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ORIGINAL ARTICLE

Synthesis, nucleic acid binding and cytotoxicity of oligonuclear ruthenium complexes containing labile ligands

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Abstract We report the synthesis, nucleic acid binding and cytotoxicity of the complexes $[Ru(terpy)(Me_2bpy)Cl]^+$, $[Ru(terpy)(phen)Cl]^+$ and dinuclear $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ {where Me_2bpy = 4,4′-dimethyl-2,2′-bipyridine; phen = 1,10-phenanthroline; and bb_n = bis[4(4′-methyl-2,2′-bipyridyl)]-1,n-alkane, with n = 7, 10, 12, 14}. The complexes were isolated from the reaction of the $[Ru(terpy)Cl_3]$ precursor with the respective bidentate and di-bidentate bridging ligands. The time-course UV–Visible spectroscopy of the reaction of the mono- and dinuclear complexes with guanosine 5-monophosphate (GMP) showed the movement of the metal-to-ligand charge transfer (MLCT) band to lower wavelengths, accompanied by a hypochromism effect. The formation of the aqua complex and phosphate-bound intermediates in the reaction were detected by the time-course ^{1}H NMR and ^{31}P NMR experiments, which also demonstrated that the complex bound to the N7 guanine was the major product. The UV–Visible and ^{1}H NMR studies showed no evidence of the interaction of the complexes with both adenosine 5-monophosphate (AMP) and cytidine 5-monophosphate (CMP). Cytotoxicity studies of these complexes against a murine leukemia L1210 cell line revealed that the dinuclear $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ complexes were significantly more cytotoxic than mononuclear $[Ru(terpy)(Me_2bpy)Cl]^+$. The $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_{14})]^{2+}$ complex appeared to be the most active (IC_{50} = 4.2 μM).

Keywords Ruthenium · Polypyridyl · Oligonuclear · DNA binding · Cytotoxicity · Labile ligands

Introduction

The development of metallopharmaceuticals in the treatment of cancers dates back to the mid 1970s when Cisplatin was approved by the US Food and Drug Administration for clinical use. Since then, much attention has been directed to Cisplatin derivatives, primarily driven by the fact that the use of Cisplatin is accompanied by major clinical drawbacks such as acquired and natural drug resistance, and severe side effects on kidney, nervous and hearing systems [1]. Complementary research has focused on the development of ruthenium complexes in an attempt to provide alternatives to the platinum-based anti-cancer therapeutics [2, 3]. In contrast to the square planar geometry of Cisplatin and its analogs, the structure of Ru(II) and Ru(III) complexes features an octahedral geometry which may provide a different type of interaction with the DNA base pairs and proteins in the cell. Ruthenium-based metallopharmaceuticals have a high potential for cancer therapy as they generally have lower toxicity and good selectivity for solid tumor metastases [2, 3]. A number of ruthenium complexes of such as KP1019 {indazolium bis(indazole)tetrachlororuthenate(III)} and NAMI-A {imidazolium *trans*-imidazole(dimethyl sulfoxide)tetrachlororuthenate(III) have entered clinical trials [4]. Other labile ruthenium complexes, such as $[Ru(phen)_2Cl_2]$, [5] and "piano-stool" organometallic complexes of the type

Dedicated to Len Lindoy—colleague, mentor and friend—on the occasion of his 75th birthday

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$[RuCl(\eta^6\text{-arene})(en)]^+$, [6] have been shown to interact with DNA through purine binding and display moderate to high activity for cancer cell inhibition.

The cytotoxicity and DNA binding studies of $[Ru(terpy)(bpy)Cl]^+$, *cis*-$[Ru(bpy)_2Cl_2]$ and $[Ru(terpy)Cl_3]$ (bpy = 2,2′-bipyridine) were reported in 1995 [7]. Very recently, a screening of the anti-proliferation properties of mononuclear $[Ru(terpy)(L)Cl]^+$ (L = derivatives of 2,2′-bipyridine) against fin mesenchymal cells of the zebra fish embryos has been conducted [8, 9].

We are interested in developing new labile oligonuclear ruthenium complexes of the type $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ {bb_n = bis[4(4′-methyl-2,2′-bipyridyl)]-1,n-alkane, with n = 2, 5, 7, 10, 12, 14, 16; Fig. 1; terpy = 2,2′:6′,2″-terpyridine}, following our recent investigation of the corresponding inert oligonuclear $[\{Ru(phen)_2\}_2(\mu\text{-}bb_n)]^{4+}$ ($Rubb_n$; phen = 1,10-phenanthroline) complexes as new anti-cancer and anti-bacterial drug candidates [10–12]. It is of a great interest to study the detailed metal complex-nucleic acid interaction of the labile complexes, particularly in multinuclear derivatives which may exhibit better cellular uptake and cytotoxicity properties compared with the mononuclear analogs. We report the synthesis, nucleic acid binding and cytotoxicity studies of the complexes $[Ru(terpy)(Me_2bpy)Cl]^+$, $[Ru(terpy)(phen)Cl]^+$ and dinuclear $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ (Fig. 2; Me_2bpy = 4,4′-dimethyl-2,2′-bipyridine).

