Controlling ligand substitution reactions of organometallic complexes: Tuning cancer cell cytotoxicity


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Organometallic compounds offer broad scope for the design of therapeutic agents, but this avenue has yet to be widely explored. A key concept in the design of anticancer complexes is optimization of chemical reactivity to allow facile attack on the target site (e.g., DNA) yet avoid attack on other sites associated with unwanted side effects. Here, we consider how this result can be achieved for monofunctional “piano-stool” ruthenium(II) arene complexes of the type \([\text{arene} \text{Ru}(\text{en})(X)]^+\), where \(X\) is a leaving group (e.g., Cl). In these pseudooctahedral “piano-stool” RuII complexes, a π-bonded arene (the “seat” of the stool) occupies three coordination sites, and the two nitrogens of ethylenediamine (en) and X fill the remaining three sites (the “legs”). Although ruthenium has a rich redox chemistry (9), the presence of an arene greatly stabilizes RuII compared with RuIII (ref. 10 and references therein). These complexes can exhibit cytotoxicity toward cancer cells, including cisplatin-resistant cells (11, 12). In nucleotide adducts, \([(\text{en})\text{Ru}(\text{en})]^{2+}\) exhibits a high selectivity for N7 of guanine and, in contrast to cisplatin, shows little interaction with adenine (13, 14). Reactions of these chloro RuII arene complexes with nucleotides appear to involve initial aquation, and Ru–OH bonds appear to be more reactive than Ru–OH bonds (15), a behavior parallel to that of PtII diammine anticancer complexes (16).

Here, we have studied the effect of both X and the arene on the kinetics and thermodynamics of hydrolysis of \([(\text{en})\text{Ru}(\text{en})\text{Ru}(\text{en})X]^+\) complexes, with X ranging from halide, pseudohalide, and pyridine (py) derivatives to thiocarbamatc (tsc) and with the arene ranging from benzene (bz) to more sterically hindered p-cymene (p-cym) and hexamethylbenzene (hmb) to multiring [indan (ind) and biphenyl (bip)] complexes. In view of the high chloride concentrations in blood plasma and cell culture media, we have also studied reactions with chloride and with guanine [as guanosine 5′-monophosphate (GMP)] as a potential target site on DNA. Density functional computational methods have been used to elucidate the mechanism of the aquation reactions, HPLC and electrospray MS. The x-ray crystal structures of six complexes are available on the PNAS web site.

Conflict of interest statement: University of Edinburgh has submitted patent applications relating to the compounds used in this study for which an exclusive license has been granted to Oncosense Ltd.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: bpy, bipyrene; bz, benzene; dcp, 3,5-dichloropyridine; dfp, 3,5-difluoropyridine; dha, dihydroxanthene; en, ethylenediamine; ESI-MS, electrospray ionization MS; hmb, hexamethylbenzene; ind, indan; pic, picoline (3-methylpyridine); pcp, p-cyanoxyridine; p-cym, p-cymene; py, pyridine; Sph, sphingohexenate; thia, tetrahydroxanthene; UV-Vis, UV-visible.

Data deposition: The atomic coordinates have been deposited in the Cambridge Structural Database, Cambridge Crystallographic Data Centre, Cambridge CB2 1EZ, United Kingdom (CSD reference nos. 288190–288197). The x-ray crystallographic data for complexes 1, 3, 5, 9, 11, and 19 can be found in Data Sets 1–6, which are published as supporting information on the PNAS web site.

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Materials and Methods

Materials. The synthesis and characterization of the ruthenium arene complexes studied here, [(η^6-arene)Ru(en)X][PF_6], arene = hmb, X = Cl (4), Br (5), I (9), N_3 (14), and thiophenolate (SPb) (21), n = 1; X = py (16), 3,5-dichloropyridine (dcp) (17), 3,5-difluoropyridine (dfp) (18), p-cyanopyridine (pcp) (19), and 3-picoline (pic) (20), n = 2; arene = bip, X = Cl (2), Br (6), I (10), and N_3 (15), n = 1; arene = ind, X = Cl (3), Br (7), and I (11); arene = bz, X = Cl (4), Br (8), I (12); arene = p-cym, X = I (13), n = 1, are described in Supporting Text, which is published as supporting information on the PNAS web site. CF_3COOH (TFAH) was purchased from Acros (Geel, Belgium), and GMP disodium salt hydrate was purchased from Aldrich.

