Organometallic chemistry, biology and medicine: ruthenium arene anticancer complexes

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Our work has shown that certain ruthenium(II) arene complexes exhibit promising anticancer activity *in vitro* and *in vivo*. The complexes are stable and water-soluble, and their frameworks provide considerable scope for optimising the design, both in terms of their biological activity and for minimising side-effects by variations in the arene and the other coordinated ligands. Initial studies on amino acids and nucleotides suggest that kinetic and thermodynamic control over a wide spectrum of reactions of Ru(II) arene complexes with biomolecules can be achieved. These Ru(II) arene complexes appear to have an altered profile of biological activity in comparison with metal-based anticancer complexes currently in clinical use or on clinical trial.

1. Introduction

Although most new drugs are carbon-based compounds, there is an increasing realisation that many metal ions are involved in natural biological processes and that there is much scope for the design of metal-based therapeutic agents.[1,2] Metal complexes, with their wide spectrum of coordination numbers, coordination geometries, thermodynamic and kinetic preferences (which cover enormous scales of magnitude) for ligand atoms, and in some cases redox activity, offer novel mechanisms of action which are unavailable to organic compounds. In general, the nature of the metal ion, its oxidation state, and the types and number of bound ligands, can all exert a critical influence on the biological activity of a metal complex.[3,4] An understanding of how these factors affect biological activity should enable the design of metal complexes with specific medicinal properties. The wide spectrum of contrasting biological activity amongst platinum complexes (Fig. 1)[1,5,6] and the clinical success of platinum(II) diam(m)ine complexes, *e.g.* cisplatin, as anticancer drugs provide a good illustration of this point. Although platinum complexes are now widely used for the treatment of cancer, the development of drug resistance, the toxic side-effects of cisplatin, and the lack of activity of platinum compounds against several types of cancer are problems which need to be overcome.[7] This provides the impetus for the search for anticancer activity amongst complexes of other metals.

Organometallic chemistry evolved rapidly during the second half of the 20th century[8] and bioorganometallic chemistry is now establishing itself as an important branch of the subject.[9] In particular, organometallic complexes, *i.e.* complexes with at least one direct metal–carbon bond, offer much potential for exploration as anticancer agents due to the large diversity of structure and bonding modes (*e.g.* π-coordination, M–C

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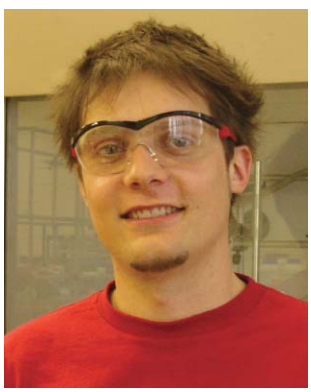

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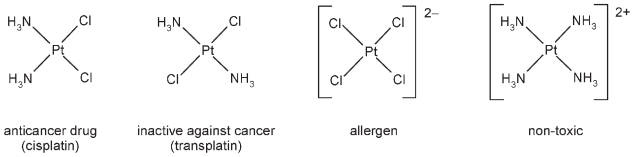

Fig. 1 The contrasting biological activities of platinum complexes.

multiple bonds) that are unique to them.[10] Despite this, few systematic attempts have been made to design organometallic complexes as therapeutic agents.[11,12] This is perhaps due to the assumption that organometallic chemistry and biology are mutually incompatible, many organometallic compounds being sensitive to water and oxygen. However, research in the past decade or so, notably by Köpf,[11] Alberto,[13] Fish[14] and Jaouen,[15] has demonstrated that these problems can be overcome, and that organometallic pharmaceuticals can be formulated. The organometallic technetium-99m complex $[Tc(MIBI)_6]^+$ (MIBI = 2-methoxy-2-methylpropyl isocyanide) (Fig. 2), for example, is widely used as a myocardial perfusion radioimaging agent.[16] Technetium complexes based on $\{Tc^I(CO)_3\}^+$ are currently being investigated for other radioimaging applications, and analogous rhenium(I) tricarbonyl complexes for radioimmunotherapy.[13] Mann and co-workers have shown that ruthenium carbonyl complexes, *e.g.* $[Ru(CO)_3Cl(glycinate)]$ (Fig. 2), can serve as CO-releasing molecules *in vivo*, thereby suppressing organ graft rejection and protecting tissues from ischemic injury and apoptosis.[17] It is also noteworthy that arsenic trioxide (As_2O_3) has been introduced recently into the treatment of acute promyelocytic leukemia, with remarkable clinical success.[18] Arsenic(III) is methylated in the liver *via* oxidative addition reactions to mono- and dimethylated metabolites, including methylarsonic acid, methylarsonous acid, dimethylarsinic acid, and dimethylarsinous acid. These organometallic metabolites are thought to contribute to the *in vivo* therapeutic effect of As_2O_3.[19]

Natural biomolecules with metal–carbon bonds have also been shown to exist, a well-established example being methylcobalamin (methyl-B_{12}), which contains a distinct Co–CH_3 bond. Methylcobalamin functions as a methylating

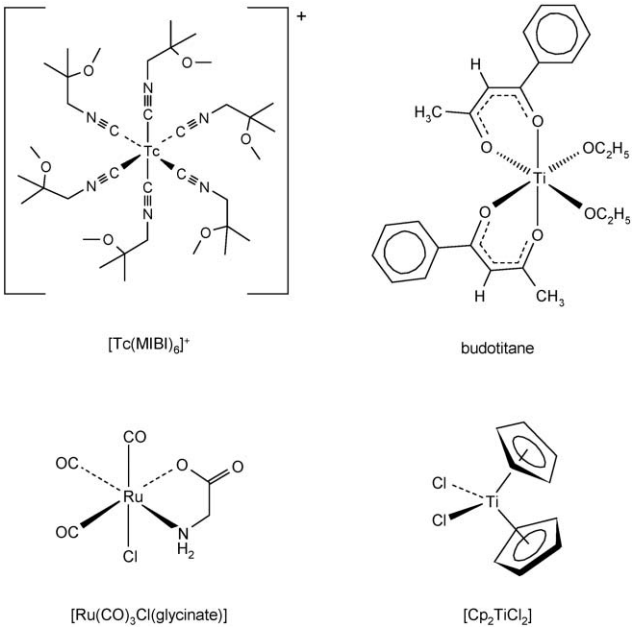

Fig. 2 Some organometallic complexes of medical interest.

agent in many important biochemical reactions, such as the synthesis of methionine from homocysteine.[20] Recently, enzymes with metal–carbon bonds have also been discovered, *e.g.* NiFe hydrogenase,[21] and acetyl-CoA synthase (also known as carbon monoxide dehydrogenase).[22] Acetyl-CoA synthase is a bifunctional enzyme that catalyzes the reversible reduction of CO_2 to CO and the synthesis of acetyl-coenzyme A from CO, coenzyme A, and a methyl group donated by a corrinoid iron–sulfur protein. The active site for acetyl-coenzyme A synthesis consists of a Fe_4S_4 cubane bridged by a cysteine thiolate sulfur to a dinuclear Ni site. During catalysis, the CO and methyl groups appear to be bound to one of the Ni atoms, on which they combine to form a Ni–$C(O)CH_3$ intermediate.

This review focuses on our work on ruthenium(II) arene anticancer complexes, and illustrates the rich structural and electronic diversity which can be incorporated into this class of

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organometallic complexes. The critical roles that the arene and ancillary ligands play in determining chemical properties, and hence biological activity of these complexes, will be discussed, together with the factors that make ruthenium(II) arene complexes good candidates as anticancer drugs. Selected organometallic anticancer complexes of other transition metals will also be briefly discussed.

2. Organometallic anticancer complexes

Titanocene dichloride, Cp_2TiCl_2 (Cp = cyclopentadienyl, Fig. 2), was originally investigated because it was believed that the *cis*-$TiCl_2$ motif would react with DNA in a similar manner to cisplatin and lead to the formation of bifunctional cross-links, which might in turn induce apoptosis and cancer cell death. However, the complex binds only weakly to DNA bases, and more strongly to the phosphate backbone.[23] There is no chemical evidence that titanocene dichloride and cisplatin have similar mechanisms of action. Titanocene dichloride is difficult to formulate for administration because of its ease of hydrolysis and ready formation of hydroxy- and oxy-bridged species. The Cp ligand is also readily displaced and readily protonated, *e.g.* on reaction with the protein transferrin in the blood.[24] Responses to titanocene dichloride in the clinic were not encouraging and the trials have now been abandoned.[25,26] Of concern is the finding that titanocene derivatives can stimulate the growth of breast cancer cells,[27] perhaps due to activation of the steroid receptor protein by Ti. Some other metallocenes are also active *in vitro*, *e.g.* Cp_2VCl_2 and Cp_2NbCl_2,[11] but have not reached clinical trials. Another Ti(IV) complex, budotitane (Fig. 2) was the first non-platinum complex to be approved for clinical trials, but poor solubility and hydrolysis made formulation difficult even in micelles, and the trials were abandoned.[28] Attempts to modify the aqueous solubility and stability of titanocenes are underway in several laboratories.[29,30]

Several series of organotin(IV) carboxylates of the general formula $[R_nSn(R'CO_2)_{4-n}]$ (R = *n*-Bu, Ph; $R'CO_2$ = benzoate, substituted benzoates, substituted salicylates; n = 2, 3) have also been found to exhibit high *in vitro* activity against MCF-7 breast cancer and WiDr colon carcinoma cells.[31,32] However, the *in vivo* activity of these compounds in tumour-bearing mice is less promising, with the compounds showing either low tumour-inhibiting activity or high toxicity. Crowe and co-workers have also screened numerous diorganotin dihalide and dipseudohalide complexes for *in vivo* activity against P388 lymphocytic leukaemia and found that the majority are inactive.[33] The reasons for the inactivity are unclear and require further investigation. Intriguingly, tin is thought to be an essential element, but its biochemistry is poorly understood.

