of ruthenium anticancer agents have been developed, centered around ruthenium arene compounds (Fig. 9). In a striking parallel to NAMI-A and KP1019, the two sets of agents are structurally very similar, yet one shows good cytotoxicity against primary tumors while the other is an antimetastatic agent of low toxicity.

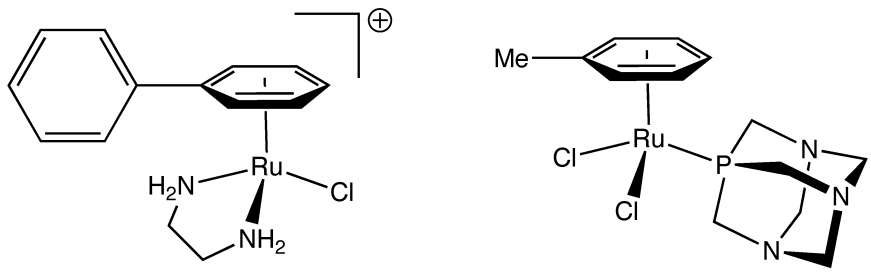


Fig. 9 Examples of the organometallic piano-stool ruthenium agents of Sadler (left) and the antimetastatic RAPTA agents of Dyson (right).

The first class has been developed by Sadler and consists of an aryl ruthenium with a "piano stool" type of conformation, with a didentate ethylenediamine and a chloride occupying the three remaining coordination sites. These stable water-soluble organometallic agents are as potent as the platinum drugs cisplatin and carboplatin in some primary cell lines, and activity in vivo has also been

demonstrated [27]. They exhibit a wide spectrum of activity and are also active against some tumors which have become resistant to cisplatin. The level of anticancer activity is dependent on the aryl unit with more extended aryls (biphenyl, tetrahydroanthracene) showing higher activity. Replacing the ethylenediamine with more bulky *N*-donor ligands such as bipyridine or *N*,*N*,*N*',*N*'-tetramethylethylenediamine reduces the activity, although with 1,2-diaminobenzene activity is retained. While the compounds can interact with a variety of different biomolecules, the biomolecular target may be DNA [28]; the chloride can be replaced by a water ligand in aqueous solution, and the complex can coordinate to the DNA bases at this position. The complexes exhibit a strong preference for G residues (binding at N7), and there is some indication that the larger aryl groups can partially insert between the DNA bases, thereby creating a bifunctional (metal coordination and noncovalent partial insertion) lesion which may account for their higher activity. Nevertheless, this lesion is distinct from that caused by cisplatin and is not recognized by HMG proteins [29]. The different mode of binding is consistent with the activity against cisplatin-resistant cell lines.

The second class are the ruthenium arene 1,3,5-triaza-7-phosphaadamantane (RAPTA) agents developed by Dyson which are similar aryl ruthenium piano-stool complexes, but in which the three remaining coordination sites are occupied by two chlorides and a monodentate 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane [30]. The compounds developed out of studies to create pH-dependent DNA-binding agents, but were shown to be of only very low toxicity toward cancer cell lines. Like NAMI-A, these agents are inactive against primary tumors but found in vivo to have activity against metastases. The RAPTA complexes are slightly less potent antimetastatic agents than NAMI-A, but (in mice) less toxic and thus can be administered in higher doses. As NAMI-A, the indications are that proteins, rather than DNA, are the biomolecular targets for action of the drug. Enhanced activity against lung metastases on coadministration of cisplatin has been demonstrated. Given the structural differences between the RAPTA and NAMI-A complexes, they might be expected to act differently with proteins, perhaps selecting different biomolecular targets or pathways. Ultimately, cocktails of different antimetastatic metallo-drugs which target different proteins might be envisaged.

