doi:10.2533/chimia.2007.704 CHIMIA **2007**, *61*, No. 11

Chimia 61 (2007) 704–715

ISSN 0009–4293

The Design of Organometallic Ruthenium Arene Anticancer Agents

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Abstract: Organometallic half-sandwich Ru^{II} anticancer complexes of the type $[(\eta^6\text{-arene})Ru(YZ)X]^n$ can exhibit interesting anticancer activity. We review the comparative aqueous solution chemistry (hydrolysis rates, pK_a values of aqua complexes), cancer cell cytotoxicities, cross-resistance, reactivity towards nucleobases, DNA and important biomolecules for complexes containing various arenes, N,N- or N,O- or O,O-chelating ligands as YZ, and monodentate leaving groups X. We show that the choice of these ligands can have a dramatic effect on reactivity. The same is true for analogous Os^{II} arene complexes. The interpretation of structure–activity relationships requires an understanding of reactions of these organometallic complexes under biological test conditions.

Keywords: Anticancer · Arene · Osmium · Organometallic complexes · Ruthenium

1. Introduction

1.1. *Ruthenium Complexes as Potential Anticancer Agents*

The anticancer properties of ruthenium were first reported in 1976 when the Ru^{III} compound *fac*-$Ru(NH_3)_3Cl_3$ was found to induce filamentous growth of *E. coli* at a concentration comparable to the concentration required for cisplatin to produce similar effects.[1] This compound, and analogues such as *cis*-$[RuCl_2(NH_3)_4]$ demonstrated the potential anticancer activity of ruthenium complexes. However, they were too insoluble for pharmacological use.[2]

Since this discovery, there have been several other reports of ruthenium complexes which exhibit anticancer activity, most of which contain Ru^{III}. Perhaps two of the most exciting Ru^{III} anticancer compounds are currently imidazolium *trans*-tetrachlorodimethylsulfoxideimidazoleruthenate(III), NAMI-A, and indazolium *trans*-tetrachlorobis(1H-indazole)ruthenate(III), KP-1019, since both compounds have entered clinical trials[3,4] as anticancer drugs.

It has been suggested that Ru^{III} complexes are 'activated by reduction' *in vivo* to Ru^{II} to coordinate more rapidly to biomolecules[5] due to the increased lability of Ru^{II}–Cl bonds.[3] There is a lower oxygen content and more acidic pH in tumours than in normal tissue and so the production of Ru^{II} relative to Ru^{III} should be favoured in tumours.[5]

1.2. *Ruthenium(II) Complexes*

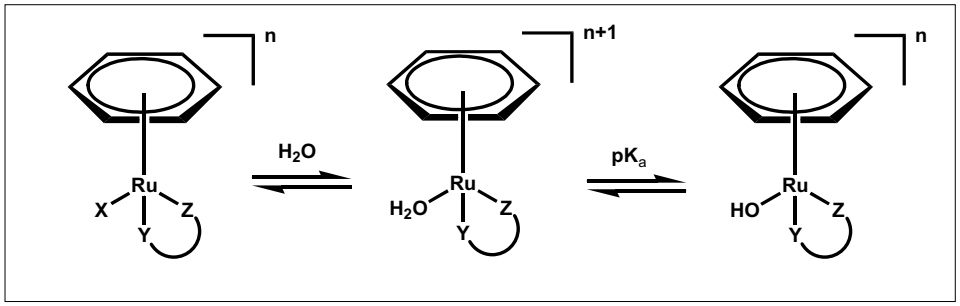
Because Ru^{II} may be an active form of ruthenium, there is now an increased effort into research on the anticancer activity of Ru^{II} complexes. Early work on ruthenium(II) complexes, for example *cis*-$[RuCl_2(DMSO)_4]$ and *trans*-$[RuCl_2(DMSO)_4]$[6–8] showed that Ru^{II} complexes are potentially interesting in the design of new drugs.

In this review we will focus on organometallic ruthenium(II) complexes of the type $[(\eta^6\text{-arene})Ru(XY)Z]$ (XY = bi-dentate ligand or two mono-dentate ligands, Z typically a halide) where XY are nitrogen or oxygen ligands (NN, NO, OO, N). Other active areas of research into the anticancer activity of Ru^{II} arenes include complexes such as $[(\eta^6\text{-arene})RuCl_2(\text{phosphine})]$ which contain phosphorus ligands such as pta (pta = 1,3,5-triaza-7-phosphaadamantane).[9]

2. Ruthenium(II) Arene Complexes as Anticancer Agents

2.1. *General Structure and Reactivity*

The general structure of the Ru^{II} arene complexes described here is shown in Scheme 1. All complexes contain an η^6-arene occupying three coordination sites, a chelating ligand occupying two and a mono-dentate ligand occupying the final site.



Scheme 1. General structure of Ru^{II} arene anticancer complexes containing an η^6 arene, a chelating ligand YZ and a monodentate labile ligand X that provides a reactive site for the molecule. In water, the Ru–X bond is subject to hydrolysis to generate the corresponding aqua adduct which can be deprotonated (depending on the pK_a) to give the hydroxo form.

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Depending on the nature of the chelated ligand, these complexes are either neutral or positively charged (and isolated as salts). Variations to all three ligand types can be achieved, so there is a great scope for the synthesis of a vast library of potential anticancer complexes. There are several synthetic routes to the complexes and these have recently been summarized.[10]

The mechanism of cytotoxic action of Ru^{II} arenes is generally thought to involve hydrolysis of the Ru–X bond generating an active Ru–OH_2 species. This species will exist over a range of pH values, but above the pH = pK_a value (the pH at which 50% of the species exists as Ru–OH_2 and Ru–OH through deprotonation of the H_2O ligand) the hydroxo Ru–OH species will predominate, and this complex is usually considered to be a less reactive species (Scheme 1). Hydroxide is a less labile ligand than water and hence will not be as easily displaced by biomolecule targets. Thus ideally pK_a values of *ca*. pH >7 for aqua adducts should ensure that the active species predominates at physiological pH (7.2–7.4). Furthermore the rate of hydrolysis is important; if the complexes hydrolyse too fast they may not reach the target site.

Hydrolysis can be suppressed extracellularly due to high [Cl^-] (*ca.* 0.1 M) but becomes possible after the complex enters the cells due a lower [Cl^-] (*ca*. 4–25 mM) found intracellularly. We thus obtain selective activation inside the cell. The primary cellular target for Ru^{II} arenes, as for many metal-based drugs is thought to be DNA[11] and so factors affecting DNA binding such as rate and extent of binding and non-covalent interactions such as hydrogen bonding and DNA intercalation become important.

2.2. *Ruthenium–Arene Bonding*

The structural and electronic features of metal–arene bonding have been thoroughly reviewed.[12] The arene is considered as a π-acid/π-acceptor ligand towards ruthenium. Evidence for this comes from

i) arene ^1H proton resonances which, upon arene coordination to Ru^{II} shift to a lower frequency due to increased electron density,[13] and
ii) from UV-Vis spectroscopic observations.[14]

Ru–arene bonds are generally stable towards hydrolysis, although recently we have reported that the photochemical displacement of the arene can occur in aqueous solution for dinuclear complexes such as [{(η^6-indan)RuCl}$_2$(μ-2,3-dpp)](PF_6)$_2$,[15] and arene lability can be induced by the presence of strong π-acceptor ligands bound elsewhere in the complex.

The aqueous solution chemistry of [(η^6-benzene)Ru(OH_2)$_3$]$^{2+}$ compared to [Ru(OH_2)$_6$]$^{2+}$ has shown that water exchange rates are three orders of magnitude faster for [(η^6-benzene)Ru(OH_2)$_3$]$^{2+}$ and this increase in rate has been attributed to a change in the transition state properties of the reaction due to the incorporation of the benzene ligand and reaction *via* an I (interchange) mechanism.[13]

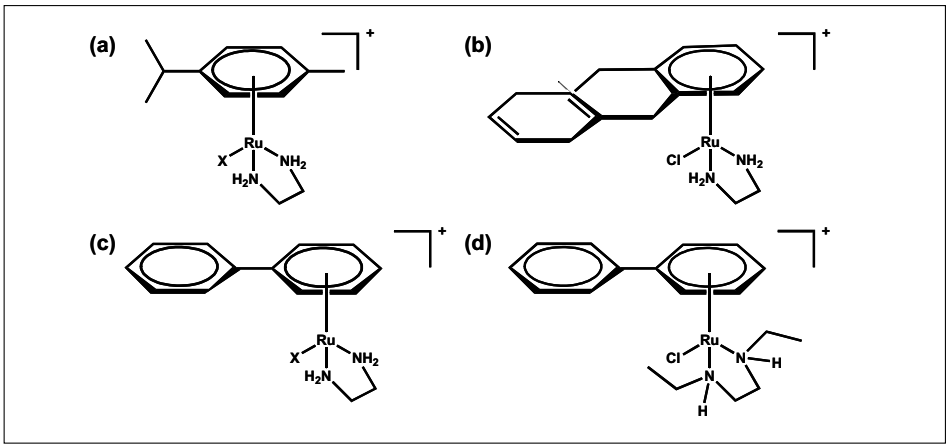

Fig. 1. Molecular Structures of (a) [(η^6-*p*-cymene)Ru(en)X]$^+$, (b) [(η^6-tetrahydronaphthalene)Ru(en)Cl]$^+$, (c) [(η^6-biphenyl)Ru(en)X]$^+$ and (d) [(η^6-biphenyl)Ru(en-Et)Cl]$^+$

3. Ruthenium(II) Arene Complexes Containing Chelating σ-Donor Nitrogen Ligands

3.1. *Initial Work*

Initially the anticancer activity of Ru^{II} arenes containing chelating nitrogen ligands such as ethylenediamine (en) or N-ethylethylenediamine (en-Et) (Fig. 1) was evaluated.[16] Several derivatives such as [(η^6-*p*-cymene)Ru(en)Cl]$^+$, [(η^6-*p*-cymene)Ru(en)I]$^+$, [(η^6-biphenyl)Ru(en)Cl]$^+$ and [(η^6-biphenyl)Ru(en-Et)Cl]$^+$, all inhibited the growth of the A2780 cancer cells with IC_{50} values of between 6–9 μM, comparable to the clinically-used anticancer drug carboplatin (6 μM). Furthermore complexes containing more hydrophobic arenes such as [(η^6-tetrahydronaphthalene)Ru(en)Cl]$^+$ were active with IC_{50} values equipotent with cisplatin (0.6 μM).[17] Interestingly ruthenium complexes containing mono-dentate ligands such as [(η^6-*p*-cymene)Ru(CH_3CN)$_2$Cl]$^+$ and [(η^6-*p*-cymene)Ru(isonicotinamde)$_2$Cl]$^+$ were inactive towards the A2780 cancer cell line (IC_{50} values >150 μM) indicating that a bi-dentate chelating ligand is required for cancer cell cytotoxicity. Structure–activity relationships thus showed that the most active complexes contain stable bi-dentate chelating ligands, a more hydrophobic arene ligand and a single ligand exchange centre (*e.g.* halide).

