Structure−Activity Relationships for Cytotoxic Ruthenium(II) Arene Complexes Containing N,N-, N,O-, and O,O-Chelating Ligands

Abraha Habtemariam,[†] Michael Melchart,[†] Rafael Fernández,[†] Simon Parsons,[†] Iain D. H. Oswald,[†] Andrew Parkin,[†] Francesca P. A. Fabbiani,[†] James E. Davidson,[†] Alice Dawson,[†] Rhona E. Aird,[‡] Duncan I. Jodrell,[‡] and Peter J. Sadler*[,†]

School of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, UK, and University of Edinburgh Centre for Cancer Research, Western General Hospital, Crewe Road South, Edinburgh EH4 2XR, UK

Received May 18, 2006

We report structure−activity relationships for organometallic Ru^{II} complexes of the type [(η^6-arene)Ru(XY)Cl]Z, where XY is an N,N- (diamine), N,O- (e.g., amino acidate), or O,O- (e.g., β-diketonate) chelating ligand, the arene ranges from benzene derivatives to fused polycyclic hydrocarbons, and Z is usually PF_6. The X-ray structures of 13 complexes are reported. All have the characteristic "piano-stool" geometry. The complexes most active toward A2780 human ovarian cancer cells contained XY = ethylenediamine (en) and extended polycyclic arenes. Complexes with polar substituents on the arene or XY = bipyridyl derivatives exhibited reduced activity. The activity of the O,O-chelated complexes depended strongly on the substituents and on the arene. For arene = *p*-cymene, XY = amino acidate complexes were inactive. Complexes were not cross-resistant with cisplatin, and cross-resistance to Adriamycin was circumvented by replacing XY = en with 1,2-phenylenediamine. Some complexes were also active against colon, pancreatic, and lung cancer cells.

Introduction

There is much current interest in the design of ruthenium complexes as anticancer agents.[1,2] Early work by Clarke[3] showed that octahedral cis-dichlorido Ru^{III} complexes such as [$RuCl_3(NH_3)_3$] are cytotoxic to cancer cells, but poor aqueous solubility prevented their further development. Ruthenium(III) complexes tend to be inert toward ligand substitution and are thought to be activated by in vivo reduction to Ru^{II}.[4] More recently other Ru^{III} and Ru^{II} complexes have been reported to exhibit potentially useful cytotoxicity, including *mer*-[Ru(terpy)Cl_3] (terpy = 2,2′:6′,2″-terpyridine), α-[Ru(azpy)$_2Cl_2$], α-[Ru(azpy)$_2$-O,O′] (azpy = 2-(phenylazo)pyridine, O,O′ = 1,1-cyclobutanedicarboxylate, oxalate, or malonate)[5,6] and also a Ru^{IV} complex [Ru(H_2cdta)Cl_2] (cdta = 1,2-cyclohexanediaminetetraacetate).[7] Most significant is the entry of two octahedral Ru^{III} complexes into clinical trials for cancer treatment.[8] The first, *trans*-[HIm][$Ru^{III}Cl_4$(DMSO)(Im)] (Im = imidazole; NAMI-A), is relatively nontoxic to primary cancer cells but exhibits antimetastatic activity,[9−11] whereas the second, *trans*-[HIn][$Ru^{III}Cl_4(Ind)_2$] (Ind = indazole),[12] appears to be active against primary tumors.

Organometallic Ru^{II} arene complexes (complexes containing metal−carbon bonds) offer much scope for the design of anticancer agents.[13,14] We have shown that complexes of the type [(η^6-arene)Ru^{II}(en)Cl]$^+$, exhibit anticancer activity both in vitro and in vivo, including activity against cisplatin-resistant cancer cells.[15,16] These complexes have pseudo-octahedral "piano-stool" structures with the neutral arene ligand occupying three coordination positions (the "seat") and chelated en and monodentate chloride the other positions (the "legs"). [(η^6-Arene)Ru^{II}(en)Cl]$^+$ complexes are monofunctional: only the Ru−Cl bond is highly reactive. The presence of the coordinated arene stabilizes ruthenium in the +2 oxidation state, and the complexes are not readily oxidized to Ru^{III}. As for (bifunctional) cisplatin, hydrolysis of the metal−chloride bond appears to be important for activation, giving aqua adducts[17] [(η^6-arene)Ru^{II}(en)(H_2O)]$^{2+}$ that can bind to DNA (or proteins), forming monofunctional adducts. Density functional calculations suggest that aquation occurs by means of a concerted ligand exchange mechanism.[18] In aqueous solution, [(η^6-biphenyl)Ru^{II}(en)Cl]$^+$ binds specifically to guanine when in competition with adenine, cytosine, and thymine nucleotides,[15] and a similar base specificity is apparent on reaction with plasmid DNA.[19] Guanine binds strongly through N7 with additional formation of a strong H-bond between C6O and en-NH.[20] Also, if the arene is extended (e.g. as in dihydroanthracene), the possibility of arene intercalation between the DNA bases arises.[21−23] Replacement of neutral ethylenediamine by anionic acetylacetonate (acac) as the chelating ligand increases the rate and extent of hydrolysis, raises the pK_a of the aqua complex, and changes the nucleobase selectivity such that the affinity for adenosine (N7 and N1 binding) is comparable to that for guanosine, and now with little binding to cytidine or thymidine.[24] Bifunctional phosphine Ru^{II} arene complexes such as [(η^6-*p*-cymene)$RuCl_2$(pta)] (pta = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane) have also been reported to be cytotoxic.[2,25]

The only organometallic complex to have entered clinical trials as an anticancer agent is the titanium(IV) complex [Cp_2TiCl_2].[26−28] However, this complex is difficult to formulate, since it is readily hydrolyzed and the negatively charged cyclopentadienyl ligands are readily displaced (via protonation).[29] Recently, benzyl- and heteroaryl-substituted titanocences have shown promising activity and are candidates for clinical trials,[30] as are ferrocenyl derivatives of hydroxy-Tamoxifen.[31]

Here we report structure−activity relationships (SAR) for monofunctional Ru^{II} complexes [(η^6-arene)Ru(chelate)Cl]$^+$, in which the arene is benzene (bz) or a functionalized phenyl ring, including fused ring systems, and the chelate is an N,N-bound (neutral aliphatic or aromatic) diamine, O,O-bound (anionic) acetylacetonate derivative, or N,O-bound amino acidate ligand.

* Corresponding author. Phone: 44-131-650-1000. Fax: 44.131.650.6453. E-mail: P.J.Sadler@ed.ac.uk.
[†] University of Edinburgh.
[‡] Western General Hospital.

10.1021/jm060596m CCC: $33.50
Published on Web 10/14/2006

Scheme 1. Method A[a]

$$RuCl_3.nH_2O \xrightarrow{a} [(\eta^6\text{-arene})RuCl_2]_2$$

[a] Reagents and conditions: (a) dihydroarene, EtOH, reflux.

Scheme 2. Method B[a]

$$[(\eta^4\text{-COD})RuCl_2]_n \xrightarrow{a} [(\eta^6\text{-arene})RuCl_2]_2$$

[a] Reagents and conditions: (a) arene, Zn powder; THF, reflux; CH_3CN, 1 M HCl in ether.

Scheme 3. Method C[a]

$$RuCl_3.nH_2O \xrightarrow{a} [(\eta^6\text{-benzene})RuCl_2]_2$$

$$[(\eta^6\text{-benzene})RuCl_2]_2 \xrightarrow{b} [(\eta^6\text{-benzene})Ru(\text{cyclophane})](BF_4)_2$$

$$[(\eta^6\text{-benzene})Ru(\text{cyclophane})](BF_4)_2 \xrightarrow{c} [(\eta^6\text{-cyclophane})RuCl_2]_2$$

[a] Reagents and conditions: (a) cyclohexadiene, EtOH, reflux; (b) acetone, $AgBF_4$, cyclophane, CF_3CO_2H, reflux; (c) THF, 273 K, red Al, H_2O, hexane, 37% HCl_{aq}.

Scheme 4. Method D[a]

$$[(\eta^4\text{-COD})RuCl_2]_n \xrightarrow{a} [(\eta^4\text{-COD})Ru(acac)_2]_2$$

$$[(\eta^4\text{-COD})Ru(acac)_2]_2 \xrightarrow{b} [(\eta^6\text{-naphthalene})Ru(\eta^4\text{-COD})]$$

$$[(\eta^6\text{-naphthalene})Ru(\eta^4\text{-COD})] \xrightarrow{c\,1 \text{ or } c\,2} [(\eta^6\text{-arene})Ru(\eta^4\text{-COD})]$$

$$[(\eta^6\text{-arene})Ru(\eta^4\text{-COD})] \xrightarrow{d} [(\eta^6\text{-arene})RuCl_2]_2$$

[a] Reagents and conditions: (a) acetylacetone, DMF, Na_2CO_3, 413 K; (b) Na(naphthalide), THF, under Ar, 16 h, Al_2O_3 + 5% H_2O filtration; (c1) arene, CH_3CN, THF; (c2) for **15**, diphenylacetylene in excess, THF; (d) HCl, acetone.

Scheme 5. Method E[a]

$$[(\eta^6\text{-}p\text{-cymene})RuCl_2]_2 \xrightarrow{a} [(\eta^6\text{-hmb})RuCl_2]_2$$

[a] Reagents and conditions: (a) hexamethylbenzene, 453 K.

Scheme 6. Method F (XY = neutral chelating ligand)[a]

$$[(\eta^6\text{-arene})RuCl_2]_2 \xrightarrow{a \text{ or } b} [(\eta^6\text{- arene})Ru(XY)Cl]$$

[a] Reagents and conditions: (a) XY, NH_4PF_6, MeOH or H_2O or MeOH/H_2O; (b) when XY is en, NH_4PF_6, CH_3CN or H_2O or MeOH.

Scheme 7. Method G (XY = anionic chelating ligand)[a]

$$[(\eta^6\text{-arene})RuCl_2]_2 \xrightarrow{a \text{ or } b} [(\eta^6\text{- arene})Ru(XY)Cl]$$

[a] Reagents and conditions: (a) Na/K diketonate, acetone, ambient temperature; (b) Na amino acidate or Na aminoamidate, acetone, ambient temperature.