More recently, Jaouen *et al.* prepared a series of ferrocenyl derivatives ("ferrocifens") of the breast cancer drug tamoxifen (Fig. 3).[34,35] Several of these compounds are highly active against both estrogen-dependent and estrogen-independent breast cancer cells. Tamoxifen itself is active against only estrogen-dependent cells. The mechanism of action of ferrocifens against estrogen-dependent breast cancer cells is likely to be similar to that of hydroxytamoxifen (active metabolite of tamoxifen), *i.e.* blocking the receptor protein, ER_α, for

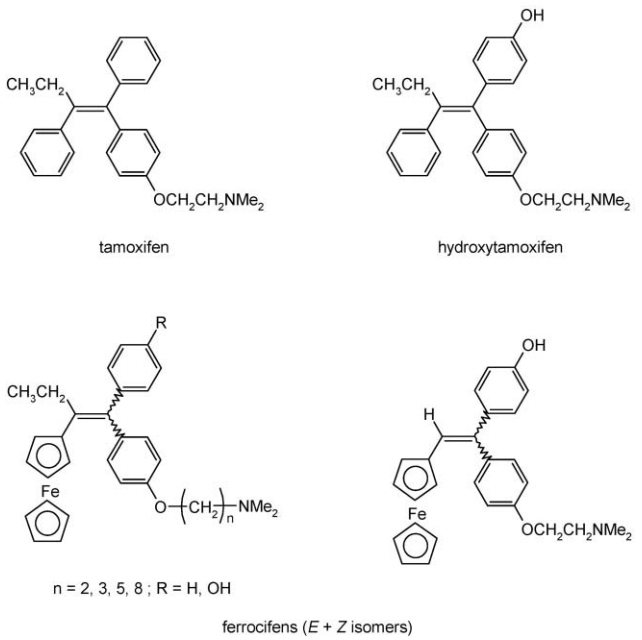

Fig. 3 Tamoxifen, hydroxytamoxifen, and ferrocifens: active against breast cancer cells.

estradiol. Ferrocifens are thought to act against estrogen-independent breast cancer cells by causing oxidative damage to DNA, after the ferrocenyl group is oxidized in the cells.[35] Other tests have shown that one of the most active ferrocifens exhibits less acute toxicity than tamoxifen.[35]

Cancer cell cytotoxicity has also been observed recently for rhenium(I) carbonyl hydroxide, alkoxide, aminoethoxide, and bromide complexes, *e.g.* $[Re_2(\mu\text{-}OR)_3(CO)_6]^-$ (R = H, Me, Et), $[Re_3(\mu_3\text{-}OH)(\mu\text{-}OH)_2(\mu\text{-}OCH_2CH_2NMe_2H)(CO)_9]$ and $[ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2CH_2OH\}]$.[36–38] These complexes generally show high *in vitro* activity against HeLa-S3 suspended uterine cells and a wide range of leukemia cell lines, but are selective in inhibiting the growth of cultures from solid tumours. It is noteworthy that many of the complexes show especially high activity against MCF-7 breast cancer cells, and that the complexes exhibit low toxicity towards non-cancerous cells (human fibroblasts).[37,38] Mode of action studies conducted on the carbonyl alkoxide and hydroxide complexes show that they interfere with DNA synthesis by inhibiting several enzymes involved in nucleic acid metabolism.[36] The complexes probably interact with the side-chains of proteins *via* substitution of the alkoxide/hydroxide ligands. Ligand exchange reactions of $[Re_2(\mu\text{-}OR)_3(CO)_6]^-$ (R = H, Me) with alcohols, phenols and thiols to form well-defined alkoxide, phenoxide and thiolate complexes have been observed by electrospray mass spectrometry.[39,40]

3. Ruthenium–arene anticancer complexes

Ruthenium compounds in general are well-suited for medicinal applications.[41] They have been investigated as immunosuppressants,[42,43] nitric oxide scavengers,[44] antimicrobial agents[45] and antimalarials.[46] Ruthenium Red, $[(NH_3)_5\text{-}Ru(III)ORu(IV)(NH_3)_4ORu(III)(NH_3)_5]^{6+}$, is known to inhibit

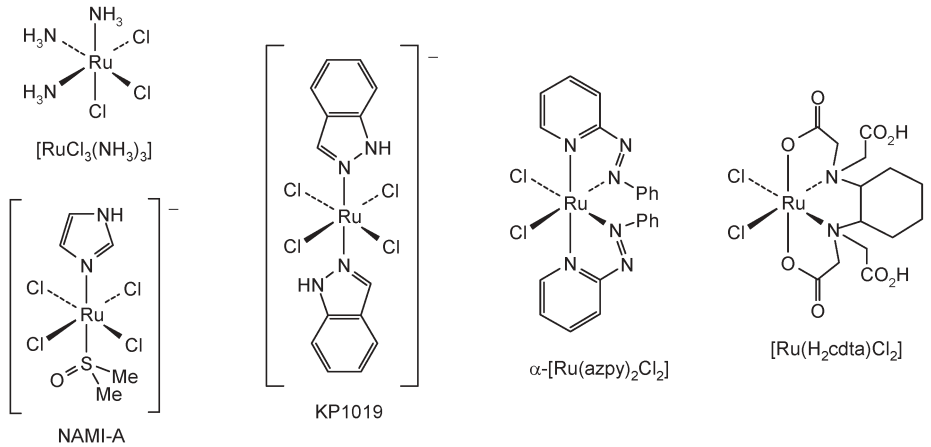

Fig. 4 Ruthenium anticancer complexes.

calcium ion uptake by mitochondria.[47] Early interest in the anticancer activity of ruthenium complexes stemmed from the observations of Clarke that Ru(III) ammines, *e.g.* $[RuCl_3(NH_3)_3]$ (Fig. 4), are active anticancer agents.[48] However, these were too insoluble for clinical use. Two other Ru(III) complexes (Fig. 4), *trans*-$[RuCl_4(DMSO)(Im)]ImH$ (NAMI-A, Im = imidazole)[49] and *trans*-$[RuCl_4(Ind)_2]IndH$ (KP1019, Ind = indazole),[50] are undergoing clinical trials. Whilst KP1019 is cytotoxic to cancer cells, NAMI-A is relatively non-toxic but has antimetastatic activity (prevents the spread of cancer). Several other Ru complexes have shown promise recently[51,52] as anticancer complexes, *e.g.* α-$[Ru(azpy)_2Cl_2]$ (azpy = 2-phenylazopyridine) and $[Ru(H_2cdta)Cl_2]\cdot 2H_2O$ (H_2cdta = 1,2-cyclohexanediaminotetraacetate), Fig. 4.

Clarke has proposed that the activity of Ru(III) complexes, which are usually relatively inert towards ligand substitution, is dependent on *in vivo* reduction to more labile Ru(II) complexes.[48,53] With this in mind, we have explored the activity of Ru(II) complexes. It should be remembered, however, that the kinetic lability of metal ions is highly dependent on the types of bound ligands. We have found that the substitution rates of Ru(II) arene complexes, for example, can vary on a timescale of many orders of magnitude. Exploration of the kinetics and mechanisms of ligand substitution and redox reactions is essential in this field.

We discovered that Ru(II) aminophosphine complexes were cytotoxic to cancer cells,[54] but they had poor aqueous solubility and were difficult to isolate and purify in large quantities. Since arenes are known to stabilise ruthenium in its 2+ oxidation state, we have investigated the potential of Ru(II) arene complexes as anticancer agents and their associated aqueous chemistry. We have found that "half-sandwich" Ru(II) mono-arene complexes often possess good aqueous solubility (an advantage for clinical use) and that the arene ligand is relatively inert towards displacement under physiological conditions.

3.1 Structure of Ru arenes

A typical structure of a half-sandwich "piano-stool" [(η^6-arene)Ru(X)(Y)(Z)] complex is shown in Fig. 5, where the arene forms the seat of the piano stool and the ligands resemble the legs. Linking the ligands Y and Z to form a bidentate chelating ligand (L) seems to be advantageous for anticancer activity. The structure of Ru(II) half-sandwich complexes allows for variations of the three main building blocks, the monodentate ligand X, the bidentate ligand L and the arene, to fine-tune the pharmacological properties of these complexes. As will be demonstrated in the subsequent sections, the chelating ligand can help to control the stability and ligand-exchange kinetics of these complexes. The nature of the arene can help to influence cell uptake and interactions with potential targets. The leaving group, which typically is chloride and occupies the biomolecule binding site on the metal centre, can be of importance to control the timing of activation of these complexes.

3.2 Anticancer activity

Reproducible cytotoxicities against A2780 human ovarian cancer cells are exhibited by the complexes [(η^6-arene)Ru(en)(Cl)]$^+$ (Fig. 6).[55] Activity appears to increase with the size of the coordinated arene: benzene (Ben) < *p*-cymene

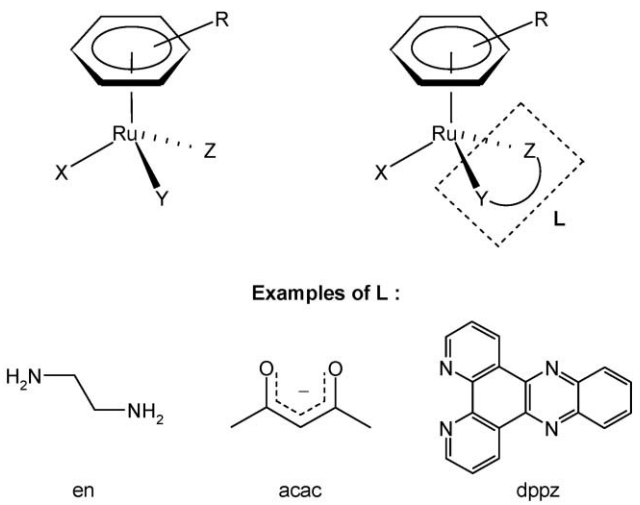

Fig. 5 Typical structures of Ru(II) half-sandwich complexes and selected examples of chelating ligands, L.

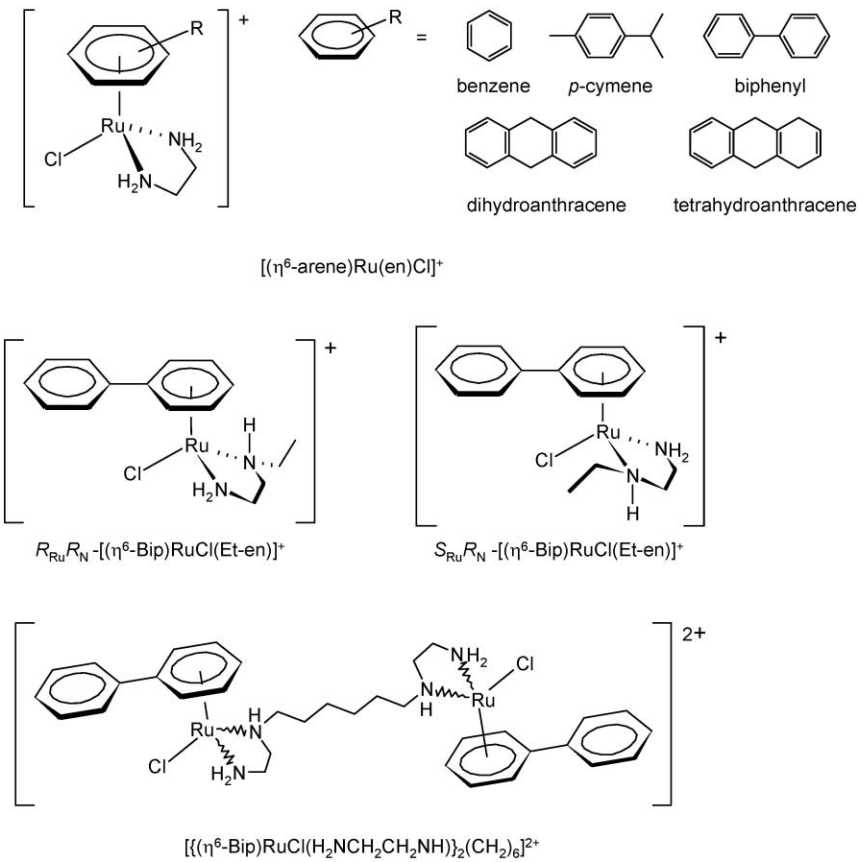

Fig. 6 Some mono- and dinuclear Ru(II) arene complexes.