In water, [(η^6-*p*-cymene)Ru(en)Cl]$^+$ undergoes rapid aquation to form [(η^6-*p*-cymene)Ru(en)H_2O]$^{2+}$. This hydrolysis is largely suppressed in 0.1 M NaCl.[16] Reaction of [(η^6-*p*-cymene)Ru(en)Cl]$^+$ with the DNA 14-mer d($A_1T_2A_3C_4A_5T_6G_7G_8T_9A_{10}C_{11}A_{12}T_{13}A_{14}$) in varying ratios showed that [(η^6-*p*-cymene)Ru(en)H_2O]$^{2+}$ ruthenates DNA specifically at guanine residues to from two mono-ruthenated adducts at G7 and G8, as well as species di-ruthenated at both G7 and G8.[16]

3.2. *Ethylenediamine (en) Ligands*

3.2.1. *Structure–Activity Relationships (SAR)*

In general, introduction of polar substituents into the coordinated benzene ring in [(η^6-arene)Ru(en)Cl]PF_6 complexes lowers their cytotoxicity towards A2780 human ovarian cancer cells.[10] Thus for mono-substituted benzenes, the activity follows the order of substituents OPh (18 μM) > H (20 μM) > $CONH_2$ (33 μM) >> COOEt (52 μM), COPh (55 μM), COOMe (56 μM), Br (60 μM) >> CH_2OH (>100 μM). On the other hand, benzene arenes substituted with relatively non-polar sterically-demanding alkyl, phenyl or benzyl groups are much more potent with IC_{50} values as low as 3 μM.

The activity against the same cell line for analogous complexes in which the arene is a fused system has also been evaluated.[10] In general, complexes with Ru^{II} coordinated to partially-saturated five-, six- or seven-membered cyclic hydrocarbons all show good activity with the exception of the cyclophane arene. The order of potency is tetrahydroanthracene (0.4 μM), > 5,6-dihydrophenanthracene (1 μM), fluorene (2 μM), dihydroanthrancene (2 μM) > dibenzosuberane (8 μM), indan (8 μM) >tetralin (20 μM) >> cyclophane (>100 μM). In general the trend is that cytotoxicity increases with arene ring size in these cases, as described earlier.

3.2.2. *Aqueous Solution Chemistry*

Hydrolysis of the Ru–X bond is thought to be central to the cytotoxicity of Ru^{II} arene

complexes since it is this step that activates the complex for potential binding to DNA or other possible cellular targets. The rates of hydrolysis of $[(\eta^6\text{-bip})Ru(en)Cl]^+$, $[(\eta^6\text{-tha})Ru(en)Cl]^+$ and $[(\eta^6\text{-dha})Ru(en)Cl]^+$ (bip = biphenyl, tha = tetrahydroanthracene, dha = dihydroanthracene) are essentially independent of ionic strength and increase with the size of the arene: 3.95×10^{-3} s^{-1}, $t_{1/2}$ = 2.92 min for arene = bip, 6.84×10^{-3} s^{-1}, $t_{1/2}$ = 1.69 min for arene = tha and 6.49×10^{-3} s^{-1}, $t_{1/2}$ = 1.78 min for arene = dha (310 K, 0.1 M $NaClO_4$, 0.3 mM bip, 0.5 mM tha and dha complexes).[18] The rates of the corresponding anation reactions (replacement of H_2O by Cl^-) were also studied and decrease by about two-fold on increasing the ionic strength from 0.015 to 0.5 M $NaClO_4$. This decrease is expected on the basis of the Brønsted equation, *i.e.* that the rate constant is expected to be independent of the ionic strength when one of the reactants remains uncharged, whereas the rate constant decreases with ionic strength if the charges on the two ions are of opposite sign.[19] The anation reactions were rapid, reaching equilibrium within *ca.* 100–1600 s. Generally the anation reactions of the $[(\eta^6\text{-tha})Ru(en)H_2O]^{2+}$ and $[(\eta^6\text{-dha})Ru(en)H_2O]^{2+}$ complexes were about 2.5 fold faster than $[(\eta^6\text{-bip})Ru(en)H_2O]^{2+}$.

The pK_a of the aqua complexes $[(\eta^6\text{-bip})Ru(en)H_2O]^{2+}$, $[(\eta^6\text{-dha})Ru(en)H_2O]^{2+}$ and $[(\eta^6\text{-tha})Ru(en)H_2O]^{2+}$ are 7.71, 7.89 and 8.01 respectively.[18] Thus in the blood plasma, where the chloride concentration is high (*ca.* 0.14 M) all three complexes exist largely (>89%) in this inactive chlorido form. Upon entering the cytoplasm and nucleus, the chloride concentrations drop to *ca.* 23 and 4 mM, respectively. Hence the extent of aquation is predicted to increase from about 30% in the cytoplasm to about 70% in the nucleus. This would represent an activation mechanism for these chloro complexes inside the cytoplasm/nucleus. Interestingly, only a small amount of Ru–OH hydroxo adducts (average <10% of total ruthenium) is predicted to exist inside cells due to the high pK_a value of the aqua adducts. This becomes important for DNA binding, since the Ru–OH bond is found to be less reactive towards DNA nucleobases compared to $Ru–OH_2$ (*vide supra*).

Density functional theory calculations[20] for aquation of $[(\eta^6\text{-benzene})Ru(en)X]^+$, X = Cl^-, Br^-, I^-, N_3^- suggests that the aquation proceeds *via* a concerted interchange pathway and does not appear to be strongly associatively or dissociatively activated. The reaction barriers and overall reaction energies for aquation follow the order Br < Cl < I < N_3. The reactions appear to occur nearer the I_a (interchange-associative) mechanistic continuum rather than the I_d (interchange-dissociative mechanism). On the basis of electronegativity, the N_3 complex would be expected to hydrolyze at a rate between the Cl^- and Br^- analogues but a much slower rate is observed. The lower rate is ascribed to the increased steric bulk of this polyatomic pseudohalide; an I_a substitution is more influenced by steric factors than an I_d pathway. Furthermore, the electron-accepting effect of the strong π-acid arene ligands is thought to be responsible for the shift towards a more associative I_a pathway. Ru^{III} complexes usually react *via* associative pathways[21] whereas Ru^{II} complexes are more suited to dissociative mechanisms.[22] The π-acid arene accepts electron density from ruthenium to produce a higher charge on the metal. Thus Ru^{II} in $\{(\eta^6\text{-arene})Ru]\}^{2+}$ may behave more like a Ru^{III} centre.

Good correlations between hydrolysis rates, hydrolysis equilibrium and cytotoxicity have been observed for the ruthenium complexes $[(\eta^6\text{-hmb})Ru(en)X]^+$, hmb = hexamethylbenzene[20] (Fig. 2). In general, a faster hydrolysis rate and a high percentage of aqua species at equilibrium correlated with good cytotoxicity towards the A2780 human ovarian cancer cell line. An exception is for the complex $[(\eta^6\text{-hmb})Ru(en)SPh]^+$ which hydrolyses very slowly and to a low extent. This complex may be activated by oxidation of bound SPh to the sulfenate or sulfinate by oxygen, since reactions of Ru^{II} arenes with GSH show similar behaviour (*vide infra*).

3.2.3. *Guanine Binding*

The interaction of $[(\eta^6\text{-arene})Ru(en)Cl]^+$ complexes (arene = biphenyl, tetrahydronapthalene, dihydronapthalene) and guanine DNA derivatives 9-ethylgusnine (9EtG), guanosine and 5-guanosine monophosphate (5-GMP) has been studied ex-

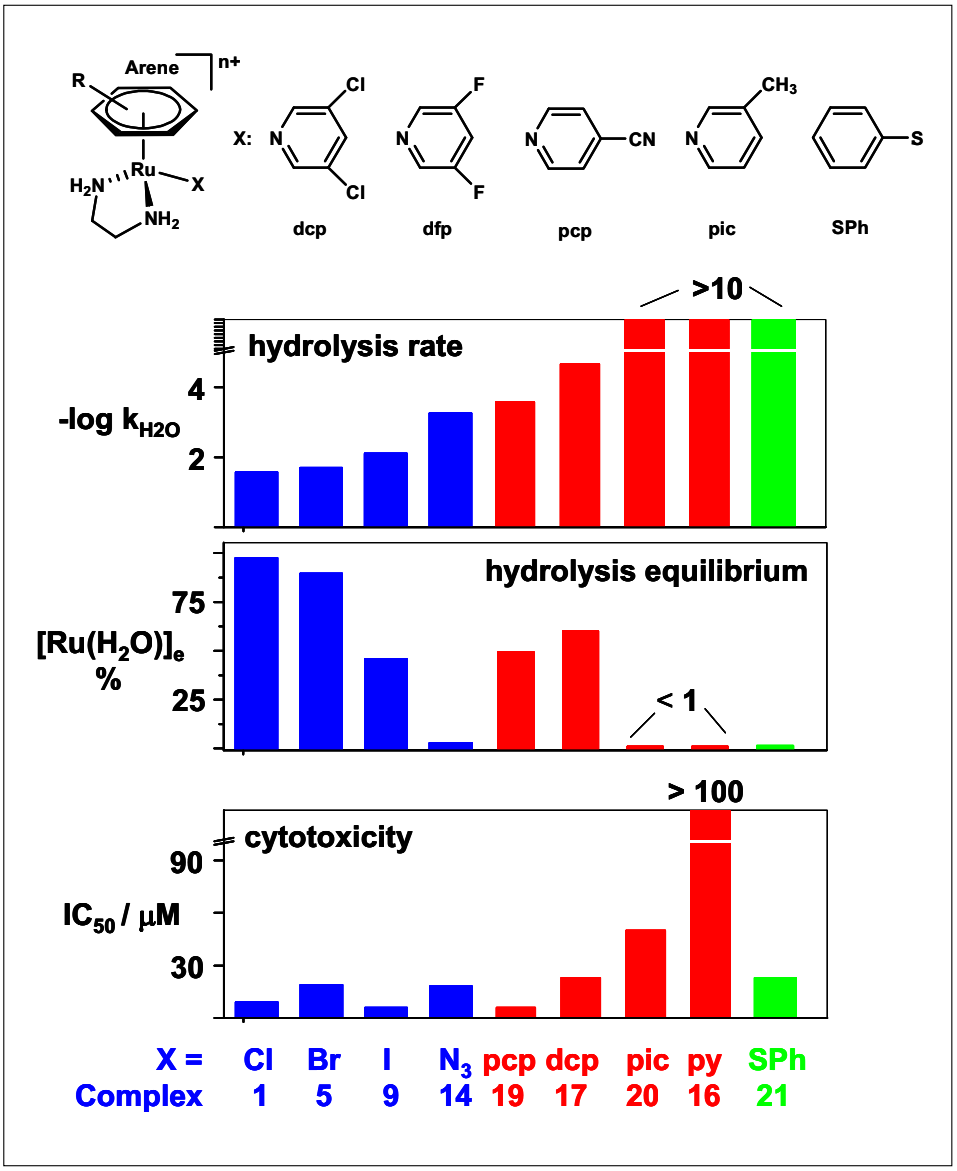

Fig. 2. Correlation of hydrolysis with cytotoxicity. (Upper) Structures of the complexes and leaving groups. (Lower) Hydrolysis rates, equilibrium percentage of total Ru as $[(\eta^6\text{-hmb})Ru(en)H_2O]^{2+}$ ($[Ru(H_2O)]_e$ %) and A2780 cancer cell cytotoxicity IC_{50} values for $[(\eta^6\text{-hmb})Ru(en)X]^{n+}$ complexes with different 'leaving groups'. Adapted from ref. [20].

tensively, in both the solid (X-ray crystal structures) and solution (NMR) state.[23] Although there is strong bonding between the DNA nucleobase and Ru^{II}, other non-covalent interactions including intercalation and hydrogen-bonding become important for DNA recognition.

