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Multinuclear ruthenium(II) complexes as anticancer agents

Anil K. Gorle, Alaina J. Ammit, Lynne Wallace,* F. Richard Keene* c d e and J. Grant Collins*a

A series of dinuclear ruthenium(II) complexes that contain labile chlorido ligands, \([\text{Ru}(\text{tpy})\text{Cl}]_{2}(\mu-\text{bb})_n\) \((n = 7, 10, 12, 14\) or 16) and derivatives containing nitro substituents on the tpy ligand and/or secondary amines within the \(\text{bb}\) linking chain have been synthesised and their potential as anticancer agents examined. Some of the \(\text{Cl-Rub}_{n}\) species showed good anticancer activity against MCF-7 and MDA-MB-231 breast cancer cell lines, with the \(\text{Cl-Rub}_{12}\) complex being four-times more active than cisplatin. Inclusion of nitro substituents on the tpy ligands of \(\text{Cl-Rub}_{12}\) resulted in significantly decreased anticancer activity. The incorporation of amine groups into the linking ligand did not increase the anticancer activity of the \(\text{Cl-Rub}_{n}\) complexes. The \(\text{Cl-Rub}_{n}\) complexes and those containing amine groups in the linking chain aquated at approximately the same rate, with 50% aquation within 120 minutes. By comparison, the complexes containing nitro substituents on the tpy ligand aquated extremely slowly, with 60% of the chlorido complex remaining 24 hours after they were dissolved in water. Cyclic voltammetry with the model mononuclear complex \([\text{Ru}(\text{NO}_2)\text{tpy}][\text{Me}_2\text{bpy}][\text{Cl}]^+\) \((\text{NO}_2)\text{tpy} = 4,4',4''-\text{trinitro-2',6',2''-terpyridine}) showed that the nitro substituents exerted a strong effect on the ruthenium centre, with the anodic peak corresponding to the \(\text{Ru}(\text{III})\) couple shifted positively by 300 mV compared to that from the non-nitrated parent complex \([\text{Ru}(\text{tpy})][\text{Me}_2\text{bpy}][\text{Cl}]^+\). 1H NMR studies of the reaction of the \(\text{Cl-Rub}_{n}\) complexes with GMP indicated that the ruthenium complexes covalently bound the nucleotide slowly, with 33% bound in 24 hours. However, the results of this study suggest that the cytotoxicity of the dinuclear ruthenium complexes is a combination of covalent and reversible binding with DNA.

Multinuclear platinum complexes, where two or more platinum coordination units are linked by a variety of organic ligand bridges, represent a genuinely new class of anticancer drug. While complexes with bi-functional platinum centres have been reported, those containing mono-functional coordinating spheres on the terminal platinum atoms \((\text{e.g. BBR 3005}, \text{see Fig. 1})\) gave the most encouraging results.8–11 Furthermore, complexes bearing a cationic charge and hydrogen-bonding capacity \((\text{e.g. amine groups or inert am(m)ineplatinum(II) centres})\) in the linking ligand were shown to be the most active in both cisplatin-sensitive and -resistant cell lines.12–20 The trinuclear complex BBR 3464, \(\left[\text{trans-}{\text{PtCl}[\text{NH}_3]_2}\right]_{2}\left[\mu-\text{trans-Pt(NH}_3)_2[H_2N(CH}_2}_6\text{NH}_2]_3\right]\), has undergone Phase II clinical trials,21–23 while dinuclear complexes linked by spermidine (BBR 3571, see Fig. 1) and spermine (BBR 3610 and BBR 3611) are cytotoxic at nanomolar concentrations.2

While the multinuclear platinum complexes are highly cytotoxic, they are also highly toxic.11,23–26 Furthermore, upon administration they bind thiol-containing plasma proteins in the bloodstream, and are subsequently degraded to non-active metabolites. Although BBR 3464 has been withdrawn from
clinical trials, there has been recent interest in “transferring the concept of multinuclearity to ruthenium complexes”. Mendoza-Ferri et al. synthesised a series of dinuclear ruthenium(II)–arene compounds containing a bis(pyridinone)alkane linking ligand that incorporated 3, 6 or 12 methylene groups in the alkane chain. The ruthenium–arene complexes showed good activity in a variety of cancer cell lines, with the activity increasing with the length of the alkane linker, and were more active than a similar mononuclear analogue. In addition, Yamada et al. synthesised [{Ru(bpy)2Cl}2{μ-BL}]2+ complexes {where bpy = 2,2′-bipyridine and BL = 1,6-diaminohexane or 1,12-diaminododecane} and examined their cytotoxicity. While the chlorido complexes showed little activity, replacement of the chlorido ligand by DMSO in the 1,12-diaminododecane-bridged complex resulted in good activity against L1210 cells.

