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Medicinal Organometallic Chemistry: Designing Metal Arene Complexes as Anticancer Agents

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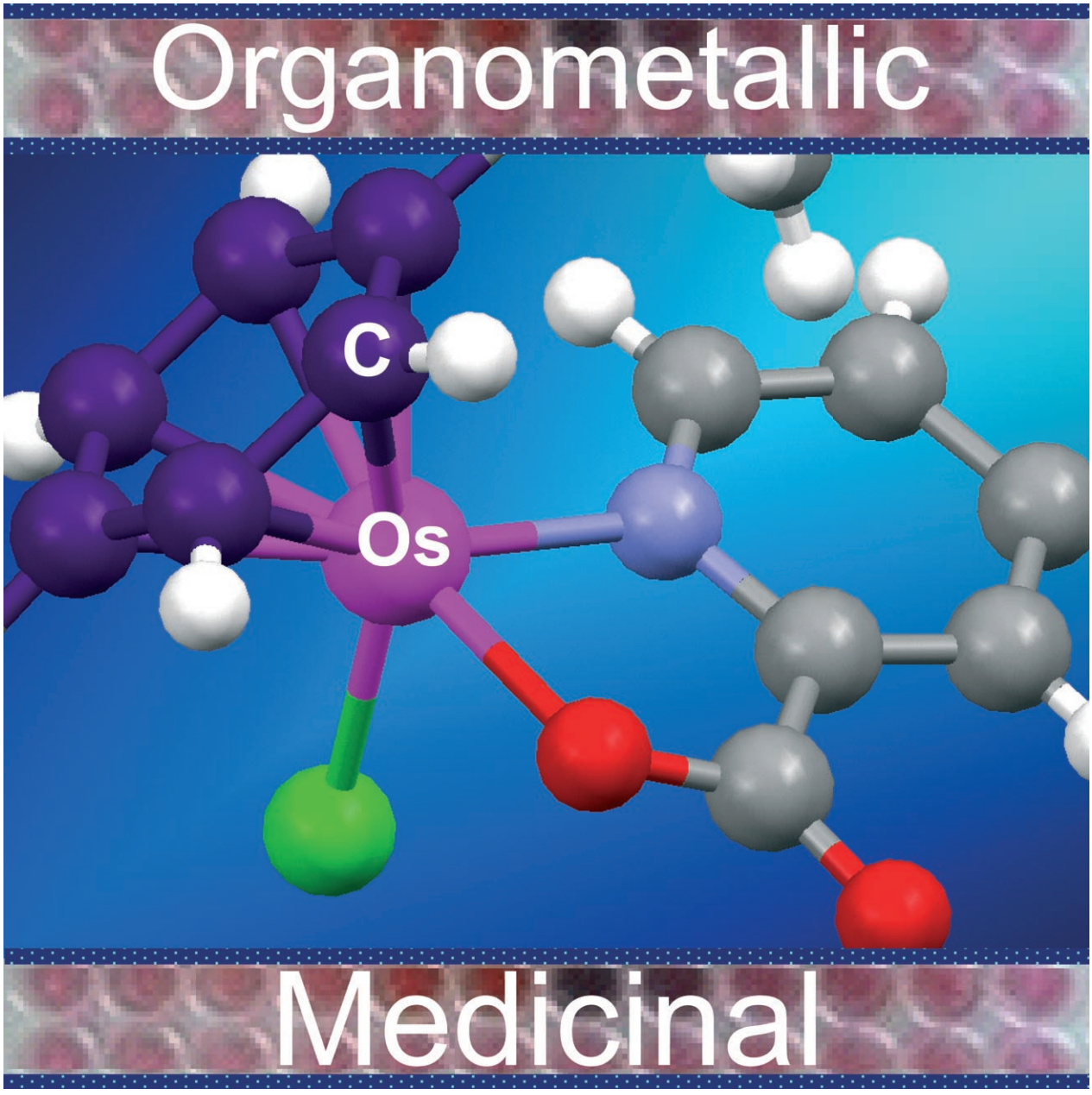

Abstract: The field of medicinal inorganic chemistry is rapidly advancing. In particular organometallic complexes have much potential as therapeutic and diagnostic agents. The carbon-bound and other ligands allow the thermodynamic and kinetic reactivity of the metal ion to be controlled and also provide a scaffold for functionalization. The establishment of structure–activity relationships and elucidation of the speciation of complexes under conditions relevant to drug testing and formulation are crucial for the further development of promising medicinal applications of organometallic complexes. Specific examples involving the design of ruthenium and osmium arene complexes as anticancer agents are discussed.

Keywords: anticancer agents · arene ligands · organometallics · osmium · ruthenium

Introduction

The study of metals in biology is a rapidly expanding field, especially the subfield of bioorganometallic chemistry, which explores the role of metal complexes containing direct metal–carbon bonds. The most notable example of a natural bioorganometallic compound is vitamin B12, which contains cobalt bound directly to a carbon atom (cyanocobalamin as isolated, C-bound adenosylcobalamin as a coenzyme).