3.2.3.1. *Arene–Nucleobase Stacking*

The X-ray crystal structures of [(η^6-tha)Ru(en)9Et-G(N(7))]$^{2+}$ and [(η^6-dha)Ru(en)9Et-G(N(7))]$^{2+}$ show the presence of strong intramolecular π–π arene–nucleobase stacking. The outer ring of tha/dha lies directly over the purine base and the centroid–centroid separation between the outer ring of the arene and the purine ring is 3.45 Å for [(η^6-tha)Ru(en)9Et-G(N(7))]$^{2+}$, and 3.31 Å for [(η^6-dha)Ru(en)9Et-G(N(7))]$^{2+}$, with dihedral angles of 3.3° and 3.1° respectively. This strong stacking is essentially optimum; the outer arene ring is parallel to and fully overlaps the purine ring (see Fig. 3). Face to face π–π stacking is reported to be optimized when both partners are electron poor[24] and so the polarization of the arene and guanine ligands by Ru^{II} may be an important factor for the stabilization for arene–nucleobase stacking. For the biphenyl complex [(η^6-biphenyl)Ru(en)9Et-G(N(7))]$^{2+}$, no intramolecular stacking between the pendant phenyl ring and the nucleobase was observed due to the biphenyl adopting an *anti* conformation with respect to the purine base. Intermolecular stacking between the pendant phenyl ring and a purine base on an adjacent cation occurs with a centroid to centroid distance of 4.0 Å and a dihedral angle of 4.5°. Thus the arene–nucleobase stacking in the biphenyl adduct is accompanied by a longer centroid–centroid distance and this is because of the higher degree of freedom for the movement of the biphenyl ring, *i.e.* the free propeller twisting of the phenyl ring in bip compared to the relatively rigid tricyclic frames of tha and dha.

In solution, ^{1}H 2D NOESY NMR experiments confirm that the pendant phenyl ring in complex [(η^6-biphenyl)Ru(en)9Et-G(N(7))]$^{2+}$ adopts a *syn* conformation with respect to the G base.[23]

3.2.3.2. *Specific Hydrogen Bonding*

In all three crystal structures of [(η^6-biphenyl)Ru(en)9Et-G(N(7))]$^{2+}$, [(η^6-tha)Ru(en)9Et-G(N(7))]$^{2+}$ and [(η^6-dha)Ru(en)9Et-G(N(7))]$^{2+}$, strong stereospecific hydrogen-bonding occurs between an en NH proton oriented away from the arene, pointing towards the DNA base (so-called NH_{down} protons) and the C6 carbonyl oxygen of the guanine (average distances 2.8 Å N···O, N–H···O 163°).[23] Such stereospecific hydrogen-bonding between the en ligand and the exocyclic oxygen atom of guanine, may play an important role in both the stability and conformation of this adduct.

3.2.3.3. *DNA Base Selectivity*

Ruthenium(II) en complexes bind preferentially to N(7) of guanine.[25] Experiments have been preformed to determine the species produced after 24 h reaction of [(η^6-biphenyl)Ru(en)Cl]$^+$ with guanosine, inosine, thymidine, cytidine and adenosine.[25] It was shown that the complex has a high selectivity for guanosine with 100% reaction to form the guanosine adduct. A significant percentage of complex binds to the N(7) and N(1) of inosine (82%) and a small percentage forms a dinuclear species in which one ruthenium binds to N(3) and another to N(1)(accounting for *ca.* 4% of the Ru). Significant binding (*ca.* 31%) was also observed to N(3) of thymidine, but contrastingly only a small percentage binds to N(3) of cytidine (12%), and even less (<3%) to adenosine. Thus the overall order of base selectivity is G (N(7)) > I (N(7)) > I (N(1)), T (N(3)) >C (N(3)) >A (N(7)), A (N(1)) > G (N(1)). The observed base selectivity can be rationalized in terms of hydrogen bonding attractions/repulsions. Thus coordination at the N(7) of guanine is stabilized by the hydrogen bonding of en N–H with guanosine C(6)-O. Coordination at N(1) of G is unfavourable due to the potentially repulsive interaction between the en NH_2 and guanine C2 NH_2 groups. Because inosine has no NH groups, coordination at either N(7) or N(1) can be stabilized by hydrogen-bonding of inosine C(6)-O resulting in significant amounts of both species being formed. For thymidine, coordination at N(3) may be favoured by hydrogen-bonds between en NH and the exocyclic oxygen's at C2 and C4, whereas for cytidine, only a small reaction by coordination at N(3) is observed. Coordination is thought to be weak partly due to a repulsive interaction of en NH with the C4 NH_2 group. For adenosine any coordination at the N(7) or N(1) is weakened by repulsive interactions with the exocyclic amino group and as a result negligible binding is observed. For reactions with mononucleotides, a similar pattern of selectivity is observed, except that significant amounts of 5´ phosphate bound species (40–60%) are present at equilibrium for 5´-TMP, 5´-CMP and 5´-AMP (but not 5´-GMP, c-GMP or c-AMP).

In competitive reactions between [(η^6-biphenyl)Ru(en)Cl]$^+$ and the cyclic nucleotide diesters c-GMP *versus* c-TMP, c-CMP or c-AMP monitored over 48 h (5 mM Ru, 1:1 nucleotides, pH 7.2, 310 K) only

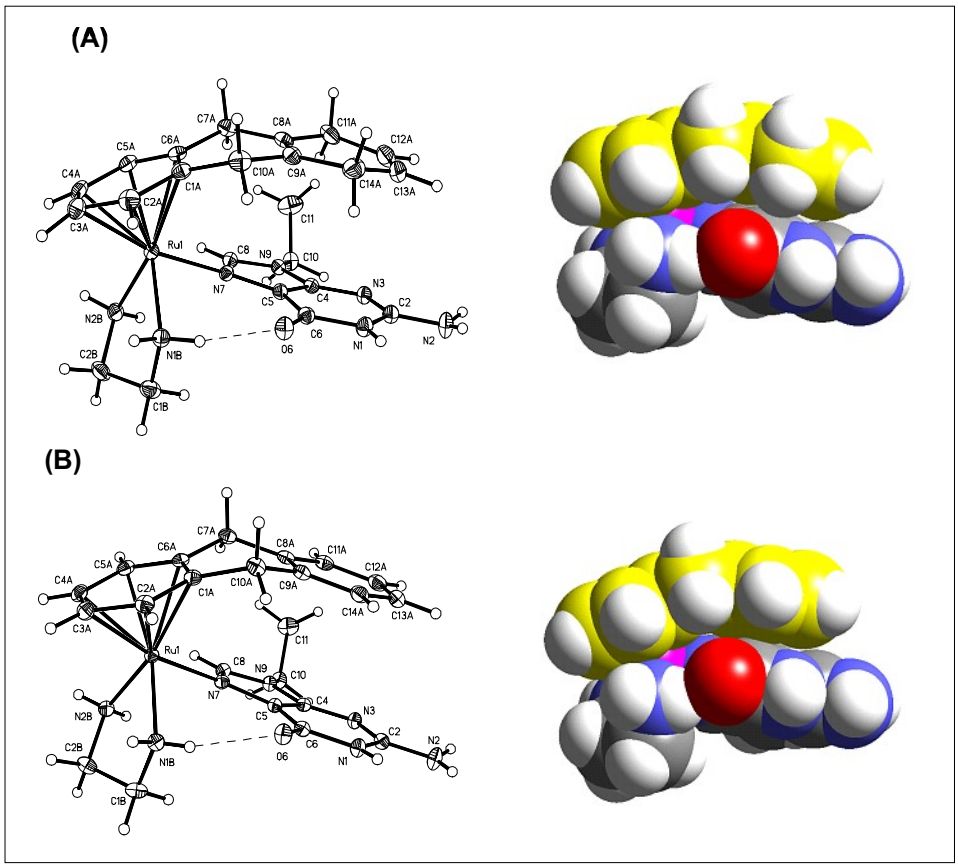

Fig. 3. X-ray structures (at 30% probability thermal ellipsoids) and atom numbering schemes for (A) [(η^6-$C_{14}H_{14}$)Ru(en)9EtG-N(7)]$^{2+}$, and (B) [(η^6-$C_{14}H_{12}$)Ru(en)9EtG-N(7)]$^{2+}$. The space-filling models show the intramolecular arene–guanine base stacking and H-bonding interactions between en NH and G O6. Colour code: C of $C_{14}H_{14}$ and $C_{14}H_{12}$ yellow, C of 9EtG and en gray, Ru purple, O red, N blue, and H white. Adapted from ref. [23].

Table. Half lives ($t_{1/2}$) for reactions of Ru^{II} arene complexes $[(\eta^6\text{-arene})Ru(en)Cl]^+$ and the corresponding aqua complexes $[(\eta^6\text{-arene})Ru(en)OH_2]^{2+}$ with cGMP[a].

$[(\eta^6\text{-arene})Ru(en)Cl]^+$	$t_{(1/2)}$ [h]	$[(\eta^6\text{-arene})Ru(en)OH_2]^{2+}$	$t_{(1/2)}$ [h]
tha	1.1	tha	0.38
bip	2.0	bip	0.69
dha	3.6	dha	0.78
p-cym	7.1	*p*-cym	2.23
bz	13	bz	4.94

[a]conditions Ru:G 1:1, 100 mM $NaClO_4$, 298 K, 5 mM for $[(\eta^6\text{-arene})Ru(en)OH_2]^{2+}$, 2 mM for $[(\eta^6\text{-arene})Ru(en)Cl]^+$.

Ru-c-GMP adduct is formed confirming the preferential binding to c-GMP.[25]

3.2.3.4. *Rates of Reactions with cGMP*

The half-lives for reaction of five ruthenium(II) arene en chloride complexes (arene = bip, tha, dha, bz and *p*-cym; bz = benzene, *p*-cym = *p*-cymene) and the corresponding aqua adducts with c-GMP are summarized in the Table.[25] The rates of reaction of c-GMP with $[(\eta^6\text{-tha})Ru(en)OH_2]^{2+}$, $[(\eta^6\text{-dha})Ru(en)OH_2]^{2+}$ and $[(\eta^6\text{-bip})Ru(en)OH_2]^{2+}$ complexes are more than three times faster than those for the $[(\eta^6\text{-}p\text{-cym})Ru(en)OH_2]^{2+}$ and $[(\eta^6\text{-bz})Ru(en)OH_2]^{2+}$ complexes. The rates of reaction of the chloride complexes are slower due to the two-step process of reaction: hydrolysis followed by Ru–N(7) binding. The same trend in reactivity was observed for the chlorido and aqua complexes. For the complexes $[(\eta^6\text{-tha})Ru(en)Cl]^+$, $[(\eta^6\text{-dha})Ru(en)Cl]^+$ and $[(\eta^6\text{-bip})Ru(en)Cl]^+$, 100% of the N(7)-bound product was observed after 2 d whereas only *ca.* 80% of the N(7) bound product formed for the *p*-cym and bz complexes after 4 d. Thus the rate of reaction of the Ru aqua complexes (and chloro complexes) with c-GMP depends markedly on the nature of the arene decreasing by over an order of magnitude as the arene is changed from tha > bip > dha >> *p*-cym > bz. Because the fastest rates are for the complexes containing the larger arene ligands, this implies lower activation energies ($\Delta G^{\ddagger}$) for formation of seven-coordinate transition states (assuming an associative mechanism). A significant contribution to $\Delta G^{\ddagger}$ may arise from π–π stacking of the arene and the purine ring in the transition state (negative $\Delta H^{\ddagger}$). Such an interaction is not possible for the *p*-cymene and benzene complexes. Thus these hydrophobic interactions could produce an additional driving force for DNA binding.