Corral et al. have recently demonstrated that the mononuclear ruthenium(II) complexes [Ru(apy)(tpy)X]+ (where apy = azobis(2-pyridine), tpy = 2,2′:6′,2′-terpyridine and X = a labile ligand such as Cl− or H2O) had good activity against a variety of cancer cell lines, but were significantly less active than cisplatin. In an attempt to increase the activity of mononuclear [Ru(tpy)(L)(Cl)]+ complexes (where L = a non-labile bidentate ligand), we previously synthesised the dinuclear ruthenium complexes [{Ru(tpy)Cl}2{μ-bbbn}]2+ {Cl-Rubb n, see Fig. 2; where bbbn = bis[4(4′-methyl-2,2′-bipyridyl)]-1, n-alkane, for n = 7, 10, 12, and 14}. The Cl-Rubb n complexes showed good activity against the highly sensitive L1210 cell line (IC50 = 5–10 μM) and were ten-times more active than the corresponding mononuclear complex [Ru(tpy)Cl{Me2bpy}Cl] (Me2bpy = 4,4′-dimethyl-2,2′-bipyridine). In this present study we sought to extend the family of Cl-Rubb n dinuclear complexes by using a similar approach to that of Farrell and co-workers for the multinuclear platinum complexes. Consequently, we have synthesised and examined the anticancer activities, rates of hydrolysis, and binding ability to guanosine 5′-monophosphate (GMP) of a series of Cl-RubbN n complexes that contain cationic groups (NH2+) in the chain of the bbbn linking ligand (Cl-RubbN n).
Furthermore, in order to determine the effect of changes in charge distribution (and hence, the rate of ligand exchange) on the ruthenium(II) complexes, we have prepared several Cl-Rubb\(_n\) and Cl-Rubb\(_n\)N\(_n\) complexes that contain three electron-withdrawing NO\(_2\) groups on the tpy ligands (Cl-Rubb\(_n\)NO\(_2\) and Cl-Rubb\(_n\)N\(_n\)NO\(_2\)).

**Results**

**Synthesis**

The synthesis of the mononuclear [Ru(tpy)(bpy)Cl\(^+\)] and the dinuclear complexes [Ru(tpy)Cl\(_2\)](μ-bb\(_n\))\(^2+\) (Cl-Rubb\(_n\) for \(n = 7, 10, 12, 14\) and 16) have been previously reported.\(^{30,31}\) In this study, we have extended the family of dinuclear complexes through the synthesis of Cl-Rubb\(_n\)NO\(_2\), Cl-Rubb\(_n\)N\(_n\), and Cl-Rubb\(_n\)N\(_n\)NO\(_2\) complexes, as shown in Schemes 1–3. For the Cl-Rubb\(_n\) complexes, the procedure used for the synthesis of the Cl-Rubb\(_n\) complexes resulted in poor yield and purity for the Cl-Rubb\(_n\) complexes. To obtain satisfactory yields the bb\(_n\) ligand was dissolved in ethanol–water and heated to 60 °C before the [Ru(tpy)Cl\(_3\)] was added, and then the mixture refluxed for a longer time period than was necessary for the synthesis of Cl-Rubb\(_n\). [Ru({NO\(_2\)}\(_3\)tpy)Cl\(_3\)] was prepared in a similar manner to that previously reported for [Ru(tpy)Cl\(_3\)],\(^{32}\) and upon addition of 4,4′-dimethyl-2,2′-bipyridine yielded [Ru({NO\(_2\)}\(_3\)tpy)(Me\(_2\)bpy)Cl]Cl in good yield. The synthesis of the new chlorido-containing dinuclear complexes Cl-Rubb\(_n\)NO\(_2\) and Cl-Rubb\(_n\)N\(_n\)NO\(_2\) were achieved using similar procedures.

**Cytotoxicity**

The *in vitro* cytotoxicities of the ruthenium complexes and the control platinum complexes cisplatin and carboplatin were determined against the MCF-7 and MDA-MB-231 breast cancer cell lines, and the results are summarised in Table 1. Cisplatin showed moderate cytotoxicity against both cell lines, while carboplatin was essentially inactive. Although IC\(_{50}\) values reported for cisplatin against MCF-7 cells can vary considerably, the results obtained for both control platinum complexes against both cell lines are consistent with previous studies.\(^{29,33–35}\) The dinuclear ruthenium complexes Cl-Rubb\(_n\) for \(n = 10, 12\) and 14 were more active than cisplatin against both cell lines. Interestingly, Cl-Rubb\(_{12}\) was the most active, with the ruthenium complexes having the shortest linking chain (Cl-Rubb\(_7\)) and longest linking chain (Cl-Rubb\(_{16}\)) being the least active. Addition of nitro substituents onto the tpy rings of Cl-Rubb\(_{12}\) and Cl-Rubb\(_{16}\) decreased the activity of the ruthenium complexes, particularly in the case of the highly active Cl-Rubb\(_{12}\). The replacement of two methylene groups by two amine groups in the ligand bridge for Cl-Rubb\(_7\) (giving Cl-Rubb\(_{N7}\)) and Cl-Rubb\(_{16}\) (Cl-Rubb\(_{N16}\)) decreased the activity of the former but had no effect on the latter complex that contained the longer linking chain. However, it was also noted that the replacement of the Me\(_2\)bpy ligand in [Ru({NO\(_2\)}\(_3\)tpy)-(Me\(_2\)bpy)Cl]\(^+\) by the bb\(_{16}\) ligand to form the mononuclear complex Cl-Rubb\(_{N16}\)NO\(_2\)-mono did significantly increase the activity in both cancer cell lines. In the one example examined, the combination of amine groups in the linking ligand and nitro substituents on the tpy ligands for Cl-Rubb\(_{N16}\)NO\(_2\) had little
Aquation and GMP binding