Organometallic chemistry offers a potentially rich field for the development of new medicinal agents with novel mechanisms of action.[1–9] The benefits include the variety of distinct molecular architectures that can be designed, owing to varying coordination numbers and geometries, choice of metals and ligands, oxidation states, and overall reactivity and charge. Organometallic complexes provide a scaffold which can be functionalized and designed. In this Focus Review, we discuss the logical design of organometallic anticancer compounds and illustrate how this process is aided by an understanding of their aqueous solution chemistry under biologically relevant conditions.

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Diagnosis and Therapy

Metals bound to organic fragments have found widespread use as both radiopharmaceuticals and imaging agents. In particular, the technetium(I) carbon monoxide fragment $[^{99m}Tc(CO)_3]^+$ is becoming widely used in radiopharmaceuticals, as are analogous rhenium compounds.[10–12] The first organometallic compound used in clinical nuclear medicine, and in particular in cardiac imaging, was cardiolite (Figure 1). The advantages of organometallic labelling tech-

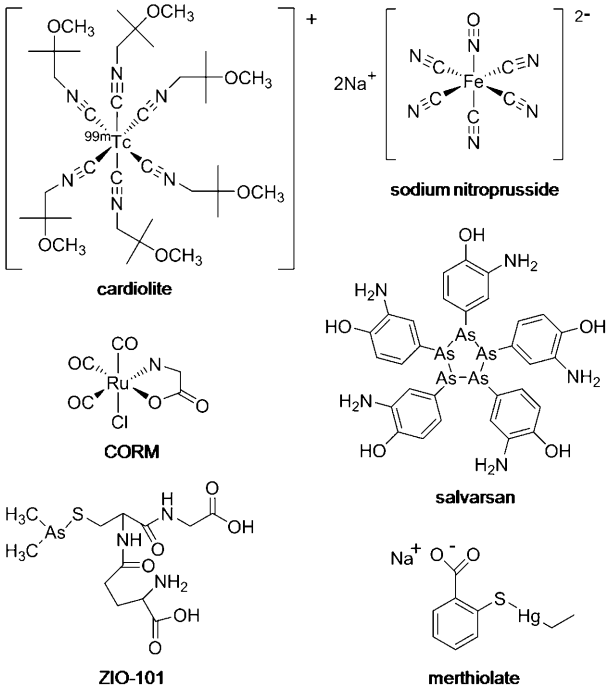

Figure 1. Examples of organometallic therapeutic complexes.

niques in the preparation of radiopharmaceuticals have recently been emphasized.[13]

One mode of action of bioorganometallic agents can involve activation by cleavage of a metal–ligand bond, to release the active component. For example, the cyanide complex sodium nitroprusside (Figure 1) delivers nitric oxide to smooth muscle to achieve vasodilation. One of its targets is the heme protein guanylate cyclase. Carbon monoxide is also increasingly being recognized as an important natural biological small molecule which can be delivered by metal ions. Like NO, CO can function as a neurotransmitter, and also has cardioprotective effects. Promising CO-releasing organometallic complexes (CORMs) include CO complexes of manganese, ruthenium, and iron (see Figure 1).[14–16] The ability to tune the thermodynamics and kinetics of CO release from these complexes in aqueous solution under biologically relevant conditions is crucial to their mode of action.

Although heavy metals are often associated with toxicity, they can have useful applications in organometallic drugs. For example, the organomercury compound merthiolate (ethylmercury mercaptobenzoate), is used as an antiseptic and antifungal agent, and organoarsenic complexes such as salvarsan, first made by Paul Ehrlich in 1909, have found application for the treatment of syphilis. The true structure of salvarsan has only recently been elucidated.[17] The success of organometallic therapeutics is illustrated by salvarsan, named one of the top 46 pharmaceuticals by *Chemical & Engineering News* in 2005.[18] A new arsenic–glutathione compound (dimethylarsenic(III) glutathione; Z10-101, darinaparsin) is currently in phase II clinical trials as a potential oral anticancer drug (see Figure 1). Organometallic complexes containing precious metals, such as gold(III) and platinum(II), also have therapeutic potential.[19–22]

Cyclopentadienyl Complexes

A range of organometallic compounds in which the organic component is a six-electron donor η^5-cyclopentadienyl ligand capable of binding to the metal center through π interactions exhibit promising anticancer activity.

The dicyclopentadienyl Ti^{IV} complex titanocene dichloride (Figure 2A) has a pseudo-tetrahedral structure with two cyclopentadienyl (Cp) rings and two chloride ligands coordinated in a *cis* geometry. Originally chosen because of the possibility of forming bifunctional cross-links on DNA similarly to cisplatin, the complex entered clinical trials. However, these were abandoned because of difficulties of formulation. Its solution chemistry is complicated by facile hydrolysis, formation of hydroxo complexes, and eventually loss of the Cp ligands, which are readily protonated. This behavior makes identification of the active species, as well as formulation, difficult.[23,24] Nonetheless, there is continuing interest in this class of metallocenes through modification of the Cp group (Figure 2B),[25] as well as various analogues containing different metals such as molybdenum, and these may lead to more controlled aqueous chemistry and to compounds more suitable for administration.[26,27]

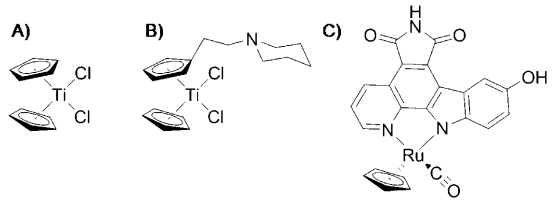

Figure 2. Examples of cyclopentadienyl metal complexes with biological activity.