3.2.3.5. *Interactions with Oligonucleotides*

Studies on the interaction of $[(\eta^6\text{-bip})Ru(en)Cl]^+$ with single strand DNA 14-mer d(ATACATGGTACATA) (I) or its complementary strand d(TATGTACCATGTAT) (II) gave either mono- or di-ruthenated species (binding at G) depending on the ratio of Ru:single strand.[26] Interestingly, the annealation of mono-ruthenated (II) with strand (I) (heat to 353 K for 2 min followed by slow cooling to 288 K over 3 h) gave a product in which all four G bases were ruthenated. Thus $[(\eta^6\text{-bip})Ru(en)Cl]^+$ is highly specific for G (N(7)) but is mobile at elevated temperatures at which migration between guanine residues is facile. This behaviour suggests that organometallic ruthenium(II) arene complexes can be readily removed from DNA, which may be beneficial for reversing DNA damage in cells. Furthermore the dynamic behaviour of arene intercalation of the pendant phenyl ring in the biphenyl arene was demonstrated (in solution by ^1H NMR) where equilibria exist between intercalated and non-intercalated forms.[26]

The interaction of $[(\eta^6\text{-bip})Ru(en)Cl]^+$ with the duplex $d(CGGCCG)_2$ revealed that there was preferential binding at G3 and G6 with no binding observed at G2, attributable to unfavourable steric interactions between the duplex and the arene.[27] So not only is sequence specificity important in the recognition process, site specificity also can play an important role.

3.2.4. *Reactions with DNA*

The interaction of several ruthenium arene complexes with DNA in a cell-free medium has been studied using several different techniques.[28] The extent of ruthenation of double helical CT-DNA (0.1 mg/ml) by the arene complexes at an r_i value of 0.1 (molar ratio of free ruthenium complex to nucleotide phosphates at the onset of incubation with DNA) in 10 mM $NaClO_4$ at 310 K was followed. The amount of ruthenium bound per DNA nucleotide phosphate increases with time and the time at which binding reaches 50% ($t_{50\%}$) is markedly dependent on the arene: 10 min ($[(\eta^6\text{-bip})Ru(en)Cl]^+$) and ($[(\eta^6\text{-tha})Ru(en)Cl]^+$), 15 min ($[(\eta^6\text{-dha})Ru(en)Cl]^+$) and 3.5 h ($[(\eta^6\text{-}p\text{-cym})Ru(en)Cl]^+$). Under the same conditions $t_{50\%}$ for cisplatin binding is *ca.* 2 h. Thus for $[(\eta^6\text{-bip})Ru(en)Cl]^+$, $[(\eta^6\text{-tha})Ru(en)Cl]^+$ and $[(\eta^6\text{-dha})Ru(en)Cl]^+$, reactions are an order of magnitude faster than for cisplatin. The binding was also nearly quantitative – for $[(\eta^6\text{-bip})Ru(en)Cl]^+$ after *ca.* 3 h *ca.* 90% of the ruthenium was bound to DNA. Transcription mapping experiments have shown that the ruthenium complexes bind mainly at guanine sites on DNA.[28]

3.2.4.1. *RNA Synthesis*

The *in vitro* RNA synthesis by RNA polymerases on DNA templates was studied using a linear DNA fragment modified by these ruthenium arene complexes, cisplatin and monofunctional [Pt(dien)Cl]Cl.[28] RNA synthesis on these modified plasmid fragments yielded fragments of defined size, which indicates that RNA synthesis on these templates was prematurely terminated (for Ru complexes mainly at guanine residues). For cisplatin the termination sites are similar as for the Ru(II) arenes (*i.e.* mainly at G) but the efficiency of the ruthenium-adducts to terminate RNA synthesis *in vitro* is reduced relative to that of cisplatin. Furthermore the efficiency of the $[(\eta^6\text{-}p\text{-cym})Ru(en)Cl]^+$ adducts was noticeably lower than for the other three Ru^{II} arenes. Interestingly, no termination of RNA synthesis by the monofunctional [Pt(dien)Cl]Cl was observed, and this inability has been observed for several monofunctional platinum adducts.[29–31] This suggests that the ruthenium complexes bind and distort DNA in a somewhat different fashion to the mononuclear Pt complex [Pt(dien)Cl]Cl.

3.2.4.2. *Circular Dichroism*

The binding of ruthenium arene compounds $[(\eta^6\text{-bip})Ru(en)Cl]^+$, $[(\eta^6\text{-tha})Ru(en)Cl]^+$, $[(\eta^6\text{-dha})Ru(en)Cl]^+$, $[(\eta^6\text{-}p\text{-cym})Ru(en)Cl]^+$, and $[(\eta^6\text{-bz})Ru(en)Cl]^+$ to CT DNA and double-stranded polynucleotide complexes poly (dG-dC) and poly (dA-dT) has been studied by circular dichroism (CD).[28] An induced CD band at 350–410 nm was observed for the interaction of $[(\eta^6\text{-bip})Ru(en)Cl]^+$, $[(\eta^6\text{-tha})Ru(en)Cl]^+$ and $[(\eta^6\text{-dha})Ru(en)Cl]^+$, with both CT-DNA and poly (dG-dC) whereas no induced band in this near UV region was observed for $[(\eta^6\text{-}p\text{-cym})Ru(en)Cl]^+$, or $[(\eta^6\text{-bz})Ru(en)Cl]^+$. This induced CD band appears to be related to intercalation of the extended arene ligands into DNA or to groove binding. The observation of such bands has been well documented for intercalation of other metal complexes.[32,33] For poly (dA-dT), only the complexes with extended π-systems ($[(\eta^6\text{-bip})Ru(en)Cl]^+$, $[(\eta^6\text{-tha})Ru(en)Cl]^+$ and $[(\eta^6\text{-dha})Ru(en)Cl]^+$) induced any spectral changes and these were small, confined to the region 260–280

nm and were ascribed to weak hydrophobic interactions between the arene and the DNA base. Because only a weak binding to A or T bases is expected (*vide supra*) this accounts for the differences between poly (dA-dT) and poly (dG-dC) spectra.

3.2.4.3. *Linear Flow Dichroism*

Linear flow dichroism studies showed that ruthenation of CT DNA and poly (dG-dC) causes bending of the DNA. The three possible intercalating complexes ([(η⁶-bip)Ru(en)Cl]⁺, [(η⁶-tha)Ru(en)Cl]⁺, [(η⁶-dha)Ru(en)Cl]⁺) cause a wavelength shift consistent with intercalation of the arene ligands but the bending precludes full intercalation. [(η⁶-*p*-cym)Ru(en)Cl]⁺ and [(η⁶-bz)Ru(en)Cl]⁺ were found to rigidify poly (dA-dT) DNA, whereas the intercalating complexes cause significant bending, with [(η⁶-dha)Ru(en)Cl]⁺ inducing the most bending. The LD observed for the binding to poly (dA-dT) is consistent with a mode involving the aromatic ligands inserted into the minor groove.

3.2.4.4. *Ethidium Bromide Experiments*

The intercalation of the arene ligands into double helical DNA has been confirmed by ethidium bromide (EtBr) fluorescence experiments.[28] EtBr is a probe which is fluorescent when bound to DNA and can be used to distinguish intercalating and non-intercalating ligands on DNA by competition.[34] Complexes [(η⁶-bip)Ru(en)Cl]⁺, [(η⁶-tha)Ru(en)Cl]⁺ and [(η⁶-dha)Ru(en)Cl]⁺ can intercalate into the double helix of DNA, whereas [(η⁶-*p*-cym)Ru(en)Cl]⁺ does not.

3.2.4.5. *DNA Unwinding*

Complexes [(η⁶-bip)Ru(en)Cl]⁺, [(η⁶-tha)Ru(en)Cl]⁺ and [(η⁶-dha)Ru(en)Cl]⁺ unwind negatively supercoiled pSP73KB plasmid DNA by 14 ± 1° per bound ruthenium and [(η⁶-*p*-cym)Ru(en)Cl]⁺ by only 7 ± 0.5°.[28] These results suggest that the extra unwinding is a result of the intercalation of the extended arene or another non-covalent interaction of these complexes with DNA upon mono-functional binding. Large unwinding angles of 15 or 19° produced for the platinum compounds *cis*-[Pt-$(NH_3)_2$(N8-ethidium)Cl]$^{2+}$ and *cis*-[Pt-$(NH_3)_2$(N(3)-ethidium)Cl]$^{2+}$ which contain the known intercalator ethidium and can form only mono-functional adducts with DNA, have also been explained this way.[35]

3.2.4.6. *DNA Melting Temperature (t_m)*

At lower salt concentrations (0.01M $NaClO_4$), [(η⁶-bip)Ru(en)Cl]⁺, [(η⁶-tha)Ru(en)Cl]⁺ and [(η⁶-dha)Ru(en)Cl]⁺ increase the melting temperature (t_m) of calf thymus (CT)-DNA. The increase becomes more pronounced with increasing r_b values, where r_b = number of Ru atoms bound per nucleotide. As the ionic strength is increased, the enhancement of t_m (Δt_m) due to the presence of the ruthenium compounds decreased and at higher salt concentrations (0.2 M) t_m decreased. The dependence of t_m of DNA modified by ruthenium at differing ionic strengths is due to competing electrostatic effects as the salt concentration is varied.[36] At low ionic strengths the increase in t_m due to the modification of DNA by [(η⁶-bip)Ru(en)Cl]⁺, [(η⁶-tha)Ru(en)Cl]⁺ and [(η⁶-dha)Ru(en)Cl]⁺ are caused by the positive charges on the ruthenium and by the intercalation.

Three factors appear to account for the change in thermal stability of DNA modified by ruthenium:

i) stabilizing effects of the positive charge on the ruthenium fragments,
ii) stabilizing effects of DNA interstrand crosslinks, and
iii) destabilizing effects of conformational distortions such as intrastrand crosslinks induced in DNA by ruthenium coordination.

A further two additional factors may be involved in DNA stabilization by intercalators; favourable stacking interactions between the base residues and the intercalator and the separation of negative backbone charges inherent to intercalation (due to elongation and unwinding of DNA).