Previous studies with mononuclear ruthenium(II) complexes that contain a chlorido ligand have shown that the first step in the binding to GMP, a simple model for DNA, is aquation. Consistent with previous studies,\textsuperscript{30} aquation of $[\text{Ru(tpy)}\{\text{Me}_2\text{bpy}\}\text{Cl}]^+$ was found to be relatively fast, with 50% of the ruthenium complex being converted to the corresponding aqua form in approximately 60 minutes. Similarly, 50% aquation of each ruthenium centre in the dinuclear complexes $\text{Cl-Rubb}_n$ and $\text{Cl-RubbN}_n$ was shown by $^1\text{H}$ NMR spectroscopy to occur in approximately 120 minutes (see Fig. 3). The aquation then proceeds to equilibrium, where approximately 90% of the ruthenium complex exists in the aqua form. The inclusion of amine groups into the linking ligand had no significant effect on the rate or equilibrium position of aquation.

Fig. 4 shows the $^1\text{H}$ NMR spectrum of $\text{Cl-RubbN}_{16}$ as a function of time after dissolution in $\text{D}_2\text{O}$ and the addition of 2 equivalents of GMP. After 120 minutes, the spectrum of the $\text{Cl-RubbN}_{16}$ is essentially identical to that in the absence of GMP, as shown in Fig. 3, with approximately 50% of the dinuclear complex aquated but with no covalent binding to GMP observed. As evidenced by the increasing intensity of the resonance at 5.36 ppm, assigned to the sugar H1 of GMP bound to a ruthenium centre, the aquated form of $\text{Cl-RubbN}_{16}$ slowly reacts with GMP, reaching an equilibrium of approximately 33% bound in 24 hours. Similar results were obtained with the $\text{Cl-Rubb}_n$ complexes (results not shown).

Table 1  The IC$_{50}$ values of the metal complexes against the MCF-7 and MDA-MB-231 breast cancer cell lines, defined as the concentration (µM) of the complex required to inhibit cell growth by 50%

<table>
<thead>
<tr>
<th>Metal complex</th>
<th>IC$_{50}$ (µM)</th>
<th>MCF-7</th>
<th>MDA-MB-231</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>34 ± 2</td>
<td>31 ± 3</td>
<td>51 ± 8</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>273 ± 7</td>
<td>451 ± 8</td>
<td>51 ± 9</td>
</tr>
<tr>
<td>$\text{Cl-Rubb}_6$</td>
<td>29 ± 4</td>
<td>24 ± 5</td>
<td>45 ± 8</td>
</tr>
<tr>
<td>$\text{Cl-Rubb}_{10}$</td>
<td>8 ± 3</td>
<td>14 ± 3</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>$\text{Cl-Rubb}_{12}$</td>
<td>8 ± 4</td>
<td>9 ± 4</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>$\text{Cl-Rubb}_{14}$</td>
<td>7 ± 4</td>
<td>13 ± 1</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>$\text{Cl-Rubb}_{16}$</td>
<td>27 ± 5</td>
<td>24 ± 6</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>$[\text{Ru}((\text{NO}_2)_3\text{tpy}){\text{Me}_2\text{bpy}}\text{Cl}]^+$</td>
<td>48 ± 4</td>
<td>105 ± 7</td>
<td>105 ± 7</td>
</tr>
<tr>
<td>$\text{Cl-RubbN}_6$</td>
<td>68 ± 3</td>
<td>35 ± 4</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>$\text{Cl-RubbN}_{16}$</td>
<td>27 ± 2</td>
<td>31 ± 4</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>$\text{Cl-Rubb}_{10}\text{NO}_2$</td>
<td>42 ± 5</td>
<td>35 ± 4</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>$\text{Cl-Rubb}_{12}\text{NO}_2$</td>
<td>36 ± 2</td>
<td>32 ± 2</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>$\text{Cl-RubbN}_{16}\text{NO}_2$</td>
<td>31 ± 2</td>
<td>36 ± 2</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>$\text{Cl-RubbN}_{16}\text{NO}_2\text{-mono}$</td>
<td>27 ± 2</td>
<td>26 ± 2</td>
<td>26 ± 2</td>
</tr>
</tbody>
</table>
was dissolved in D₂O. Unlike the corresponding non-nitrated complex [Ru(tpy)(Me₂bpy)Cl]⁺, where >95% of the ruthenium complex was converted into the aqua form well within 24 hours, 60% of the [Ru([NO₂]tpy)(Me₂bpy)Cl]⁺ remained unchanged.
after 24 hours. This indicates that the incorporation of the nitro substituent on the tpy ligand significantly slowed the aquation reaction. Even after 216 hours, 25% of the original \([\text{Ru}\{(\text{NO}_2)_3\text{tpy}\}(\text{Me}_2\text{bpy})\text{Cl}\}]^+\) remained in the chlorido form. Interestingly however, 10% of the \([\text{Ru}\{(\text{NO}_2)_3\text{tpy}\}(\text{Me}_2\text{bpy})\text{Cl}\}]^+\) was rapidly converted into another form after being dissolved. This new complex then appeared to slowly aquate. Based upon the observations of Fallahpour et al.,\textsuperscript{36} it is proposed that one of the three nitro substituents on the tpy ligand is reduced to an amine. This new "(NO\textsubscript{2})\textsubscript{2}(NH\textsubscript{2})-tpy" complex then slowly aquates.