UV-Vis Spectroscopy. A PerkinElmer Lambda-16 UV-Vis spectrophotometer was used with 1-cm path-length quartz cuvettes (0.5 ml) and a PTP1 Peltier temperature controller. Spectra were processed by using UVWINLAB software for Windows 95.

HPLC and ESI-MS. The procedures were similar to those we reported in ref. 18 and are described in Supporting Text.

X-Ray Crystallography. Details of the structure determinations of complexes 1, 3, 5, 9, 11, and 19 are provided in Tables 4–7 and Figs. 3–8, which are published as supporting information on the PNAS web site.

Hydrolysis Equilibria. Normalized areas of HPLC peaks detected by UV absorption at wavelengths where the absorption of aqua complexes and their respective parent species presented minimum differences were used to calculate the hydrolysis equilibrium constants (K) according to Eq. 1:

\[ K = \frac{(C_0 A_{aq})^2}{C_0 (1 - A_{aq})}, \]

where C_0 is the initial concentration of the parent complex, and A_{aq} is the normalized area of the HPLC peak for the aqua complex.

Kinetics. Details of the procedures used to determine rate constants for substitution of X by H_2O, Cl, or GMP by UV-Vis spectroscopy are given in Supporting Text.

IC_50 Values. The concentrations of the complexes that caused 50% inhibition of the growth of human ovarian A2780 cancer cells were determined as described in the Supporting Text.
basis I with bz as the arene ligand is a suitable model system, we then explored the effect of varying X, assuming that the same interchange mechanism applies. The results show that the reaction barriers and overall reaction energies for the aquation of the halide (Cl, Br, and I) and pseudohalide N₃ in ([(η⁷-bz)Ru(en)]²⁺ complexes follow the order Br < Cl < I (Table 1).

Next, the anionic X ligand was replaced by a neutral py donor, either py itself or pep, dep, dp, or pic. A similar concerted mechanism was used with entering and leaving groups remaining in the second coordination sphere. The calculated order of hydrolysis rates based on the forward reaction barrier heights is pcp > dep > dp = pep > py, although it should be noted that the barriers for the last four ligands span only ~2 kcal mol⁻¹ (Table 1).

These data suggested that the nature of X could control the hydrolysis of these complexes, and this hypothesis was confirmed by the following experiments.

Synthesis and Characterization of Complexes. The syntheses followed previously described routes (12, 22–24). The x-ray crystal structures of complexes 2, 13, 22, and 23 have already been reported (12, 13); those of the halo complexes [η⁷-hmb)Ru(en)X][PF₆]₂, X = Cl (1), Br (5), I (9), [η⁷-ind)Ru(en)X][PF₆] (X = Cl (3), and I (11), as well as the pcp complex [η⁷-hmb)Ru(en)(pcp)][PF₆] (19), are reported here (details are provided in Supporting Text). All adopt the familiar piano-stool structure (Figs. 3–8).

Hydrolysis Products. Aqueous solutions of [(η⁷-hmb)Ru(en)]²⁺[PF₆]₂, [X = Cl (1), Br (5), I (9), N₃ (14), dcp (17), dp (18), pep (19), and pic (20)] were allowed to equilibrate for 24–48 h at ambient temperature and were then analyzed by HPLC. Two well-separated peaks were observed for each complex corresponding to the aqua adduct and the respective parent cation (Fig. 9), as identified by the subsequent ESI-MS assays (Figs. 10 and 11). As an example, for complex 9 [(η⁷-hmb)Ru(en)][PF₆], the fraction eluting at 3.76 min gave rise to two ion peaks at m/z 323.0 and 437.1, assignable on the basis of mass-to-charge ratios and isotopic models (18) (Fig. 10) to the aqua complex [(η⁷-hmb)Ru(en)(H₂O)]²⁺ (calculated m/z of 323.1 for (η⁷-hmb)Ru(en)(H₂O)²⁺ and its trifluoroacetato (TFA) adduct (calculated m/z of 437.1 for (η⁷-hmb)Ru(en)(TFA)⁻)), respectively. The fraction eluting at 11.32 min gave a peak at m/z ~ 350.8 corresponding to the intact cation of 9 (calculated m/z of 451.0 for ⁹⁺). Analogous products in aqueous equilibrium solutions of complexes 1, 3, 12, 13, 14, 15, 17, 18, 19, and 20 were also identified by ESI-MS analysis of their HPLC fractions (Figs. 11–15).