(Cym) < biphenyl (Bip) < dihydroanthracene (DHA) < tetrahydroanthracene (THA), such that, in this cell line, the Bip complex has similar cytotoxicity to the anticancer drug carboplatin (IC_{50}, the dose which inhibits growth of 50% of the cells, 6 μM) and the THA complex is as active as cisplatin (IC_{50} 0.6 μM) (Table 1).[56] The complexes [(η^6-Cym)Ru(X)(Y)(Z)] (X, Y or Z = halide, acetonitrile or isonicotinamide), with 3 monodentate ligands, are however inactive towards A2780 human ovarian cancer cells *in vitro*.[55] These complexes may be too reactive with components of the cell culture medium and/or the cells and are deactivated by biomolecules before they reach their target sites. Substitution of chloride by other halides such as iodide has only a small effect on cytotoxicity.[56] From the above results, it appears that a more hydrophobic arene ligand and a single ligand exchange site (occupation of the other two coordination sites by a stable bidentate chelating ligand) are associated with high cytotoxicity. Recent cytotoxicity tests on a more extensive range of Ru(II) arene complexes have indicated, however, that the structure–activity relationship is more complex.[57] For example, when en is replaced by acetylacetonate (acac, Fig. 5), the Cym and Bip complexes are much more cytotoxic than the DHA complex. Replacing en by *N*,*N*,*N*′,*N*′-tetramethylethylenediamine or 2,2′-bipyridine results in complexes with insignificant cytotoxicity, whilst complexes with 1,2-diaminobenzene as the chelating ligand show comparable or enhanced cytotoxicity compared to the en analogues.[57]

Significantly, the [(η^6-arene)Ru(en)Cl]$^+$ complexes are equally potent towards wild-type (A2780) and cisplatin-resistant (A2780cis) human ovarian cancer cells in culture.[56] This suggests that their mechanism of action is different from that of cisplatin. Varying degrees of cross-resistance are observed, however, between Ru(II) arene complexes and adriamycin (doxorubicin). Adriamycin-resistant A2780^{AD} cells over-express the 170 kDa plasma membrane glycoprotein P-gp, which is responsible for drug efflux from cells and has a high specificity for molecules which are hydrophobic and positively-charged, features present in [(η^6-arene)Ru(en)-(Cl/H_2O)]$^{+/2+}$ complexes. Administration of verapamil, a

Table 1 IC_{50} values of Ru(II) arene complexes [(η^6-arene)Ru-(X)(Y)(Cl)]A [A = PF_6^- for positively-charged complexes], carboplatin and cisplatin in A2780 human ovarian cancer cells after 24 h drug exposure[56]

Arene/*Pt complex*	X	Y	IC_{50} (μM)
p-cymene	CH_3CN	CH_3CN	> 100
p-cymene	Cl	Isonicotinamide	> 100
$C_6H_5CO_2CH_3$	$H_2NCH_2CH_2NH_2$		56
benzene	$H_2NCH_2CH_2NH_2$		17
p-cymene	$H_2NCH_2CH_2NH_2$		10
Carboplatin			6
$C_6H_5C_6H_5$	$H_2NCH_2CH_2NEtH$		6
$C_6H_5C_6H_5$	$H_2NCH_2CH_2NH_2$		5
dihydroanthracene	$H_2NCH_2CH_2NH_2$		2
Cisplatin			0.6
tetrahydroanthracene	$H_2NCH_2CH_2NH_2$		0.5

known inhibitor of P-gp, completely restores sensitivity of the $A2780^{AD}$ cells to Ru(II) arene complexes, confirming the involvement of P-gp in resistance against Ru(II) arene complexes. Knowledge of the active sites of such multi-drug resistance proteins[58] could allow design of Ru(II) arene complexes which circumvent this efflux mechanism. Interestingly, no adriamycin cross-resistance is observed for Ru(II)–arene complexes of 1,2-diaminobenzene, a diamine which is significantly larger and more hydrophobic than en, and which possibly makes the complex a poorer fit for the protein binding pocket.[57]

Ruthenium arene complexes are also cytotoxic towards a wide spectrum of other cancer cells. For example, the complexes $[(\eta^6\text{-Bip})Ru(en)Cl]PF_6$ and $[(\eta^6\text{-DHA})Ru(en)Cl]PF_6$ are active against HT29 colon, Panc-1 pancreatic and NX02 lung cancer cells, with IC_{50} values in the range 1–13 μM.[57]

The patterns of activity established *in vitro* for $[(\eta^6\text{-Bip})Ru(en)Cl]PF_6$ are mirrored to a large degree *in vivo*, with the compound effecting significant growth delays against both A2780 and A2780cis tumours grafted on mice (xenografts) whilst being inactive against the $A2780^{AD}$ xenograft.[56] Mice are also able to tolerate the ruthenium complex better than cisplatin (up to 25 mg per kg body mass of $[(\eta^6\text{-Bip})Ru(en)Cl]PF_6$ injected on days 1 and 5 without significant weight loss, compared to 10 mg kg^{-1} cisplatin as a single injection on day 1).[56] With the respective dose regimes above, $[(\eta^6\text{-Bip})Ru(en)Cl]PF_6$ produced a growth delay on A2780 xenografts represented by a T/C value of 46%, much higher than that of 23% for cisplatin, on day 16 of the experiment. [T/C (%) = (mean tumour volume of the drug-treated group/mean tumour volume of the control group) × (100)] In the A2780cis xenograft $[(\eta^6\text{-Bip})Ru(en)Cl]PF_6$ gave a T/C value of 51% on day 13.

4. Interaction with biologically-relevant molecules

In biological systems, $[Ru(\eta^6\text{-arene})(X)(L)]^{n+}$ complexes will encounter an array of biomolecules with which they could potentially react. Hence it is important to gain a detailed understanding of such interactions with ligands ranging from water and chloride to nucleobases, oligonucleotides, DNA, amino acids and proteins. Reactions in media with low dielectric constants may also be relevant to the passage of the complexes across membranes.

4.1 Aqueous chemistry

In aqueous media, the chloride ligand of $[(\eta^6\text{-arene})Ru(L)Cl]^+$ complexes can exchange with water to form aqua complexes $[(\eta^6\text{-arene})Ru(L)H_2O]^{2+}$. For L = en, the chloride-containing complexes generally undergo substitution reactions very much more slowly than the corresponding aqua compounds,[59] hence it is important to understand the thermodynamics and kinetics of formation of the aqua complexes (*i.e.* activated form of the ruthenium arene complexes). The rates of aquation of $[(\eta^6\text{-arene})Ru(en)Cl][PF_6]$ (arene = Bip, DHA and THA) at 310 K and ionic strength (*I*, $NaClO_4$) of 0.1 M (k_{H_2O} 3.95–6.84 × 10^{-3} s^{-1}) are an order of magnitude faster than that of cisplatin.[60] The reverse, anation reactions in the presence of 100 mM NaCl (similar concentration to that in blood plasma) are also very rapid (k_{Cl} 0.435–0.722 M^{-1} s^{-1}, 310 K, I = 0.1 M). The aquation and anation reactions are *ca.* 2 times faster for the DHA and THA complexes compared to the Bip complex, suggesting that variations in the steric and electronic effects of the arene ligands modulate the ligand exchange reactions. The exchange reactions appear to occur *via* an associative pathway, $\Delta S^{\ddagger}$ being negative.[60] Since the anation reactions are rapid, the equilibrium constants for aquation (k_{H_2O}/k_{Cl}) are small, 9.0–11.7 × 10^{-3} M. Hence at physiologically-relevant concentrations of the ruthenium(II) arene complexes (0.5–5 μM), the complexes should be present in blood plasma largely as the less reactive chloro complexes (> 89%), whereas in the cell nucleus ($[Cl^-]$ = 4 mM)[61,62] significant amounts (45–65%) of the more reactive aqua species would be formed readily (Fig. 7).

The coordinated aqua ligand of $[(\eta^6\text{-arene})Ru(en)(H_2O)]^{2+}$ undergoes acid dissociation to give the hydroxo complex $[(\eta^6\text{-arene})Ru(en)(OH)]^+$, which is less susceptible to substitution reactions than the aqua complex.[59] The pK_a values range from 7.71 to 7.89 and 8.01 for the Bip, DHA and THA aqua complexes, respectively.[60] Since the pK_a values are high, only small amounts of the hydroxo species (< 10% of the total Ru arene complex) would be present at biological pH (7.2–7.4) (Fig. 7), *cf.* for cisplatin, the dominant species in the cell nucleus are the less reactive hydroxo forms.[62]

In order to optimize the efficacy of ruthenium(II) arene compounds, it is desirable to be able to manipulate the rate and extent of aquation and the pK_a value of coordinated water. We have found that the bidentate chelating ligand L has a major influence on the rate and extent of aquation. For example, the acac complex $[(\eta^6\text{-Cym})Ru(acac)Cl]$[63] undergoes aquation more rapidly and to a much greater extent than the en analogue, and its aqua derivative $[(\eta^6\text{-Cym})Ru(acac)(H_2O)]^+$ has a pK_a value (9.41) which is considerably higher than that

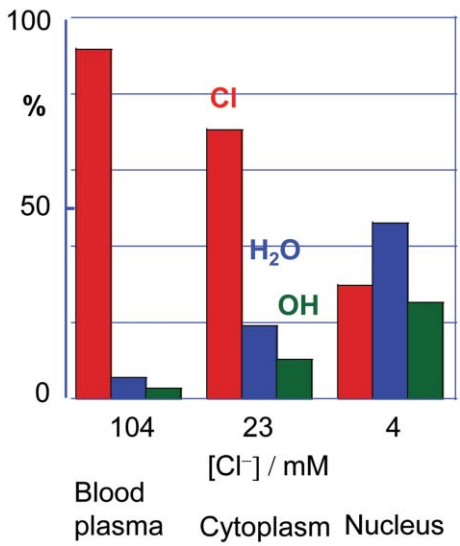

Fig. 7 Speciation of $[(\eta^6\text{-Bip})Ru(en)Cl]^+$ [5 μM] in blood plasma, cytoplasm and nucleus at equilibrium, based on the chloride concentration and pH in these environments and the equilibrium constant of aquation and pK_a of the complexes.[60]

for the en analogue (8.24).[59] These differences can be attributed to the stronger electron-donating ability of the acac ligand.[64] Therefore the ancillary ligands in Ru(II) arene complexes can be used to fine-tune the electronic properties. This allows control of the rate and extent of formation of the reactive species, which can be important in drug design.