Ferrocifens

The drive to create organometallic metallo-drugs is not limited to ruthenium(II) chemistry. Indeed, one of the big successes underpinning the emerging subfield of bioorganometallic drugs [31] has been a series of ferrocene derivatives of tamoxifen which have emerged from the laboratories of Jaouen [32]. Tamoxifen is an organic drug that acts on the estrogen receptor and has had a very significant impact in the treatment of breast cancer. However, a significant proportion of breast cancers (ca. 30 %) fail to respond to tamoxifen treatment. In part, this is due to the presence of two different estrogen receptors in humans (ERα and ERβ); tamoxifen is effective only against cancers which are rich in ERα (ERα +ve). The ferrocifens are agents in which one phenyl ring of tamoxifen is replaced with ferrocene (Fig. 10). These agents are highly active against breast cancer cells that are ERα –ve (ERβ +ve) as well as breast cancers that are ERα +ve. The role of the ferrocene is believed not to be solely restricted to that of a structural unit. Rather, it appears that the ferrocene could be oxidized to ferrocenium in the cell and subsequently cause oxidative damage through Fenton-type mechanisms. Intriguingly, a ferrocene derivative of chloroquine is being developed as an antiparasitic agent [33].

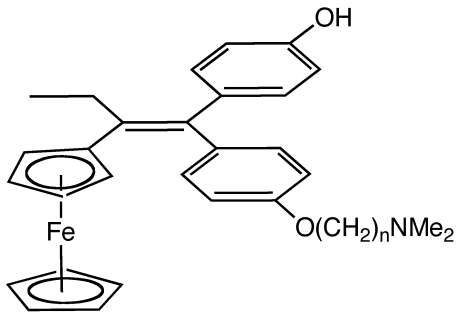


Fig. 10 The organometallic ferrocifen agents developed by Jaouen.

Titanium and gallium drugs

In the 1990s, the organometallic agent titanocene dichloride [Cp_2TiCl_2] (Fig. 11) entered clinical trials as an anticancer agent. While the *cis*-dichloride structure of this agent makes intriguing parallels with cisplatin, in fact the agent is hydrolyzed in water to a variety of species and it is still unclear what the active component is, whether the biomolecular target is indeed DNA, and if so whether there is a specific binding mode or random backbone binding [34]. Bioorganometallic chemists have recently been focusing on substituted titanocenes that might offer greater aqueous stability, water solubility, or cytotoxicity [35,36]. One such agent is Titanocene Y, which contains methoxyphenyl substituents of the Cp rings (Fig. 11) which confer the agent with higher potency [35]. When the current screening for activity of multiple substituted variants of the initial lead (titanocene dichloride) becomes coupled with studies of their different effects on specific biomolecular targets, a better understanding of the biomolecular-level activity of these drugs may result. At the present time, the published work seems to be in a phase of lead optimization.

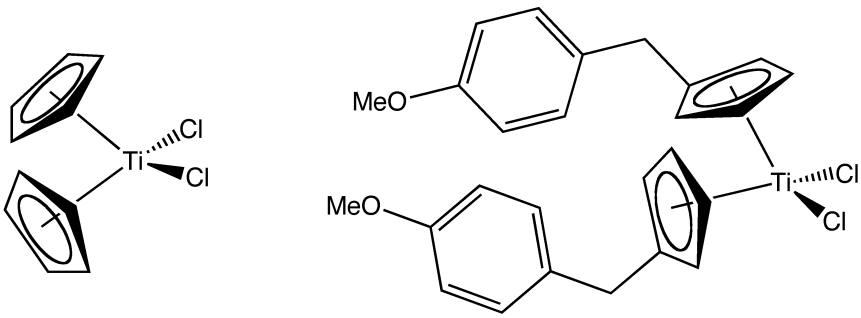


Fig. 11 Titanocene dichloride (left) and "Titanocene Y" (right).

Gallium complexes are known to inhibit tumor growth. In large part, their action seems to be a consequence of the similarity of gallium(III) to iron(III): Gallium interferes with the cellular transport of iron by binding to transferrin, and also interferes with the action of ribonucleotide reductase, which then results in inhibition of DNA synthesis. The key to activity is making gallium(III) bioavailable, and work is focused on ligands which stabilize gallium against hydrolysis and facilitate membrane permeation. Tris(8-quinolinolato)gallium(III) (KP46/FFC11) has entered clinical trials [36].