Contrastingly, the melting behaviour of DNA modified by [(η⁶-*p*-cym)Ru(en)Cl]⁺, results in a similar decrease in t_m at all ionic strengths.[28] It is not known as yet why the modification at higher salt concentrations appears to result in a smaller thermal stabilization or even destabilization, although some possibilities have been suggested.[28] The melting behaviour of [(η⁶-*p*-cym)Ru(en)Cl]⁺ decreases t_m even at a lower salt concentration, indicating that the factors responsible for thermal stabilization of DNA are notably reduced. Thus it is likely that the stabilizing effects of the positive charge on the ruthenium atom are markedly reduced so that the destabilization effect of conformational alterations induced by [(η⁶-*p*-cym)Ru(en)Cl]⁺ predominates already at low salt concentrations.

3.2.4.7. *Differential Pulse Polarography*

CT-DNA modified by ruthenium complexes was analyzed by differential pulse polarography (DPP).[28] Intact double helical DNA is polarographically inactive because its reduction sites are involved in hydrogen bonds and thus cannot be electrochemically reduced. However, electroreduction of adenosine or cytosine residues present on distorted but still double stranded (nondenatured) DNA generates a small DPP peak (peak I). The appearance of more negative peaks in the DPP curves indicates the presence of single stranded denatured regions in the DNA molecule in which hydrogen bonds between complementary bases have been broken and so are readily electrochemically reduced (peak II). Results show that [(η⁶-bip)Ru(en)Cl]⁺, [(η⁶-tha)Ru(en)Cl]⁺ and [(η⁶-dha)Ru(en)Cl]⁺ induce non-denaturational conformational distortions of CT-DNA at low levels of global modification (r_b values of 0.0005–0.01) with an increase in intensity of peak I with increasing values of the modification. This behaviour is similar to that observed for cisplatin and its analogues.[37,38] In contrast, for Ru-*p*-cym an increase in intensity of peak I was observed at low concentrations of the DNA modification ($r_b \leq 0.005$) and higher levels of DNA modification peak II were observed. This supports the view that the DNA binding mode of the [(η⁶-*p*-cym)Ru(en)Cl]⁺ is different from that of the [(η⁶-bip)Ru(en)Cl]⁺, [(η⁶-tha)Ru(en)Cl]⁺ and [(η⁶-dha)Ru(en)Cl]⁺ analogues and that modification of DNA by [(η⁶-*p*-cym)Ru(en)Cl]⁺ may even lead to denaturational distortions of DNA.

3.2.4.8. *Isothermal Titration Calorimetry*

The conformation of DNA modified by the ruthenium arene complexes [(η⁶-tha)Ru(en)Cl]⁺ and [(η⁶-*p*-cym)Ru(en)Cl]⁺ has been further studied by isothermal titration calorimetry (ITC).[39] Data show that ruthenation of the duplex 5'-CTCTCTT-GTCTTCTC-3' is exothermic and results in a large decrease in enthalpy of duplex formation by 4.4 and 7.4 kcal mol^{-1} for the tha and *p*-cym complexes, respectively, and in a substantial decrease in the entropy of the duplex of 11.6 or 18.2 cal $K^{-1}mol^{-1}$ ($T\Delta\Delta S$ 3.6 and 5.4 kcal mol^{-1}), respectively. Thus we have enthalpic destabilization of the duplex relative to its non-modified analogue, but entropic stabilization of the duplex. The net result is that the formation of these mono-functional adducts with the duplex induces a decrease in duplex thermodynamic stability of 0.8 or 2.0 kcal mol^{-1}, with this destabilization being enthalpic in origin. The [(η⁶-*p*-cym)Ru(en)Cl]⁺ adduct was more destabilized than that of [(η⁶-tha)Ru(en)Cl]⁺. The higher thermodynamic stability of the DNA adducts of [(η⁶-tha)Ru(en)Cl]⁺ may be associated with intercalation since various intercalators have been known to thermodynamically stabilize DNA.[40,41]

3.2.4.9. *DNA Repair Synthesis*

Considerably different levels of damage-induced DNA repair synthesis were detected in plasmids modified by [(η⁶-tha)Ru(en)Cl]⁺, [(η⁶-*p*-cym)Ru(en)Cl]⁺ and cisplatin.[39] The level of synthesis detected

in the plasmid modified by [(η⁶-tha)Ru(en)Cl]⁺ is *ca.* 6 times lower than in that in the plasmid modified by [(η⁶-*p*-cym)Ru(en)Cl]⁺. DNA repair synthesis can occur by various repair mechanisms including cisplatin, are the nucleotide excision repair (NER) mechanism (the usual mechanism for cisplatin). Compared to cisplatin, the monofunctional adducts of [(η⁶-tha)Ru(en)Cl]⁺ and [(η⁶-*p*-cym)Ru(en)Cl]⁺ are excised with a significantly lower efficiency than the major intrastrand crosslink of cisplatin, and [(η⁶-*p*-cym)Ru(en)Cl]⁺ adducts are excised slightly more than those of [(η⁶-tha)Ru(en)Cl]⁺. The HMG (High Mobility Group) protein plays a role in sensitizing the cells to cisplatin. For example it has been shown that the HMG domain proteins recognize and bind to DNA adducts formed by cisplatin.[42] An important structural motif recognized by HMG domain proteins on DNA modified by cisplatin is a directional bend of the helix axis towards the major groove. No recognition of the DNA monofunctional adducts of [(η⁶-tha)Ru(en)Cl]⁺ or [(η⁶-*p*-cym)Ru(en)Cl]⁺ by HMGB1 has been observed, suggesting that these adducts do not afford structural motifs recognized by HMG domain proteins. Thus the mechanism of antitumour activity of Ru^{II} arenes does not involve recognition of its DNA adducts by HMG domain proteins as a crucial step, in direct contrast to cisplatin.

3.2.5. *Mechanism of Action*

3.2.5.1. *A2780 and 2780AD Cellular Resistance*

A high degree of cross resistance has been observed between Ru^{II} arene complexes and adriamycin, as observed in the 2780AD cell line.[17] This cell line displays the classic Multi Drug Resistance (MDR) phenotype *via* over-expression of the 170 kD plasma membrane glycoprotein P-gp and reduced cellular drug accumulation.[43,44] P-gp has a substrate specificity for naturally-occurring hydrophobic molecules, particularly those carrying a positive charge and so the cationic hydrophobic Ru^{II} arene complexes exhibit both these features. Thus it is likely that the cross resistance to Ru^{II} arene complexes is due, at least partly, to their recognition and active efflux by P-gp. This has been confirmed by co-administering verapamil (a drug known to abrogate effectively P-gp mediated active efflux by competitive inhibition of drug transport and hence reverse MDR) with the ruthenium complex [(η⁶-bip)Ru(en)Cl]⁺ when the fold resistance decreased from 38-fold to three-fold, *i.e.* almost a complete reversal of drug resistance.

In contrast Ru^{II} arene complexes are completely non-cross resistant towards the A2780cis cell line. This is interesting as it suggests that the mechanism of action differs from that of cisplatin.

In vivo antitumour activity in human ovarian A2780, 2780AD and A2780cis xenografts for the complex [(η⁶-bip)Ru(en)Cl]⁺ showed that it produced a significant growth delay in A2780 cells, maintained the growth inhibitory activity in the A2780cis xenograft, but was inactive against A2780AD cells.[17] Thus, encouragingly, the patterns established *in vitro* were mirrored to a large degree *in vivo*.

3.2.5.2. *Downstream Effects*

The downstream mechanism of action of [(η⁶-bip)Ru(en)Cl]⁺ in (un)derivatised HCT116 colon cells has been investigated[45] since prediction or enhancement of tumoricidal effect, based on knowledge of cellular response determinants could improve the clinical utility of these types of complexes. HCT116-p53 null cells were two-fold more resistant than HCT116-WT (WT = wild-type) cells to short-time [(η⁶-bip)Ru(en)Cl]⁺ mediated growth suppression (IC_{50} HCT116-WT 8 μM, IC_{50} HCT116-p53null 16 μM). In contrast, longer term clonogenic assays (where colonies were counted on days 16–18) showed no statistically significant differences in [(η⁶-bip)Ru(en)Cl]⁺-induced loss of clonogenicity between both cell lines.[45]

Immunoblots demonstrated that [(η⁶-bip)Ru(en)Cl]⁺ induced accumulation of p53 and p21/WAF1 at 24 h and 48 h in a concentration-dependent manner and Bax at 48 h. Cell cycle analysis demonstrated a mixed G1 and G2 growth arrest by 48 h in HCT116-WT and HCT116-Bax-null cells, but this growth arrest was not observed in the p53-null or in the p21/WAF1 null cells, indicating that the mixed G1/G2 growth arrest was p53- and p21/WAF1-dependent, but independent of Bax.[45] Annexin-v apoptosis assays demonstrated the induction of increased apoptosis within 24 h of treatment in WT and p21/WAF1 null cells.[45] On the other hand, no significant increase in apoptosis was induced in p53-null or bax-null cells. Whilst a sub-G1 peak appeared in the WT and p21/WAF1 null cells (consistent with the onset of internucleosomal DNA cleavage in late apoptosis), no sub-G1 peak appeared in the p53-null or bax-null cells. Thus p53 and Bax are required to mediate [(η⁶-bip)Ru(en)Cl]⁺-induced apoptosis in human colon cancer cells within the first 48 h of treatment.

3.2.5.3. *Topiosomerase Activity*

Neither [(η⁶-*p*-cym)Ru(en)Cl]⁺ nor [(η⁶-bip)Ru(en)Cl]⁺ inhibited topiosomerase I (Topo I) or topiosomerase II (Topo II) enzyme activity, as characterized by the conversion of pBR322 plasmid DNA from the supercoiled conformation to the fully relaxed conformation, up to 50 μM concentration.[16] It is thus unlikely that inhibition of these enzymes is responsible for their anticancer activity. Interestingly inhibition of Topo II enzyme was thought to be critical for antiproliferative activity of the bifunctional Ru^{II} arene [(η⁶-bz)Ru(DMSO)Cl_2],[46] which may act as a tri-functional ion in solution due to hydrolysis of the Ru–Cl and Ru–DMSO bonds. Thus monofunctional complexes *e.g.* [(η⁶-*p*-cym)Ru(en)Cl]⁺ may act in a different manner.