**Cyclic voltammetry of \([\text{Ru}\{(\text{NO}_2)_3\text{tpy}\}(\text{Me}_2\text{bpy})\text{Cl}\}]^+\)**

Electrochemical measurements were carried out on the \([\text{Ru}\{(\text{NO}_2)_3\text{tpy}\}(\text{Me}_2\text{bpy})\text{Cl}\}]^+\) and \([\text{Ru}\{(\text{tpy})\text{Me}_2\text{bpy}\text{Cl}\}]^+\) complexes to assess the electronic effect of the nitro substituents on the ruthenium centre, and the electrode potentials are listed in Table 2.

The electrochemical response of the \([\text{Ru}\{(\text{tpy})\text{Me}_2\text{bpy}\text{Cl}\}]^+\) complex as a hexafluorophosphate salt has previously been investigated;\textsuperscript{37} the results here are consistent with that report: two ligand-based reductions are observed in the cathodic region ([tpy/tpy\textsubscript{0} to −0.4 V followed by Me\textsubscript{2}bpy/Me\textsubscript{2}bpy\textsubscript{0} to −1.5 V]), while the anodic region shows a reversible Ru(II/III) peak at +0.90 V. In the present case, an irreversible peak is also seen at +1.28 V, corresponding to oxidation of the chloride counter-ion. The \([\text{Ru}\{(\text{NO}_2)_3\text{tpy}\}(\text{Me}_2\text{bpy})\text{Cl}\}]^+\) complex shows several important changes compared to the non-nitrated parent complex. Three closely-spaced reductions appear at low potentials in the cathodic region (−0.4 to −0.7 V), followed by further irreversible peaks at more negative potentials. Previous work on the electrochemical behaviour of nitrated bipyridines and their platinum complexes has shown analogous cathodic behaviour: for example \([\text{Pt}\{4,4'-\text{(NO}_2)_2\text{bpy}\}\text{Cl}_2]\) displayed two closely-spaced reductions, and the LUMOs for that complex were shown to be localised largely on the "NO\textsubscript{2}-py" units.\textsuperscript{38} Further reduction of the complex occurred at −1.05 V,\textsuperscript{38} very close to the potential of −1.06 V observed for the first reduction (bpy/bpy\textsubscript{0}) of the non-nitrated complex \([\text{Pt}(\text{bpy})\text{Cl}_2]\) under the same conditions.\textsuperscript{38} Based on these observations, the first three cathodic peaks for \([\text{Ru}\{(\text{NO}_2)_3\text{tpy}\}(\text{Me}_2\text{bpy})\text{Cl}\}]^+\) are assigned here to reductions involving the NO\textsubscript{2}-py moieties. The next two peaks are assigned to further reduction of the (NO\textsubscript{2})\textsubscript{2}tpy ligand and reduction of the Me\textsubscript{2}bpy ligand, probably in that order.