4.2 Nucleobase binding

Binding studies of ruthenium(II) arene complexes with nucleobases are of special interest since DNA is the primary target of the archetypal metal-based drug, cisplatin.[65] Therefore we have investigated reactions of complexes $[(\eta^6\text{-arene})Ru(en)X]^{n+}$, where arene = Bip, THA, DHA, Cym and Ben, X = Cl^- or H_2O, with nucleic acid derivatives (Fig. 8) as models of DNA.[59] For mononucleosides, $\{(\eta^6\text{-Bip})Ru(en)\}^{2+}$ binds only to N7 of guanosine (G) and to N3 of thymidine (T). Binding to N3 of cytidine (C) is weak, and almost no binding to adenosine (A) is observed. The reactivity of the various binding sites of nucleobases towards Ru(II) at neutral pH decreases in the order G(N7) > T(N3) > C(N3) > A(N7), A(N1). Although this parallels the preference of cisplatin for binding with guanine over adenine,[66] the diamino Ru(II) arene complexes are more highly discriminatory between G and A bases than Pt(II) complexes. This site-selectivity appears to be enhanced by the en NH_2 groups, which H-bond with exocyclic oxygens (*e.g.* C6O of G, see Fig. 9) but are non-bonding and repulsive towards exocyclic amino groups of the nucleobases (*e.g.* C6NH_2 of A, Fig. 9).

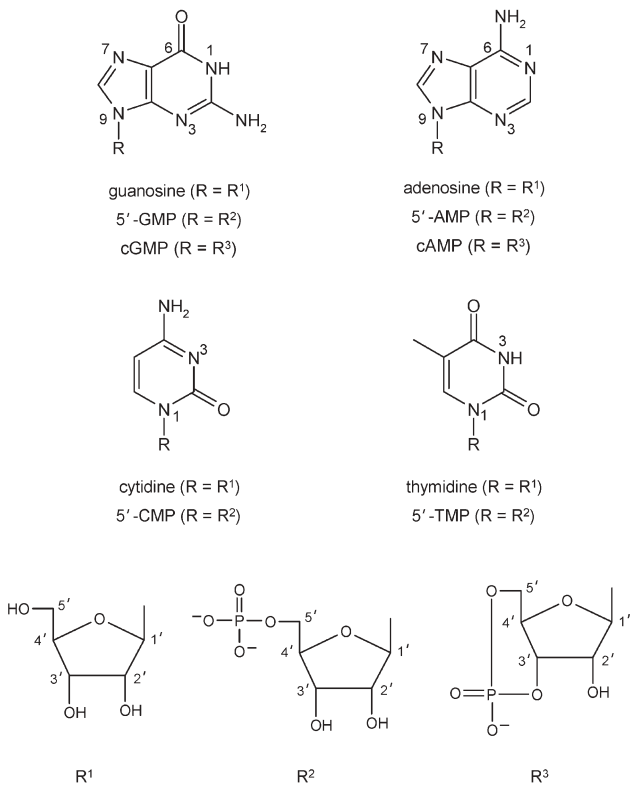

Fig. 8 Structures of mononucleosides (guanosine, adenosine, cytidine, thymidine), mononucleotides (5′-GMP, 5′-AMP, 5′-CMP, 5′-TMP), and cyclic nucleotides (cGMP, cAMP).

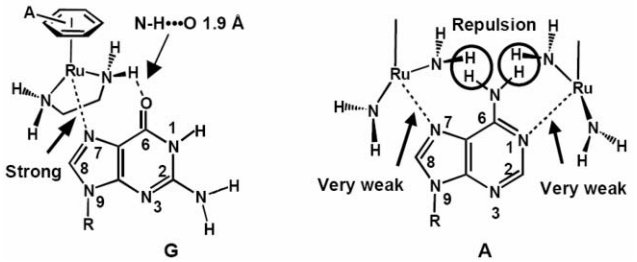

Fig. 9 H-bonding and steric interactions which give rise to strong binding of $\{(\eta^6\text{-arene})Ru(en)\}^{2+}$ to guanine but very weak binding to adenine. For clarity, the arene and en ring are omitted in the right-hand (A) structure.[59]

The strong preference for G bases may allow Ru(II) complexes to target selectively G-rich regions of DNA, such as telomeres which play key roles in cell division. Telomeres occur as guanine-rich overhangs at the 3′ ends of eukaryotic chromosomes and typically contain repeat sequences such as $(TTAGGG)_n$.[67]

For mononucleotides,[59] the same pattern of site selectivity is observed; in competitive reactions of $[(\eta^6\text{-Bip})Ru(en)Cl]^+$ with 5′-GMP, 5′-AMP, 5′-CMP and 5′-TMP, the only final adduct is $[(\eta^6\text{-Bip})Ru(en)(N7\text{-GMP})]^{2+}$. Significant amounts of the 5′-phosphate-bound species (40–60%) are also present at equilibrium for 5′-TMP, 5′-CMP and 5′-AMP. Reactions with nucleotides proceed *via* aquation of $[(\eta^6\text{-arene})Ru(en)Cl]^+$, followed by rapid binding to the 5′-phosphate group, and then rearrangement to give N7, N1 or N3-bound products. Binding of $\{(\eta^6\text{-Bip})Ru(en)\}^{2+}$ to N7 of 5′-GMP lowers the pK_a of N1H of the purine ring by 1.4 units. Such a lowering is also observed for Pt(II)–G adducts.[68,69] Metallation of N7 of G, which is accessible from the major groove of B-DNA, can therefore lead to significant electronic perturbations at N1H which is an H-bond donor in G–C base-pairs in the DNA double helix. This may influence the stability of the double helix. No binding to the phosphodiester groups of 3′,5′- cyclic guanosine monophosphate (cGMP) or cAMP (Fig. 8) is detected, suggesting that Ru(II) arene complexes do not bind to the phosphodiester groups of the DNA backbone.

Strong stereospecific intramolecular H-bonding between an en NH proton oriented away from the arene and the C6O carbonyl of G is present in the crystal structures of Ru–arene adducts of 9-ethylguanine (9EtG) and guanosine (Fig. 10; average N···O distance 2.8 Å, N–H···O angle 163°).[70] In solution, NMR studies have provided evidence that en NH protons of the 5′-GMP adduct are involved in strong

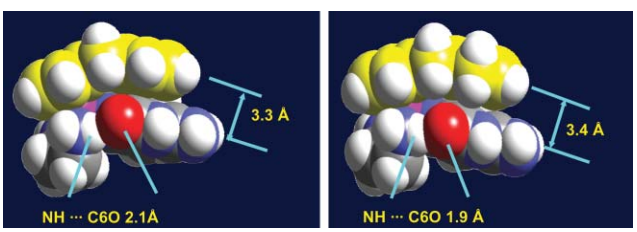

Fig. 10 Crystal structures of $[(\eta^6\text{-DHA})Ru(en)(9EtG)]^{2+}$ (left) and $[(\eta^6\text{-THA})Ru(en)(9EtG)]^{2+}$ (right), showing the arene–purine π-stacking and hydrogen bonding between en NH and G C6O.[70]

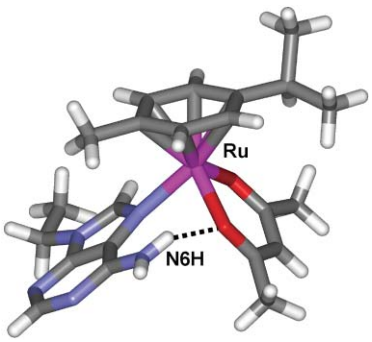

Fig. 11 Molecular model of $[(\eta^6\text{-Cym})Ru(acac)(9EtA)]^+$.[63] The hydrogen bond between acac O and A N6H is represented by a dashed line.

H-bonding with the 5′-phosphate and C6O of 5′-GMP. Such H-bonding from G C6O to en NH protons is an important factor contributing to the high preference for binding of $\{(\eta^6\text{-arene})Ru(en)\}^{2+}$ to G *versus* A (adenine). Accordingly, replacement of en (NH as H-bond donor) by the acac ligand (O as H-bond acceptor) changes the nucleobase selectivity. The complex $[(\eta^6\text{-Cym})Ru(acac)Cl]$ binds equally well to adenosine as to guanosine [A(N7) ≈ G(N7) > A(N1)][63] and anticancer activity *in vitro* (towards A2780 human ovarian cancer cells) is retained.[57] It is also worth noting that no binding of this acac complex to either thymidine or cytidine was observed. Molecular modeling[63] (Fig. 11) and X-ray crystallography[71] confirmed that coordination of adenine to $[(\eta^6\text{-Cym})Ru(acac)]^+$ is stabilized by hydrogen bonding between $N6H_2$ of A as a hydrogen-bond donor and an oxygen atom of acac as the acceptor. Furthermore, the structure of $[(\eta^6\text{-Cym})Ru(Ph_2acac)(9EtG)]^+$ (Ph_2acac = 1,3-diphenyl-1,3-propanedionate) reveals a close contact of 3.08 Å between the oxygen of Ph_2acac and exocyclic oxygen C6O of 9EtG.[71] This suggests that steric repulsion exerted by an appropriate chelating ligand can help to overcome the preference for G(N7) binding.