3.2.5.4. *Broad Activity Spectrum*

The *in vitro* activity of [(η⁶-bip)Ru(en)Cl]⁺ has been determined in a 14 cell panel line and the mean IC_{50} value was 3 μM confirming that it has a broad spectrum of activity.[47] Particular sensitivity (*ca.* tenfold lower than mean IC_{50}) was noted in the breast cancer line 401NL and a non-small lung cancer cell line LXFL 529L. In addition the IC_{50} values against the human lung A549 and H520 cell lines were determined for [(η⁶-bip)Ru(en)Cl]⁺ (3 μM, 3.5 μM) and [(η⁶-tha)Ru(en)Cl]⁺ (0.53 μM, 0.5 μM) confirming the increase in potency as the arene ring size is increased.[47] The *in vivo* anticancer activity of these two complexes in A549 xenografts demonstrated a significant growth delay although studies showed that, especially for [(η⁶-tha)Ru(en)Cl]⁺, heptatoxicity may be a problem.[47]

3.2.6. *Reactions with Biologically Important Molecules*

3.2.6.1. *L-Cysteine*

The reaction of [(η⁶-bip)Ru(en)Cl]⁺ and L-cysteine (1 mM: 2 mM) has been followed by HPLC over a 48 h period and six products were identified.[48] After the time period, *ca.* 50% of the complex had still not reacted with L-cysteine. Two intermediates corresponding to the S- and O-bound monosubstituted complexes initially increased in intensity for 12 h but disappeared after 48 h. The final products, however, corresponded to unusual dinuclear ruthenium complexes from which half or all of the chelated ethylenediamine had been displaced to form predominantly [(η⁶-biphenyl)Ru(H_2O)(μS,N-L-cys)Ru(η⁶-biphenyl)(en)]²⁺ but also [(η⁶-biphenyl)Ru(O,N-L-cys-S)(S-L-Cys-N)Ru(η⁶-biphenyl)(H_2O)]. A small amount of oxidized [(η⁶-biphenyl)Ru(O-Cys_2H_2)(en)]²⁺ was also detected. Further studies[48] showed that the course of the reaction of [(η⁶-bip)Ru(en)Cl]⁺ with L-cysteine strongly depends on pH and the molar ratio of reagents. For example, below pH 5 the final dinuclear adducts are detected but above this value, the hydrolyzed product is the predominant species. Furthermore when the mixture is at pH >7, more L-cysteine was oxidized to cystine and so less bound to ruthenium.

3.2.6.2. *L-Methionine*

The reaction of [(η⁶-bip)Ru(en)Cl]⁺ with L-methionine was analyzed in a similar manner, although only one product cor-

responding to the S-bound [(η⁶-biphenyl)Ru(S-L-MetH)(en)]²⁺ was detected.[48] This reaction was slow with a $t_{1/2}$ *ca.* 2.3 h. After 48 h, only approximately 27% of the ruthenium complex had reacted with L-methionine.

3.2.6.3. *Histidine*

Reactions with [(η⁶-bip)Ru(en)Cl]⁺ and histidine have also been investigated, including the 1.6 Å resolution crystal structure of the half-sandwich ruthenium [(η⁶-*p*-cymene)Ru(lysozyme)Cl_2], which showed selective ruthenation of Nε the imidazole ring of His15.[49] The reaction of [(η⁶-bip)Ru(en)Cl]⁺ and histidine (2 mM:4 mM, 310 K) reached equilibrium after approximately 24 h, but only approximately 22% of the complex had reacted, with hydrolysis (59%) being the preferred reaction.[50] The products formed were confirmed by ESI-MS and NMR as [(η⁶-biphenyl)Ru(en)(Nε-L-His]²⁺ and [(η⁶-biphenyl)Ru(en) Nδ-L-His]²⁺.

Thus overall the reactivity of [(η⁶-bip)Ru(en)Cl]⁺ towards amino acids follows the order L-Cys (K_{Cys} = 0.6 mM^{-1}) > L-Met (K_{Met} = 0.34 mM^{-1}) > L-His (K_{His} = 0.14 mM^{-1}).

3.2.6.4. *Cytochrome-c*

The reactions of [(η⁶-bip)Ru(en)Cl]⁺ with cytochrome-c, an electron transfer haem protein which contains two solvent-accessible histidine residues, His26 and His33, with [(η⁶-bip)Ru(en-^{15}N)Cl]⁺ gave two mono-ruthenated protein adducts that were thought to contain ruthenium bound to the N-terminus or to a carboxylate group.[50] The reaction reached equilibrium after approximately 2 h, by which time approximately 50% of cytochrome c had been ruthenated.

Competitive reactions of [(η⁶-bip)Ru(en)Cl]⁺ (0.2 mM) with the 14-mer oligonucleotide d(TATGTACCATGTAT) (0.1 mM) in the absence and presence of cytochrome-c (0.1 mM) or histidine (0.4 mM) showed that the presence of either of these two reagents had little effect on the reaction of [(η⁶-bip)Ru(en)Cl]⁺ with the oligonucleotide, and that in all cases *ca.* 90% of the oligonucleotide had reacted to give rise to mono-ruthenated and diruthenated adducts.[50] This suggests that in the cells, DNA (or RNA) may be the favoured reaction site for this class of organometallic ruthenium(II) ethylenediamine anticancer complex.

3.2.6.5. *Glutathione*

Similar competitive reactions between [(η⁶-bip)Ru(en)Cl]⁺ glutathione and cGMP have been studied under physiologically-relevant conditions ([(η⁶-bip)Ru(en)Cl]⁺ 20 μM, 250 mol equiv. GSH, 25 mol equiv. cGMP, 10 mM phosphate buffer, 22 mM NaCl, 310 K).[51] Initially, reactions with GSH alone under physiologically relevant conditions yielded two main products: the monoruthenated GSH adduct [(η⁶-bip)Ru(en)SG]⁺ and, surprisingly, the corresponding oxidized sulfenato complex [(η⁶-bip)Ru(en)SOG]⁺, the intensity of which increased from about 11% of the total ruthenium after 12 h to 19% after 48 h. The presence of this adduct was confirmed by ESI-MS (m/z 638.1, calcd m/z 638.1 for {(η⁶-bip)Ru(en)SOG + H}⁺ and infrared spectroscopy (band at 1018 cm^{-1} assignable to S=O stretching). The oxygen atom is thought to originate from molecular O_2 since reactions performed under O_2 yielded a higher proportion of sulfenato adduct compared to those carried out under Ar. In the competitive reactions (reactions purged with N_2 to minimize oxygen content) after 30 h of reaction three products formed corresponding to [(η⁶-bip)Ru(en)SG]⁺, [(η⁶-bip)Ru(en)SOG]²⁺ and [(η⁶-bip)Ru(en)cGMP-N(7)]⁺ in ratios of 36:30:26. A similar reaction sampled in air yielded [(η⁶-bip)Ru(en)cGMP-N(7)]²⁺ as the major product after 72 h. Thus the thiolato adduct appeared to be oxygenated by O_2 to the sulfenato complex and this sulfenato ligand appeared to be readily displaced by cGMP to give the cGMP adduct as the dominant product of the reaction. This implies that whilst the sulfenato ligand is readily substituted by cGMP, the thiolato ligand is not. This observed oxidation of the coordinated glutathione to the sulfenate appears to be a facile route for displacement of S-bound glutathione, since a Ru–S(sulfenate) bond would be expected to be weaker than a Ru–S(thiolate) bond.[52]

3.2.6.6. *Nicotinamide Adenine Dinucleotide NAD⁺*

Ru^{II} arenes can catalyze the regioselective reduction of nicotinamide adenine dinucleotide NAD⁺ by formate to form 1,4-NADH in aqueous solutions.[53] NAD⁺ is an important co-enzyme found in cells and is involved in the transfer of electrons. A plausible mechanism of reduction is thought to involve formation of the Ru^{II} hydride complex [(η⁶-arene)Ru(en)H]⁺ with formate as the hydride donor and then transfer of hydride to NAD⁺ regenerates [(η⁶-arene)Ru(en)H_2O]²⁺. For example, the complex [(η⁶-hmb)Ru(en)Cl]⁺ can reduce NAD⁺ with a maximum TOF of 1.46 h^{-1} and a K_m of 58 mM. Other organometallic complexes such as [Cp*Rh(bipy)(H_2O]²⁺ have higher TOF (77.5 h^{-1}) and higher K_m values (140 mM).[54] The rate was found to be independent of NAD⁺ and the rate-determining step is hydride transfer from formate to ruthenium. Cell growth of A549 cancer cells was unaffected by formate concentrations up to 2.5 mM. The cytotoxicity of the ruthenium complexes appeared to be unaffected by the co-administration of formate. For the proposed *in vivo* catalysis is to succeed, more active catalysts are probably required.

3.3. *Other Chelating Nitrogen Ligands*

3.3.1. *Aliphatic σ-Donor Diamines*

Structure–activity relationships have revealed that replacement of the ethylenediamine chelating ligand with the N,N-dimethyl-ethylenediamine derivative result in a dramatic loss of cytotoxicity (*e.g.* [(η⁶-*p*-cym)Ru(en)Cl]⁺, IC_{50} = 5 μM, [(η⁶-bip)Ru(N,N-dimethyl-en)Cl]⁺, IC_{50} >100 μM).[10] This may be related to the inability of the complex to form strong stereospecific C(6)O..HN hydrogen bonds with guanine bases, an interaction thought to stabilize and enhance the recognition (*vide supra*). The reaction of this [(η⁶-bip)Ru(N,N-dimethyl-en)Cl]⁺ with 9-Et-G was followed by ^{1}H NMR spectroscopy and no binding was observed. Thus steric effects of the methyl groups (preventing approach by 9-Et-G) as well as a lack of potential hydrogen bonding towards DNA bases may contribute to the loss of activity.[10]

Changing the chelate from ethylenediamine to propylene-diamine (prop) (*i.e.* changing the chelate ring size from five to six members) ring did not affect the cancer cell cytotoxicity [(η⁶-*p*-cym)Ru(en)Cl]⁺ IC_{50} = 10 μM, [(η⁶-*p*-cym)Ru(prop)Cl]⁺ IC_{50} = 10 μM.[10] However, addition of a polar OH substituent on the propylenediamine backbone decreased the activity. Further derivatisation of en ligands has varying effects on the cytotoxicity; mono-methylation of en does not affect IC_{50} values, whereas dialkylation *via* ring formation lowers the activity three-fold and cyclisation of en to give homopiperazine results in an inactive compound (IC_{50} >50 μM).[10]

3.3.2. *Aromatic σ-Donor Diamines*

Good cytotoxicity is retained when ethylenediamine ligands are replaced with chelating 1,2-diaminobenzene ligands.[10] A slightly different trend was observed with cytotoxicity on changing the arene in 1,2-diaminobenzene complexes; the IC_{50} value increased in the order biphenyl (5 μM), dha (7 μM) < *p*-cymene (11 μM), tetralin (13 μM) <tha (23 μM). Interestingly these complexes are able to overcome cross-resistance to the 2780AD cell line, in contrast to en complexes, see Fig. 4.[17]

3.3.3. *Dinuclear Chelating Ligands*

The dinuclear complex [((η⁶-bip)RuCl(en))$_2$-$(CH_2)_6$Cl]²⁺, in which two Ru^{II} arene centres are linked with a flexible chain contains four stereogenic centres (Ru, N, N, Ru) which gives rise to ten potential configurations.[55] In aqueous solution the complex exists as a diastereomeric mixture of AA/AB/BB (A = (R*RuR*N), B = (S*RuR*N)) as 67.7:24.0:8.3 and hydrolysis of Ru–Cl appears to have a negligible influence on the configurational abundance ratios. The A (R*RuR*N) configuration