NONCOVALENT METALLO-DRUGS

While cisplatin is perhaps the highest-profile anticancer drug that targets DNA, other anticancer drugs also act on DNA and can do so in quite distinct ways. For example, the anthracycline antibiotics (Fig. 12) such as doxorubicin (trade name "Adriamycin" or "Rubex") are important tools in the clinic for cancer treatment [38]. These organic molecules contain planar, aromatic units and bear cationic charge. They bind to DNA by intercalation between the bases. Minor groove DNA-binders such as berenil and pentamidine have also been investigated as clinical anticancer agents, although they are more commonly used as antiparasitic agents [39]. Whereas clinical "alkylating agents" such as cisplatin or chlorambucil (a nitrogen mustard) form a direct bond to the nitrogens bases, these minor groove binders and intercalators bind to the DNA through noncovalent interactions [11]. Electrostatic attraction between these cationic agents and DNA makes a substantial contribution to the energy of the noncovalent binding.

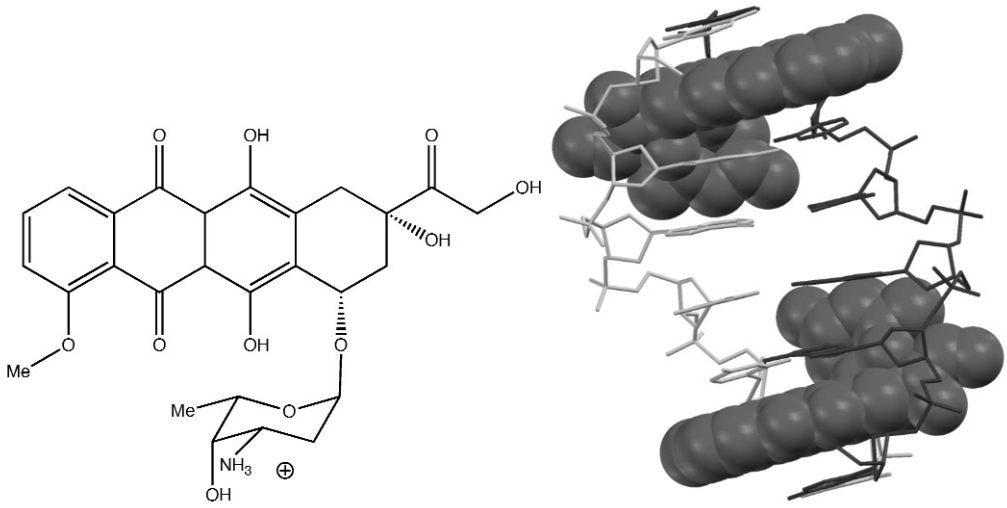


Fig. 12 Structure of doxorubicin (left) and of a DNA oligonucleotide with two doxorubicin drugs (shown in space-fill) intercalated between the base pairs (PDB ref. 1D12) [40].

As well as other anticancer drugs, proteins also bind to and regulate DNA through noncovalent interactions, using a variety of different structural motifs [10]. Noncovalent DNA recognition might, therefore, be a powerful way for metal complexes to exert an anticancer effect through unique molecular actions, different from that of cisplatin. Very recently, three completely new types of anticancer agents have emerged which are based on noncovalent recognition of DNA. These approaches, described below, represent a complete step-change away from metallo-drug designs inspired by cisplatin.

Metallo-intercalators

Since metal centers can be used to impart cationic charge to reagents, as well as other exciting properties such as redox, color, and luminescence, they have much to offer in noncovalent DNA recognition designs. There has been considerable interest in the design of metallo-intercalators as DNA probes [41]. It is perhaps, therefore, surprising (especially given that organic intercalators are used in the clinic) that the anticancer action of these agents has not been widely explored.