There is evidence that N7-binding of guanine is also promoted by favourable arene–purine hydrophobic interactions in the associative transition state.[59] The rates of reaction of 3′,5′-cyclic guanosine monophosphate with $[(\eta^6\text{-arene})Ru(en)X]^{n+}$ (where X = Cl^- or H_2O) (pH 7.0, 298 K, 100 mM $NaClO_4$) decrease in the order THA > Bip > DHA ≫ Cym > Ben. Strong arene–nucleobase π-stacking is present in the crystal structures of the 9-ethylguanine (9EtG) complexes $[(\eta^6\text{-DHA})Ru(en)(9EtG\text{-}N7)][PF_6]_2$ and $[(\eta^6\text{-THA})Ru(en)(9EtG\text{-}N7)][PF_6]_2$, (Fig. 10).[70] The outer ring of the DHA ligand stacks over the purine base at a distance of 3.45 Å, and for THA at 3.31 Å, with dihedral angles of 3.3° and 3.1°, respectively. In the crystal structure of $[(\eta^6\text{-Bip})Ru(en)(9EtG\text{-}N7)][PF_6]_2$, there is intermolecular stacking between the pendent phenyl ring and the purine six-membered ring of an adjacent cation at a distance of 4.0 Å (dihedral angle 4.5°). The guanosine (Guo) adduct $[(\eta^6\text{-Bip})Ru(en)(Guo\text{-}N7)][PF_6]_2$ exhibits intramolecular stacking of the pendent phenyl ring with the purine five-membered ring (3.8 Å, 23.8°), and intermolecular stacking of the purine six-membered ring with an adjacent pendent phenyl ring (4.2 Å, 23.0°). Although the orientation of arene and purine in the crystal structure of $[(\eta^6\text{-Bip})Ru(en)(9EtG\text{-}N7)][PF_6]_2$ is *anti*, in solution a *syn* orientation predominates for all the Bip adducts as revealed by NMR NOE studies. The predominance of the *syn* orientation can be attributed to hydrophobic interactions between the arene and purine rings. Binding to guanine is accompanied by significant re-orientations and conformational changes of the arene ligands with respect to the parent chloro-complexes $[(\eta^6\text{-arene})Ru(en)Cl]^+$.[70] The arene ligands are flexible through rotation around the arene–Ru π-bonds, through twisting about the Ph–Ph bond (for Bip), and ring bending (for THA and DHA), so as to maximise intra- or inter-molecular stacking with the purine ring.

We have also shown that dynamic chiral recognition of guanine can occur. For example, the $R^*_{Ru}R^*_N$ and $S^*_{Ru}R^*_N$ diastereomers of $[(\eta^6\text{-Bip})Ru(Et\text{-}en)(Cl)]^+$ (Et-en = $EtNHCH_2CH_2NH_2$) (Fig. 6) react with 9EtG to give selectively (95%) the $S^*_{Ru}R^*_N$ adduct, although for the chloro complex, the $R^*_{Ru}R^*_N$ diastereomer (73%) is more stable than the $S^*_{Ru}R^*_N$ diastereomer (27%).[72] This highly diastereoselective binding of 9EtG probably proceeds *via* epimerization of $R^*_{Ru}R^*_N$ to $S^*_{Ru}R^*_N$-$[(\eta^6\text{-Bip})Ru(Et\text{-}en)(Cl)]^+$, which occurs slowly in water ($t_{½}$ *ca.* 2 h at 298 K, pH 6.2). X-ray crystallographic analysis of $R^*_{Ru}R^*_N$ and $R^*_{Ru}R^*_N$-$[(\eta^6\text{-Bip})Ru(Et\text{-}en)(Cl)]^+$ and the $R^*_{Ru}R^*_N$ 9EtG adduct showed that the latter is stabilized by stereospecific hydrogen bonding between en NH and G C6O. Thus, the concept of induced-fit recognition of DNA by organometallic Ru(II) arene complexes containing dynamic stereogenic centres *via* dynamic epimerization may be useful in the design of anticancer drugs.

In summary, direct coordination to the bases, intercalation, and stereospecific H-bonding are useful features to incorporate into the design of Ru(II) arene complexes to optimise the recognition of DNA.

4.3 Interactions with oligonucleotides and DNA

The complex $[(\eta^6\text{-Cym})Ru(en)Cl][PF_6]$ binds selectively to G bases on DNA oligonucleotides, forming Ru–G_7 and Ru–G_8 monoruthenated and G_7(Ru)–G_8(Ru) diruthenated adducts on the 14-mer d(ATACATG$_7$G$_8$TACATA).[55] The reaction of the complementary strand d(TATG$_4$TACCATG$_{11}$TAT) with $[(\eta^6\text{-Bip})Ru(en)Cl][PF_6]$ in triethylammonium acetate buffer (pH 7.03) also gives mono- and diruthenated oligonucleotides, as indicated by LC-ESI-MS.[73] At 310 K *ca.* 92% of the latter oligonucleotide is ruthenated, of which *ca.* 52% forms the diruthenated product, further suggesting a high affinity of $[(\eta^6\text{-Bip})Ru(en)Cl][PF_6]$ for G bases. The presence of cytochrome c (1 mole equivalent) or L-histidine (4 mole equivalents) (see Section 4.4) has little effect on the amounts of mono- and diruthenated oligonucleotide products formed, and no $\{(\eta^6\text{-Bip})Ru(en)\}$–histidine or $\{(\eta^6\text{-Bip})Ru(en)\}$–cytochrome c adducts are detected. This suggests that in cells DNA (or RNA) may be the favoured reaction site for $[(\eta^6\text{-arene})Ru(en)Cl]^+$ anticancer complexes.

The dinuclear complex $[\{(\eta^6\text{-Bip})RuCl(H_2NCH_2CH_2NH)\}_2(CH_2)_6]^{2+}$ (Fig. 6), like $[(\eta^6\text{-Bip})Ru(Et\text{-}en)(Cl)]^+$ (*vide supra*) exhibits dynamic chiral recognition of 9EtG.[72] It binds rapidly and strongly to calf thymus DNA (*i.e.* natural B-DNA), preferentially at the G sites, and effectively inhibits

Table 2 Unwinding of supercoiled plasmid DNA by Ru(II) arene complexes[72,74]

Complex	Unwinding angle
$[(\eta^6\text{-Cym})Ru(en)Cl]^+$	7°
$[(\eta^6\text{-Bip})Ru(en)Cl]^+$	14°
$[(\eta^6\text{-THA})Ru(en)Cl]^+$	14°
$[(\eta^6\text{-DHA})Ru(en)Cl]^+$	14°
$[(\eta^6\text{-Bip})Ru(Et\text{-}en)Cl]^+$	14°
$[\{(\eta^6\text{-Bip})RuCl(H_2NCH_2CH_2NH)\}_2(CH_2)_6]^{2+}$	31°

DNA-directed RNA synthesis *in vitro*.[72] This binding probably involves dynamic chiral recognition similar to that observed with 9EtG. The complex $[\{(\eta^6\text{-Bip})RuCl(H_2NCH_2CH_2NH)\}_2(CH_2)_6]^{2+}$ also induces a large unwinding (31°) of supercoiled plasmid DNA. This unwinding angle is more than twice that induced by the mononuclear complex $[(\eta^6\text{-Bip})Ru(Et\text{-}en)(Cl)]^+$ (Table 2) and is attributable to cross-linking of the DNA *via* the two Ru centres and perturbation of the DNA structure by the pendent phenyl rings (Fig. 12). Independent evidence for DNA cross-linking by $[\{(\eta^6\text{-Bip})RuCl(H_2NCH_2CH_2NH)\}_2(CH_2)_6]^{2+}$ is obtained from its reaction with a 213-bp plasmid DNA fragment, on which the complex generates interstrand cross-links with similar efficiency to cisplatin, and with 20-mer DNA duplexes, where 1,3-GG interstrand and 1,2-GG and 1,3-GTG intrastrand cross-links are formed.[72]

Comparative studies have shown that $[(\eta^6\text{-arene})Ru(en)Cl]^+$ complexes (arene = Bip, DHA, THA or Cym) bind relatively rapidly to calf thymus (CT) DNA at 310 K, with 50% binding in 3 h for the Cym complex and 10–15 min for the others.[74] Circular dichroism (CD) and differential pulse polarography data suggest that the Bip and anthracene complexes cause non-denaturational changes in DNA conformation (like cisplatin), in contrast to the Cym complex, which distorts the DNA more severely (like transplatin).[74] The CD data also suggest that intercalation and/or minor groove binding are involved in the binding of the Bip and anthracene derivatives, but not the Cym complex, to CT DNA. Flow linear dichroism (LD) data suggested that all the Ru(II) arene complexes (arene = Bip, DHA, THA or Cym) cause bending of DNA. The LD data are also consistent with partial intercalation of the Bip, DHA and THA complexes into DNA. In agreement with this conclusion, these complexes induce a loss of fluorescence of DNA–ethidium bromide adducts, consistent with displacement of the intercalated ethidium cations by the Ru(II) arenes. Furthermore, at ionic strengths similar to that found in cells, the Bip and anthracene derivatives all increase the melting temperature (t_m, temperature at which transition from duplex to single-stranded DNA occurs) of CT DNA, whilst the Cym complex induces a decrease of t_m. The difference in melting behaviour is consistent with the intercalating ability of the former, and the lack of this ability in the latter. The intercalating complexes also produce an unwinding angle (14°) on supercoiled plasmids that is twice that produced by $[(\eta^6\text{-Cym})Ru(en)Cl]^+$ (7°).[74] Overall, the above results provide strong evidence for combined intercalative and coordinative binding modes for the Bip and anthracene complexes. It is also worth noting that DNA intercalation can also be achieved by incorporation of a polycyclic aromatic chelating ligand *e.g.* dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz, Fig. 5).[75]

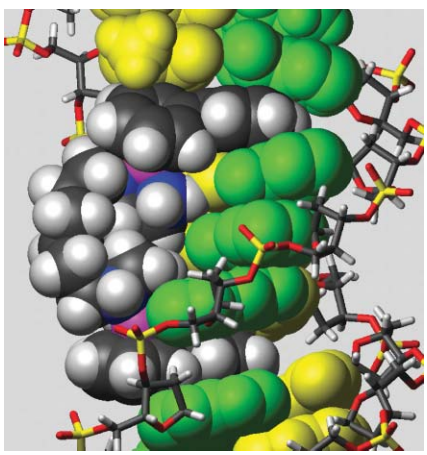

Fig. 12 Model of the interaction of $[\{(\eta^6\text{-Bip})RuCl(H_2NCH_2CH_2NH)\}_2(CH_2)_6]^{2+}$ with DNA to form a 1,3-interstrand G–G cross-link *via* the two Ru centres.[72] There is also partial intercalation of the pendent phenyl rings and G C6O···HN hydrogen-bonding. The C6 linker of the dinuclear complex sits in the major groove of the DNA double helix. The sugar–phosphate backbones of the DNA strands are shown in capped-stick representation (P – yellow, O – red, C – black, H – grey); colour code for space-filling model: nucleobases – green and yellow (complementary strand), Ru – magenta, C – black, N – blue, H – grey.