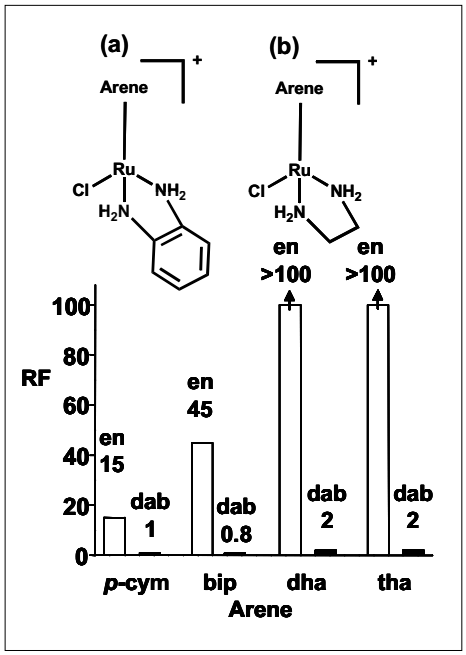

Fig. 4. Effect of changing the chelating ligand from ethylenediamine (en) to 1,2-diaminobenzene on the cytotoxicity towards A2780 and 2780AD cells for ruthenium complexes $[(\eta^6\text{-arene})Ru(N,N)Cl]^+$ containing the arenes *p*-cym, bip, dha and tha. The resistance factor (RF) = IC_{50} (2780AD)/$IC_{50}(2780^{AP})$.

is thermodynamically preferred. Analysis of the complex after reaction with 9-EtG (to give $[((\eta^6\text{-bip})Ru(N(7)\text{-}9\text{-}EtG)(en)2\text{-}(CH_2)_6Cl]^{2+}$) showed that the B (S*RuR*N) configuration is highly favoured (*ca.* 95%) due to the A configuration being strongly destabilized by steric interactions between G and the en alkyl substituent whereas the B configuration is stabilized by stereospecific hydrogen bonding between en NH and G O(6). Thus upon reacting with 9-EtG, a facile epimerization at Ru/N centres occurs to allow dynamic switching between these configurations leading to a high selectivity in the formation of G adducts.[55] Substitution of an en NH proton by an alkyl group had little effect on the kinetics of the reactions with CT-DNA indicating that the alkyl substituent does not significantly hinder G binding when epimerization is facile.

DNA-directed RNA synthesis experiments supported that $[((\eta^6\text{-bip})Ru(en)Cl)_2\text{-}(CH_2)_6]^{2+}$coordinates preferentially to G bases of DNA and it was assumed that this binding involves dynamic chiral recognition, with preferential formation of the B configuration and this allows 84% to coordinate to the DNA.[55] Interestingly this dinuclear complex inhibits RNA synthesis more effectively than the mononuclear complex $[(\eta^6\text{-bip})Ru(en)Cl]^+$. Binding to CT-DNA induced a positive CD band centred at 370–380 nm suggesting intercalation of the extended phenyl ring into DNA (as observed previously for the mononuclear complexes (*vide supra*)). The dinuclear complex was able to unwind pS/P73KB DNA with an angle of 31° which was over double that for $[(\eta^6\text{-bip})Ru(en)Cl]^+$ (14°) suggesting that cross-linking of DNA and perturbation of DNA structure by the two pendant phenyl rings is important. Evidence of cross-linking came from the observations

i) that the efficiency of the interstrand cross-linking on a 213-bp EcoRI fragment of pSP73 randomly modified by the dinuclear complex was similar to that of the known DNA cross-linker cisplatin and
ii) a site-specifically ruthenated 20-mer formed a 1,3-GG interstrand cross-link (20% frequency) and 1,2-GG and 1,2-GTG cross-links were also detected.[55]

3.3.4. *Chelating σ-Donor π-Acceptor Ligands*

3.3.4.1. *Bipyridine and Bipyridine Derivative Ligands*

Ruthenium(II) chloride arenes with indan as the arene containing 2,2'-bipyridine, 4,4'-bipyridine derivatives or phenanthroline (phen) as the chelating nitrogen ligand are all inactive towards the A2780 cancer cell line (IC_{50} >50 μM).[10] The reason for inactivity may be the absence of suitable NH donors to form favourable hydrogen bond interactions with guanine and the increased bulk around the ruthenium, perhaps preventing binding to G, as observed for the $[(\eta^6\text{-bip})Ru(N,N\text{-dimethyl-en})Cl]^+$ complexes. The rate of hydrolysis may also be slower and this may reduce cytotoxicity; slow hydrolysis has been reported for $[(\eta^6\text{-arene})Ru(phen)Cl]^+$complexes and isolation of aqua adducts was assisted by the use of Ag-salts,[56] and the incorporation of bipy into $[(\eta^6\text{-bz})Ru(en)OH_2]^+$ slows down the rate of water exchange by a factor of 174 (*c.f.* $[(\eta^6\text{-bz})Ru(OH_2)_3]^{2+}$).[13]

3.3.4.2. *Phenylazopyridine Ligands*

Ruthenium(II) arene chloride complexes containing chelating 2-phenylazopyridine (azpy) ligands have been synthesized and evaluated for cytotoxicity against the A2780 human ovarian and A549 human lung cancer cell lines and moderate cytotoxicity was observed for some derivatives (IC_{50} values 18–88 μM).[57] These phenyazopyridine ligands are superior in terms of π-acceptor ability compared to bipyridine.[58] The complexes undergo a mixture of slow hydrolysis and arene loss ($t_{1/2}$ 9–21 h) at physiologically relevant concentrations (100 μM, 310 K). Arene loss is rarely observed for Ru^{II} arenes, since the ruthenium–arene bonds are usually considered to be inert to hydrolysis. Literature reports of arene loss for $[(\eta^6\text{-bz})Ru(bipy)Cl]^+$ exist, but much stronger reaction conditions are required.[59] Comparison of the ruthenium–arene centroid distances in the X-ray crystal structures of $[(\eta^6\text{-bip})Ru(en)Cl]^+$ and $[(\eta^6\text{-bip})Ru(azpy)Cl]^+$ reveals that the arene is not as strongly bound to the ruthenium in the azpy complex (ruthenium–arene centroid distance 1.662(3) Å *vs* 1.707(2) Å) and this suggested that the arene and the chelating azo ligand are effectively acting as competitive π-acceptors for Ru $4d^6$ electrons resulting in a more labile arene.

The inertness towards hydrolysis may indicate that these complexes have a novel mechanism of action; surprisingly replacement of the chloride ligand by iodide to give $[(\eta^6\text{-arene})Ru(azpy\text{-}NMe_2)I]^+$ or $[(\eta^6\text{-arene})Ru(azpy\text{-}OH)I]^+$ results in complexes that are inert to both hydrolysis and arene loss over 24 h (310 K) and yet display good cytotoxicity in both the A2780 and A549 cancer cell lines.[60] Work into the mechanism of action of these complexes is currently being investigated.

The stability of Ru(II)–arene bonds towards hydrolysis can be increased by changing the chelating phenylazopyridine ligand to a phenylazopyrazole derivative.[60] The pyrazole substituent renders the whole ligand a less efficient π-acceptor since pyrazole is formally classed as a five-membered π-excessive/π-neutral heterocycle, *c.f* pyridine (six-membered π–electron deficient). Rates for hydrolysis (determined by UV-Vis spectroscopy 50 μM, 310 K, pH 2.25) are 2–2.7 h (arene = bz, bip, tetralin, *p*-cym). In the biphenyl case slow arene loss is also observed.[57,61] These complexes were found to be more cytotoxic towards A2780 ovarian and A549 lung cancer cell lines than their phenylazopyridine analogues.[57] Interestingly the pK_a of the coordinated water in $[(\eta^6\text{-}p\text{-cym})Ru(azpy\text{-}NMe_2)(OH_2)]^{2+}$ is 4.60 so at physiological pH the complex exists almost exclusively in the hydroxo form. Reaction of $[(\eta^6\text{-}p\text{-cym})Ru(azpy\text{-}NMe_2)(OH_2)]^{2+}$ with one mol equiv of 9EtG at 310 K over 24 h gave *ca.* 26% binding and equillibrium was reached after *ca*. 3 h.

4. Ruthenium(II)–Arene Complexes Containing Chelating σ-Donor π-Donor Oxygen Ligands

4.1. *Acetylacetonate Ligands*

Changing the chelating ligand from a dinitrogen σ-donor such as ethylenediamine to a σ-donor π-donor oxygen chelating ligand dramatically changes the reactivity of the ruthenium arene with respect to hydrolysis, cytotoxicity and DNA base specificity. The chemistry of ruthenium(II) arenes containing the anionic O,O-chelating ligand acetylacetonate (acac) has been reported.[62] Complexes such as $[(\eta^6\text{-}p\text{-cym})Ru(acac)Cl]$ hydrolyze rapidly in water and equilibrium is reached in less than 5 min (298 K). The pK_a of the coordinated water molecule

in the aqua adduct [(η⁶-*p*-cym)Ru(acac)OH_2]⁺ is 9.41, which means that at physiological pH (pH 7.4) the complex exists almost exclusively in the reactive aqua form. The increased pK_a value is due to the acac ligands being strong σ-donor and π-donors towards the Ru^{II} centre, increasing the electron density and hence decreasing the acidity of the ruthenium centre.[63] In the reaction of [(η⁶-*p*-cym)Ru(acac)Cl] with guanosine (2 mM 1:1) at equilibrium *ca.* 80% of the guanosine is bound. A similar result is obtained for adenosine (*ca.* 80% is bound ratio of adoN(7):adoN(1) 4:1). This selectivity is in stark contrast to the corresponding ethylenediamine analogues for which negligible binding to adenosine is observed (*vide supra*). In competitive reactions with adenosine and guanosine at equimolar ratios, the ratios of Ru^{II} bound to guanosine/adenosine is *ca.* 4:5 suggesting a slight preference for adenosine over guanosine. The formed adducts are, however, kinetically labile and nucleoside exchange reactions within the system are facile. The complex [(η⁶-*p*-cym)Ru(acac)Cl] does not form adducts with cytidine and thymidine over the pH range of 2.4–10.4 which, again, is in contrast to the en analogues. This lack of binding may be due to unfavourable steric and electronic interactions of the nucleobase carbonyl groups with the acac ligands.

The complexes [(η⁶-arene)Ru(acac)Cl] (arene = pcym, bip, bz, indan and dha) exhibit moderate activity towards A2780 ovarian cancer cells (IC_{50} 19–70 μM).[10] In general the acac complexes hydrolyze rapidly and have poor aqueous solubility. The reason for the reduced cytotoxicity compared to the en analogues may in part be due to the protonation and irreversible displacement of the chelated acac derivative under some conditions; the aqueous solution chemistry of the osmium analogue [(η⁶-*p*-cym)Os(acac)Cl] was studied using conditions mimicking the cytotoxicity testing[64] and this revealed that only one species was present, assignable to the hydroxo-bridged dimer [(η⁶-*p*-cym)Os(μ$_2$-OD)$_3$Os(η⁶-*p*-cym)]⁺, *i.e.* acac is readily lost from the complex. This complex is inactive towards the A2780 cancer cell line (IC_{50} > 50 μM).