Most importantly, the nitro substituents are observed to exert a strong effect on the ruthenium centre, as the anodic
The results of this study show that the dinuclear ruthenium(n) complexes Cl-Rubb\textsubscript{n} have potential as drugs against breast cancer. The most active complex, Cl-Rubb\textsubscript{12}, was almost four times more active than cisplatin. Furthermore, Cl-Rubb\textsubscript{12} is more active than the mononuclear [Ru(apy)(tpy)Cl\textsuperscript{+}] and dinuclear ([Ru(bpy)\textsubscript{2}Cl\textsubscript{2}({\mu-\textsuperscript{BL}})]\textsuperscript{2+} complexes previously reported by other groups\textsuperscript{,26,29} and of similar activity to the most active dinuclear ruthenium-arene complex linked by a bis(pyridinone)alkane chain reported by Mendoza-Ferri et al.\textsuperscript{27} Interestingly, the Cl-Rubb\textsubscript{n} complexes with the shortest (Cl-Rubb\textsubscript{2}) or the longest linking chain (Cl-Rubb\textsubscript{16}) were the least active against both breast cancer cell lines. Insertion of three nitro substituents onto the tpy ligand of Cl-Rubb\textsubscript{12} significantly decreased the activity against both breast cancer cell lines. Incorporation of amine groups into the linking bridging ligand of Cl-Rubb\textsubscript{7} decreased the activity, whereas it had little effect on the activity of Cl-Rubb\textsubscript{16}.

In previous studies with chlorido-containing dinuclear ruthenium(II) complexes\textsuperscript{,27,28,30,40} the cytotoxicity has always increased as the number of methylene groups in the flexible alkane chain increased. Interestingly, in the present study the Cl-Rubb\textsubscript{16} complex was the least active of the Cl-Rubb\textsubscript{n} complexes. The decreased activities of Cl-Rubb\textsubscript{7} and Cl-Rubb\textsubscript{16}, compared to Cl-Rubb\textsubscript{12} suggest two competing factors govern the anticancer activity. While it is yet to be confirmed, it is assumed that the major mechanism of anticancer activity is related to DNA binding, analogous to the corresponding dinuclear platinum complexes. Increasing the number of methylene groups in the linking chain should increase the lipophilicity of the dinuclear complex, and hence the ease with which it can pass through the cellular membrane. While aquation is the necessary first step in DNA binding, as determined by the GMP binding experiments, all the Cl-Rubb\textsubscript{n} complexes exhibited similar rates of aquation and percentage of the aqua form at equilibrium. Consequently, the relative cytotoxicity results could imply that the range of possible DNA cross-linked adducts formed have significantly different biological outcomes, and/or the anticancer activity is controlled by both covalent and reversible binding to DNA. For the corresponding inert Rubb\textsubscript{n} complexes, the DNA binding affinity decreases with increasing methylene groups in the linking chain.\textsuperscript{43} Furthermore, based purely upon polycation condensation of polyanionic DNA, it would also be expected that the cytotoxicity of the Cl-Rubb\textsubscript{n} complexes would decrease with increasing chain length.

The inclusion of three nitro substituents on the tpy ligand significantly increased the IC\textsubscript{50} value for the more cytotoxic Cl-Rubb\textsubscript{12} but had a relatively small effect with the less cytotoxic Cl-Rubb\textsubscript{16}. It was determined that the [Ru([NO\textsubscript{2})\textsubscript{3}tpy]-([Me\textsubscript{2}bpy]Cl\textsuperscript{+}] complex aquated significantly more slowly than the non-nitrated parent complex [Ru(tpy)(Me\textsubscript{2}bpy)Cl\textsuperscript{+}]. This observation is consistent with the results from the cyclic voltammetry study, from which it was concluded that there was a significant reduction in the electron density on the ruthenium centre, making oxidation to Ru(m) more difficult.

**Discussion**

**Conclusions**

In conclusion, the results of this study support the idea of developing a new class of anticancer agent by transferring from platinum to ruthenium the concept of gaining advantages in efficacy through the use of multinuclear complexes, as proposed by Mendoza-Ferri et al.\textsuperscript{27} Dinuclear ruthenium complexes – containing...
a single chlorido ligand on each metal centre – were synthesised and found to be significantly more active than cisplatin against two breast cancer cell lines. The anticancer activity appears to be due to a combination of covalent and reversible binding with DNA. The IC₅₀ results indicated that the Cl-Rubb₁₂ complex was the most active of the dinuclear complexes. The superior activity of Cl-Rubb₁₂ might be due to the best compromise between lipophilicity (for cellular uptake) and the cytotoxic effects of the covalent adducts formed with DNA. Given the vast array of ligands that can be utilised for the Cl-Rubb₂ complexes, it should be possible to optimise cellular uptake and the kinetics of DNA binding, and thereby produce dinuclear ruthenium(II) complexes with significant clinical potential.

**Experimental**

**Physical measurements**

1D and 2D ¹H NMR spectra were recorded on a Varian Advance 400 MHz spectrometer at room temperature in D₂O (99.9%, Cambridge Isotope Laboratories (CIL)), CDCl₃ (99.8%, CIL), or CD₃CN (>99.8%, Aldrich). Microanalyses were performed by the Microanalytical Unit, Research School of Chemistry, Australian National University, Canberra.