Chemistry

Synthesis. The synthesis of the complexes is outlined in Schemes 1−7. The synthetic routes for these half-sandwich organometallic Ru^{II} complexes usually involved the use of dimeric Ru^{II} arene complexes of the type $[(\eta^6\text{-arene})RuCl_2]_2$ as starting materials. These Ru^{II} dimers, containing mono- and disubstituted arenes, were generally prepared by reaction of $RuCl_3{\cdot}xH_2O$ with the appropriate 1,3- or 1,4-cyclohexadiene (Scheme 1, method A)[32,33] or with the reduced form of the desired arene readily obtained by Birch reduction (use of alkali metals in liquid ammonia as a source of solvated electrons, in the presence of a proton source, e.g. alcohol).[34−36] However, the Birch reduction was suitable for the preparation of only a limited number of precursor dienes. Ru^{II} dimers containing hexamethylbenzene (hmb) were readily obtained by heating or fusing $[(\eta^6\text{-}p\text{-cymene})RuCl_2]_2$ with a large excess of the neat arene (Scheme 5, method E).[37] Arenes bearing functional groups were prepared[38] via the Ru^0 intermediate $[(\eta^6\text{-naphthalene})(\eta^4\text{-}1,5\text{-cyclooctadiene})Ru]$. Displacement of the labile naphthalene by the appropriate arene in acetonitrile and treatment with hydrochloric acid yielded the corresponding dimer $[(\eta^6\text{-arene})Ru^{II}Cl_2]_2$.[39,40] $[(\eta^6\text{-Naphthalene})(\eta^4\text{-}1,5\text{-cyclooctadiene})Ru]$ was also used as a template for stoichiometric alkyne cyclotrimerization, giving $[(\eta^6\text{-}C_6Ph_6)Ru^{II}Cl_2]_2$ (Scheme 4, method D).[41,42] In addition, several Ru^{II} dimers containing polycyclic arenes were prepared via in situ reduction of $[(\eta^4\text{-}1,5\text{-cyclooctadiene})RuCl_2]_n$ with zinc dust in the presence of the appropriate arene (Scheme 2, method B).[43] However, this method generally gave low yields of 20% or less. The dimer $[(\eta^6\text{-cyclophane})RuCl_2]_2$ was also prepared via a Ru^0 intermediate (Scheme 3, method C).[44] The $[(\eta^6\text{-arene})RuCl_2]_2$ dimers were converted into the half-sandwich Ru^{II} complexes containing the N,N-, N,O-, or O,O-chelating ligands as shown in Schemes 6 and 7.

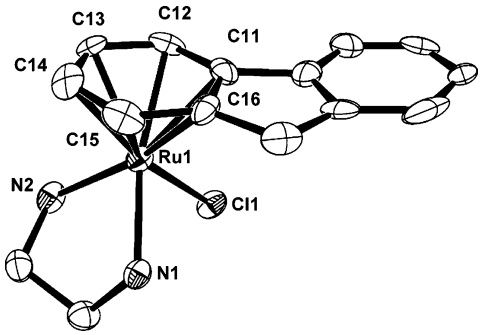


Figure 1. X-ray crystal structure of complex **16**, showing the atom numbering scheme (hydrogens are omitted for clarity). The ellipsoids enclose 50% probability surfaces. The fluorene ligand is disordered over two orientations.

X-ray Crystal Structures. The X-ray crystal structures of complexes $[(\eta^6\text{-bip})Ru^{II}(en)Cl]PF_6$ (**9**)[15] (bip = biphenyl, en = ethylenediamine), $[(\eta^6\text{-hmb})Ru^{II}(en)Cl]PF_6$ (**12**), $[(\eta^6\text{-tha})Ru^{II}(en)Cl]PF_6$ (**17**) (tha = tetrahydroanthracene), $[(\eta^6\text{-dha})Ru^{II}(en)Cl]PF_6$ (**19**)[20] (dha = dihydroanthracene), $[(\eta^6\text{-indan})Ru^{II}(en)Cl]PF_6$ (**21**),[18] and $[(\eta^6\text{-}p\text{-cymene})Ru(H_3CCOCHCOCH_3)Cl]$ (**54**)[24] have been reported previously. Some of the features of the complexes $[(\eta^6\text{-flu})Ru^{II}(en)Cl]PF_6$ (**16**) (flu = fluorene), $[(\eta^6\text{-tetralin})Ru^{II}(en)Cl]PF_6$ (**22**), $[(\eta^6\text{-tha})Ru^{II}(dab)Cl]Cl$ (**36**) (dab = 1,2-diaminobenzene), and $[(\eta^6\text{-}p\text{-cymene})Ru(Me_3CCOCHCOCMe_3)Cl]$ (**60**) are described below. The X-ray crystal structures for $[(\eta^6\text{-indan})Ru(1,3\text{-diamino-2-propanol-}N,N)Cl]PF_6$ (**27**), $[(\eta^6\text{-indan})Ru(\text{homopiperazine})Cl]PF_6$ (**28**), $[(\eta^6\text{-indan})Ru(\text{bipy})Cl]PF_6$ (**38**), $[(\eta^6\text{-bip})Ru(\text{bipy})Cl]PF_6$ (**39**), $[(\eta^6\text{-indan})Ru(\text{phen})Cl]PF_6$ (**43**), $[(\eta^6\text{-cymene})Ru(C_9H_6NO)Cl]PF_6$ (**53**), $[(\eta^6\text{-}p\text{-cymene})Ru(CF_3CCOCHCOCCF_3)Cl]$ (**61**), and $[(\eta^6\text{-}p\text{-cymene})Ru(C_{13}H_9O_2)Cl]$ (**62**) are described in the Supporting Information.

Crystallographic data for $[(\eta^6\text{-flu})Ru^{II}(en)Cl]PF_6$ (**16**) and $[(\eta^6\text{-tetralin})Ru^{II}(en)Cl]PF_6$ (**22**) are listed in Tables S2a and S2b and selected bond lengths and angles in Tables S3a and S3b, respectively (see Supporting Information). The structures of the cations of **16** and **22** are shown in Figures 1 and 2, respectively. Both complexes adopt the familiar half-sandwich three-legged piano-stool geometry with an η^6-arene ring forming the seat and σ-bonded N atoms of ethylenediamine and Cl the legs of the stool. In complex **16**, the fluorene ring is disordered over the two possible orientations. The coordinated phenyl ring in **16** is only slightly hinged above the methylene bridge as was also observed in the sandwich complex $[(\eta^6\text{-flu})_2Ru^{II}](BF_4)_2$.[43] In contrast, the unconstrained phenyl ring in $[(\eta^6\text{-bip})$-

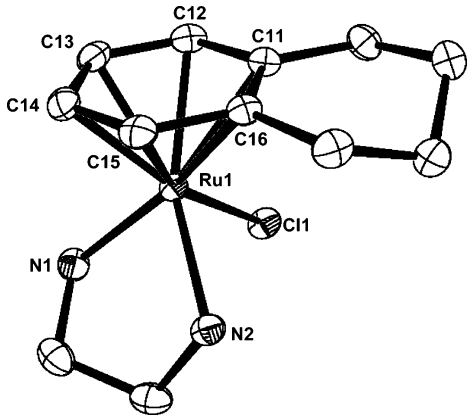


Figure 2. X-ray crystal structure of the cation of complex **22** containing tetralin as the arene ligand, showing the atom numbering scheme at 50% probability thermal ellipsoids (hydrogens are omitted for clarity).

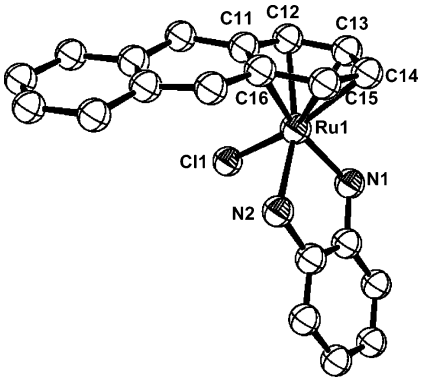


Figure 3. X-ray crystal structure of the cation of complex **36**, containing tetrahydroanthracene as arene and 1,2-diaminobenzene as chelating ligand, showing the atom numbering scheme at 50% probability of thermal ellipsoids (hydrogens are omitted for clarity). One of the two independent cations in the asymmetric unit is shown.

Ru^{II}(en)Cl]PF_6 (**9**) is twisted from coplanarity by ca. 25.0°.[15] The Ru−N bond lengths for **16** and **22** are 2.120, 2.121 and 2.125, 2.137 Å and for Ru−Cl are 2.413 and 2.411 Å, respectively. The Ru−C(arene) bond lengths are in the range of 2.181−2.211 and 2.161−2.229 Å, respectively. The alicyclic ring of tetralin in complex **22** is bent away from the Cl ligand, giving a twist conformation rather than pointing toward Cl to form the more energetically favorable chair conformation. This is in contrast to [(η^6-indan)Ru^{II}(en)Cl]PF_6 (**21**), for which the terminal five-membered ring adopts a chair conformation.[18]

The structure of the cation [(η^6-tha)Ru^{II}(dab)Cl]$^+$ (**36**) is shown in Figure 3. The crystallographic data are listed in Table S2b and selected bond lengths and angles are in Table S3e (Supporting Information). There are two independent molecules in the asymmetric unit. The Ru−N bond lengths are 2.120, 2.131 and 2.120, 2.125 Å and for Ru−Cl are 2.38 and 2.42 Å, the latter showing a significant difference between the two molecules. The Ru−C(arene) bond lengths are in the range of 2.163−2.203 and 2.158−2.222 Å, again showing some difference in the two molecules. As a consequence, the tricyclic ring system appears to be relatively planar in one of the molecules but in the other slightly hinged above the methylene bridges of the middle ring. However, the phenylene ring of dab is planar in both cases. There is a network of H-bonding interactions between NH protons of the chelating ligand, chloride, counterions, and solvent molecules in the unit cell, but no intermolecular arene ring stacking is observed.

Crystals of [(η^6-*p*-cymene)Ru(Me_3CCOCHCOCMe_3)Cl] (**60**) suitable for X-ray diffraction were obtained from diethyl ether/hexane at 253 K. The crystal data are shown in Table S2c and the respective structure in Figure 4. The β-diketonate ligand forms a six-membered chelate ring with the ruthenium center. Selected bond lengths and angles are shown in Table S3c. The Ru−O bond lengths are 2.06 and 2.07 Å, and the Ru−Cl bond length is 2.41 Å. These values are comparable to those of known structures of Ru^{II} arene complexes containing acac-type ligands.[45] The Ru−C(arene) bond lengths are between 2.16 and 2.19 Å, all within the known range for ruthenium arene complexes, but spanning a comparatively narrower range. The Ru−centroid distance is 1.65 Å. The structures of the Ru^{II} *p*-cymene β-diketonato complexes **54**, **60**, and **61** do not differ greatly from each other, despite the different nature of the substituents on the chelate rings. The Ru−O bond lengths in the β-diketonato complexes are comparable, with the exception of compound **61** (see Supporting Information), where the distance is significantly longer ($>3\sigma$), probably due to the presence of the electron-withdrawing fluorine atoms on the backbone of the chelate ring.

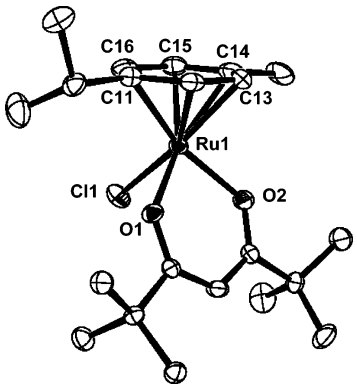


Figure 4. X-ray crystal structure of the β-diketonato complex **60** with *p*-cymene as arene ligand, showing the atom numbering scheme at 50% probability of thermal ellipsoids (hydrogens are omitted for clarity).