Cyclopentadienyl rhodium[28] and ruthenium complexes with potential therapeutic applications have also been reported. In particular the organometallic Ru^{II} Cp complexes derived from the class of indolocarbazole alkaloids (for example, staurosporine) have been designed as inhibitors for certain protein kinases (Figure 2C).[29,30]

Modified ferrocene ($[Fe(C_5H_5)_2]$) sandwich complexes are of interest owing to their redox activity, which is within the range of biologically accessible potentials. A notable example is that of ferrocifen, which consists of ferrocene modified by incorporation of tamoxifen, and its derivatives (Figure 3). These, and their Ru analogues, are being explored as poten-

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Peter Sadler obtained his BA, MA, and DPhil at the University of Oxford and then spent two years as a Medical Research Council Research Fellow at the National Institute for Medical Research and University of Cambridge. In 1973 he was appointed as a Lecturer in Chemistry at Birkbeck College, University of London, where he subsequently became Reader in Biological Inorganic Chemistry, and Professor of Chemistry. In 1996 he was appointed to the Crum Brown Chair of Chemistry at the University of Edinburgh, and in June 2007 took up a Chair at the University of Warwick, where he is also Head of Department. He is a Fellow of the Royal Society of Edinburgh (FRSE) and the Royal Society of London (FRS). His research interests are centered on the chemistry of metals in medicine.

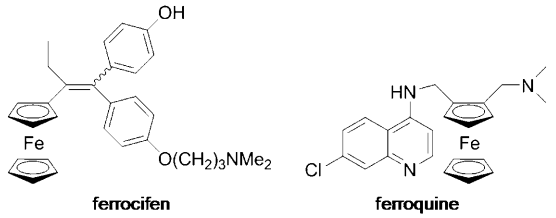

Figure 3. Ferrocene-based ferrocifen and ferroquine complexes with anticancer and antimalarial activity, respectively.

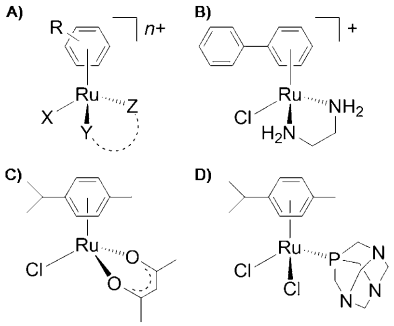

Figure 4. Half-sandwich metal arene complexes which exhibit cytotoxicity towards cancer cells. A) General chemical structure of Ru^{II} arene "piano-stool" complexes, $[(\eta^6\text{-arene})Ru(X)(Y)(Z)]^{n+}$. B) $[(\eta^6\text{-bip})Ru(en)Cl]^+$; bip = biphenyl, en = ethylenediamine. C) $[(\eta^6\text{-}p\text{-cym})Ru(acac)Cl]$; *p*-cym = *p*-cymene, acac = acetylacetonate. D) $[(\eta^6\text{-}p\text{-cym})RuCl_2(pta)]$; pta = 1,3,5-triaza-7-phosphatricyclo[3,3,1,1]decane.[41]

tial breast cancer drugs.[31,32] The systematic study of selective estrogen receptor modulators (SERMs) combining organometallic chemistry and biology may well lead to novel drugs.[33] Similarly, the antimalarial drug chloroquine exhibits enhanced activity when bound to the ferrocene unit in ferroquine (Figure 3).[34,35]

However, cyclopentadienyl derivatives can be relatively unstable in aqueous media since the negatively charged cyclopentadiene ligand is readily protonated and displaced from the metal. Neutral six-membered arene rings cannot be protonated and consequently are more inert to displacement in aqueous solution. The following sections are focussed on recent data from our research group concerned with the chemistry and anticancer activity of metal arene complexes with an emphasis on aqueous solution chemistry that can be fed into the drug design process.