In vitro RNA synthesis by RNA polymerases on DNA templates (plasmid fragments) bound to $\{(\eta^6\text{-arene})Ru(en)\}^{2+}$ units (arene = Bip, DHA, THA or Cym) can be prematurely terminated at the level or in the proximity of the adducts.[74] The major stop sites are similar to those exhibited by cisplatin–DNA adducts (mainly at G sites), although in general the efficiency of the Ru adducts in terminating RNA synthesis is lower than that of cisplatin. In addition, the efficiency of the Cym complex is noticeably lower than that of the other complexes.

In a further collaboration with Viktor Brabec's group, we also analysed DNA duplexes (15–20 bp) singly-modified at central guanine residues by the complexes $[(\eta^6\text{-Cym})Ru(en)Cl]^+$ (Ru-CYM, non-intercalating) and $[(\eta^6\text{-THA})Ru(en)Cl]^+$ (Ru-THA, intercalating) for conformational distortions, recognition by DNA-binding proteins and repair.[76] We observed substantial differences in conformation and thermodynamic stability between duplexes modified by Ru-CYM and by Ru-THA. The distortion induced by Ru-CYM, detected using chemical probes of DNA conformation ($KMnO_4$ and diethyl pyrocarbonate), extends over at least 7 bp, whereas that induced by Ru-THA is less extensive. Ru-CYM also destabilizes duplex DNA more than Ru-THA, as shown by isothermal titration calorimetry. Neither distortion is recognized by the DNA-binding HMGB1 protein, however, indicating that the mechanism of antitumour activity of Ru(II) arene complexes does not involve recognition of their DNA adducts by HMG domain proteins as a crucial step, in contrast to the proposals for cisplatin and its direct analogs.[77,78] The adducts of Ru-CYM are removed from DNA more efficiently than those of Ru-THA, which is consistent with the latter being more cytotoxic to cancer cells. Interestingly, both adducts are removed from DNA preferentially by mechanisms

other than nucleotide excision repair (a major mechanism contributing to cisplatin resistance).[79–81] This provides additional support for a different mechanism for antitumour activity of Ru(II) arene complexes compared to cisplatin. Both Ru-CYM and Ru-THA constitute fairly strong blocks to DNA polymerization when bound to the template strand, but these blocks are not absolute, allowing DNA synthesis across the block site with limited efficiency.

4.4 Reactions with amino acids and proteins

Reactions between the sulfur-containing amino acids cysteine and methionine (Fig. 13) and ruthenium(II) arene anticancer complexes are of much interest in view of the strong influence of sulfur amino acids on the intracellular chemistry of platinum drugs, in particular, their involvement in detoxification and resistance mechanisms.[82] Protein targets may also play a role in the mechanism of action of Ru(II) arene complexes, including the possibility that ruthenium can substitute for iron in proteins.

We found that [(η^6-Bip)Ru(en)Cl][PF_6] reacts slowly, and only to about 50% completion, with the thiol amino acid L-cysteine in aqueous solution at 310 K, pH 2–5, and a 1 : 2 molar ratio.[83] Reactions appeared to involve aquation as the first step followed by initial formation of 1 : 1 adducts *via* substitution of water by S-bound or O-bound cysteine. Two dinuclear complexes were also detected as products from the reaction. These arise from the loss of chelated ethylenediamine, and contain one or two bridging cysteines. The unusual cluster species {(Bip)Ru}$_8$ was also formed, especially at higher cysteine concentrations. Reactions with cysteine are suppressed in 50 mM triethylammonium acetate solution at pH > 5 or in 100 mM NaCl, suggesting that thiols may not readily inactivate Ru(II)–en arene complexes in blood plasma or in cells. Similarly, interaction with the thioether sulfur of methionine appears to be relatively weak. Only 27% of [(η^6-Bip)Ru(en)Cl][PF_6] reacted with L-methionine (L-MetH) at an initial pH of 5.7 after 48 h at 310 K, and gave rise to only one adduct, [(η^6-Bip)Ru(en)(L-MetH-*S*)]$^{2+}$. In recent work we have detected surprisingly facile oxidation of the sulfur of coordinated glutathione (γ-L-Glu-L-Cys-Gly) to give sulfenato complexes.[84] Ruthenium-bound sulfenato ligands appear to be readily displaced by guanine. Hence, there may be redox-mediated pathways for the ruthenation of DNA (and RNA) *via* glutathione intermediates. Glutathione is present in cells at millimolar concentrations.[85]

Histidine residues are also possible binding sites for ruthenium arene complexes in proteins. Hence, we also studied the reaction of [(η^6-Bip)Ru(en)Cl][PF_6] with L-histidine (L-His, Fig. 13) in aqueous solution at 310 K.[73] This reaction is also slow, and gives two isomeric imidazole-bound L-His adducts, [(η^6-Bip)Ru(en)(N_δ-L-His]$^{2+}$ and [(η^6-Bip)Ru(en)(N_ε-L-His]$^{2+}$. Considering the two isomers together, an equilibrium constant of 0.14 mM^{-1} was determined for the reaction between L-His and the aquated species [(η^6-Bip)Ru(en)(H_2O)]$^{2+}$. Comparison of this value to those obtained for L-cysteine (0.60 mM^{-1}) and L-methionine (0.34 mM^{-1})[83] suggests that the affinity of the [(η^6-Bip)Ru(en)]$^{2+}$ fragment for these amino acids decreases in the order L-Cys > L-Met > L-His.

Reactions between [(η^6-Bip)Ru(en)Cl][PF_6] and the haem protein cytochrome c have also been studied.[73] Cytochrome c has a buried (His26) and an exposed surface histidine residue (His33). Electrospray mass spectrometry indicated that in both water (pH 8.7) and triethylammonium acetate buffer (pH 7.6) only monoruthenated cytochrome c products are formed, even when [(η^6-Bip)Ru(en)Cl][PF_6] is present in ten-fold molar excess. Analysis by ICP-AES revealed that 50% of cytochrome c was ruthenated. Interestingly, 2D [^{1}H, ^{15}N] HSQC NMR data show that the ruthenium complexes are bound to carboxylate groups (*ca.* 30%) and the amino terminus (*ca.* 70%), instead of the histidine residues, of cytochrome c. This is probably due to the steric constraints imposed on the single coordination site of {(η^6-Bip)Ru(en)}$^{2+}$ by the arene and en ligands.

In contrast, for the arene ruthenium–enzyme complex [(η^6-Cym)Ru(lysozyme)Cl_2], X-ray crystallography (at 1.6 Å resolution) showed that the ruthenium atom is selectively bonded to N_ε of the imidazole ring of His15, situated at the surface of the protein (Fig. 14).[86] Ruthenation has little effect on the rest of the enzyme structure, the root-mean-square-difference fit of all backbone atoms being 0.22 Å compared with the native lysozyme structure (193L). The only significant structural perturbations are the displacement of the main chain atoms of residues Arg14 and His15 (maximum 0.4 Å), and conformational changes of the side-chains of these two residues to accommodate the Ru complex. These structural changes provide the Ru complex with a rather hydrophobic

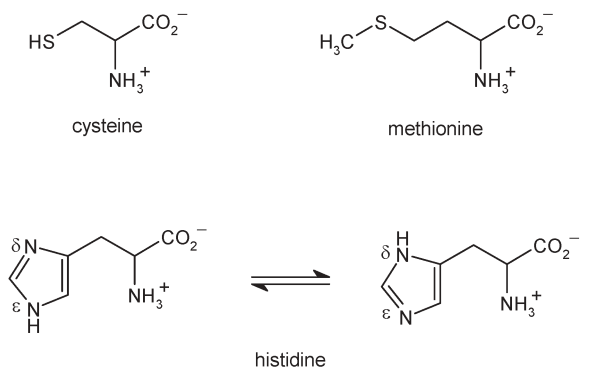

Fig. 13 Structures of cysteine, methionine and histidine.

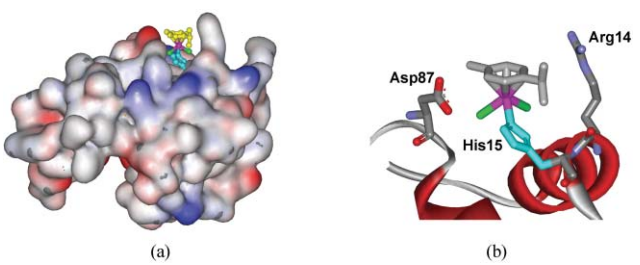

Fig. 14 Crystal structure of [(η^6-Cym)Ru(lysozyme)Cl_2]: (a) space-filling model (with surface colouring to indicate the electrostatic potential: red – negative, blue – positive) showing the position of the Ru complex (ball-and-stick model) in the protein; and (b) details of the binding pocket showing the side-chains of the His15, Asp87 and Arg14 residues.[86]

binding pocket (Fig. 14). The specificity of the binding of {(η^6-Cym)$RuCl_2$} to lysozyme and the ability of Ru(II) arene complexes to catalyse transfer hydrogenation reactions (*vide infra*) suggest that ruthenium–protein complexes might provide a basis for the design of enantioselective artificial metalloenzymes.[87] It is possible that the histidine-bound Ru–lysozyme complex is the kinetic product of the reaction between the enzyme and [(η^6-*p*-cymene)$RuCl_2(H_2O)$], and that slow conversion to a thermodynamically more stable complex, with Ru π-bonded to an aromatic side-chain, could occur. Under some conditions it is possible to form π-complexes between {(arene)Ru(II)}/{CpRu(II)} and aromatic amino acid side-chains (Phe, Trp),[88,89] although such reactions are often blocked when competitive binding to other side-chain donors (*e.g.* His, Asp, Glu) is possible.