**Materials and methods**

4,4'-Dimethyl-2,2'-bipyridine (Me₂bpy), 2,2':6',2''-terpyridine (tpy), sodium borohydride, phosphorus trichloride, 1,3-diaminopropane, 1,12-diaminopropanol, guanosine 5’-monophosphate disodium salt (GMP), ammonium hexafluorophosphate (NH₄PF₆), potassium hexafluorophosphate (KPF₆) and Amberlite IRA-400 (chloride form) anion-exchange resin were purchased from Aldrich Health Care Bioscience, RuCl₃ supplied; Sephadex (chloride form) and potassium hexafluorophosphate (KPF₆) were obtained from American Elements, SeO₂ was obtained from Ajax Chemicals. The syntheses of ligands bbₙ (n = 7, 10, 12, 14 and 16) and [Ru(tpy)Cl₃]₂ were performed according to reported literature methods.

**Cyclic voltammetry**

Cyclic voltammetry was carried out using an eDAQ EA161 potentiostat operated via an eDAQ ED401 e-corder. A glassy carbon working electrode, platinum wire counter electrode and Ag/AgCl reference electrode were used. HPLC grade acetonitrile was used as solvent and the supporting electrolyte was 0.1 mol L⁻¹ tetra-n-butyl ammonium hexafluorophosphate (Aldrich).

**Cytotoxicity assays**

Cytotoxicity data was obtained using the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT) to formazan as described by Guh et al. Metal complex solutions, including the control platinum complexes cisplatin and carboplatin, were made to the required concentrations in warm Milli-Q water. Growth inhibition assays were carried out over a 72 h continuous exposure period.

**Synthesis of ligands**

**Trinitro-terpyridine**

2,2',6',2''-Terpyridine trioxide. A solution of 2,2',6',2''-terpyridine (4.0 g, 17.1 mmol) in glacial acetic acid (21 mL) and 30% hydrogen peroxide (14 mL) was heated for 2 h at 80 °C after addition of further hydrogen peroxide (14 mL) the temperature was raised to 90 °C and maintained for 18 h. The mixture was then poured into acetone (200 mL). After standing for 4–6 h, the precipitate was filtered and washed with acetone (2 × 40 mL) to obtain 4.2 g of pure product (yield 88%). ¹H NMR (400 MHz, CDCl₃): δ 8.35 (t, J = 9.3 Hz, 2H); 7.81 (d, J = 6.9 Hz, 2H); 7.77 (t, J = 10.4 Hz, 2H); 7.45 (t, J = 14.5 Hz, 1H); 7.36 (m, 4H).

4,4',4''-Trinitro-2,2',6',2''-terpyridine trioxide. Fuming nitric acid (90%, 7.2 mL) was added slowly to a cooled mixture of 2,2',6',2''-terpyridine trioxide (4.2 g, 15.1 mmol), conc. sulfuric acid (15 mL) and fuming sulfuric acid (30%, 3.6 mL) at 0–5 °C. The mixture was then stirred at 100 °C for 1 h and at 120 °C for 4 h. The contents of the flask were then poured into ice water and filtered. The precipitate, after washing first with sodium bicarbonate solution (40 mL) and then with water (40 mL), was dried and crystallised from 50% aqueous pyridine (50 mL) to yield 1.3 g of a light yellow coloured product (yield 21%). ¹H NMR (400 MHz, CDCl₃): δ 8.66 (s, 2H); 8.55 (d, J = 3.0 Hz, 2H); 8.39 (d, J = 7.4 Hz, 2H); 8.25 (dd, J = 2.9 Hz, 3.2 Hz, 2H).

4,4',4''-Trinitro-2,2',6',2''-terpyridine trioxide. A mixture of 4,4',4''-trinitro-2,2',6',2''-terpyridine trioxide (1.3 g) and phosphorus trichloride (15 mL) was refluxed for 18 h under an Ar atmosphere, and the hot solution was then poured on ice and made alkaline with 40% ammonium hydroxide solution. The precipitate was filtered, dried under vacuum, and crystallised from benzene to obtain 0.64 g of the pure product (yield 56%). ¹H NMR (400 MHz, CDCl₃): δ 9.30 (s, 2H); 9.28 (d, J = 2.0 Hz, 2H); 9.08 (d, J = 5.2 Hz, 2H); 8.18 (dd, J = 2.0 Hz, 1.9 Hz, 2H).