η^6-Arene Ruthenium Complexes

A number of ruthenium complexes have been shown to display promising anticancer activity, and two Ru^{III} complexes are currently in clinical trials, [ImH][*trans*-$RuCl_4$(dmso)Im] (NAMI-A) and [ImH][*trans*-$RuCl_4Im_2$] (KP1019).[36,37] It has been proposed that their mode of action involves their in vivo reduction to the more reactive Ru^{II} species, and this has led to increased interest in pseudo-octahedral organometallic Ru^{II} arene complexes (see Figure 4) in which the arene stabilizes ruthenium in the +2 oxidation state.[38–41]

These half-sandwich "piano-stool" type constructs (Figure 4) offer much scope for design, with the potential for modifications to the arene and its substituents (R), the monodentate leaving group (X), chelating ligand (YZ), and overall charge of the complex ($n+$). These features provide handles for the control of both the thermodynamics and kinetics of these systems as well as their overall structural architecture. They also provide an ability to fine-tune the chemical reactivity of the complexes, potentially allowing control of pharmacological properties including cell uptake, distribution, interactions with biomolecules, toxic side effects, and detoxification mechanisms. These structural variables have been systematically investigated in our laboratory and are discussed in the following sections.[2,3,42]

Ruthenium arene complexes containing the chelating ligand ethylenediamine, for example $[(\eta^6\text{-arene})Ru(en)Cl]^+$, show promising activity both in vitro and in vivo, and are thought to have a mode of action that is analogous to that of cisplatin. The intact chloro adduct is a "pro-drug" which is activated in vivo by hydrolysis of the Ru–Cl bond (replacement of Cl^- by a water molecule). This is largely suppressed in the blood where high chloride concentrations are found (ca. 100 mM), whereas in the nucleus (4 mM Cl^-) the complex is largely hydrolyzed to give the reactive species, $[(\eta^6\text{-arene})Ru(en)(OH_2)]^{2+}$.[43] The aqua species is then thought to bind to nuclear DNA with a high affinity for the N7 position of guanine (G) bases.[44,45] This high affinity for G has even been observed in the presence of a 250-fold excess of glutathione.[46] Despite similarities in the proposed mode of action, there are clearly differences from cisplatin. A different mode of binding to DNA is observed, as the ruthenium arene complexes can form only monofunctional adducts (compared to the bifunctional adducts formed by cisplatin), and, intriguingly, DNA treated with $[(\eta^6\text{-arene})Ru(en)Cl]^+$ is more difficult for enzymes to repair than DNA treated with cisplatin.[47,48] Excitingly, ruthenium arene complexes containing chelating en ligands, $[(\eta^6\text{-arene})Ru(en)Cl]^+$, are found to be active against cisplatin-resistant cell lines, indicating a different detoxification mechanism.[49]

Arene Hydrophobicity

The biological activity of Ru^{II} arene complexes, $[(\eta^6\text{-arene})Ru(en)Cl]^+$, has been shown to be highly dependent on the nature of the bound arene, with increasing hydrophobicity correlating with increased cytotoxicity (where IC_{50} values correspond to the drug concentrations that inhibit growth of cells by 50%; see Table 1 and Figure 5).[49]

Not only does the arene stabilize ruthenium in the +2 oxidation state, but it also provides a hydrophobic face to the molecule, which may assist passage across cell membranes and play a role in biological recognition processes. The increase in activity with increase in hydrophobicity is primarily thought to be due to the ability of the extended arenes to intercalate into DNA, thus causing further distortion of the

Table 1. IC_{50} values for ruthenium arene complexes and platinum drugs in human ovarian A2780 cancer cells after 24 h drug exposure (data from reference [49]).

Arene/Pt Drug	IC_{50} [μM]
bz	17
p-cym	10
bip	5
dha	2
tha	0.5
cisplatin	0.6
carboplatin	6

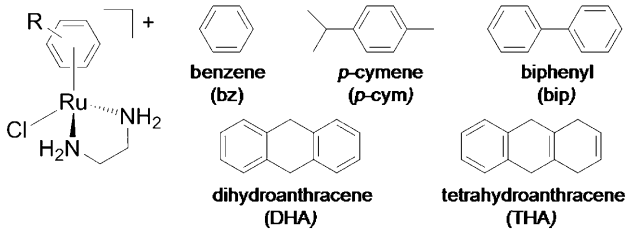

Figure 5. Ruthenium(II) complexes containing various arenes, $[(\eta^6\text{-arene})Ru(en)Cl]^+$.

DNA structure. NMR studies have shown that the biphenyl (bip) analogue, $[(\eta^6\text{-bip})Ru(en)Cl]^+$, binds to DNA through a combination of both coordination bonds and noncovalent intercalation.[50,51] Furthermore, complexes with extended arenes capable of intercalation are able to compete effectively and displace the intercalator ethidium bromide from DNA, as monitored by quenching of ethidium fluorescence.[47] This is supported by the observation that in the X-ray crystal structure of the 9-ethylguanine (9EtG) adduct with the dihydroanthracene (dha) arene complex, $[(\eta^6\text{-dha})Ru(en)(9EtG)]^{2+}$, the extended arene base-stacks with the coordinated 9EtG (Figure 6 A).[52] Therefore, the flexibility of the extended arene to bend and twist is likely to affect the anticancer activity of this class of compound. In addition, the rate of reaction with cyclic guanosine monophosphate (cGMP) is highly dependent on the coordinated arene, so that complexes containing an extended arene bind to cGMP more rapidly than those containing an unsubstituted arene like benzene.[53] This suggests that binding is promoted by favorable arene–purine hydrophobic stacking interactions.