Results and Discussion

1. Variations in the Arene Ring. First we studied the effects of variations in the coordinated arene in [(η^6-arene)Ru^{II}(en)Cl]-PF_6 complexes on cytotoxicity toward A2780 human ovarian cancer cells. The arenes included a benzene ring bearing polar (e.g., halide, hydroxyl, ester, amide) or nonpolar (aliphatic and aromatic hydrocarbon) substituents, as well as fused ring systems.

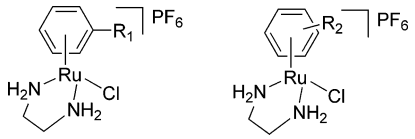
1.1. Substituted Benzenes. In general, introduction of polar substitutents into the coordinated benzene ring lowers the cytotoxicity (Table 1). For monosubstituted benzenes, IC_{50} values follow this order of substitutents: OPh (**1**) < H (benzene) (**8**) < $CONH_2$ (**3**) ≪ COOEt (**7**), COPh (**2**), COOMe (**6**), Br (**5**) ≪ CH_2OH (**4**), and activities range from moderate (18 μM) to inactive (>100 μM).

Complexes **9**−**15** with benzene ligands bearing relatively nonpolar, sterically demanding alkyl, phenyl, or benzyl groups were more potent, with IC_{50} values as low as 3 μM, and follow the order 1,3,5-Ph (**14**), Ph_6 (**15**), Ph (**9**) < Me_6 (**12**), 1-Me_3-3-^{i}Pr (*p*-cymene) (**10**), CMe_2Ph (**11**) ≪ 1,2-Ph (**13**) (Table 1).

The inactivity of the 1,2-diphenylbenzene complex **13** is surprising, since structurally related complexes such as the biphenyl complex **9** and the triphenylbenzene complex **14** exhibit good activity (Table 1).

As seen for the selection of complexes in Table 2, and noted previously,[16] no cross-resistance with cisplatin was observed for these or the other ruthenium arene complexes tested. In

Table 1. Effect of Benzene Ring Substitutions on A2780 IC_{50} Values



complex	R_1	R_2	IC_{50} (μM)	synthetic method
1	OPh		18	D + F
2	COPh		55	D + F
3	$CONH_2$		33	D + F
4	CH_2OH		>100	D + F
5	Br		60	D + F
6[a]	COOMe		56	A + F
7[a]	COOEt		52	A + F
8[a]	H		20	A + F
9[a]	Ph		5	A + F
10[a]		1-Me,3-iPr (p-cymene)	10	A + F
11	CMe_2Ph		11	B + F
12		Me_6	9	E + F
13		1,2-Ph	>100	A + F
14		1,3,5-Ph	3	D + F
15		Ph_6	3	D + F

[a] ref 15.

Table 2. Comparison of the Cytotoxicity of Selected Complexes toward A2780 Cisplatin-Resistant ($A2780^{cis}$ Cells Used in These Studies) and Adriamycin-Resistant ($A2780^{AD}$) Human Ovarian, HT29 Human Colon, PANC-1 Human Pancreatic, and NX002 Human Lung Cancer Cells

complex	IC_{50} (μM) A2780	RF[a] $A2780^{cis}$	RF[b] $A2780^{AD}$	IC_{50} (μM) HT29	IC_{50} (μM) PANC-1	IC_{50} (μM) NX002
cisplatin	0.5[c]	12	8	0.9	0.6	1
12	9	0.9	10	>100	39	52
30	11	1	0.6	50	21	>100
16	2	1	14	16	–	–
11	11	1	25	>100	>100	99
18	1	1	78	8	–	–
15	3	0.7	13	2	–	–
14	3	2	9	11	–	–
17	0.4	1	>100	4	–	–
19	2	0.5	92	11	1	5
33	5	1	0.8	22	–	–
9	5	1	45	12	7	8

[a] RF = $IC_{50}(A2780^{cis})/IC_{50}(A2780)$ [b] RF = $IC_{50}(A2780^{AD})/IC_{50}(A2780)$, also determined for **10**, **35**, **36** as 10, 2, and 2, respectively. [c] Carboplatin, IC_{50} = 6 μM

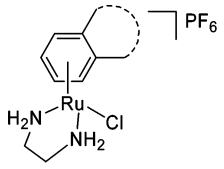
A2780 cells, cisplatin resistance is associated with loss of proficiency of mismatch repair due to methylation and silencing of the MLH1 gene.[46,47]

1.2. Fused Rings. In general, complexes with Ru^{II} coordinated to benzene fused to a partially saturated five-, six- or seven-membered cyclic hydrocarbon all showed good activity against A2780 cells (Table 3). The cyclophane complex **23** was an exception, being inactive. The two aromatic decks (phenyls) in this complex can stack to give one overall arene system, but this enhanced hydrophobicity confers poor aqueous solubility. The indan complex **21**, which lacks the terminal phenyl ring found in **16**, showed reduced cytotoxic activity compared to **16**. Similarly complex **22**, which lacks the terminal phenyl ring found in **18** and **19**, showed a substantially reduced potency.

IC_{50} values varied with the arene in the following order: tetrahydroanthracene, 5,6-dihydrophenanthrene, fluorene, dihydroanthracene < dibenzosuberane, indan < tetralin ≪ cyclophane.

These results are consistent with our previous observations that the cytotoxicity of this class of compounds increases with the size of the arene ring system in the order arene = benzene < *p*-cymene < biphenyl < dihydroanthracene < tetrahydroanthracene.[16] Compounds containing polycyclic aromatic hydrocarbons have the greatest potency. The presence and nature of the terminal ring in the polycyclic arenes appear to be crucial, especially when held rigidly by a methylene or ethylene bridge. This arrangement gives rise to enhanced potency and may be related to the degree and ease of involvement in hydrophobic interactions with nucleobases. Thus these complexes are well-suited to probe the effects of shape, size, flexibility, and degree of hydrophobicity on activity.

Table 3. A2780 IC_{50} Values for Complexes Containing Fused Ring Arenes



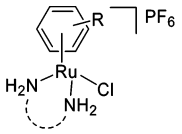
Complex	Arene	IC_{50} (μM)	Synthetic Method
16	fluorene (flu)	2	B + F
17[a]	tetrahydroanthracene (tha)	0.4	A + F
18	5,6-dihydrophenanthrene (dhp)	1	B + F
19[a]	dihydroanthracene (dha)	2	A + F
20	dibenzosuberane (dbs)	8	B + F
21	indan	8	A + F
22	tetralin	20	A + F
23	cyclophane	>100	C + F

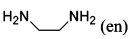
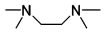
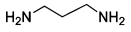
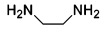
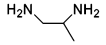
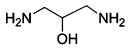
[a] ref 16.

One role of the arene ring in these Ru^{II} arene complexes may be to confer lipophilic character to the complex, thereby enhancing uptake into cells. It may also play an important role in biomolecular interactions and recognition processes. For example, hydrophobic interactions between the arene ligand in $\{(\eta^6\text{-arene})Ru^{II}(en)\}^{2+}$ and DNA could produce a driving force for DNA binding.[48] Hydrophobic interactions with nucleobases have been shown to play a key role in determining the recognition of cisplatin-modified DNA by the high-mobility group protein (HMG1)[49] and also contribute to the formation of stereoselective GG intrastand cross-links on DNA by the anticancer drug *cis*-$[PtCl_2(NH_3)(2\text{-picoline})]$.[50] Strong $\pi-\pi$ arene−nucleobase stacking was also found to be present in the crystal structures of $[(\eta^6\text{-tha})Ru^{II}(en)(9\text{-EtG-}N7)][PF_6]_2$ and $[(\eta^6\text{-dha})Ru^{II}(en)(9\text{-EtG-}N7)][PF_6]_2$, where 9-EtG = 9-ethylguanine.[20]

2. Variations in the N,N-Chelating Ligand. We also studied the effects of variation of the substituents on N as well as the chelating backbone of the aliphatic and aromatic N,N-chelating

Table 4. Effect of Variations in the Aliphatic Diamine Chelating Ligand on A2780 IC_{50} Values



Complex	Arene	Diamine	IC_{50} (μM)	Synthetic Method
10[a]	*p*-cymene	 (en)	10	A + F
24	*p*-cymene		>100	A + F
25	*p*-cymene		10	A + F
21	indan		8	A + F
26	indan		8	A + F
27	indan		>100	A + F
28	indan		>50	A + F
29	indan		24	A + F

[a] ref 15.

ligands in $[(\eta^6\text{-arene})Ru^{II}(N,N)Cl]PF_6$ complexes on their cytotoxicity toward A2780 human ovarian cancer cells. The aromatic backbone in these N,N-chelating ligands contained either polar (OH) or nonpolar (Me) substituents.

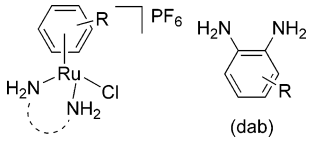
2.1. Aliphatic Diamines. With *p*-cymene as the arene, activity decreased dramatically when all the NH hydrogens of en were replaced by methyl groups (**24**) (Table 4), whereas mono-N-ethylation of complex **9** (to give $[(\eta^6\text{-bip})Ru^{II}(Et\text{-en})Cl]PF_6$) had no significant effect on activity (6 vs 5 μM).[15] Increasing the chelate ring size from five- to six-members (**25**) had little effect. For indan complexes, monomethylation of the en backbone had no effect (**26**), whereas dialkylation via ring formation (**29**) lowered the activity 3-fold. Cyclization of en via addition of a C3 linker (to give homopiperazine) (**28**) and introduction of a polar hydroxyl substitutent on the propylenediamine backbone (**27**) gave rise to little or no activity (IC_{50} > 50 μM).

The complete loss of potency in **24** may be attributed to the lack of NH H-bond donors in the complex. We have shown that strong stereospecific C6O···HN (en) H-bonding stabilizes guanine adducts of these Ru^{II} complexes.[20] This interaction may play a role in the biological activity, since DNA is a potential target site for these complexes.[18] Such H-bonding is also thought to play a role in the recognition of DNA by cisplatin.[51] Indeed, no binding of complex **24** to 9-ethylguanine was detected by ^{1}H NMR spectroscopy. Thus steric effects of the methyl groups as well as lack of H-bonding capability may have contributed to the loss of activity of complex **24**.

2.2 Aromatic Diamines. In general, good activity was retained when en was replaced by 1,2-diaminobenzene (dab) for a range of mono-, di- and tricyclic arenes (hydrocarbons) (Table 5). For dab complexes, the IC_{50} values increase with the arene in the order biphenyl (**33**), dihydroanthracene (**35**) < *p*-cymene (**30**), tetralin (**34**) < tetrahydroanthracene (**36**).