Hydrolysis Kinetics. Dissolution of compounds 1–21 in 19:1 mixtures of water and methanol at 298 K gave rise to hydrolysis as indicated by the concomitant changes in UV-Vis absorption bands. A typical time evolution for [(η⁷-ind)Ru(en)][PF₆] (11) at 298 K is shown in Fig. 16. The first-order exponential decay of the absorbance of 11 at 270 nm gave a hydrolysis rate constant k₁ of 5.38 × 10⁻⁴ s⁻¹. The hydrolysis rate constants and half-lives for compounds 1–21 are shown in Table 2 and listed in Tables 2, 9, and 10.

The hydrolysis rates vary over several orders of magnitude. Within each group of complexes containing the same coordinated arene, the hydrolysis rate decreases in the order Cl ≈ Br > I. Replacement of chloride (in complex 1 or 2) by the pseudohalide N₃ (to give complexes 14 and 15) has an even greater effect on the hydrolysis rate (Fig. 2 and Table 2), slowing it down by ~40-fold. Changing the leaving group to py, pic, or SPh, as in 16, 20, and 21, slows down hydrolysis dramatically, such that it was too slow to observe. The introduction of a substituted py, however, such as dep in 17 and dpf in 18, led to observable hydrolysis, albeit slower than the corresponding Cl complex by a factor of ~1,400. The hydrolysis rate of the pcp complex 19 was similar to that of the N₃ complex 14.

For complexes containing the same leaving group Cl, the hydrolysis rate decreased with variation of the coordinated arene in the order hmb ≫ tetrahydroanthracene (tha) ~ dihydroanthracene (dha) (bip) > ind > bz > bip, and for I complexes the order was hmb > p-cym > ind > bip > bz (Tables 2 and 9).

Hydrolysis Equilibria. The Cl and Br complexes hydrolyzed to >75% at equilibrium, but the I complexes (9, 12, and 13) were <50% hydrolyzed, the lowest being for the p-cym complex 13 (5%). Similarly, the azide compounds hydrolyzed only to a very small extent, <5% for complexes 14 and 15 (Fig. 2 and Table 2).

Although the dpf, dp, and pcp complexes 17, 18, and 19 hydrolyze only slowly, the extent of their hydrolysis at equilibrium is significant (32–60%). However, no significant aquation (<1%) of the py and pic complexes 16 and 20 was detected by HPLC.

Substitution of I, N₃, dp, py, and SPh by Cl. The possibility that some complexes might be transformed into their chloro analogs in high-chloride (biological) media was investigated. At 310 K, significant changes in the UV-Vis absorption of complexes 13 (p-cym/I), 15 (bip/N₃), 17 (hmb/dcp), and 21 (hmb/SPh) were observed in 104 mM NaCl solution (Figs. 17, 18, 20, and 24). HPLC separations (Figs. 17, 18, 20, and 25) indicated that the majority of the I leaving group in 13 was substituted by Cl, but N₃ in 15 and dpf in 17 were only partially substituted after 24 h and for SPh < 3%. The variation of absorption with time gave rise to the reaction half-lives listed in Table 3. Both UV-Vis spectra and HPLC assays (Fig. 19) showed that no substitution of the py ligand in 16 by Cl occurred within 24 h.

Partition Coefficients and Cell/DNA Uptake. The octanol/water partition coefficients (log Poct) of 1 and 21 were determined to
be \(-1.53 \pm 0.02\) and \(-0.17 \pm 0.02\), respectively. Treatment of A2780 human ovarian cancer cells with 20 \(\mu\)M \(1\) or \(21\) for 24 h gave rise to cell/DNA-bound [Ru] levels of 74 \(\pm\) 3/7.1 \(\pm\) 4.0 and 136 \(\pm\) 5/4.2 \(\pm\) 2.7 pmol per 10\(^6\) cells, respectively.