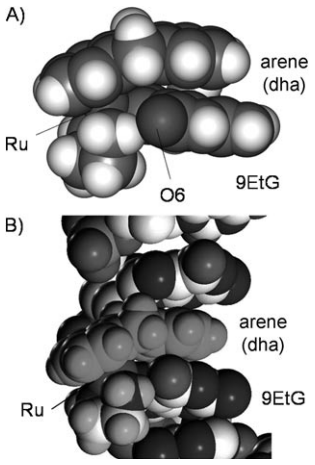

Figure 6. Base stacking of an extended arene and intercalation into DNA. A) Space-filling representation of the X-ray crystal structure of the dihydroanthracene 9-ethylguanine adduct, $[(\eta^6\text{-dha})Ru(en)(9EtG\text{-}N7)]^{2+}$. B) Model of the ruthenium complex bound to B-DNA, illustrating how the coordinated arene could intercalate between G base pairs. Figure adapted from reference [52].

Furthermore, the choice of arene affects both the rate of hydrolysis of the Ru–Cl bond and the acidity of the resulting coordinated aqua ligand. Hydrolysis is twice as fast when the arene is tetrahydroanthracene (tha) compared to bip, and the acidity of the aqua ligand decreases (pK_a 8.01 and 7.71 for tha and bip, respectively).[43] The rate of hydroysis is important as hydrolysis is thought to be the activation step for this class of complexes. The acidity of the coordinated water determines the speciation of $[(\eta^6\text{-arene})Ru(en)(OH_2/OH)]^{+/2+}$ at physiological pH values, with the hydroxo complex thought to be less reactive than the aqua complex.

Leaving Group (X)

The role of the leaving group has been investigated by Wang et al.[54] in a systematic study. There is a correlation between the rate of hydrolysis for $[(\eta^6\text{-arene})Ru(en)X]^{n+}$ and the cytotoxicity of the complex towards cancer cells, with high activity for complexes that aquate readily (e.g., X = halide) and inactivity for those that do not aquate (e.g., X = pyridine). An interesting exception to this rule is when X is thiophenolate. This complex is active towards A2780 human ovarian cancer cells (IC_{50} 23 μM), despite being relatively inert to hydrolysis. The mechanism of activation may be different and involve oxidation of the thiolate, followed by hydrolysis of the sulfenate group.[55,56]

Chelating Ligand (YZ)

The reactivity of ruthenium(II) arene complexes is highly dependent on the nature of the YZ chelating ligand in $[(\eta^6\text{-arene})Ru(YZ)Cl]^{n+}$ complexes. Replacing the neutral en chelate with an anionic O,O-chelator like acetylacetonate (acac; Figure 4) significantly increases the rate and extent of hydrolysis (too rapid to measure by NMR spectroscopy for acac complexes).[57,58] The increased electron density on the ruthenium center manifests itself in a considerable increase in the pK_a value (increase in basicity) of the coordinated water (from 8.25 to 9.41 for arene = *p*-cym) of the aqua adduct.[57] The chelated ligand also influences the rate of binding to DNA nucleobases (which is rapid when XY is acac), but more significantly also influences the nucleobase selectivity. When the chelated ligand is a hydrogen-bond donor such as ethylenediamine (en), binding occurs to N7 of guanine, N7/N1 of inosine, and N3 of thymine, with weak and almost no binding to the N3 and N7 atoms of cytosine

and adenine, respectively. In competition with other nucleobases, $[(\eta^6\text{-arene})Ru(en)Cl]^+$ binds selectively to the N7 atom of guanine. This appears to be due to the favorable hydrogen bonds formed between the NH protons of en and the C6O exocyclic oxygen atom of guanine (observed in the X-ray crystal structures of G adducts), and to the unfavorable steric clashes with the $C6NH_2$ group on binding to adenine. Replacing en with an aprotic N,N-chelating ligand, like bipyridine, which is incapable of forming such hydrogen bonds, generally leads to inactive complexes.

In contrast, complexes containing acac as a chelating ligand have a similar affinity for both G and A nucleobases (though do not bind to C or T). The chelating oxygen atoms of acac can act as hydrogen-bond acceptors towards the $C6NH_2$ group of adenine. Molecular models of the guanine adduct suggest that binding would be stabilized if N1H-C=O6 adopts the tautomeric form, N1=C-O6-H, allowing hydrogen bonding between the acac oxygen atom and the OH group.[57]

Charge

The choice of both the chelating ligand YZ and leaving group X determines the overall charge on these complexes. It is likely that positively charged complexes will be electrostatically attracted to negatively charged DNA; however, their high charge may hamper passage across cell membranes. Neutral arene complexes, which cross cell membranes more readily, acquire a positive charge upon activation by replacement of the negatively charged chloride ligand by a neutral water molecule once inside the cancer cell. The activated complex is then attracted electrostatically to DNA.