Indan complexes with mono- or dimethylation of dab, **31** and **32**, respectively, also exhibited good activity. Hydroxylation of dab decreased activity 3-fold (**37**). An interesting feature of these dab complexes is their ability to overcome cross-resistance with Adriamycin (doxorubicin). In general, the more hydrophobic the complex, the higher the intrinsic drug potency in A2780 parental cells, but also the greater the degree of cross resistance in $A2780^{AD}$ cells. Multidrug resistant $A2780^{AD}$ cells overexpress the plasma membrane transporter P-170 glycoprotein and the multidrug-associated protein MRP2 is also upregulated in $A2780^{AD}$ cells, which are 45-, 100-, and 100-fold resistant toward complexes **9**, **17**, and **19**, respectively.[16] As shown in Figure 5, several dab analogues of en complexes show little cross resistance with Adriamycin, the most dramatic examples being the reduction in the resistance factors [RF = $IC_{50}(A2780^{AD})/IC_{50}(A2780)$] for **17** and **19** from >100 to 2.

Table 5. A2780 IC_{50} Values for Complexes Containing Substituted 1,2-Diamino Benzenes (dab) as the Chelating Ligand



complex	arene	diamine	IC_{50} (μM)	synthetic method
30	*p*-cymene	dab	11	A + F
31	indan	4-Me-dab	4	A + F
32	indan	4,5-Me_2-dab	14	A + F
33	biphenyl (bip)	dab	5	A + F
34	tetralin	dab	13	A + F
35	dha	dab	7	A + F
36[a]	tha	dab	23	A + F
37	*p*-cymene	3-hydroxy-dab	32	A + F

[a] Chloride salt.

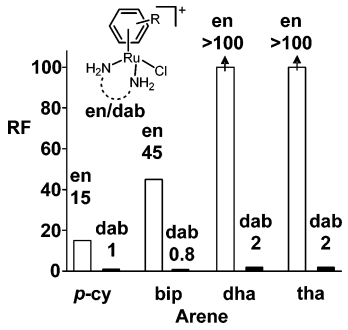
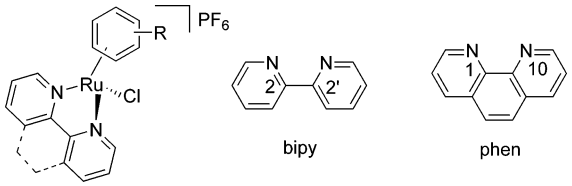


Figure 5. Effect of changing the N,N-chelating ligand from ethylenediamine to 1,2-diaminobenzene on the cytotoxicity toward A2780 and $A2780^{AD}$ cells (plotted as a resistance factor RF = $IC_{50}(A2780^{AD})/IC_{50}(A2780)$) for complexes containing various arenes: *p*-cymene (*p*-cy; comparison of complexes **10** and **30**), biphenyl (bip; **9**, **33**), dihydroanthracene (dha; **19**, **35**), and tetrahydroanthracene (tha; **17**, **36**).

Proteins of the p-glycoprotein class, which are responsible for pumping drugs out of cells, appear to possess binding cavities lined on opposite faces with nonpolar (e.g., Phe) and negatively charged (e.g., Asp) residues.[52] Such a cavity might accommodate favorably positively charged $\{(\eta^6\text{-arene})Ru^{II}(en)\}^{2+}$ adducts, which have hydrophobic arene and polar en faces. Substitution of the polar en by nonpolar dab might lead to much weaker interactions at this site and also introduce unfavorable steric restraints.

It is of interest to consider the relationship between hydrophobicity (as measured by partition coefficient) and cytotoxicity. We have investigated this in detail for many of the complexes reported in this paper, but no general correlation has emerged.[53] This implies that specific biomolecular interactions, e.g with DNA, play an important role in the activity. This is consistent

Table 6. A2780 IC_{50} Values for Complexes Containing Bipyridine Derivatives



complex	arene	bipyridine	IC_{50} (μM)	synthetic method
38	indan	bipy	>100	A + F
39	bip	bipy	>100	A + F
40	indan	4,4′-Me_2-bipy	>100	A + F
41	indan	4,4′-$(CO_2Me)_2$-bipy	>100	A + F
42	indan	4,4′-$(CH_2OH)_2$-bipy	>100	A + F
43	indan	phen	52	A + F
44	indan	2,9-Me_2-phen	>50	A + F
45	indan	$(PhSO_3Na)_2$-phen	>100	A + F

with the findings of Scolaro et al.,[54] who reported little correlation between the extent of cell uptake of triazaphosphaadamantane Ru^{II} arene complexes and cytotoxicity, and with the data of Wang et al.,[18] which suggests that the cytotoxicity of ethylenediamine-containing Ru^{II} arene complexes correlates more closely with DNA ruthenation levels.

2.3. Bipyridine Derivatives. With indan as the arene, complexes containing 2,2′-bipyridine (**38**) or 4,4′-bipyridine derivatives (**40**−**42**) as well as the biphenyl complex containing 2,2′-bipyridine (**39**) were all inactive against A2780 cells (Table 6).

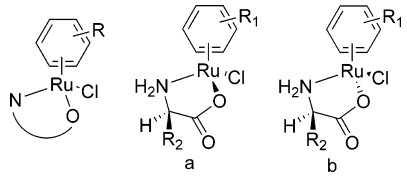
The phenanthroline complexes tested (with either neutral or negatively charged substitutents) showed either poor (**43**) or no activity (**44**, **45**). This suggests that N−H groups on the bound N atoms of the chelated ligand can play important roles in activity, as suggested by the inactivity of complex **24**, e.g., in DNA H-bonding interactions.

3. N,O-Chelating Ligands. The neutral *p*-cymene Ru^{II} N,O-chelating complexes of glycine [(η^6-*p*-cymene)Ru(H_2CNH_2(COO)-*N*,*O*)Cl] (**46**), L- and D-alanine [(η^6-*p*-cymene)Ru(CH(CH_3)NH_2(COO)-*N*,*O*)Cl] (**47**, **48**), β-alanine [(η^6-*p*-cymene)-Ru($H_2NCH_2CH_2COO$)-*N*,*O*)Cl] (**49**), L- and D-phenylalanine [(η^6-*p*-cymene)Ru(CH(CH_2Ph)NH_2(COO)-*N*,*O*)Cl] (**50**, **51**), and [(η^6-bip)Ru(L-proline)Cl] (**52**) all have stereogenic Ru centers and were obtained as racemic mixtures. The amino acidato complexes were all inactive toward A2780 human ovarian cancer cells (IC_{50} > 100 μM) (Table 7). The N,O-chelated complex containing the aminophenolate 8-hydroxyquinoline (**53**) was inactive (IC_{50} > 100 μM) but was poorly soluble. Although there is a report[55] that the amino acidato complex [(η^6-bz)Ru-(L-proline)Cl] is active toward P388 leukemia cells, no experimental data to support this appear to have been published.

For N,N- and some O,O-chelated Ru^{II} arene complexes, activation by hydrolysis and binding to biomolecules such as guanine nucleotides may play roles in the activity.[15,20,24] We therefore investigated the hydrolysis, pK_a of aqua adducts, and binding to nucleobases of the amino acidato complexes, since they were unexpectedly inactive, in case such complexes exhibit unusual reactivity in comparison to the active *N*,*N*- and O,O-chelated complexes.

Hydrolysis. The ^{1}H NMR spectrum of [(η^6-*p*-cymene)Ru(H_2-CNH_2(COO)-*N*,*O*)Cl] (**46**), in 10% D_2O/90% H_2O at pH 6.8 contained two sets of peaks, suggesting that partial hydrolysis had occurred and that the chloro and aqua adducts coexist in the equilibrium, in the ratio 1:3.7. The hydrolysis of **46** appeared

Table 7. A2780 IC_{50} Values for Neutral Complexes Containing Anionic N,O-Chelating Ligands



complex	arene	N,O-chelating ligand	IC_{50} (μM)	synthetic method
46	*p*-cymene	glycine	>100	A + G
47	*p*-cymene	L-alanine	>100	A + G
48	*p*-cymene	D-alanine	>100	A + G
49	*p*-cymene	β-alanine	>100	A + G
50	*p*-cymene	L-phenylalanine	>100	A + G
51	*p*-cymene	D-phenylalanine	>100	A + G
52	bip	L-proline	>100	A + G
53	*p*-cymene	8-hydroxyquinoline	>100	A + G

to be very fast, equilibrium being reached by the time the first ^{1}H NMR spectrum was recorded (<5 min). Addition of 100 mM chloride partially reversed the hydrolysis, giving a 1.2:1 ratio of Cl:H_2O species, but even with 500 mM Cl^- present, ca. 30% of the ruthenium complex was still present as the aqua species. This can be compared with en chelates for which hydrolysis is largely suppressed in 100 mM Cl^-.[17] The high stability of the aqua complex compared to the chloro complex may account for the low cytotoxicity of the amino acidato complexes. The highly reactive aqua species may interact with other cellular biomolecules before they reach target sites, thus effectively being deactivated. For example, the tripeptide glutathione (γ-L-Glu-L-Cys-Gly), which is abundant in cells at millimolar concentrations, can form O-bound and thiolate S-bound adducts with Ru^{II} arene complexes and these can undergo oxygen atom addition reactions at Ru-bound sulfur.[56]

pK_a of Aqua Complex. A ^{1}H NMR pH titration of the aqua adduct of complex **46** [(η^6-*p*-cymene)Ru(H_2CNH_2(COO)-*N*,*O*)-H_2O]$^+$ (Figure 6) gave a pK_a of 8.65 for the coordinated water ligand. Hence at pH 7.4, the hydrolyzed complex would be present largely in the aqua form as opposed to the potentially less reactive hydroxo adduct. This pK_a value is similar to that of [(η^6-*p*-cymene)Ru(en)H_2O]$^{2+}$ (pK_a = 8.25)[23] but lower than that for [(η^6-*p*-cymene)Ru($H_3CCOCHCOCH_3$-*O*,*O*)H_2O]$^+$ (pK_a = 9.41).[24]

Nucleobase Binding. ^{1}H NMR studies showed that the glycine complex [(η^6-*p*-cymene)Ru(H_2CNH_2(COO)-*N*,*O*)Cl] (**46**) reacts rapidly with 9-ethylguanine in D_2O. The H8 peak of 9EtG shifted from δ 7.69 to 7.90, indicative of the formation of a 9EtG-coordinated adduct (**46G**). Equilibrium was reached within minutes and ca. 75% of **46** reacted. The X-ray crystal structure of complex **46G** was determined (Figure 7). The structure is similar to a related complex reported previously.[55] Coordination of Ru^{II} is through the N7 nitrogen of 9EtG and strong H-bond formation between the ligand NH_2 and G C6O are observed (d(N···O6) = 2.855 Å). This is consistent with a previously reported en adduct.[20]

No reaction between complex **46** and adenosine was observed at ambient temperature, as was the case for the analogous en complex, but in contrast to the acac complex.[24] Since 9-EtG adducts of Ru^{II} arene complexes containing α-amino acidato and en ligands have similar structures, the biological inactivity of the former complexes may be due to factors other than the nature of possible DNA lesions, e.g. the strength of binding. A fast hydrolysis rate and the presence of a relatively large proportion of a reactive aqua species at physiological chloride

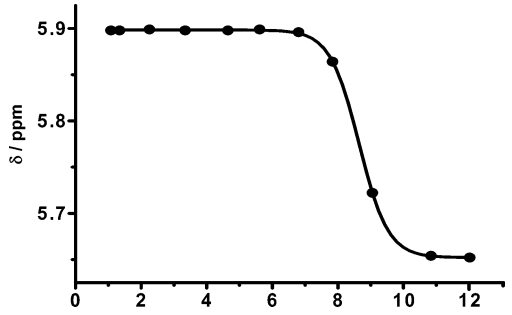


Figure 6. Dependence on pH of the ^{1}H NMR chemical shift of one of the resonances of the aromatic protons of *p*-cymene in [(η^6-*p*-cymene)Ru(H_2CNH_2(COO)-*N*,*O*)(H_2O)]$^+$. The line is a computer best fit giving pK_a(H_2O) = 8.65 ± 0.01.