**bbₙ ligands**

4-Formyl-4'-methyl-2,2'-bipyridine. 4,4'-Dimethyl 2,2'-bipyridine (2.0 g, 10.8 mmol) and SeO₂ (1.8 g, 16.7 mmol) were refluxed in 1,4-dioxane (45 mL) under a N₂ atmosphere for 24 h. The solution was filtered while hot to remove the solid selenium and the filtrate was evaporated to obtain pale yellow solid. The crude product was dissolved in ethylacetate (150 mL), the undissolved solid was removed by filtration and the filtrate was evaporated to obtain pale yellow solid. The crude product was dissolved in minimal volume of DCM and impregnated with silica gel (230–400 mesh, 5 g) the impregnated mixture was then loaded on a silica gel column (230–400 mesh; 3 cm diam. × 15 cm), the unreacted Me₂bpy was eluted with 5% (v/v) ethyl acetate in n-hexane and the product was eluted using 20–30% (v/v) ethyl acetate in n-hexane. The purity of each fraction was monitored by TLC, using 30% (v/v) ethyl acetate in n-hexane as the mobile phase. The purest fractions were combined and the solvent was evaporated in vacuo to obtain white solid. A final recrystallisation with n-pentane.
gave 0.82 g of the pure product as a white powder (yield 38%).

1H NMR (400 MHz, CDCl3): δ 10.17 (s, 1H); 8.89 (d, J = 5.1 Hz, 1H); 8.85 (s, 1H); 8.57 (d, J = 4.9 Hz, 1H); 8.28 (s, 1H); 7.72 (d, J = 5.0 Hz, 1H); 7.20 (d, J = 4.2 Hz, 1H); 2.46 (s, 3H).

**bbN7.** A mixture of 4-formyl-4'-methyl-2,4'-bipyridine (0.74 g, 3.76 mmol) and the 1,3-diaminopropane (0.16 mL, 1.88 mmol) was stirred in methanol (50 mL) at room temperature under N2 atmosphere for 4 h. Sodium borohydride (0.57 g, 15.07 mmol) was added to the reaction mixture and stirred at 65°C for 1–2 h. The solvent was evaporated from the reaction mixture and water (10 mL) added to the crude residue. The organic component was extracted with dichloromethane (3 x 50 mL), and the organic phase was then washed with water (20 mL) and brine (20 mL). After removing the solvent, the crude residue was purified by column chromatography using silica gel, the unreacted starting material and other impurities were eluted with 1–2% (v/v) MeOH in DCM and the bbN7 was eluted with 5–8% (v/v) MeOH and 0.1% (v/v) triethylamine in DCM. Yield: 0.38 g, 23%. 1H NMR (400 MHz, CDCl3): δ 8.61 (d, J = 5.0 Hz, 2H); 8.52 (d, J = 4.9 Hz, 2H); 8.30 (s, 2H); 8.22 (s, 2H); 7.36 (bs, 2H); 7.13 (d, J = 3.9 Hz, 2H); 3.89 (s, 4H); 2.63 (t, J = 10.9 Hz, 4H); 2.44 (s, 6H); 1.66–1.52 (m, 2H).

**Synthesis of metal complexes**

**[Ru(tpy)Cl2]_2([μ-bb]_n)_2** (Cl-Rubb). The ruthenium(II) complexes Cl-Rubb were synthesised using a slight modification of methods previously described.

**[Ru(NO2)3tpy]Cl2.** 4,4',4'-Trinitro-2,2',6',2'-terpyridine (0.44 g, 1.7 mmol) was stirred in absolute ethanol (220 mL) with gentle heating until dissolution. RuCl3-3H2O (0.63 g, 1.7 mmol) was added and the solution refluxed for 3 h with stirring under nitrogen atmosphere. After the mixture was cooled to room temperature, the violet brown precipitate was filtered, washed with excess of ethanol and ether, and dried under vacuum to yield 0.85 g of the product (yield 59%). 1H NMR (400 MHz, DMSO-d6): δ 9.91 (s, 2H); 8.36 (d, J = 2.5 Hz, 2H); 9.70 (d, J = 6.3 Hz, 2H); 8.30 (dd, J = 2.5 Hz, 2.4 Hz, 2H). 13C NMR (DMSO-d6): δ 160.0, 158.1, 157.3, 154.2, 153.2, 120.8, 117.9, 117.5, 56.4, 19.0.

**[Ru(NO2)3tpy]PF6.** A solution of [Ru(NO2)3tpy]Cl2 (0.10 g, 0.17 mmol) and Me6-ppy (0.032 g, 0.17 mmol) in EtOH/H2O (4:1; 20 mL) was refluxed under an N2 atmosphere for 5 h. After cooling, the solvent mixture was evaporated to approximately half of the original volume and saturated aqueous NH4PF6 was added slowly to precipitate a dark violet-purplish material, which was filtered and washed with ethanol (2 x 15 mL) followed by diethyl ether (2 x 15 mL). The crude product was dissolved in a minimum amount of acetone and loaded onto a column of Sephadex LH-20 (2 cm diam. x 30 cm), and using acetone as the eluent, the major first band was collected and acetone was evaporated to obtain [Ru(NO2)3tpy][Me6-ppy]Cl]PF6 complex as a dark violet-brown material and was crystallised using acetonitrile–toluene. Anal. calc. for [Ru(NO2)3tpy]-