η^6-Arene Osmium Complexes

Our laboratory, and more recently others,[59,60] have explored a new class of potential anticancer agents containing the heavier congener of ruthenium, osmium. Osmium has the reputation of being highly toxic and relatively substitution inert, in keeping with the normal behavior of a third-row transition metal, and as a consequence has been relatively little investigated for use in therapeutic agents. Though the nature of the arene was found to have some impact on the chemistry of osmium arene half-sandwich complexes, it was found to be very subtle and subsequently our efforts have been focused on systematically varying the nature of the chelating ligands, as this was found to have the most dramatic effect on the kinetics and thermodynamics of the ruthenium analogues. The following section discusses how we were able to tune both the kinetics and thermodynamics of these half-sandwich osmium(II) arene complexes based on the structure–activity relationships established previously for ruthenium(II) arene anticancer complexes, allowing us to design successfully a new class of cytotoxic complexes which are potential anticancer agents.

N,N- and O,O-Chelating Ligands

Osmium analogues of the active ruthenium complexes containing N,N-chelating ligands were prepared (Figure 7). Though the Os and Ru complexes were isostructural in the solid state (Figure 8), the osmium complexes were much more inert, with ligand exchange rates 40 to 100 times (depending on the pH value) slower than for the active ruthenium analogues, and consequently exhibited slower rates of binding to G nucleobases.

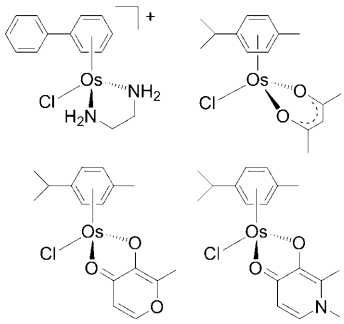

Figure 7. Osmium(II) arene complexes with various chelating ligands.

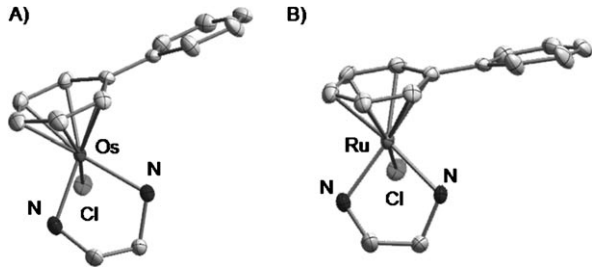

Figure 8. X-ray crystal structure of Os^{II} and Ru^{II} complexes $[(\eta^6\text{-bip})M(en)Cl]^+$. They are isostructural in the solid state (50% probability ellipsoids). Hydrogen atoms and counterions have been omitted for clarity.

In addition, water bound to osmium was significantly more acidic by 1–2 pK units than when bound to analogous ruthenium complexes.[61,62] In our efforts to design complexes with faster kinetics, that is, rates similar to ruthenium analogues, we replaced the neutral N,N-chelate en with the anionic O,O-chelate acac, as this was found to both increase the rate of hydrolysis and reduce the acidity of coordinated water bound to ruthenium.[62] This change was successful in increasing the rate of hydrolysis (too fast to measure by NMR spectroscopy) as well as the pK_a value of the bound water molecule in the aqua complex (ΔpK_a 1.3 units). As for ruthenium, the nucleobase specificity also changed and binding to both the N7 and N1 atoms of adenosine was also observed. Despite achieving rapid hydrolysis and a higher pK_a value for bound water, we observed the formation of an

hydroxo-bridged dimer, $[(\eta^6\text{-arene})Os(\mu\text{-}OH)_3Os(\eta^6\text{-arene})]^+$, formed by loss of the acac chelate. These dimers were found to be inert with respect to reactions with nucleobases and therefore represented a route by which this class of compounds could be deactivated in vivo. In addition, it was established that these compounds form readily at low concentrations of osmium, and notably at the micromolar concentrations typically used in both the cytotoxicity assays and in chemotherapy. Under these conditions, all the osmium was found to be present as the inert hydroxo-bridged dimer, which appeared to form much more readily for osmium than for the analogous ruthenium complexes.[63]

Our efforts then turned towards maintaining the reactivity of this class of compounds (high rates of hydrolysis, pK_a of bound water, and reactivity of the aqua complex), while stabilizing the complex towards loss of the chelated ligand and avoiding subsequent formation of the inert hydroxo-bridged dimer.

Our first strategy was to replace the six-membered chelate from acac with the related anionic O,O-chelating ligands 3-oxy-2-methyl-4-pyrone (maltolate) and 3-hydroxy-1,2-dimethyl-4(1H)pyridine (pyridonate, unpublished results), which on binding to osmium form more stable five-membered chelate rings (see Figure 7). The osmium arene complexes containing this class of ligands retained the high rate of hydrolysis (still too fast to measure by ^{1}H NMR spectroscopy), similar pK_a values for the coordinated water ligand, and the aqua complexes reacted rapidly with both G and A nucleobases. On exploring aqueous stability, it was found that they were indeed more stable than the six-membered acac chelate analogue (Figure 9) with the pyridonate complex showing very high stability (a lone pair of electrons located on the N atom in the ligand ring plays a role in stabilizing resonance structures). However, the maltolate complex showed no activity towards human cancer cells up to 100 μM test concentrations, though the pyridonate complex showed moderate activity towards human ovarian A2780 cancer cells with an IC_{50} value of 30 μM.[64] These observations suggest that the higher stability of the complexes improves the biological activity.