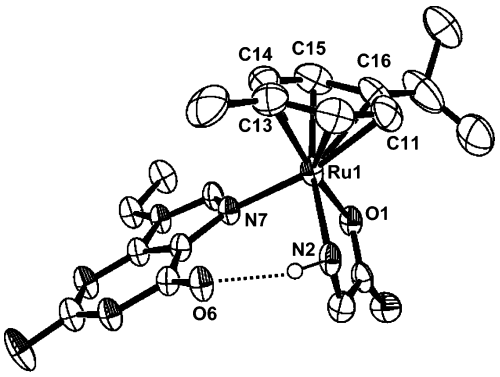
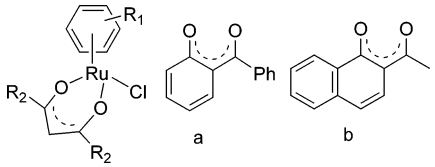


Figure 7. The X-ray crystal structure of the 9-ethylguanine adduct (**46G**), showing the atom numbering scheme and 50% probability of thermal ellipsoids. The H-bonding interaction of glycine NH with guanine O6 is indicated by a dotted line.

Table 8. A2780 IC_{50} Values for Neutral Complexes Containing Anionic O,O-Chelating Ligands (Acetylacetonate Derivatives)



complex	arene	R_2	IC_{50} (μM)	synthetic method
54	*p*-cymene	CH_3	19	A + G
55	bip	CH_3	21	A + G
56	benzene (bz)	CH_3	>50	A + G
57	indan	CH_3	>50	A + G
58	dha	CH_3	70	A + G
59	*p*-cymene	Ph	11	A + G
60	*p*-cymene	CMe_3	14	A + G
61	*p*-cymene	CF_3	>100	A + G
62	*p*-cymene	**a**	>100	A + G
63	*p*-cymene	**b**	83	A + G

concentrations might favor a deactivation pathway, preventing the complex from reaching its target site. Carmona et al. have suggested that amino acidato complexes can undergo ring-opening reactions.[57] Thus, chelate ring opening could also provide an additional mechanism by which these complexes are deactivated under biological conditions.

4. O,O-Chelating Ligands. The IC_{50} values of neutral Ru acetylacetonate (acac) complexes against A2780 human ovarian cancer cells showed the following dependence on the arene (Table 8): *p*-cymene, biphenyl ≪ dihydroanthracene < benzene, indan. The most potent complex, [(η^6-*p*-cymene)Ru(PhCOCHCOPh)Cl] (**59**), had an IC_{50} value of 11 μM. The dependence on the arene contrasts with en analogues (bip, *p*-cymene), with [(η^6-bip)Ru($H_3CCOCHCOCH_3$)Cl] (**55**) being ca. 4 times less active than the en complex, and the *p*-cymene complex **54** being ca. 2 times less active than its en analogue (**10**).

For *p*-cymene complexes containing 1,3-disubstituted acac derivatives, the IC_{50} values vary with acac substitutents in the order: Ph, *tert*-butyl < Me ≪ Me/naphthyl < CF_3, Ph/phenyl (Table 8), with the latter two complexes being inactive. In general, these acac-type complexes have poor aqueous solubility and hydrolyze rapidly. It seems likely that their lower potency compared to the en analogous is due partly to the possibility of the protonation and irreversible displacement of the chelated acac derivative under some conditions.[58] The weakness of the Ru−O bond in complex [(η^6-cymene)Ru($F_3CCOCHCOCF_3$)Cl] (**61**), which contains strongly electron-withdrawing CF_3 substituents, is evident from the X-ray structures. The Ru−O bond lengths are significantly longer than for [(η^6-*p*-cymene)Ru($H_3CCOCHCOCH_3$)Cl] (**54**)[24] and [(η^6-*p*-cymene)Ru(Me_3-CCOCHCOCMe_3)Cl] (**60**). Of the complexes tested, those containing symmetrical acac-type ligands are active, with the exception of complex **61**. The inactivity of **61** could be as a result of the potential loss of the chelated ligand.

Steric bulk around the metal center appears to enhance the activity of these acac complexes. Both *p*-cymene (pendant isopropyl group) and bip (pendant phenyl ring) contain rotating components that shield the metal center, whereas the arenes bz, dha, and indan are more constrained. In addition, introduction of more sterically demanding substituents on the β-diketonate ligands increases activity. The complexes **62** and **63** contain planar substituents in the chelate backbone, which could also be involved in delocalization of charge, possibly weakening the binding of the chelating ligand to the metal. Potentially sterically demanding substituents could slow deactivation especially by bulky bioligands, e.g. proteins, by limiting their access to the metal center. The acac-type complexes hydrolyze rapidly and the aqua adducts are more stable than the chlorido adducts compared to en analogues. However, the nucleobase adducts (although formed with both G and A) are less stable than those of the en analogues, as found for the complex [(η^6-*p*-cymene)Ru($H_3CCOCHCOCH_3$)Cl] (**54**).[24]

Conclusions

We have shown that organometallic ruthenium(II) half-sandwich complexes containing an η^6-arene ligand, a chelated diamine, and chloride as a leaving group can be as cytotoxic as cisplatin or carboplatin toward A2780 human ovarian cancer cells. Moreover, no cross-resistance with cisplatin is observed. The most active compounds contain extended polycyclic arene ligands and ethylenediamine as the chelating ligand. Polar substituents on the arene decreased activity significantly. Introduction of substituents on the N atom or the backbone of en, in general, decreased activity, which was lost entirely on substitution of all N−H groups (e.g. methylation or bipyridines). Increasing the chelate ring size from five to six appears to have no effect on activity. As for cisplatin, the Ru−NH group may play an important role as H-bond donor in DNA interactions, e.g. with guanine C6O. Cross-resistance with Adriamycin can be circumvented by introducing an aromatic backbone on the diamine chelate. Curiously, all the N,O chelated complexes tested here (mostly amino acidates) were inactive, despite an apparent ability to form stable G adducts, perhaps due to the greater extent of hydrolysis of the chloro complex, potentially giving rise to deactivating reactions with biomolecules. Although the O,O-chelated complexes tested possess only H-bond ac-

ceptors on the chelating ligand and bind more weakly to nucleobases,[24] some possess reasonable activity toward A2780 cells.

This work has demonstrated that the nature of the arene and the chelating ligand can play crucial roles in activity. Elsewhere we have shown that variation of the leaving group can also have a dramatic effect on chemical and biological activity.[18] Hence, there is much scope within this class of compounds for controlling the kinetics and thermodynamics of biologically important reactions and for tuning their behavior to optimize the properties required for a candidate for clinical trials.

Experimental Section

Materials. $RuCl_3 \cdot xH_2O$ was purchased from Alfa-Aesar and PMA Ltd.; indan, terpenene, fluorene, diaminophenylene, *O*-terphenyl, tetrahydronaphthalene, (1*R*,2*R*)-(−)-1,2-diaminocyclohexane, bipy, 4,4′-Me_2-bipy, phen, $(PhSO_3Na)_2$-phen, 1,3 diamino-2-hydroxypropane, and homopiperazine were obtained from Aldrich. Fluorene and diaminophenylene were further purified by sublimation. 4,4′-$(CO_2Me)_2$-bipy and 4,4′-$(CH_2OH)_2$-bipy were prepared according to a published procedure.[59] Acetonitrile was dried over CaH_2, and alcohols were dried and distilled from Mg/I_2. THF was dried over Na/benzophenone. Diethyl ether, hexane, and ethylenediamine were distilled over Na metal prior to use. All other reagents were obtained from commercial suppliers and used as received.

Methods and Instrumentation. IC_{50} Values. The doses of the complexes that caused 50% inhibition of the growth of human ovarian A2780 cancer cells were determined as previously described.[16]

NMR Spectroscopy. ^1H NMR spectra were acquired on either Bruker DMX 500 (^1H = 500 MHz) or Bruker DPX 360 (^1H = 360 MHz) instruments. All data processing was carried out using XWIN NMR version 3.6 (Bruker U.K. Ltd.). ^1H NMR chemical shifts were internally referenced to 1,4-dioxane (3.75 ppm) or to the methyl singlet of TSP (0 ppm) in aqueous solutions or to CD_2HCOCD_3 (2.06 ppm) in acetone-d_6 solution.

X-ray Crystallography. With the exception of **16**, diffraction data were collected with Mo Kα radiation on a Bruker Smart Apex CCD diffractometer equipped with an Oxford Cryosystems LT device; absorption corrections were applied with the multiscan procedure SADABS.[60] Data for **16** were collected on a Nonius Kappa-CCD diffractometer mounted on a rotating anode generator at the EPSRC National Crystallography Service at the University of Southampton; an absorption correction was applied using SORTAV.[61] Structures were solved by direct methods (SHELX97,[62] SIR92[63] or SIR97[64]) or Patterson methods (DIRDIF[65] or SHELX97[62]) and refined by full-matrix least-squares against F^2 (SHELX97 or CRYSTALS[66]). Unless otherwise noted, all non-H atoms were refined with anisotropic displacement parameters, with H-atoms in idealized positions.

In **16**, the fluorene ligand is disordered over two orientations, for which the occupancies refined to 0.76(1) and 0.26(1). The minor component was treated as a rigid body with a geometry based on that of the major component. Anisotropic displacement parameters (adps) were refined for all non-H atoms, except those in the minor disorder component of the fluorene ring, for which a single overall isotropic displacement parameter was refined. In **27** and **39** the two components of disorder were refined subject to similarity restraints. In **28**, H1 and H2 were located in a difference map and refined freely. H-atoms attached to the methanol of crystallization in **36** were located in difference syntheses calculated about the locus of possible H-atom positions; the methyl and hydroxyl groups were then allowed to rotate about the C−O vector during refinement. The H-atoms attached to the water of crystallization (O16) were located using the CALCOH procedure.[67] The water molecule was then treated as a freely rotating rigid group. In **53** the methyl group attached to C411 is disordered, the two components being related by a 60° rotation about C41−C411. In the 9EtG adduct, the cymene ligand is disordered over two orientations. It was modeled with four half-weight methyl groups (the terminal groups of the ^{i}Pr substituents), with all other atoms being full weight. Similarity restraints were applied to the distances and angles in the disordered groups.