4.2. *Maltolato (mal) Ligands*

Both [(η⁶-*p*-cym)Ru(mal)Cl] and the corresponding osmium complex [(η⁶-*p*-cym)Os(mal)Cl] complexes are non-toxic towards A549 human lung and A549 human ovarian cells.[65] The inactivity of these complexes is due to hydroxo-bridged dimer formation, which persists at low metal concentrations, see Fig. 5. This feature is also observed in the acac derivatives [(η⁶-*p*-cym)Ru(acac)Cl] and [(η⁶-*p*-cym)Os(acac)Cl]. The most stable complex towards dimer formation is [(η⁶-*p*-cym)Ru(mal)Cl] and the least stable [(η⁶-*p*-cym)Os(acac)Cl].

Although introduction of the five-membered mal chelate ring provides stabilization towards hydroxo-bridged dimer formation compared with the six-membered acac ring, the dominant species at biologically relevant conditions are still the hydroxo-bridged dimers. A possible mechanism for dimer formation of [(η⁶-*p*-cym)Ru(mal)Cl] and [(η⁶-*p*-cym)Os(mal)Cl] may involve protonation of one of the maltolate oxygen atoms followed by ring opening and eventual loss of maltolate.[65]

Both [(η⁶-*p*-cym)Ru(mal)Cl] and [(η⁶-*p*-cym)Os(mal)Cl] react rapidly with N(7)-G (guanosine and 9EtG) and N(1) and N(7) of A (adenosine). Isolation of [(η⁶-*p*-cym)Os(mal)(EtG)]⁺ and subsequent stability studies (concentrations 20 μM–2 mM, after 24 h at 310 K) revealed that at higher concentrations the Os-N(7)-9EtG complex persisted (*ca.* 69% of [(η⁶-*p*-cym)Os(mal)(EtG)]⁺ at 2 mM), but that as the concentration of osmium decreased, the hydroxo-bridged dimer increased in intensity (0% of [(η⁶-*p*-cym)Os(mal)(EtG)]⁺ at 20 μM).[65] *In situ* preparation of [(η⁶-*p*-cym)Ru(mal)(EtG)]⁺ and [(η⁶-*p*-cym)Os(mal)(EtG)]⁺ from the chloride analogues and equimolar 9etG in aqueous solution allowed the formation constants to be determined (log K = 4.41 (Os), 3.87 (Ru)).[65] Whilst Os binds more strongly than Ru, the binding is only moderate in strength, and the formation of hydroxo-bridged dimers provides a driving force for the dissociation of nucleobase adducts since the dimers appear to be the thermodynamically stable products from the dissociation of nucleobases.

$Ruthenium^{II}$ arene complexes containing the monoanionic O,O chelating ligands acetato and tropolonato have been synthesised and their interactions with nucleobases studied.[66]

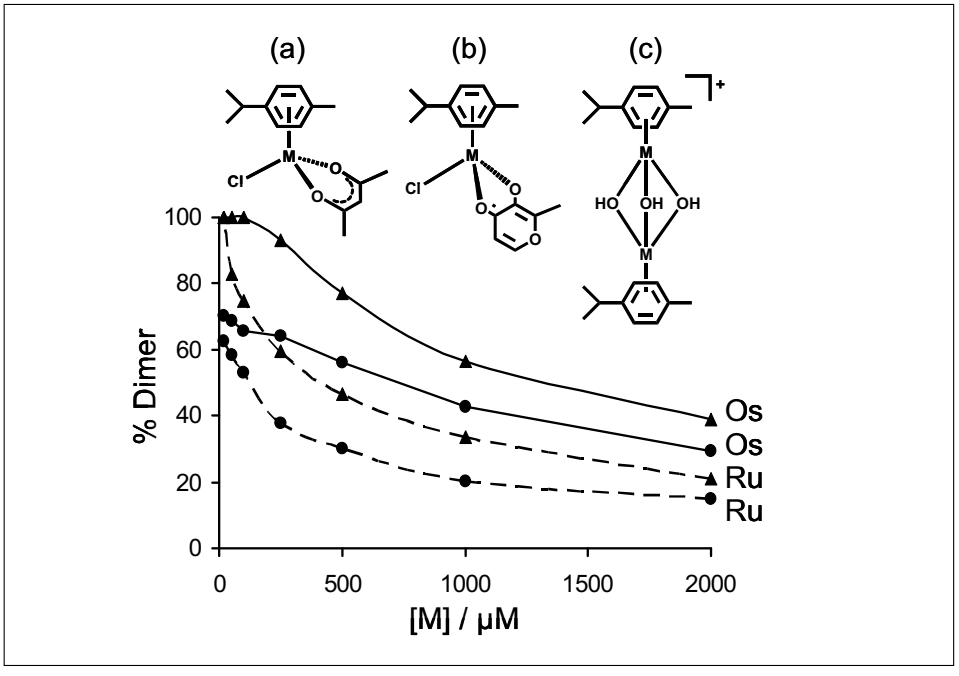

Fig. 5. Dimer formation in [(η⁶-*p*-cym)M(acac)(Cl)] and [(η⁶-*p*-cym)M(mal)(Cl)] systems (M = Ru, Os). Upper: the molecular structures of (a) [(η⁶-*p*-cym)M(acac)(Cl)], (b) [(η⁶-*p*-cym)M(mal)(Cl)] and the triply hydroxo-bridged dimer (c) [(η⁶-*p*-cym)M(OH)$_3$M(η⁶-*p*-cym)]⁺ Lower: Dependence on the formation of hydroxo-bridged dimers on total metal concentrations of the ruthenium and osmium-mal and acac complexes in D_2O after incubation at 310 K for 24 h. - - - - M = Ru, ——— M = Os, ▲ = acac, ● = mal.

5. Ruthenium(II) Arene Complexes Containing Mixed Chelating Nitrogen-Oxygen Ligands

Several ruthenium arene complexes of the type [(η⁶-*p*-cymene)Ru(N,O)Cl] containing N,O chelating ligands such as gylcine, L-alanine, D-alanine, β-alanine, L-phenylalanine, D-phenylalanine and 8-hydroxyquinoline have all been found to be inactive towards the A2780 human ovarian cancer cell line (IC_{50} >100 μM).[10]

The pK_a of the coordinated water in [(η⁶-*p*-cymene)Ru(glycine-N,O)H_2O]⁺ is 8.25, hence at physiological pH the complex will exist in the more reactive aqua form (*c.f.* the inactive hydroxo form). The hydrolysis of [(η⁶-*p*-cymene)Ru(glycine-N,O)Cl], studied by ^1H NMR, was very fast (equilibrium reached in <5 min) and contained two sets of peaks indicating that at equilibrium, aqua and chlorido adducts exist in a 3.7:1 ratio.[10] The addition of 500 mM NaCl was not sufficient to suppress

the hydrolysis fully, in contrast to the corresponding en-type complexes. The higher stability of the aqua complex may account for the lack of observed cytotoxicity since this highly reactive aqua complex may be deactivated by reaction with biomolecules before it reaches its target site. Such a phenomenon is observed for Pd^{II} analogues of active Pt^{II} complexes which are inactive on account of their higher reactivity.[67]

Sheldrick and Heeb have previously reported that reaction of [(η^6-C_6H_6)Ru(L-alanine)9EtG] with 9EtG afforded the complex [(η^6-C_6H_6)Ru(L-alanine)9EtG]Cl.[68] In this report it was mentioned that [(η^6-C_6H_6)Ru(L-proline)Cl] showed significant activity towards P388 leukaemia, but no data on this were published.

Recently we have reported that osmium arene analogues containing anionic N,O chelating ligands picolinate (pico) or 8-hydroxyquinolate (oxine) ([(η^6-bip)Ru(pico)Cl], [(η^6-*p*-cym)Ru(pico)Cl] and [(η^6-*p*-cym)Ru(oxine)Cl]) are cytotoxic towards both the A549 (IC_{50} 8–60 μM) and A2780 (IC_{50} 4–15 μM).[69] Interestingly the corresponding ruthenium complex [(η^6-*p*-cym)Ru(oxine)Cl] is inactive,[10] indicating that not only the choice of ligand but also the choice of metal can influence the cytotoxicity. Os^{II} complexes are generally believed to be more inert than their Ru^{II} counterparts.[70] Os^{II} arenes containing ethylenediamine chelating ligands (e.g. the complex ([(η^6-bip)Os(en)Cl]$^+$ were found to hydrolyze slowly ($t_{1/2}$ *ca.* 6.3 h, 298 K)[64] and those containing acac-type ligands hydrolyzed rapidly but formed inert hydroxo-bridged dimers under physiologically relevant conditions (*vide supra*) hence both sets of complexes were not cytotoxic towards A549 cancer cells. The N,O chelates display aqueous solution chemistry intermediate to N,N and O,O complexes with half lives for hydrolysis of [(η^6-bip)Os(pico)Cl] and [(η^6-*p*-cym)Os(pico)Cl] of 0.52 h and 0.20 h (298 K).[69] Interactions of [(η^6-*p*-cym)Os(pico)Cl] with nucleotides showed binding to both G and A , but with a strong preference for G, and little or no reaction with C or T. Under physiologically relevant concentrations (μM) >40% of the osmium is bound to purine nucleobases and there is an increased kinetic stability of the 9-EtG adduct compared with 9-EtA and such kinetic stability may make G adducts less susceptible to repair compared to A adducts.

6. Conclusions

The chemistry of ruthenium(II)–arene complexes (as well as the heavier osmium(II)–arene analogues) is diverse and the choice of chelating ligand (YZ) and 'leaving group' (X) can influence the reactivity and hence the cytotoxicity of this class of complexes. We have shown that complexes such as [(η^6-arene)Ru(en)Cl]$^+$ hydrolyse quickly in aqueous solution. The extent of hydrolysis is suppressed in high [Cl^-] media as found extracellularly, and the aqua adducts have pK_a values which allow the complex to exist as its 'active' OH_2 form at physiological pH and interact with DNA bases. Subtle changes, *e.g.* changing the arene from *p*-cym to tha can influence the cytotoxicity since these extended arenes allow the complex to interact into DNA, and changes to the X group can render the complex inert to aquation but some can still retain cytotoxicity. Some en analogues have also been shown to exhibit *in vivo* anticancer activity and are thought to act in a manner distinct from cisplatin.

Other N,N chelating systems have been studied, and interestingly, when the ligand is 1,2-diaminobenzene, no cross resistance is observed in the 2780AD cell line, whereas this was not the case for the en analogues.

Changing the ligand to O,O chelating ligands, for example ([(η^6-arene)Ru(acac)Cl] increases the hydrolysis rate and the pK_a of the coordinated water and can change the base selectivity on account of steric interactions, and whilst some derivatives exhibit anticancer activity, deactivation *via* the hydroxo-bridged dimer [(η^6-arene)Ru(μ_2-OH)$_3$Ru(η^6-arene)]$^+$ is found to occur, and this was also observed for osmium analogues. N,O chelating ligands have also been investigated, and interestingly the Os complexes [(η^6-bip)Os(pico)Cl], [(η^6-*p*-cym)Os(pico)Cl] and [(η^6-*p*-cym)Os(oxine)Cl] have been found to be active towards both A2780 and A549 cancer cell lines, whilst the ruthenium analogue[(η^6-*p*-cym)Ru(oxine)Cl] was found to be inactive. This illustrates that the choice of metal as well as the choice of arene, chelating ligand and leaving group are all important in the design of this class of organometallic anticancer agents.

Received: July 30, 2007

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