**Reactions of N\(_3\), py, dcp, and SPh Complexes with GMP.** The possibility that slowly aquating complexes could react directly with guanine was investigated. Reactions of 0.5 mM \(15, 16, 17, \) or \(21\) with 0.5 mM GMP were followed by UV-Vis spectrometry at 298 K. The py complex \(16\) did not react (Fig. 22). Reaction half-lives were determined from absorbance-versus-time plots for \(15, 17, \) and \(21\) and, for comparison, for \(1\) and \(13\), which undergo fast aquation (Figs. 21, 23, 24, and 26 and Table 3).

Positive-ion mass spectra of HPLC fractions (Figs. 21–23 and 25) indicated that the second fractions for reactions of \(15\) and \(17\) (identical to that for \(21\)) with GMP contain GMP adducts, giving rise to singly charged ion peaks at \(m/z = 678.2\) (calculated \(m/z\) of 678.1 for \([(\eta^6\text{-hmb})\text{Ru(en)}(\text{GMP})] + \text{H}^+\)) and 686.3 (calculated \(m/z\) of 686.2 for \([(\eta^6\text{-hmb})\text{Ru(en)}(\text{GMP})] + \text{H}^+\)), respectively. No GMP adduct of \(16\) was detectable (Fig. 22). Hence, it appears that \(N_3\), dcp, and SPh can all be substituted by GMP.

**Cytotoxicity.** The concentrations of complexes that achieved 50% inhibition of the growth of human ovarian A2780 cancer cells (IC\(_{50}\)) values) were determined (Fig. 2 and Table 2). The activity of most of the complexes against this cancer cell line is at least comparable to that of carboplatin (IC\(_{50}\) = 6 \(\mu\)M), and several approach that of cisplatin (IC\(_{50}\) = 0.6 \(\mu\)M). Exceptions are the hmb/py and hmb/pic complexes \(16\) and \(20\) (for which no significant hydrolysis was observed); the former is inactive, and the latter is weakly active. For complexes containing the same leaving group, the cytotoxicity appears to be strongly associatively or dissociatively activated, because the Ru–Cl bond at the transition state extends by 0.81 Å because the Ru–Cl bond at the transition state extends by 0.81 Å.

**Discussion**

**Mechanism of Hydrolysis.** The density functional theory calculations (Fig. 1) suggested that aquation of \([(\eta^6\text{-bz})\text{Ru(en)}]^{2+}\) complexes proceeds via a concerted interchange pathway rather than a stepwise dissociation/coordination process. The reaction does not appear to be strongly associatively or dissociatively activated, because the Ru–Cl bond at the transition state extends by 0.81 Å relative to the reactant species, whereas the Ru–O bond is 0.79 Å longer than in the aqua product (Table 1).

In an interchange associative pathway (\(I_a\)), bond-breaking alone is not rate controlling, and a heavier halide makes crowding of the central Ru atom unfavorable in associative states.

**Table 2. Hydrolysis data for \([(\eta^6\text{-arene})\text{Ru(en)}X]\text{[PF}_6\text{]}\text{n} (n = 1 or 2) complexes at 298 K and cytotoxicity (IC\(_{50}\)) toward A2780 human ovarian cancer cells**

<table>
<thead>
<tr>
<th>Complex (arene)</th>
<th>X</th>
<th>(k_{H2O}, 10^{-3} \text{s}^{-1})</th>
<th>([\text{Ru(H}_2\text{O)}\text{]e, %}]</th>
<th>IC(_{50}), (\mu)M</th>
</tr>
</thead>
<tbody>
<tr>
<td>(18) hmb</td>
<td>dp</td>
<td>0.0208 (\pm) 0.0001</td>
<td>32.6</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>bip</td>
<td>Cl</td>
<td>1.24 (\pm) 0.13</td>
<td>76.2</td>
</tr>
<tr>
<td>6</td>
<td>bip</td>
<td>Br</td>
<td>1.05 (\pm) 0.04</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>bip</td>
<td>I</td>
<td>0.321 (\pm) 0.045</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>bip</td>
<td>N(_3)</td>
<td>0.0315 (\pm) 0.0043</td>
<td>4.5</td>
</tr>
<tr>
<td>3</td>
<td>ind</td>
<td>Cl</td>
<td>2.29 (\pm) 0.14</td>
<td>76.3</td>
</tr>
<tr>
<td>7</td>
<td>ind</td>
<td>Br</td>
<td>2.16 (\pm) 0.05</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>ind</td>
<td>I</td>
<td>0.512 (\pm) 0.046</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>bz</td>
<td>Cl</td>
<td>1.98 (\pm) 0.02</td>
<td>98.1</td>
</tr>
<tr>
<td>8</td>
<td>bz</td>
<td>Br</td>
<td>1.50 (\pm) 0.02</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>bz</td>
<td>I</td>
<td>0.294 (\pm) 0.003</td>
<td>20.5</td>
</tr>
<tr>
<td>13</td>
<td>p-cym</td>
<td>I</td>
<td>0.948 (\pm) 0.008</td>
<td>5.0</td>
</tr>
<tr>
<td>22</td>
<td>dha</td>
<td>Cl</td>
<td>2.23 (\pm) 0.02</td>
<td>80.4*</td>
</tr>
<tr>
<td>23</td>
<td>tha</td>
<td>Cl</td>
<td>2.36 (\pm) 0.02</td>
<td>80.0*</td>
</tr>
</tbody>
</table>