**[Ru(NO2)3tpy]Cl2.** The syntheses of [Ru(NO2)3tpy]Cl2-(n-bb)2Cl2 (n = 12, 16) complexes were adapted from literature methods.30,31 [Ru(NO2)3tpy]Cl2 (70 mg, 0.12 mmol) was dissolved in EtOH/H2O (4:1; 15 mL), the appropriate bb ligand (0.06 mmol) added and the mixture was refluxed under an N2 atmosphere for 5–6 h. After cooling, the solvent from the reaction mixture was evaporated to approximately half of the original volume and then cooled, after which a saturated aqueous NH4PF6 solution was slowly added until no further precipitation occurred. The dark violet-purple precipitate was then filtered and washed with ethanol (2 x 20 mL) followed by diethyl ether (2 x 20 mL). The crude product was dissolved in a minimum amount of acetone and loaded onto a column of Sephadex LH-20 (2 cm diam. x 30 cm); on elution with acetone the major first band collected. The pure [Ru(NO2)3tpy][Cl]2-(n-bb)2[PF6]2 complex was isolated as dark violet-purplish material.
8.07 (m, 8H); 7.92–7.89 (m, 2H); 6.88 (d, J = 5.6 Hz, 2H); 6.81 (m, 2H); 3.07–3.06 (m, 2H); 2.81 (s, 3H); 2.60–2.59 (m, 2H); 2.34 (s, 3H); 1.61–1.08 (m, 20H). 13C NMR (CD3CN): δ 160.8, 159.9, 157.8, 156.6, 155.7, 154.95, 154.4, 152.4, 152.33, 152.29, 152.24, 151.3, 150.9, 129.4, 128.7, 128.2, 127.5, 125.6, 124.9, 124.7, 122.2, 118.9, 118.7, 36.0, 35.3, 31.1, 30.76, 30.73, 30.4, 30.3, 30.19, 30.13, 30.09, 29.99, 29.96, 29.89, 29.72, 29.66, 21.4 and 20.9.

The chloride salts were obtained by stirring the PF6− salt in water using Amberlite IRA-400 (chelate form) anion-exchange resin. The resin was removed by filtration, and the solution was freeze-dried to obtain a fluffy dark purple-brown powder of [Ru(tpy)Cl2(μ-bbH2N6)]Cl4. Yield: 20–25%.

[Ru(NO3)3tpy][Cl]2(μ-bbH2N16)]Cl4. The synthesis of [Ru(NO3)3tpy][Cl]2(μ-bbH2N16)]Cl4 complex was prepared as described for [Ru(tpy)Cl2(μ-bbH2N6)]Cl4. Typical yield ~20%.

1H NMR (400 MHz, CD3CN): δ 9.22–9.11 (m, 1H); 8.84 (m, 1H); 8.53 (m, 1H); 8.38 (m, 1H); 8.23 (m, 1H); 8.17 (m, 1H); 8.06 (m, 1H); 7.86 (m, 1H); 7.66–7.64 (m, 1H); 7.43 (m, 1H); 7.32 (m, 1H); 7.28 (m, 1H); 7.17 (m, 1H); 7.06 (m, 1H); 6.96 (m, 1H); 6.91 (m, 1H); 6.88 (m, 1H); 6.84 (m, 1H); 6.52 (m, 1H); 3.99 (m, 1H); 2.98 (m, 1H); 2.43 (m, 1H); 2.17 (m, 1H); 1.95 (m, 1H); 1.78 (m, 1H); 1.65–1.62 (m, 1H); 1.53 (m, 1H); 1.36 (m, 1H); 1.27 (m, 1H); 1.18 (m, 1H); 1.09 (m, 1H); 0.98 (m, 1H); 0.79 (m, 1H); 0.72 (m, 1H); 0.68 (m, 1H); 0.61 (m, 1H); 0.55 (m, 1H); 0.47 (m, 1H); 0.39 (m, 1H); 0.31 (m, 1H); 0.24 (m, 1H); 0.16 (m, 1H); 0.08 (m, 1H); 0.01 (m, 1H). 13C NMR (CD3CN): δ 160.6, 159.6, 159.5, 158.9, 158.7, 158.4, 157.5, 156.2, 153.6, 153.1, 153.0, 152.9, 152.6, 152.2, 149.9, 149.1, 137.9, 134.7, 134.5, 128.9, 128.2, 128.1, 127.8, 126.8, 125.1, 124.45, 124.42, 124.1, 123.4, 123.3, 51.2, 50.7, 49.5, 49.3, 30.0, 29.9, 29.6, 29.5, 27.3, 27.0, 26.9, 21.4 and 20.9.

The chloride salt was obtained by stirring the PF6− salt in water with Amberlite IRA-400 (chelate form) anion-exchange resin. The resin was removed by filtration, and the solution was freeze-dried to obtain a fluffy dark purple-brown powder of [Ru(tpy)Cl2(μ-bbH2N6)]Cl4. Yield: 20–25%.

References