Lincoln and Nordén have reported bis-intercalation for the $[\Delta,\Delta\text{-}\mu\text{-C4(cpdppz)-(phen)}_4\text{Ru}_2]^{4+}$ (Fig. 13) [42]. The complex contains two linked $[\text{Ru(phen)}_2\text{(dppz)}]^{2+}$ motifs. That motif binds to DNA by inserting the intercalating dppz between the base pairs. However, in this linked bis-intercalator the intercalating units are linked at the point through which they would usually insert into the DNA. Therefore, to intercalate, part of the molecule must thread through the DNA. This is indeed what happens with the dppz groups of the molecule intercalating from one groove and the bridging chain lying in the opposite groove. The agent has been shown to have activity in some cancer cell lines, with a three- to four-fold increase in potency over the mononuclear analog $[\text{Ru(phen)}_2\text{(dppz)}]^{2+}$ [43].

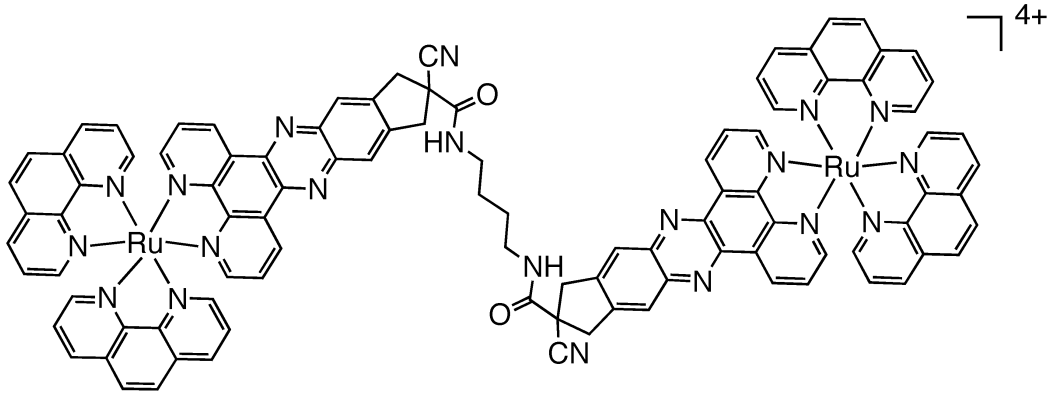


Fig. 13 The threading bis-intercalator $[\Delta,\Delta\text{-}\mu\text{-C4(cpdppz)-(phen)}_4\text{Ru}_2]^{4+}$.

The simple tris-chelate $[\text{Ru(bpy)}_3]^{2+}$ is a groove binder (rather than an intercalator) and is reported to be inactive, although some related azopyridine-containing compounds with potential to (partially) insert between the bases do display some activity [44]. Activity of related thiosemicarbazone $[\text{Ru(phen/bpy)}_2\text{L}]^{2+}$ complexes has been reported in mice, although the precise nature of their interaction with DNA has not been determined [45].

Metallo-supramolecular cylinders

In my laboratories, we have focused on DNA recognition by cylindrical metallo-supramolecular agents [46–51]. The compounds we have studied are the first to bridge the fields of metallo-supramolecular architecture and anticancer drug design. The concept we have explored and developed is that supramolecular chemistry should allow construction of large synthetic structures that closely mimic the dimensions of protein DNA recognition motifs, that such agents should have novel DNA-binding properties as a result, and hence should display new types of activity in biological systems. In particular, our initial focus was to create agents that would be the right size and shape to bind in the DNA major groove. In this way, we would move beyond established synthetic intercalators and minor groove binding drugs. We designed a tetracationic triple-stranded cylinder (a helicate) ~2 nm in length and ~1 nm in diameter (Fig. 14). These dimensions are similar to those of the alpha-helical DNA recognition unit of zinc fingers, which bind in the major groove of DNA. Importantly, the size is too great to fit readily into the minor groove. The agent not only binds to the major groove, but in addition causes unexpected and dramatic intramolecular DNA coiling, which gives rise to small coils of DNA (Fig. 15) [46]. By stepping up to the size-scale of nature, quite remarkable and new effects are observed.