Synthesis. Routes via Ruthenium(0). Naphthalene Displacement (Scheme 4, Method D). Complexes **1**−**5** and **14** were prepared by displacing coordinated naphthalene from [(η^6-naphthalene)Ru(1,5-cyclooctadiene)] in the presence of excess arene and acetonitrile. The general procedure is outlined below, and the experimental details are given in the Supporting Information.

[(η^6-Naphthalene)Ru(1,5-cyclooctadiene)], the desired arene ligand, and acetonitrile were dissolved in THF, and the mixture was stirred at ambient temperature for 2 d. Solvent, excess arene, and naphthalene were removed in vacuo, again at ambient temperature. The resulting solid was dissolved in hexane and chromatographed on Al_2O_3/5% H_2O. Removal of the solvent under reduced pressure yielded the pure yellow complexes [(η^6-arene)-Ru(1,5-cyclooctadiene)], which were recrystallized from pentane. These complexes were dissolved in acetone, and concentrated HCl was added dropwise with stirring. After 30 min the precipitated red-brown dimer [(η^6-arene)$RuCl_2$]$_2$ was filtered off, washed with acetone, and dried in vacuo. This dimer was suspended in methanol and heated at 333 K for 1 h. At this temperature, 1.5 mol equiv of ethylenediamine was added and the mixture was left to react for 1 h. The solution was filtered and the volume of the filtrate was reduced to about 10 mL. To this solution was added 3 mol equiv of NH_4PF_6, and a precipitate appeared after shaking the flask. The solid was filtered off, washed, and dried in vacuo.

[(η^6-Hexaphenylbenzene)Ru(en-*N*,*N*)Cl]PF_6 (15). [(η^6-Hexaphenylbenzene)Ru(1,5-cyclooctadiene)] (744 mg, 1 mmol) was dissolved in acetone (25 mL) and concentrated HCl (0.25 mL) was added dropwise with stirring. After 30 min the precipitated dimer [(η^6-hexaphenylbenzene)$RuCl_2$]$_2$ was filtered off, washed with acetone, and dried in vacuo. This dimer was suspended in methanol and heated at 333 K for 1 h. At this temperature, 1.5 mol equiv of ethylenediamine was added and the mixture was left to react for 1 h. The solution was filtered and the volume of the filtrate was reduced to about 10 mL. To this solution 3 mol equiv of NH_4PF_6 was added and a precipitate appeared after shaking the flask. The solid was filtered off, washed, and dried in vacuo. Yield: 45%. ^1H NMR (DMSO-d_6): δ 7.28 (m, 5H), 7.03 (m, 5H), 6.96 (m, 5H), 6.92 (m, 5H), 6.84 (m, 10H), 5.61 (b, 1H), 4.62 (b, 1H), 5.55 (b, 1H), 5.33 (b, 1H), 2.80 (m, 2H), 2.75 (m, 2H). MS (ESI) for $C_{44}H_{38}ClN_2Ru$ calcd 731.3, found 731.4.

In Situ Reduction (Scheme 2, Method B). Complexes **11**, **16**, **18**, and **20**, were prepared via in situ reduction of $[(COD)RuCl_2]_n$. The general procedure is outlined below, and the experimental details are given in the Supporting Information.

A three-neck round-bottom flask fitted with a condenser was flame dried. To this were added $[(COD)RuCl_2]_x$, arene (3.4 mol equiv), and Zn powder (ca 40 mol equiv), and the flask was put under vacuum and then under Ar. The cycle was repeated three times. To this was added dry and freshly distilled THF and the reaction mixture was heated under reflux for 36 h. The solvent was then taken off under vacuum and left sealed under Ar overnight. Dry and freshly distilled hexane was then added to the reaction mixture and the mixture was stirred. The solution was then transferred via a cannula equipped with a filter paper on one end to a Schlenk tube under a pressure of Ar. The clear hexane solution appeared brick red. The hexane was taken off under reduced pressure to give a yellowish solid. This was redissolved in dry and freshly distilled acetonitrile, and 1 M HCl in ether was added to the solution. The solution turned red and after a few minutes a red precipitate started to appear. The mixture was left stirring overnight, ether was added, and the solid was collected by filtration, washed with ether, and dried in vacuo. A mixture of [(η^6-(arene)$RuCl_2$]$_2$ and NH_4PF_6 (2.2 mol equiv) was suspended in dry and freshly distilled acetonitrile and the reaction mixture left stirring at ambient temperature for 18 h. This was filtered to give an orange filtrate. The solvent was removed on a rotary evaporator to give an orangey oily residue. Ether was added and trituration yielded an orangey

powder, [(η^6-arene)Ru($CH_3CN)_2$Cl]PF_6. This was dissolved in acetonitrile to give an orange solution. One molar equivalent of etheylenediamine was then added in one portion and the reaction mixture was left stirring at ambient temperature for ca. 1 h. The solution was filtered and after rotary evaporation gave a reddish oil. Ether was then added and after triturating and storage at 253 K, a red solid was obtained, which was washed with ether and air-dried to give [(η^6-arene)Ru(en)Cl]PF_6.

Phenylenediamine (dab) Complexes (Scheme 6, Method F). Representative syntheses are given here (complexes **30** and **33**), and details of the syntheses of complexes **31**, **32**, and **34**−**37** are in the Supporting Information.

[(η^6-*p*-Cymene)Ru(phenylenediamine-*N,N*)Cl]PF_6 (30). [(η^6-*p*-Cymene)Ru($CH_3CN)_2$Cl]PF_6 (0.12 g 0.25 mmol) was dissolved in CH_3CN (20 mL) to give a yellowish solution. To this was added *o*-phenylenediamine (0.151 g, 1.40 mmol) and the reaction mixture stirred at ambient temperature for 18 h to give a deep red solution. The solvent was removed on a rotary evaporator to give a brownish/red oily solid. This was washed several times with ether and triturated to give a reddish powder (0.143 g, 54%). ^{1}H NMR (DMSO-d_6): δ 7.81 (m, 2H) 7.47 (m, 3H) 7.39 (NH, 4H), 5.9 (d, 2H), 5.55 (d, 2H), 2.9 (m, 1H), 2.25 (s, 3H), 1.15 (M, 6H). Anal. ($C_{16}H_{22}ClF_6N_2PRu$) C, H, N.

[(η^6-Bip)Ru(phenylenediamine-*N,N*)Cl]PF_6 (33). The dimer [(η^6-bip)$RuCl_2]_2$ (0.220 g, 0.35 mmol) was suspended in MeOH/H_2O (50 mL/10 mL), heated under reflux for 1 h, and cooled to ambient temperature. *o*-Phenylenediamine (0.065 g, 0.60 mmol) in MeOH (5 mL) was then added dropwise and the reaction mixture further heated under reflux for 15 min and filtered. To the filtrate was added NH_4PF_6 (0.122 g, 0.75 mmol) and the volume of the filtrate reduced on a rotary evaporator to about 20 mL, and storage at 253 K overnight gave a brownish microcrystalline solid. The product was collected by filtration, washed with MeOH and ether, and dried in air. It was recrystallized from MeOH. Yield: 0.180 g, 54%. ^{1}H NMR (DMSO-d_6): δ 8.49 (d, NH, 2H), 7.81 (m, 2H) 7.47 (m, 3H) 7.18 (m, 2H), 7.15 (m, 2H), 6.46 (d, NH, 2H) 6.28 (m, 2H), 6.00 (m, 1H), 5.86 (m, 2H). Anal. ($C_{18}H_{18}ClF_6N_2PRu$) C, H, N.

Bipyridyl Complexes (Scheme 6, Method F). Representative syntheses are given here (complexes **38** and **39**), and details of the syntheses of complexes **40**−**45** are in the Supporting Information.

[(η^6-Indan)Ru(bipy-*N,N*)Cl]PF_6 (38). The dimer [(η^6-indan)$RuCl_2]_2$ (0.254, 0.44 mmol) was suspended in MeOH (40 mL) and to this was added bipyridine (0.148 g, 0.95 mmol) in MeOH (10 mL) dropwise and the resulting clear solution turned yellowish-green. The reaction mixture was left stirring at ambient temperature for 1 h. It was then filtered and the volume of the filtrate was reduced on a rotary evaporator to ca. 10 mL, NH_4PF_6 (0.225 g, 1.37 mmol) was added, and the flask was shaken. Precipitate started to appear almost immediately. The flask was kept at 253 K overnight. The solid obtained was collected by filtration, washed with cold methanol and ether, and dried in air to give an intense yellow solid. Yield: 425 mg, 88%. Crystals suitable for X-ray analysis were obtained by slow evaporation of a methanolic solution at ambient temperature. ^{1}H NMR (DMSO-d_6): δ 9.85 (m, 2H), 8.9 (m, 2H), 8.26 (s, 2H), 8.14 (m, 2H) 6.47 (m, 2H), 6.02 (m, 2H), 2.72 (m, 4H) 1.86 (m, 1H), 1.41 (m, 1H). Anal. ($C_{19}H_{18}ClF_6N_2$-PRu) C, H, N.

[(η^6-Bip)Ru(bipy-*N,N*)Cl]PF_6 (39). The dimer [(η^6-bip)$RuCl_2]_2$ (0.226 g, 0.35 mmol) was suspended in freshly distilled methanol (50 mL). Deionized and degassed water (10 mL) was added and the reaction mixture heated under reflux for 1 h. It was then cooled for about 10 min, bipridyl (0.78 g, 0.50 mmol) was added, and the reaction mixture was heated under reflux for ca. 20 min. The solution was then hot filtered to give a reddish/yellow solution and NH_4PF_6 was added. A few minutes later a precipitate started to appear. After standing at ambient temperature and slow solvent evaporation, the microcrystalline solid was filtered off. Yield: 0.25 g, 90%. ^{1}H NMR (DMSO-d_6): δ 9.30 (m, 2H), 8.57 (d, 2H), 8.20 (t, 2H), 7.61 (m, 2H), 7.40 (m, 1H), 7.38 (m, 2H), 6.61 (d, 2H), 6.40 (m, 2H), 6.18 (m, 1H). Anal. ($C_{22}H_{18}ClF_6N_2PRu$) C, H; N.

Amino Acidate Complexes (Scheme 7, Method G). The general procedure is outlined below, and the experimental details are given in the Supporting Information.