Overall, in comparison with DNA, amino acids and proteins appear to have lower reactivity towards ruthenium arene complexes (see Section 4.3). This may account for the low toxic side effects of such complexes.[56] On the other hand, the relatively weak binding of amino acids and proteins to these complexes may aid the transport and delivery of the latter to cancer cells, and allow some amino acids, peptides and proteins to serve as drug reservoirs for DNA ruthenation, as has been proposed for cisplatin.[82] Recent cell distribution studies show that RNA is also ruthenated in cancer cells treated with Ru(II) arene complexes,[90] but the consequences of this are unknown. Some short strands of RNA (si-RNA) are now known to be directly involved in gene silencing.[91]

5. Biocatalysis by Ru(II)–arene complexes

An interesting feature of the chemistry of Ru(II) arene complexes is their ability to form stable hydride complexes in aqueous solution with formate as the hydride donor:

$$[(\eta^6\text{-}C_6Me_6)Ru(bpy)(H_2O)]^{2+} + HCO_2^- \rightarrow [(\eta^6\text{-}C_6Me_6)Ru(bpy)(H)]^+ + CO_2$$

(bpy = 2,2′-bipyridine)

This system can catalyse the reduction of ketones (*e.g.* cyclohexanone and acetophenone) to alcohols, although the conditions for optimum turnover are not biologically compatible (pH 4, 70 °C).[92]

Steckhan *et al.*[93] and Fish *et al.*[94] have shown that Rh(III) pentamethylcyclopentadienyl complexes can catalyse the reduction of NAD^+ (an enzyme cofactor) in the presence of formate (Fig. 15). This reduction is regioselective, giving the biologically relevant 1,4-NADH isomer, and can drive enzymatic reactions relying on NADH as cofactor (*e.g.* stereoselective reduction of $PhCH_2CH_2COMe$ catalyzed by alcohol dehydrogenase[95]). We have recently shown that anticancer complexes such as [(η^6-C_6Me_6)Ru(en)(H_2O)]$^{2+}$ can also catalyse regioselective NAD^+ reduction under biological conditions (37 °C and pD 7.2)[96] *via* formation of the hydride complex [(η^6-C_6Me_6)Ru(en)(H)]$^+$. However, such reactions are slow and formate is scarce in eukaryotic cells. It seems unlikely therefore that these reactions are of importance for the anticancer activity of Ru(II) arene complexes. Further work is underway in our laboratory to explore biocatalytic properties of Ru(II) arene complexes and their relevance to biology.

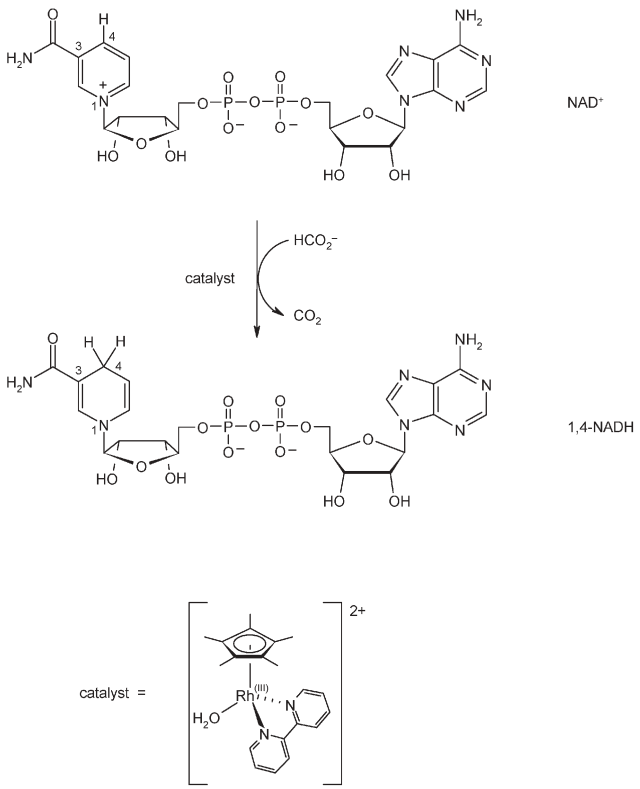

Fig. 15 Regioselective reduction of NAD^+ by formate, catalysed by [(η^5-C_5Me_5)Rh(bpy)(H_2O)]$^{2+}$.[94]

6. Osmium analogues

It is intriguing to consider the possible design of Os(II) arene anticancer complexes. In Group 10 of the periodic table, the stark contrast between the rapid reaction kinetics (often *ca.* $10^4\times$ faster) of the 4*d* ion Pd^{II} compared to the 5*d* ion Pt^{II} has led to difficulties in designing active palladium(II) complexes. If there is a parallel in Group 8, then strategies for the kinetic activation of Os^{II} will be required. Indeed, we have synthesized [(η^6-Bip)Os(en)Cl]$^+$, the Os(II) analogue of an active Ru(II) anticancer complex, and found that it is inactive.[97] The osmium complex is much less reactive towards guanine in solution, in line with the expectation that Os(II) is more kinetically inert than Ru(II), perhaps too inert to allow attack on DNA. However, as with Ru(II), we have found that it is possible to activate Os(II) arene complexes towards ligand substitution reactions by appropriate choice of other bound ligands.[97] Although others have alluded to anticancer activity for an Os(II) arene complex,[98] no IC_{50} values appear to have been published.

7. Epilogue

Our work has shown that certain ruthenium(II) arene complexes exhibit promising anticancer activity *in vitro* and *in vivo*. The complexes are stable and water-soluble, and their frameworks provide considerable scope for optimising the

design, both in terms of their biological activity and for minimising side-effects by variations in the arene and the other coordinated ligands. We have synthesized a number of such complexes with different arenes, chelating ligands and leaving groups, and have obtained preliminary data on structure–activity relationships. Initial studies on amino acids and nucleotides suggest that kinetic and thermodynamic control over a wide spectrum of reactions of Ru^{II} arene complexes with biomolecules can be achieved. These Ru^{II} arene complexes appear to have an altered profile of biological activity in comparison with metal-based anticancer complexes currently in clinical use or on clinical trial. It is hoped that rapid progress can be made towards clinical trials of ruthenium(II) arene anticancer complexes. Our current work is aimed at optimizing the pharmacological profiles of these complexes and investigating their molecular mechanism of action.

It is clear that exploration of the aqueous chemistry of organometallic complexes has the potential to provide exciting new applications not only in cancer therapy, but also in fields as diverse as radioimaging, immunosuppression, protection from ischemia and transplant rejection, and biocatalysis. Such future developments will benefit greatly from close collaborations between chemists, biologists and clinicians.

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Notes and references

1 P. J. Sadler, *Adv. Inorg. Chem.*, 1991, **36**, 1.
2 M. J. Clarke, *Coord. Chem. Rev.*, 2003, **236**, 209.
3 Z. Guo and P. J. Sadler, *Angew. Chem., Int. Ed.*, 1999, **38**, 1512.
4 Z. Guo and P. J. Sadler, *Adv. Inorg. Chem.*, 2000, **49**, 183.
5 M. J. Cleare and J. D. Hoeschele, *Bioinorg. Chem.*, 1973, **2**, 187.
6 R. D. Murdoch and J. Pepys, *Int. Arch. Allergy Appl. Immunol.*, 1985, **77**, 456.
7 H. M. Pineto and J. H. Schornagel (Eds.), *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy*, Plenum, New York, 1996.
8 J. Halpern, *Pure Appl. Chem.*, 2001, **73**, 209.
9 R. H. Fish and G. Jaouen, *Organometallics*, 2003, **22**, 2166.
10 C. Elschenbroich and A. Salzer, *Organometallics: a concise introduction*, VCH, Weinheim, 2nd edn., 1992.
11 P. Köpf-Maier and H. Köpf, in *Metal Compounds in Cancer Therapy*, S. P. Fricker (Ed); Chapman & Hall, London, 1994, p. 109.
12 G. Jaouen, A. Vessières and I. S. Butler, *Acc. Chem. Res.*, 1993, **26**, 361.
13 R. Alberto, R. Schibli, R. Waibel, U. Abram and A. P. Schubiger, *Coord. Chem. Rev.*, 1999, **190–192**, 901.
14 R. H. Fish, *Coord. Chem. Rev.*, 1999, **185–186**, 569.
15 F. Le Bideau, M. Salmain, S. Top and G. Jaouen, *Chem. Eur. J.*, 2001, **7**, 2289.
16 R. Alberto, in *Comprehensive Coordination Chemistry II*, Vol. 5, E. C. Constable and J. R. Dilworth (Eds), Elsevier, Amsterdam, 2004, Ch. 5.2, p. 248.
17 T. R. Johnson, B. E. Mann, J. E. Clark, R. Foresti, C. J. Green and R. Motterlini, *Angew. Chem., Int. Ed.*, 2003, **42**, 3722.
18 T. Bachleitner-Hofmann, M. Kees and H. Gisslinger, *Leukemia Lymphoma*, 2002, **43**, 1535.
19 G.-Q. Chen, L. Zhou, M. Styblo, F. Walton, Y. Jing, R. Weinberg, Z. Chen and S. Waxman, *Cancer Res.*, 2003, **63**, 1853.
20 G. T. Burke, J. H. Mangum and J. D. Brodie, *Biochemistry*, 1970, **9**, 4297.
21 J. C. Fontecilla-Camps and S. W. Ragsdale, *Adv. Inorg. Chem.*, 1999, **47**, 283.
22 P. A. Lindahl, *J. Biol. Inorg. Chem.*, 2004, **9**, 516.
23 M. Guo, Z. Guo and P. J. Sadler, *J. Biol. Inorg. Chem.*, 2001, **6**, 698.
24 M. Guo, H. Sun, H. J. McArdle, L. Gambling and P. J. Sadler, *Biochemistry*, 2000, **39**, 10023.
25 K. Mross, P. Robben-Bathe, L. Edler, J. Baumgart, W. E. Berdel, H. Fiebig and C. Unger, *Onkologie*, 2000, **23**, 576.
26 N. Kröger, U. R. Kleeberg, K. Mross, L. Edler, G. Saß and D. K. Hossfeld, *Onkologie*, 2000, **23**, 60.
27 S. Top, E. B. Kaloun, A. Vessières, I. Laïos, G. Leclercq and G. Jaouen, *J. Organomet. Chem.*, 2002, **643–644**, 350.
28 T. Pieper, K. Borsky and B. K. Keppler, in *Topics in Biological Inorganic Chemistry*, Vol. 1, M. J. Clarke and P. J. Sadler (Eds.), Springer Verlag, Berlin, 1999, p. 171.
29 M. M. Harding and G. Mokdsi, *Curr. Med. Chem.*, 2000, **7**, 1289.
30 O. R. Allen, L. Croll, A. L. Gott, R. J. Knox and P. C. McGowan, *Organometallics*, 2004, **23**, 288.
31 M. Gielen, *Coord. Chem. Rev.*, 1996, **151**, 41.
32 M. Gielen, *Appl. Organomet. Chem.*, 2002, **16**, 481.
33 A. J. Crowe, P. J. Smith and G. Atassi, *Inorg. Chim. Acta*, 1984, **93**, 179.
34 S. Top, A. Vessieres, C. Cabestaing, I. Laios, G. Leclercq, C. Provot and G. Jaouen, *J. Organomet. Chem.*, 2001, **637–639**, 500.
35 S. Top, A. Vessieres, G. Leclercq, J. Quivy, J. Tang, J. Vaissermann, M. Huche and G. Jaouen, *Chem. Eur. J.*, 2003, **9**, 5223.
36 Y. K. Yan, S. E. Cho, K. A. Shaffer, J. E. Rowell, B. J. Barnes and I. H. Hall, *Pharmazie*, 2000, **55**, 307.
37 W. Wang, Y. K. Yan, T. S. A. Hor, J. J. Vittal, J. R. Wheaton and I. H. Hall, *Polyhedron*, 2002, **21**, 1991.
38 J. Zhang, J. J. Vittal, W. Henderson, J. R. Wheaton, I. H. Hall, T. S. A. Hor and Y. K. Yan, *J. Organomet. Chem.*, 2002, **650**, 123.
39 C. Jiang, T. S. A. Hor, Y. K. Yan, W. Henderson and L. J. McCaffrey, *J. Chem. Soc., Dalton Trans.*, 2000, 3197.
40 C. Jiang, T. S. A. Hor, Y. K. Yan, W. Henderson and L. J. McCaffrey, *J. Chem. Soc., Dalton Trans.*, 2000, 3204.
41 C. S. Allardyce, A. Dorcier, C. Scolaro and P. J. Dyson, *Appl. Organomet. Chem.*, 2005, **19**, 1.
42 D. S. Dwyer, K. Gordon and B. Jones, *Int. J. Immunopharmacol.*, 1995, **17**, 931.
43 M. J. Clarke, V. Bailey, P. Doan, C. Hiller, K. J. LaChance-Galang, H. Daghlian, S. Mandal, C. M. Bastos and D. Lang, *Inorg. Chem.*, 1996, **35**, 4896.
44 T. W. Hayton, P. Legzdins and W. B. Sharp, *Chem. Rev.*, 2002, **102**, 935.
45 C. S. Allardyce, P. J. Dyson, D. J. Ellis, P. A. Salter and R. Scopelliti, *J. Organomet. Chem.*, 2003, **668**, 35.
46 R. A. Sanchez-Delgado, M. Navarro, H. Perez and J. A. Urbina, *J. Med. Chem.*, 1996, **39**, 1095.
47 K. C. Reed and F. L. Bygrave, *Biochem. J.*, 1974, **140**, 143.
48 M. J. Clarke, *Met. Ions Biol. Syst.*, 1980, **11**, 231.
49 G. Sava, E. Alessio, A. Bergamo and G. Mestroni, in *Topics in Biological Inorganic Chemistry*, Vol. 1, M. J. Clarke and P. J. Sadler (Eds.), Springer Verlag, Berlin, 1999, p. 143.