The precise size and shape of the cylinder are important for the coiling process: increasing the cylinder dimensions by around 10 % leads to a dramatic reduction in DNA coiling [46].

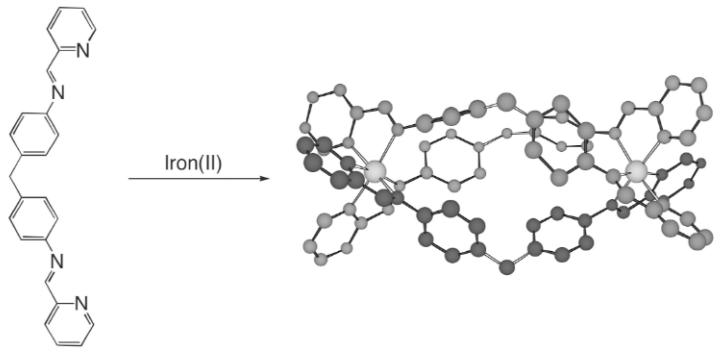


Fig. 14 Design of the tetracationic triple-stranded supramolecular cylinder.

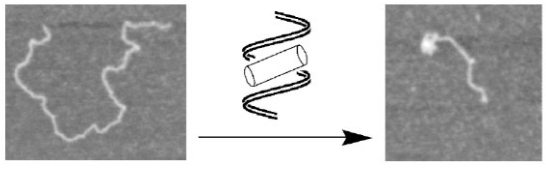


Fig. 15 Intramolecular DNA coiling induced by the supramolecular cylinder.

Still more remarkable is a second mode of binding discovered when the cylinder was crystallized with a palindromic hexanucleotide in a collaboration with Miquel Coll [47]. The crystal structure revealed the cylinder to be located at the heart of a DNA three-way junction (3WJ) (Fig. 16). This unexpected DNA structure is possible because, for a palindromic DNA, Watson–Crick hydrogen bonding can be satisfied either through a duplex structure, or through junction structures (e.g., 3WJ, 4WJ, 5WJ…). The six phenyl rings of the cylinder face–face π-stack onto the 6 DNA bases that are placed at the junction point, and the tetracation sits at the junction point where significant anionic charge is located. The cylinder and the junction are almost perfectly matched, like a hand and a glove, with neither significantly perturbed by the binding. NMR studies in a collaboration with Einar Sletten, established

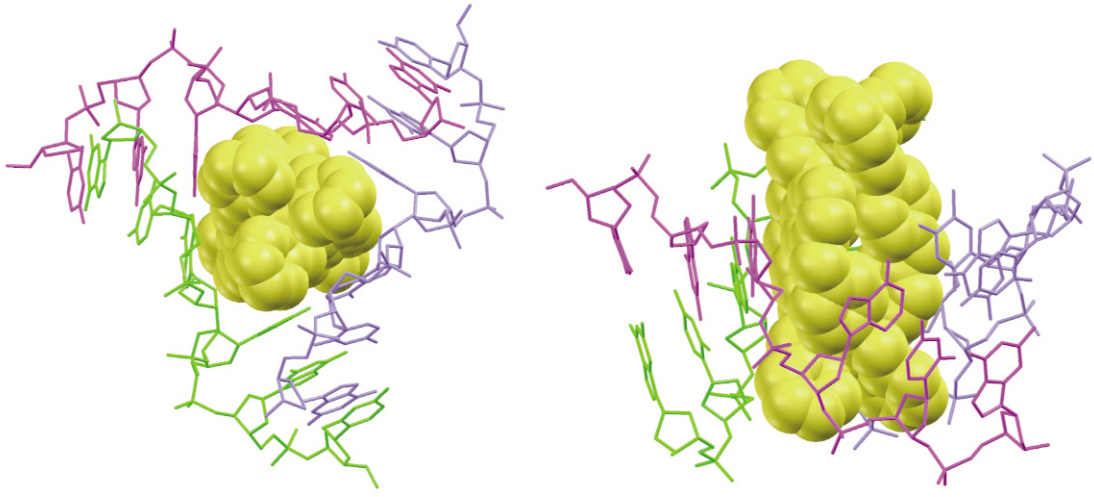


Fig. 16 Two perpendicular views of a DNA hexanucleotide 3WJ with a supramolecular cylinder (space-filling) bound in the center of the junction (PDB ref. 2ET0) [47].