These complexes were prepared following published methods for analogous complexes.[55,68] When the amino acidate salt was not available commercially, the amino acid was reacted with a 0.1 M solution of KOH. Two molar equivalents of the amino acidate salt were reacted with [(η^6-*p*-cymene)$RuCl_2]_2$ (for complexes **46**−**51** or [(η^6-biphenyl)$RuCl_2]_2$ (for complex **52**). The reaction mixture was stirred overnight at ambient temperature. The solvent was then removed on a rotary evaporator and the resulting orange solid extracted with CH_2Cl_2 and filtered. The solvent was removed again, the resulting solid was redissolved with the minimum amount of 2-propanol, and the saturated solution was left in the freezer for crystallization.

Acetylacetonate-Type Complexes (Scheme 7, Method G). A representative synthesis is given here (complex **60**), and details of the syntheses of complexes **54**−**59** and **61**−**63** are in the Supporting Information.

[(η^6-*p*-Cymene)Ru(($CH_3)_3CCOCHCOC(CH_3)_3$-*O,O*)Cl] (60). 2,2,6,6-Tetramethyl-3,5-heptanedione (299 mg, 1.62 mmol) and sodium methoxide (68 mg, 1.26 mmol) were stirred in methanol (40 mL) for 5 h at ambient temperature. The solvent and residual starting material were removed on a rotary evaporator. The resulting sodium salt (233 mg, 1.13 mmol, 89.6% yield) and [(η^6-*p*-cymene)-$RuCl_2]_2$ (258 mg, 0.42 mmol) were stirred in acetone (25 mL) for 40 min at ambient temperature. The solvent was removed on a rotary evaporator and the residue extracted with dichloromethane. After filtration and removal of the solvent on a rotary evaporator, the residue was dissolved in diethyl ether, and the solution was concentrated on a rotary evaporator and then diluted with hexane. The solution was stored at 253 K overnight and orange needles (215 mg, 0.47 mmol, 56.0% yield), suitable for X-ray diffraction, were collected by filtration and dried in vacuo.^{1}H NMR ($CDCl_3$): δ 5.41 (d, 2H, J = 6 Hz), 5.40 (s, 1H), 5.13 (d, 2H, J = 6 Hz), 2.91 (sp, 1H, J = 7 Hz), 2.24 (s, 3H), 1.36 (d, 6H, J = 7 Hz), 1.13 (s, 18H). Anal. ($C_{21}H_{33}ClO_2Ru$) C; H.

X-ray Crystallography. Standard data relating to the X-ray crystal structures of compounds **16**, **22**, **27**, **28**, **36**, **38**, **39**, **43**, **46G**, **53**, **60**, **61**, and **62** have been deposited in the Cambridge Crystallographic Data Centre with CCDC reference numbers 607563, 607565, 607573, 607562, 607564, 607570, 607572, 607571, 607569, 607567, 607574, 607566, and 607568, respectively.

Acknowledgment. We thank the Edinburgh Technology Fund, European Community (Marie Curie award for R.F.), Cancer Research UK, and Oncosense Ltd. for support and Ian Hanson (University of Edinburgh), Prof. M. B. Hursthouse, and Dr. S. Coles (University of Southampton) for assistance with X-ray data collection for compounds **60**, **61**, and **16**. We are also grateful for COST D20 for stimulating discussions.

Supporting Information Available: Details of synthesis, characterization, X-ray crystallographic data, and structures of complexes **27**, **28**, **38**, **39**, **43**, **53**, **61**, and **62**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Clarke, M. J.; Zhu, F.; Frasca, D. R. Non-platinum chemotherapeutic metallopharmaceuticals. *Chem. Rev.* **1999**, *99*, 2511−2533.
(2) Allardyce, C. S.; Dyson, P. J. Ruthenium in medicine: Current clinical uses and future prospects. *Platinum Met. Rev.* **2001**, *45*, 62−69.
(3) Clarke, M. J. Oncological implication of the chemistry of ruthenium. *Met. Ions Biol. Syst.* **1980**, *11*, 231−83.
(4) Clarke, M. J.; Bitler, S.; Rennert, D.; Buchbinder, M.; Kelman, A. D. Reduction and subsequent binding of ruthenium ions catalyzed by subcellular components. *J. Inorg. Biochem.* **1980**, *12*, 79−87.
(5) Velders, A. H.; Kooijman, H.; Spek, A. L.; Haasnoot, J. G.; De Vos, D.; Reedijk, J. Strong differences in the in vitro cytotoxicity of three isomeric dichlorobis(2-phenylazopyridine)ruthenium(II) complexes. *Inorg. Chem.* **2000**, *39*, 2966−2967.

(6) Hotze, A. C. G.; Bacac, M.; Velders, A. H.; Jansen, B. A. J.; Kooijman, H.; Spek, A. L.; Haasnoot, J. G.; Reedijk, J. New cytotoxic and water-soluble bis(2-phenylazopyridine)ruthenium(II) complexes. *J. Med. Chem.* **2003**, *46*, 1743−1750.

(7) Vilaplana, R. A.; Gonzalez-Vilchez, F.; Gutierrez-Puebla, E.; Ruiz-Valero, C. The first isolated antineoplastic Ru(IV) complex: Synthesis and structure of [Cl_2(1,2-cyclohexanediaminotetraacetate)-Ru]·2H_2O. *Inorg. Chim. Acta* **1994**, *224*, 15−18.

(8) Rademaker-Lakhai, J. M.; Van Den Bongard, D.; Pluim, D.; Beijnen, J. H.; Schellens, J. H. M. A phase I and pharmacological study with imidazolium-trans-DMSO-imidazole-tetrachlororuthenate, a novel ruthenium anticancer agent. *Clin. Cancer Res.* **2004**, *10*, 3717−3727.

(9) Alessio, E. Synthesis and reactivity of Ru-, Os-, Rh-, and Ir-halide-sulfoxide complexes. *Chem. Rev.* **2004**, *104*, 4203−4242.

(10) Sava, G.; Gagliardi, R.; Bergamo, A.; Alessio, E.; Mestroni, G. Treatment of metastases of solid mouse tumors by NAMI-A: Comparison with cisplatin, cyclophosphamide and dacarbazine. *Anticancer Res.* **1999**, *19*, 969−972.

(11) Alessio, E.; Mestroni, G.; Bergamo, A.; Sava, G. Ruthenium anticancer drugs. *Met. Ions Biol. Syst.* **2004**, *42*, 323−351.

(12) Peti, W.; Pieper, T.; Sommer, M.; Keppler, B. K.; Giester, G. Synthesis of tumor-inhibiting complex salts containing the anion trans-tetrachlorobis(indazole)ruthenate(III) and crystal structure of the tetraphenylphosphonium salt. *Eur. J. Inorg. Chem.* **1999**, 1551−1555.

(13) Yan, Y. K.; Melchart, M.; Habtemariam, A.; Sadler, P. J. Organometallic chemistry, biology and medicine: Ruthenium arene anticancer complexes. *Chem. Commun.* **2005**, 4764−4776.

(14) Melchart, M.; Sadler, P. J. Ruthenium arene anticancer complexes. In *Bioorganometallics: Biomolecules, Labeling, Medicine*; Jaouen, G., Ed.; Wiley-VCH Verlag: Weinheim, 2006; pp 39−64.

(15) Morris, R. E.; Aird, R. E.; Murdoch, P. d. S.; Chen, H.; Cummings, J.; Hughes, N. D.; Parsons, S.; Parkin, A.; Boyd, G.; Jodrell, D. I.; Sadler, P. J. Inhibition of cancer cell growth by ruthenium(II) arene complexes. *J. Med. Chem.* **2001**, *44*, 3616−3621.

(16) Aird, R. E.; Cummings, J.; Ritchie, A. A.; Muir, M.; Morris, R. E.; Chen, H.; Sadler, P. J.; Jodrell, D. I. In vitro and in vivo activity and cross resistance profiles of novel ruthenium(II) organometallic arene complexes in human ovarian cancer. *Br. J. Cancer* **2002**, *86*, 1652−1657.

(17) Wang, F.; Chen, H.; Parsons, S.; Oswald, I. D. H.; Davidson, J. E.; Sadler, P. J. Kinetics of aquation and anation of ruthenium(II) arene anticancer complexes, acidity and X-ray structures of aqua adducts. *Chem.-Eur. J.* **2003**, *9*, 5810−5820.

(18) Wang, F.; Habtemariam, A.; van der Geer Erwin, P. L.; Fernandez, R.; Melchart, M.; Deeth, R. J.; Aird, R.; Guichard, S.; Fabbiani, F. P. A.; Lozano-Casal, P.; Oswald, I. D. H.; Jodrell, D., I.; Parsons, S.; Sadler, P. J. Controlling ligand substitution reactions of organometallic complexes: Tuning cancer cell cytotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 18269−18274.

(19) Novakova, O.; Chen, H.; Vrana, O.; Rodger, A.; Sadler, P. J.; Brabec, V. DNA interactions of monofunctional organometallic ruthenium(II) antitumor complexes in cell-free media. *Biochemistry* **2003**, *42*, 11544−11554.

(20) Chen, H.; Parkinson, J. A.; Parsons, S.; Coxall, R. A.; Gould, R. O.; Sadler, P. J. Organometallic ruthenium(II) diamine anticancer complexes: Arene-nucleobase stacking and stereospecific hydrogen-bonding in guanine adducts. *J. Am. Chem. Soc.* **2002**, *124*, 3064−3082.

(21) Liu, H.; Wang, F.; Parkinson, J. A.; Bella, J.; Sadler, P. J. Ruthenation of duplex and single-stranded d(CGGCCG) by organometallic anticancer complexes. *Chem.-Eur. J.* **2006**, *12*, 6151−6165.

(22) Chen, H.; Parkinson, J. A.; Novakova, O.; Bella, J.; Wang, F.; Dawson, A.; Gould, R.; Parsons, S.; Brabec, V.; Sadler, P. J. Induced-fit recognition of DNA by organometallic complexes with dynamic stereogenic centers. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14623−14628.

(23) Chen, H.; Parkinson, J. A.; Morris, R. E.; Sadler, P. J. Highly selective binding of organometallic ruthenium ethylenediamine complexes to nucleic acids: Novel recognition mechanisms. *J. Am. Chem. Soc.* **2003**, *125*, 173−186.

(24) Fernandez, R.; Melchart, M.; Habtemariam, A.; Parsons, S.; Sadler, P. J. Use of chelating ligands to tune the reactive site of half-sandwich ruthenium(II)−arene anticancer complexes. *Chem.-Eur. J.* **2004**, *10*, 5173−5179.

(25) Allardyce, C. S.; Dyson, P. J.; Ellis, D. J.; Heath, S. L. [Ru(η^6-*p*-cymene)Cl_2(pta)] (pta = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]-decane): A water soluble compound that exhibits pH dependent DNA binding providing selectivity for diseased cells. *Chem. Commun.* **2001**, 1396−1397.

(26) Carso, F.; Rossi, M. Antitumor titanium compounds. *Mini-Rev. Med. Chem.* **2004**, *4*, 49−60.