Data for other hmb complexes are provided in Fig. 2. \([\text{Ru(H}_2\text{O)}\text{]e, %}] \text{equilibrium percentage (average of three independent measurements) of aqua complex in 2 mM solution of Cl complex; ND, not determined.}

*In 15 mM NaClO\(_4\) (18).

†Data are from ref. 11.
with a higher coordination number (seven) (25, 26). The effect of halides on the hydrolysis rate of Ru(VI) arene complexes is opposite to that reported for platinum compounds [PtX2(H2O)Cl]2+ (19), for which Br analogs of Cl complexes hydrolyze faster in all three hydrolysis steps (27).

The effective electronegativity of N3 lies between that of Cl and Br (28); however, the hydrolysis rates of N3 complexes 14 and 15 are much lower than those of the Cl and Br analogs. This lower rate may be due to the increased steric bulk of this polyatomic pseudohalide; an Ia substitution pathway is more influenced by steric factors than an I2 (dissociative) pathway (18).

For arene en Ru(VI) halide complexes, the hydrolysis rates decrease with increase in the electron-accepting ability (29) of the arene (hmb < p-cym < tha < dha < indan < bip < bpy), with the exception that aquation of bz complexes is slightly faster than for the bip analogs, perhaps due to sterical hindrance from the pendant phenyl ring being twisted toward X (12, 18). We used 19:1 H2O:MeOH unbuffered solutions for hydrolysis (as well as Cl and GMPII) studies because the aqua adducts have high pKw values (∼8.0) and hydrolysis reduces the pH only slightly [from 7 to ∼6.2 for 2, 22, and 23 (18)]. The calculated reaction barriers and overall reaction energies for the aquation of the halide and azide ([η4-bz](η4-bz)Ru(en))2+ complexes follow the order Br < Cl < I < N3 (Table 1), in agreement with the experimental hydrolysis rates, Cl > Br > I > N3 (Fig. 1), confirming that the higher activation energies are responsible for the slower hydrolysis of the I and N3 complexes. The larger leaving groups py, pic, and SPh in 16, 19, and 21 make the Ru center less accessible to an incoming ligand. However, electron-accepting substituents on the py ring such as Cl, F, or CN, as in 17, 18, and 19, weaken the donor ability of the py nitrogen, as seen by the much lower pKw values of dfp, dcp, and pcp (<2) compared with pic and py (5–6) (30) and lead to an increase in the hydrolysis rate.

Given the different charge states, direct comparisons of calculated activation energies for negative (Cl, Br, I, and N3) and neutral (py and py derivatives) X ligands (Table 1) are problematic. For example, the hydrolysis rate of the hmb/Cl complex 1 is ∼100-fold faster than that of the hmb/pic complex 19, but the forward reaction barrier for 19 is only half that of 1. However, among the py and py-derivative complexes, the calculated order of hydrolysis rates based on the forward reaction barrier heights (cpd⇒dfp⇒pic⇒py) is in agreement with the experimental data. The hydrolysis rate of the hmb/pic complex 19 is ∼12 times faster than that of the hmb/dfp and hmb/dcp compounds 17 and 18, and no hydrolysis was observed for the hmb/py complex 16, although the difference between the barriers for the four complexes is only ∼2 kcal mol−1. An exception is that no hydrolysis was observed for the hmb/pic compound 20 by UV-Vis spectroscopy, although the calculated reaction barrier is the same as that for 18 (half-life of 555 min). However, the very small equilibrium constant (Table 10) and very small change in the UV-Vis absorption made the rate difficult to determine.