that this structure is not a crystallographic artefact but is the exclusive solution species formed when the cylinder and a palindromic DNA (the hexamer or a dodecamer) are mixed [48].

This binding mode, a perfect shape-fit in the heart of a Y-shaped, three-way DNA junction, is an unprecedented DNA binding-mode, and quite different from those by which other synthetic drugs interact with DNA. Moreover, there seems to be no direct analog either in the way that proteins or other biomolecules bind to DNA. Yet, what is particularly tantalizing is that the DNA replication fork is a form of Y-shaped junction. Indeed, whenever the DNA duplex is opened to process the genetic information (replication or transcription) a Y-shaped junction is created (albeit not a perfectly paired 3WJ as seen in this structure). Since cancer cells are de facto replicating and transcribing their DNA more frequently than normal cells, agents that recognize this particular "unusual" DNA structure and interfere with those processes could be a very powerful tool.

In collaboration with Jarek Malina and Victor Brabec, we have studied the recognition of a variety of 3WJs by cylinders using gel electrophoresis and shown that the recognition is not restricted to palindromic 3WJs, but that nonpalindromic 3WJs, 3WJs containing unpaired nucleotides and other Y-shaped junctions, such as that formed at a fraying point in duplex DNA (e.g., a replication fork), can indeed be recognized [49].

Alongside (and consistent with) these remarkable and unprecedented DNA-binding modes, we have shown that these iron(II) supramolecular cylinders and their fluorescent ruthenium(II) analogs are active against cancer cell lines, with potencies comparable with the threading bis-intercalators and cisplatin [50]. Studies are underway to correlate this activity with the different noncovalent DNA-binding modes and to gain a deeper understanding of their exact effects on the biological systems and pathways.

We were also intrigued to push the approach further and to explore whether the cylinder design could be combined with a cisplatin-type design to afford still better activity. In the cylinders used above, the metal centers are fully coordinated by the bridging ligands; these are termed "saturated" cylinders (or "saturated" helicates). To combine the two types of design, we developed "unsaturated" double-stranded analogs, in which there are vacant coordination sites that offer the additional possibility of the metal center interacting directly with DNA [51]. We selected ruthenium, rather than platinum, as our metal center of choice for its higher coordination number and because mononuclear azopyridine ruthenium(II) complexes with two vacant coordination sites have been shown to exhibit cytotoxic activity [52]. An azo analog of the imine ligand used to create the saturated cylinders enabled different isomers of the unsaturated $[Ru_2Cl_4L_2]$ compounds to be isolated (Fig. 17) [51]. The compounds are very active against cancer cell lines: with their potencies ranging from being similar to that of cisplatin to being as much as 100 times more potent. Further biological studies are in progress to fully understand and correlate the biological activity and DNA binding of these new classes of complex.

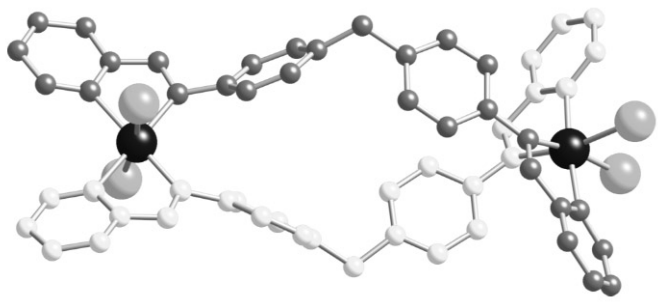


Fig. 17 An example of unsaturated $[Ru_2Cl_4L_2]$ supramolecular helicate which is active against cancer cell lines.