The mechanism of dimer formation may involve bond cleavage of one of the Os–O bonds and subsequent loss of the chelated ligand. This is supported by the fact that all bi-

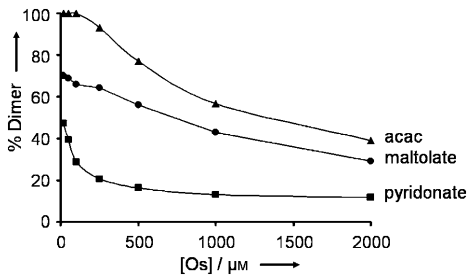

Figure 9. Dependence of hydroxo-bridged dimer formation on total osmium concentration for solutions of the osmium acac (▲), maltolate (●), and pyridonate (■) complexes $[(\eta^6\text{-}p\text{-cym})Os(XY)Cl]$ (incubation at 310 K for 24 h, based on ^{1}H NMR peak integrals).[63]

functional complexes $[(\eta^6\text{-arene})Os(X)Cl_2]$ show significant instability with respect to hydroxo-bridged dimer formation, independent of the nature of the donor ligand X.[61]

N,O-Chelating Ligands

We continued to modify our design of the complexes so as to introduce stability towards formation of the hydroxo-bridged dimer, to investigate whether this would lead to higher activity. Such stability might also be important for potential drug formulation and administration. This led us to use mixed-atom chelators containing both N- and O-donor atoms, in an attempt to harness both the stability and reactivity associated with N,N and O,O chelators, respectively.[65]

The use of five-membered chelating ligands with a primary amine -NH_2 donor, such as glycine (Figure 10) and L-alanine, resulted in complexes which hydrolyzed rapidly (too fast to measure by ^{1}H NMR spectroscopy), but which

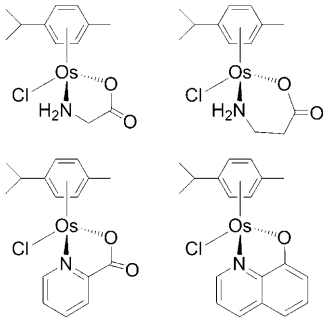

Figure 10. Osmium(II) arene complexes with various N,O-chelating ligands.

were unstable with respect to formation of the hydroxo-bridged dimer, though these did demonstrate greater stability than the six-membered chelate analogue β-alanine. As a result of their instability, these complexes were inactive towards human cancer cells. However, replacement of the primary amine by the π acceptor pyridine as the N donor, as in picolinate (pico), reduced the rate of hydrolysis as well as the pK_a value of bound water. Most significantly, this resulted in complexes which were stable in aqueous solution even at the micromolar concentrations used in the cytotoxicity tests, and which show promising activity (e.g., the IC_{50} value of $[(\eta^6\text{-arene})Os(pico)Cl]$ against A2780 cells is 4–5 μM), in some cases comparable to that of the anticancer drug carboplatin.[66]

In addition to potent activity, it was found that hydrolysis of $[(\eta^6\text{-}p\text{-cym})Os(pico)Cl]$ was suppressed at chloride concentrations typically found in the blood (ca. 100 mM), suggesting that the unreactive chloride complex, or the "pro-drug", would be present in the blood. Hydrolysis only occurred at lower chloride concentrations typical of the cell nucleus (ca. 4 mM), suggesting that selective activation by

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hydrolysis could occur in the cell nucleus where the possible biological target, nuclear DNA, is found. Remarkably, the "pro-drug" chlorido form of the *p*-cymene picolinate complex, [(η^6-*p*-cym)Os(pico)Cl], was stable for two months in isotonic saline solutions (stored in the dark), demonstrating favorable potential for drug formulation.[66]

Nuclear DNA is a likely biological target and DNA binding often correlates well with the cytotoxicity of metal anticancer complexes. The N,O-chelated pico complexes were found to bind to N7 of G, as well as to N7 and N1 of A. Both the kinetic and thermodynamic stability of the G adduct were greater than that of the A adduct, and a strong preference for G was observed in competition experiments. Notably, at the micromolar concentrations typical of cytotoxicity tests (50 μM), Os was found to bind primarily to N7 of G. We reported the first crystal structures of osmium bound to G and A nucleobases, and these are shown in Figure 11. These complexes were prepared as racemic mixtures. The X-ray crystal structures suggest that interligand