Within this family of (arene)Ru(VI) complexes, therefore, the hydrolysis rates are tunable, which is potentially useful in the design of anticancer complexes. Hydrolysis is known to be an important mechanism of activation for the anticancer drug cisplatin (6), which has hydrolysis rate constants of 7.56 × 10−3 and 6.32 × 10−3 s−1 for the first and second chloride ligands, respectively (31), significantly lower than the rate constants of Ru–Cl hydrolysis in these arene complexes. However, replacing Cl by pyridyl ligands can readily effect a 40- to 1,400-fold deceleration. In the case of complexes 17, 18, and 19, these alterations result in compounds with hydrolysis rates comparable to cisplatin. A variety of other py derivatives could be used for fine-tuning.

In ([η4-arene]Ru(VI)(en))2+ complexes, the chelated en and arene ligands also influence hydrolysis rates. Our previous work (18) has shown that en cis to the leaving group (Cl) slows down aquation of Ru(VI) arene (bip, dha, and tha) complexes, just as 2,2′-bipyridine (bpy) slows down substitution of the aqua ligand in ([η4-
than the GMP binding (Table 3) but to a very low extent (~3% under [Cl] = 104 mM). Therefore, 21 may react directly with guanine, especially in the cell nucleus where [Cl] is low (4 mM; ref. 45). This reaction pathway is analogous to that of the second-generation platinum drug carboplatin, which is stable in water and reacts with chloride only very slowly and directly with nucleophiles. It seems likely that 21 is activated by means of oxidation of the bound SPh to the sulfenate or sulfinate by oxygen, analogous to our recent finding for glutathione complexes (40). Glutathione sulfenate is readily displaced from Ru by N7 of guanine.

Conclusions

We have shown that ligand substitution reactions of organometallic Ru\textsuperscript{11} arene complexes can be finely controlled, not only by the choice of the arene but also by the other ligands, in particular a chelated ethylenediamine and a monofunctional leaving group (X). The hydrolysis rates of [{(\textsuperscript{18}F,\text{aren}e)Ru(\text{en})(X)}\textsuperscript{+}] complexes vary over many orders of magnitude, from half-lives of seconds (hmb/Cl) to minutes (e.g., hmb/N\textsubscript{3}) to hours (hmb/dfp, dcp; bip/N\textsubscript{3}) or half-lives that are too slow to be measured (hmb/py/SPh) at 298 K. Density functional theory calculations suggest that aquation occurs via a more associative pathway in a L\textsubscript{2} \textrightarrow{} L\textsubscript{i} mechanistic continuum for which bond-making is of greater importance than bond-breaking. For bz complexes, the calculated reaction barriers and overall reaction energies follow the order Cl \textless{} Br \textless{} I \textless{} N\textsubscript{3} in agreement with experimental data. Hydrolysis provides a pathway for activation. In general, complexes that are readily hydrolyzed are cytotoxic (e.g., hmb/ halide), and those that do not hydrolyze (e.g., hmb/py or pic) are inactive or weakly active.

The Br and I complexes may be produgs for the Cl complex in view of the high concentration of Cl in extracellular biological media (~104 mM), but py in the biologically inactive hmb complex 16 cannot readily be substituted by Cl, and the half-lives for substitution of N\textsubscript{3} and dcp by Cl are longer than those for hydrolysis. For the latter complexes, the hydrolysis activation pathway predominates, as it does for the Cl complexes.

An unexpected exception is the moderately active hmb/SPh complex 21, which undergoes little hydrolysis or reaction with Cl yet reacts with GMP at a similar rate to the hmb/dcp complex. It seems likely that the thiolate ligand in 21 is oxidized to sulfenate or sulfinate, which provides a facile route for displacement by guanine (DNA; ref. 40).

These findings provide a potential strategy for optimizing the anticancer activity of half-sandwich Ru(II) arene complexes whilst minimizing side effects by tuning substitution reactions, a strategy that has previously been successful for Pt anticancer drugs. Wider exploration of the kinetics and thermodynamics of metal–ligand substitution reactions should aid the tuning of the biological activity of many other families of metal complexes and of organometallic complexes in particular.

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