Metal-based DNA-backbone binders

Very recently, Farrell has explored the DNA binding of an octacationic trinuclear platinum compound, which is an analog of BBR3464 but in which the reactive Pt-Cl groups have been replaced with amines (Fig. 18). This molecule can thus bind to DNA only through noncovalent (hydrogen bonding and electrostatic) interactions and cannot form direct bonds from Pt to N7 of the purine bases. An X-ray crystallographic study has revealed that the compound forms hydrogen bonds from two *cis*-amine groups to one oxygen phosphate. This is a repeated motif in the structure with the complex able to track along the phosphate backbone or stretch across the minor groove, making contacts with the phosphate backbones on either side (Fig. 19) [53]. This synthetic agent has parallels with the threading bis-intercalators and supramolecular cylinders in that it has an unprecedented mode of DNA binding and one that is noncovalent and completely distinct from that of cisplatin or other platinum-based metallo-drugs. Excitingly, this agent also shows good activity against cancer cell lines [54].

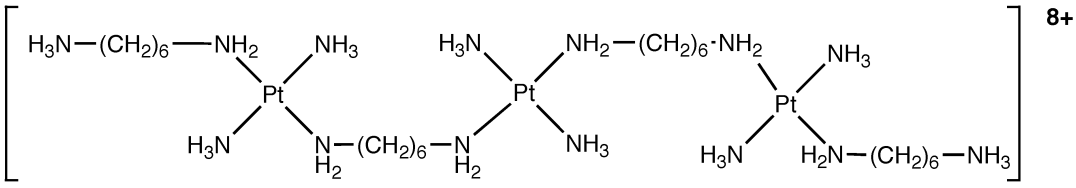


Fig. 18 Farrell's octacationic backbone binder.

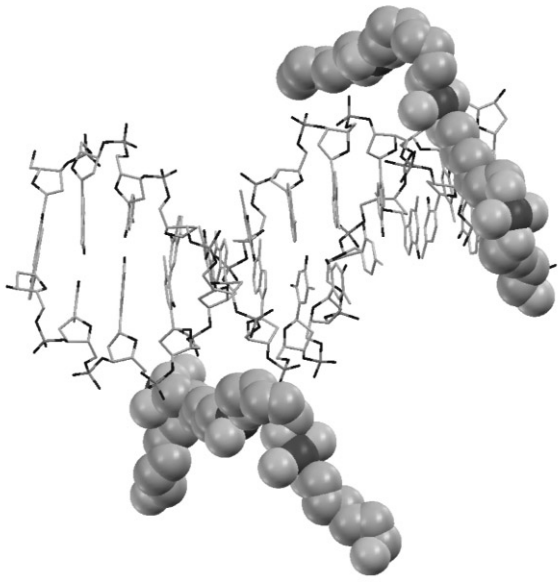


Fig. 19 Structure of Farrell's octacationic trinculear platinum compound (shown in space-filling) binding to DNA illustrating the interactions with the phosphate backbone and the backbone tracking and groove spanning binding modes that can result (PDB ref. 2DYW) [53].

OUTLOOK

The existing clinical platinum drugs have dramatically improved the prognosis for patients with a variety of cancers. They seem likely to remain at the forefront of combination therapies to treat cancers. Their unpleasant side effects are much better controlled, through different administration regimens, than