50 M. Galanski, V. B. Arion, M. A. Jakupec and B. K. Keppler, *Curr. Pharm. Des.*, 2003, **9**, 2078.
51 A. H. Velders, H. Kooijman, A. L. Spek, J. G. Haasnoot, D. de Vos and J. Reedijk, *Inorg. Chem.*, 2000, **39**, 2966.
52 R. A. Vilaplana, F. González-Vílchez, E. Gutierrez-Puebla and C. Ruiz-Valero, *Inorg. Chim. Acta*, 1994, **224**, 15.
53 M. J. Clarke, S. Bitler, D. Rennert, M. Buchbinder and A. D. Kelman, *J. Inorg. Biochem.*, 1980, **12**, 79.
54 Z. Guo, A. Habtemariam, P. J. Sadler and B. R. James, *Inorg. Chim. Acta*, 1998, **273**, 1.
55 R. E. Morris, R. E. Aird, P. del S. Murdoch, H. Chen, J. Cummings, N. D. Hughes, S. Parsons, A. Parkin, G. Boyd, D. I. Jodrell and P. J. Sadler, *J. Med. Chem.*, 2001, **44**, 3616.
56 R. E. Aird, J. Cummings, A. A. Ritchie, M. Muir, R. E. Morris, H. Chen, P. J. Sadler and D. I. Jodrell, *Br. J. Cancer*, 2002, **86**, 1652.
57 A. Habtemariam, M. Melchart, H. Chen, R. Fernández, R. E. Aird, D. I. Jodrell and P. J. Sadler, unpublished.
58 S. Murakami, R. Nakashima, E. Yamashita and A. Yamaguchi, *Nature*, 2002, **419**, 587.
59 H. Chen, J. A. Parkinson, R. E. Morris and P. J. Sadler, *J. Am. Chem. Soc.*, 2003, **125**, 173.
60 F. Wang, H. Chen, S. Parsons, I. D. H. Oswald, J. E. Davidson and P. J. Sadler, *Chem. Eur. J.*, 2003, **9**, 5810.
61 M. Jennerwein and P. A. Andrews, *Drug Metab. Dispos.*, 1995, **23**, 178.
62 R. B. Martin, in *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, B. Lippert (Ed), Wiley-VCH, Zürich, 1999, p. 183.
63 R. Fernández, M. Melchart, A. Habtemariam, S. Parsons and P. J. Sadler, *Chem. Eur. J.*, 2004, **10**, 5173.
64 T. Hasegawa, T. C. Lau, H. Taube and W. P. Schaefer, *Inorg. Chem.*, 1991, **30**, 2921.
65 S. E. Sherman and S. J. Lippard, *Chem. Rev.*, 1987, **87**, 1153.
66 M.-H. Baik, R. A. Friesner and S. J. Lippard, *J. Am. Chem. Soc.*, 2003, **125**, 14082.
67 S. Neidle, *Nucleic Acid Structure and Recognition*, Oxford University Press, Oxford, 2002, p. 76.
68 K. Inagaki and Y. Kidani, *J. Inorg. Biochem.*, 1979, **11**, 39.
69 G. Schröder, B. Lippert, M. Sabat, C. J. L. Lock, R. Faggiani, B. Song and H. Sigel, *J. Chem. Soc., Dalton Trans.*, 1995, 3767.
70 H. Chen, J. A. Parkinson, S. Parsons, R. A. Coxall, R. O. Gould and P. J. Sadler, *J. Am. Chem. Soc.*, 2002, **124**, 3064.
71 M. Melchart, A. Habtemariam, S. Parsons and P. J. Sadler, unpublished.
72 H. Chen, J. A. Parkinson, O. Novakova, J. Bella, F. Wang, A. Dawson, R. Gould, S. Parsons, V. Brabec and P. J. Sadler, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 14623.
73 F. Wang, J. Bella, J. A. Parkinson and P. J. Sadler, *J. Biol. Inorg. Chem.*, 2005, **10**, 147.
74 O. Novakova, H. Chen, O. Vrana, A. Rodger, P. J. Sadler and V. Brabec, *Biochemistry*, 2003, **42**, 11544.
75 A. Frodl, D. Herebian and W. S. Sheldrick, *J. Chem. Soc., Dalton Trans.*, 2002, 3664.
76 O. Novakova, J. Kasparkova, V. Bursova, C. Hofr, M. Vojtiskova, H. Chen, P. J. Sadler and V. Brabec, *Chem. Biol.*, 2005, **12**, 121.
77 S. M. Cohen and S. J. Lippard, in *Progress in Nucleic Acid Research and Molecular Biology*, Vol. 67, K. Moldave (Ed), Academic Press, San Diego, 2001, p. 93.
78 V. Brabec, in *Progress in Nucleic Acid Research and Molecular Biology*, Vol. 71, K. Moldave (Ed), Academic Press, San Diego, 2002, p. 1.
79 T. Furuta, T. Ueda, G. Aune, A. Sarasin, K. H. Kraemer and Y. Pommier, *Cancer Res.*, 2002, **62**, 4899.
80 D. Wang, R. Hara, G. Singh, A. Sancar and S. J. Lippard, *Biochemistry*, 2003, **42**, 6747.
81 M. Selvakumaran, D. A. Pisarcik, R. Bao, A. T. Yeung and T. C. Hamilton, *Cancer Res.*, 2003, **63**, 1311.
82 J. Reedijk, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 3611.
83 F. Wang, H. Chen, J. A. Parkinson, P. del S. Murdoch and P. J. Sadler, *Inorg. Chem.*, 2002, **41**, 4509.
84 F. Wang, J. Xu, A. Habtemariam, J. Bella and P. J. Sadler, unpublished.
85 N. S. Kosower and E. M. Kosower, *Int. Rev. Cytol.*, 1978, **54**, 109.
86 I. W. McNae, K. Fishburne, A. Habtemariam, T. M. Hunter, M. Melchart, F. Wang, M. D. Walkinshaw and P. J. Sadler, *Chem. Commun.*, 2004, 1786.
87 C. M. Thomas and T. R. Ward, *Chem. Soc. Rev.*, 2005, **34**, 337.
88 D. B. Grotjahn, *Coord. Chem. Rev.*, 1999, **190–192**, 1125.
89 A. Schlüter, K. Bieber and W. S. Sheldrick, *Inorg. Chim. Acta*, 2002, **340**, 35.
90 F. Wang, B. Zeitlin, A. Habtemariam, L. Eades, R. Aird, D. I. Jodrell and P. J. Sadler, unpublished.
91 S. Agrawal and E. R. Kandimalla, *Nat. Biotechnol.*, 2004, **22**, 1533.
92 S. Ogo, T. Abura and Y. Watanabe, *Organometallics*, 2002, **21**, 2964.
93 E. Steckhan, S. Herrmann, R. Ruppert, E. Dietz, M. Frede and E. Spika, *Organometallics*, 1991, **10**, 1568.
94 H. C. Lo, C. Leiva, O. Buriez, J. B. Kerr, M. M. Olmstead and R. H. Fish, *Inorg. Chem.*, 2001, **40**, 6705.
95 D. Westerhausen, S. Herrmann, W. Hummel and E. Steckhan, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 1529.
96 Y. K. Yan, M. Melchart, A. Habtemariam and P. J. Sadler, unpublished.
97 A. F. A. Peacock, A. Habtemariam, R. Fernández, S. Parsons, R. E. Aird, D. I. Jodrell and P. J. Sadler, unpublished.
98 A. Dorcier, P. J. Dyson, C. Gossens, U. Rothlisberger, R. Scopelliti and I. Tavernelli, *Organometallics*, 2005, **24**, 2114.