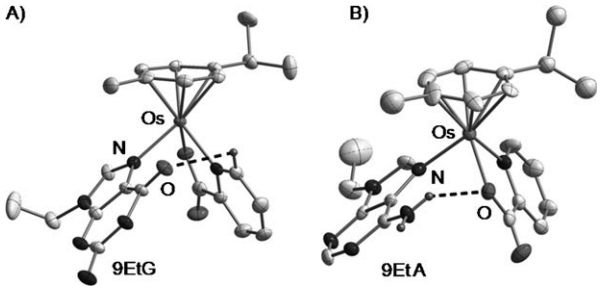

Figure 11. X-ray crystal structures of osmium adducts of G and A nucleobases. A) [(η^6-*p*-cym)Os(pico)(9EtG-N7)]$^+$ and B) [(η^6-*p*-cym)Os(pico)-(9EtA-N7)]$^+$ (50% probability ellipsoids). Remaining hydrogen atoms and counterions have been omitted for clarity.

interactions may play an important role in the biological recognition of G and A nucleobases on DNA, as the nucleobase functionality lies on opposite sides of the chelate for G and A bases, with potential short-range interactions between the chelate donor group and the nucleobase functionality.

[(η^6-arene)Os(YZ)Cl]$^{n+}$ complexes containing the π-acceptor pyridine group as the N-donor group bind irreversibly to polymeric DNA at rates that compare well with that for the anticancer drug cisplatin.[67] Binding involves both coordinative bonds, inhibiting RNA synthesis with stop-sites formed predominantly at G and A residues (which agrees well with the small-molecule binding studies), and noncovalent interactions between the arene and DNA. In the case of extended arene ring systems, for example, biphenyl, this corresponds to intercalation, as these complexes are able to displace ethidium bromide effectively from DNA. Notably, these noncovalent interactions are greater for [(η^6-bip)Os(en)Cl]$^+$ than those of its ruthenium analogue. Binding to DNA induces distortions that extend four base pairs around the adduct. However, this did not lead to bending of the DNA, unlike the anticancer drug cisplatin, but does result in a large degree of unwinding, more so than for either cisplatin or ruthenium complexes of the type [(η^6-arene)-Ru(en)Cl]$^+$.

These osmium complexes are non-cross-resistant with cisplatin towards cancer cells, and in one case even more active against cisplatin-resistant cells, suggesting promise for tackling the common problem of developed (and intrinsic) drug resistance in chemotherapy. These studies imply that though the cytotoxicty of Os^{II} arene complexes correlates with DNA binding, they are a potential new class of novel anticancer agents with both a different mechanism of action and of detoxification compared to the anticancer drug cisplatin.

Conclusions and Perspectives

The field of bioorganometallic chemistry, and in particular the development of organometallic therapeutics, is developing rapidly and has much potential. We have shown that there are parallels between the structure–activity relationships for ruthenium(II) and osmium(II) half-sandwich piano-stool complexes of the type [(η^6-arene)M(YZ)X]$^{n+}$, but also some significant differences. Figure 12 illustrates

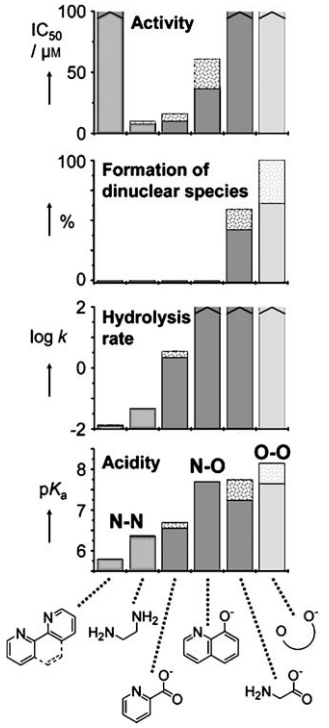

Figure 12. Bar charts illustrating the relationship between cytotoxicity towards human A2780 ovarian cancer cells, stability with respect to inert hydroxo-bridged dimer formation, rates of hydrolysis, and acidity of the aqua adduct for osmium arene complexes [(η^6-arene)Os(XY)Cl]$^{n+}$ containing different XY = N,N-, N,O-, and O,O-chelating ligands (adapted from reference [66]). There appears to be an optimum window of activity in which hydrolysis rates and acidity of coordinated water in the aqua adduct are intermediate in value, thus minimizing loss of the chelated ligand and formation of the inactive hydroxo-bridged dimer. This appears to be achieveable especially by the use of N,O-chelating ligands, with N,N chelates being at one end of the scale (slow hydrolysis, low pK_a value) and O,O chelates at the other (fast hydrolysis, high pK_a value).

the relationships between the cytotoxic activity, stability with respect to formation of inert hydroxo-bridged dimers in aqueous solution, kinetics of hydrolysis, and acidity of coordinated water for osmium arene complexes containing different chelating ligands.[61–63,66] A systematic approach and rational chemical design have been employed to fine-tune both the kinetics and thermodynamics of reactions of these complexes in aqueous solution, so as to access the appropriate reactivity and stability windows which lead to cancer cell cytotoxicity. A detailed understanding of how these organometallic complexes behave in aqueous solution under biologically relevant conditions has been crucial in these studies, and further advances in this field may lead to the development of many more potential organometallic therapeutic agents.

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