when they were first launched, but these have left an ongoing prejudice against heavy-metal-containing drugs. It is certainly true that the big pharmaceutical companies, still rooted in traditional organic synthesis, have not embraced the field with any enthusiasm. The platinum drugs remain broad cytotoxics active against DNA, in an age when drug research seems focused almost exclusively on specific protein targets and pathways. However, the recent launch of oxaliplatin has served to highlight the size of the potential market (and the associated financial rewards) and some reappraisal (or at least lowering of prejudices) might be anticipated. The platinum compounds in development address issues, particularly oral delivery, that are very important for patients and some may widen the spectrum of diseases that can be treated. Whether they make it to market will rest on commercial considerations; the level of sales for oxaliplatin may influence those decisions. While valuable to patients, the work being undertaken does not represent a scientific step-change from the original cisplatin drug and its action. Rather, it is more of a lead-optimization process. The step-change would come from an approach that targets the platinum drug selectively to the cancer cells, which could dramatically reduce the side effects. The use of liposomes as carriers has been explored for some time, and it is perhaps disappointing that an appropriate formulation sufficient to project such an agent into the clinic has not yet been developed. Conjugates of platinum drugs with a variety of biomolecular targeting vectors have been explored in the laboratory, but none have yet shown sufficiently dramatic effects to impact on patients [55].

For a prolonged period, the metallo-drug field seemed obdurately anchored in cis diamine platinum(II) chemistry. Freed from these chains, the field is now evolving rapidly with a variety of alternate and very different designs being explored. A particularly exciting feature is the emergence of antimetastatic agents (especially NAMI-A), which, along with the ferrocifens, act not at the DNA level but through protein targets. The ability to target and bind to specific biomolecules is largely an issue of size and shape. Metal complexes have much to offer because they offer a much greater diversity of shape than organic molecules (where sp, sp^2, and sp^3 hydridization restricts the angles available for construction) and because they enable large nanostructures to be assembled quickly in simple mixing steps [56]. There is every reason to expect that metal complexes may be as effective as (or more effective than) organic drugs in acting against specific protein targets. Such designs will draw heavily on supramolecular chemistry principles and noncovalent interactions. Already, such principles are being applied to target DNA and leading to new modes of action, and this is a rich seam the full potential of which remains to be tapped.

The key to the continuing rapid evolution of the field now lies in its integration with the emerging post-genomic knowledge and technologies from fields, including systems biology, genomics, proteomics, and structural biology. For example, information on the effect of existing and new agents on global and specific gene expression and on the mechanisms and the biochemical pathways whereby interference with gene expression and cell proliferation occur, will afford new biomolecular-level information about the action of metallo-drugs which can be used to guide design. Post-genome research is now opening up many exciting new approaches to molecular-level design in medicine and will pinpoint new therapeutic targets and pathways; identification of such targets is key to design of metallo-drugs with different molecular-level actions. In the long term, "individualized" medical treatments with optimized drugs and clinical regimes tailored to the individual can be envisaged. These might involve cocktails of metallo-drugs aimed at different pathways and targets. Harnessing this knowledge and responding to and taking advantage of this new environment requires teams committed to integrating chemistry and biology research and knowledge. These advances will revolutionize the field of metallo-drugs, taking it far beyond its origins in simple platinum compounds toward sophisticated and modular molecular designs with different and predicted modes of action.

ACKNOWLEDGMENTS

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Cancer and Viral Diseases: 2000–2006) which have networked and integrated the activities of the key European laboratories in the field of metallo-drugs. My own work in the field owes much to the support and encouragement of the many EU scientists in these Actions. These and other EU funding schemes have enabled our laboratory to work with and exchange ideas, compounds, and people with the laboratories of Virtudes Moreno, Anna Grandas (Barcelona), Miquel Coll (CSIC Barcelona), Einar Sletten (Bergen), Zoe Pikramenou, Kevin Chipman, Nik Hodges (Birmingham), Nils Metzler-Nolte (Bochum), Victor Brabec, Olga Novakova, Jarek Malina (Brno), Bengt Nordén, Per Lincoln (Chalmers, Gothenberg), Bernhard Lippert (Dortmund), Jorge Navarro (Granada), Jan Reedijk (Leiden), Carmen Navarro, Dori Quiroga, Felix Zamora (Madrid), Gerard Jaouen, Anne Vessieres (Paris), Peter Sadler, Alison Rodger, and Mark Rodger (Warwick); to all of whom go much appreciation and thanks.

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