(27) Kopf-Maier, P.; Kopf, H. Tumor inhibition by titanocene complexes. Activity against B16 melanoma and colon 38 carcinoma. *Arzneimittel-Forschung.* **1987**, *37*, 532−534.

(28) Kröger, N.; Kleeberg, U. R.; Mross, K.; Edler, L.; Sass, G.; Hossfeld, D. K. Phase II clinical trial of titanocene dichloride in patients with metastatic breast cancer. *Onkologie* **2000**, *23*, 60−62.

(29) Guo, M.; Sun, H.; McArdle, H. J.; Gambling, L.; Sadler, P. J. Uptake and release by human serum transferrin and recognition of Ti(IV)-transferrin by cancer cells: Understanding the mechanism of action of the anticancer drug titanocene dichloride. *Biochemistry* **2000**, *39*, 10023−10033.

(30) Fichtner, I.; Pampillon, C.; Sweeney, N. J.; Strohfeldt, K.; Tacke, M. Antitumor activity of Titanocene Y in xenografted Caki-1 tumors in mice. *Anti-Cancer Drugs* **2006**, *17*, 333−336.

(31) Jaouen, G.; Top, S.; Vessières, A. Organometallics targeted to specific biological sites: Development of new therapies. In *Bioorganometallics: Biomolecules, Labeling, Medicine*; Jaouen, G., Ed.; Wiley-VCH Verlag: Weinheim, 2006; pp 65−95.

(32) Zelonka, R. A.; Baird, M. C. Benzene complexes of ruthenium(II). *Can. J. Chem.* **1972**, *50*, 3063−3072.

(33) Bennett, M. A.; Smith, A. K. Arene ruthenium(II) complexes formed by dehydrogenation of cyclohexadiene with ruthenium(III) trichloride. *J. Chem. Soc., Dalton Trans.* **1974**, 233−241.

(34) Birch, A. J.; Rao, G. S. Reductions by metal-ammonia solutions and related reagents. *Adv. Org. Chem.* **1972**, *8*, 1−65.

(35) Harvey, R. G. Metal−ammonia reduction of aromatic molecules. *Synthesis* **1970**, *2*, 161−172.

(36) Rabideau, P. W. The metal−ammonia reduction of aromatic compounds. *Tetrahedron* **1989**, *45*, 1579−1603.

(37) Bennett, M. A.; Huang, T. N.; Matheson, T. W.; Smith, A. K. (η^6-Hexamethylbenzene)ruthenium complexes. *Inorg. Synth.* **1982**, *21*, 74−78.

(38) Bennett, M. A.; Neumann, H.; Thomas, M.; Wang, X. Q.; Pertici, P.; Salvadori, P.; Vitulli, G. (η^6-Naphthalene)(η^4-1,5-cyclooctadiene)-ruthenium(0): Efficient synthesis, chemistry, and catalytic properties. *Organometallics* **1991**, *10*, 3237−3245.

(39) Vitulli, G.; Pertici, P.; Salvadori, P. Arene ligand exchange reactions in (η^4-Cyclo-octa-1,5-diene)(η^6-naphthalene)ruthenium(0): A convenient route to new ruthenium(0) complexes. *J. Chem. Soc., Dalton Trans.* **1984**, 2255−2257.

(40) Pertici, P.; Vitulli, G.; Lazzaroni, R.; Salvadori, P.; Barili, P. L. A simple preparation for (η^6-arene)(η^4-cycloocta-1,5-diene)ruthenium-(0) complexes and their conversion into the corresponding arene-dichlororuthenium(II) complexes. *J. Chem. Soc., Dalton Trans.* **1982**, 1019−1022.

(41) Pertici, P.; Verrazzani, A.; Vitulli, G.; Baldwin, R.; Bennett, M. A. Stoichiometric alkyne cyclotrimerization at a ruthenium center: A new synthetic route to Ru(η^6-arene)(η^4-cycloocta-1,5-diene) complexes. *J. Organomet. Chem.* **1998**, *551*, 37−47.

(42) Pertici, P.; Verrazzani, A.; Pitzalis, E.; Caporusso, A. M.; Vitulli, G. Stoichiometric cyclotrimerization of chiral alkynes at a ruthenium center: Preparation of optically active (η^6-arene)(η^4-cycloocta-1,5-diene)ruthenium(0) complexes. *J. Organomet. Chem.* **2001**, *621*, 246−253.

(43) Porter, L. C.; Bodige, S.; Selnau, H. E., Jr.; Murray, H. H.; McConnachie, J. M. Preparation of new bis(arene)ruthenium(II) complexes. X-ray crystal structures of [(η^6-biphenyl)$_2$Ru][BF_4]$_2$ and the syn and anti isomers of [(η^6-fluorene)$_2$Ru][BF_4]$_2$. *Organometallics* **1995**, *14*, 4222−4227.

(44) Swann, R. T.; Hanson, A. W.; Boekelheide, V. Ruthenium complexes of [2*n*]cyclophanes. A general synthesis of bis(η^6-[2*n*]cyclophane)-ruthenium(II) compounds and related chemistry. *J. Am. Chem. Soc.* **1986**, *108*, 3324−3334.

(45) Govindaswamy, P.; Mobin, S. M.; Thöne, C.; Kollipara, M. R. Syntheses and characterization of η^6-hexamethylbenzeneruthenium-(II)-β-diketone complexes: Their reactions with mono- and bidentate neutral ligands. *J. Organomet. Chem.* **2005**, *690*, 1218−1225.

(46) Brown, R.; Hirst, G. L.; Gallagher, W. M.; McIlwrath, A. J.; Margison, G. P.; van der Zee, A. G.; Anthoney, D. A. hMLH1 expression and cellular responses of ovarian tumour cells to treatment with cytotoxic anticancer agents. *Oncogene* **1997**, *15*, 45−52.

(47) Plumb, J. A.; Strathdee, G.; Sludden, J.; Kaye, S. B.; Brown, R. Reversal of drug resistance in human tumor xenografts by 2′-deoxy-5-azacytidine-induced demethylation of the hMLH1 gene promoter. *Cancer Res.* **2000**, *60*, 6039−6044.

(48) Ren, J.; Jenkins, T. C.; Chaires, J. B. Energetics of DNA intercalation reactions. *Biochemistry* **2000**, *39*, 8439−8447.

(49) He, Q.; Ohndorf, U.-M.; Lippard, S. J. Intercalating residues determine the mode of HMG1 domains A and B binding to cisplatin-modified DNA. *Biochemistry* **2000**, *39*, 14426−14435.

(50) Chen, Y.; Parkinson, J. A.; Guo, Z.; Brown, T.; Sadler, P. J. A new platinum anticancer drug forms a highly stereoselective adduct with duplex DNA. *Angew. Chem., Int. Ed.* **1999**, *38*, 2060−2063.
(51) Reedijk, J. Improved understanding in platinum antitumor chemistry. *Chem. Commun.* **1996**, 801−806.
(52) Yu, E. W.; McDermott, G.; Zgurskaya, H. I.; Nikaido, H.; Koshland, D. E., Jr. Structural basis of multiple drug-binding capacity of the AcrB multidrug efflux pump. *Science* **2003**, *300*, 976−980.
(53) Wang, F.; Habtemariam, A.; Melchart, M.; Fernandez, R.; van der Geer Erwin, P. L.; Aird, R.; Jodrell, D., I.; Sadler, P. J. Unpublished results.
(54) Scolaro, C.; Geldbach, T. J.; Rochat, S.; Dorcier, A.; Gossens, C.; Bergamo, A.; Cocchietto, M.; Tavernelli, I.; Sava, G.; Rothlisberger, U.; Dyson, P. J. Influence of hydrogen-bonding substituents on the cytotoxicity of RAPTA compounds. *Organometallics* **2006**, *25*, 756−765.
(55) Sheldrick, W. S.; Heeb, S. Synthesis and structural characterization of η^6-arene-ruthenium(II) complexes of alanine and guanine derivatives. *Inorg. Chim. Acta* **1990**, *168*, 93−100.
(56) Wang, F.; Xu, J.; Habtemariam, A.; Bella, J.; Sadler, P. J. Competition between glutathione and guanine for a ruthenium(II) arene anticancer complex: Detection of a sulfenato intermediate. *J. Am. Chem. Soc.* **2005**, *127*, 17734−17743.
(57) Carmona, D.; Vega, C.; Lahoz, F. J.; Atencio, R.; Oro, L. A.; Lamata, M. P.; Viguri, F.; San Jose, E. Synthesis and stereochemistry of half-sandwich alkynyl amino acidate complexes of rhodium(III), iridium-(III), and ruthenium(II). *Organometallics* **2000**, *19*, 2273−2280.
(58) Peacock, A. F. A.; Habtemariam, A.; Fernández, R.; Walland, V.; Fabbiani, F. P. A.; Parsons, S.; Aird, R. E.; Jodrell, D. I.; Sadler, P. J. Tuning the reactivity of osmium(II) and ruthenium(II) arene complexes under physiological conditions. *J. Am. Chem. Soc.* **2006**, *128*, 1739−1748.
(59) Wiederholt, K.; McLaughlin, L. W, A 2,2″-bipyridine ligand for incorporation into oligodeoxynucleotides: Synthesis, stability and fluorescence properties of ruthenium−DNA complexes. *Nucleic Acids Research* **1999**, *27*, 2487−2493.
(60) Sheldrick, G. M., University of Göttingen, Germany, 2001−2004.
(61) Blessing, R. H., An empirical correction for absorption anisotropy. *Acta Crystallogr., Sect. A* **1995**, *A51*, 33−38.
(62) Sheldrick, G. M., Programs for crystal structure analysis (Release 97-2); Institüt für Anorganische Chemie der Universität, Tammanstrasse 4, D-3400 Göttingen, Germany, 1998.
(63) Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. Completion and refinement of crystal structures with SIR92. *J. Appl. Crystallogr.* **1993**, *26*, 343−350.
(64) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R., SIR97: A new tool for crystal structure determination and refinement. *J. Appl. Crystallogr.* **1999**, *32*, 115−119.
(65) Beurskens, P. T.; Beurskens, G.; Bosman, W. P.; de Gelder, R.; Garcia-Granda, S.; Gould, R. O.; Israel, R.; Smits, J. M. M. Crystallography Laboratory, University of Nijmegen, The Netherlands, 1996.
(66) Betteridge, P. W.; Carruthers, J. R.; Cooper, R. I.; Prout, K.; Watkin, D. J., CRYSTALS version 12: Software for guided crystal structure analysis. *J. Appl. Crystallogr* **2003**, *36*, 1487.
(67) Nardelli, M., Modeling hydroxyl and water H atoms. *J. Appl. Crystallogr.* **1999**, *32*, 563−571.
(68) Dersnah, D. F.; Baird, M. C. Chiral η^6-C_6H_6 ruthenium complexes. *J. Organomet. Chem.* **1977**, *127*, C55−C58.

JM060596M