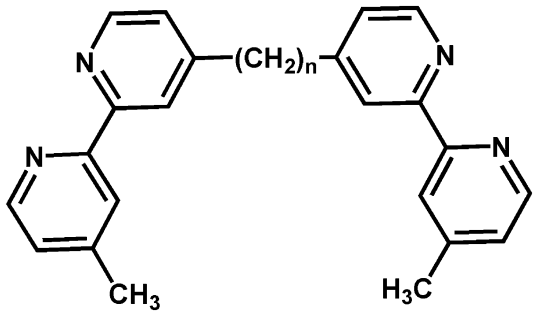

Fig. 1 The structure of bb_n (where n = 2, 5, 7, 10, 12, 14 or 16)

Result and discussion

Synthesis

The synthesis and aquation of mononuclear $[Ru(terpy)(bpy)Cl]^+$ were studied in the late 1960s [13]. While subsequent reports of studies of analogous mononuclear complexes incorporating other types of the bidentate and tridentate ligands have appeared, [8, 9, 14, 15] only very few are focused on the synthesis of dinuclear analogs. These include two recently reported dinuclear complexes with a bridging (rigid) di-bpy ligand of the type tetrapyrido[3,2-α:2′,3′-c:3″,2″-h:2″,3″-j]phenazine (tpphz) [16] and with a bridging di-terpy ligand of the type 4,6-(diterpyridine)dibenzofuran (DTD)

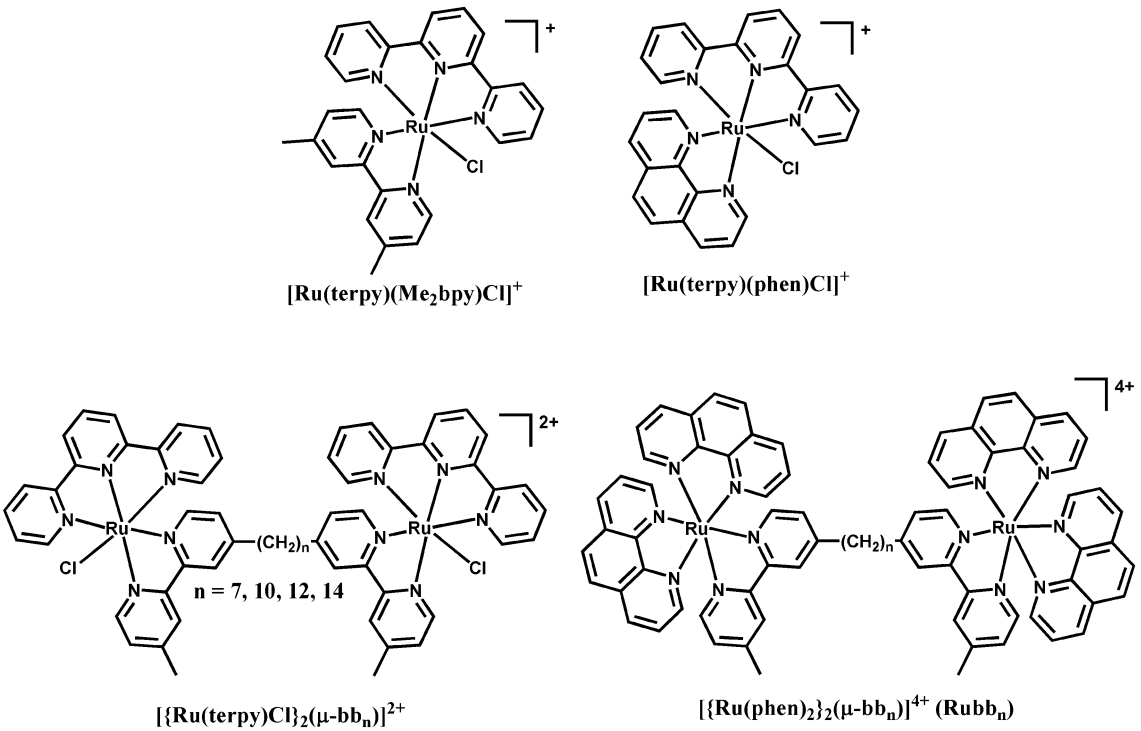

Fig. 2 $[Ru(terpy)(Me_2bpy)Cl]^+$, $[Ru(terpy)(phen)Cl]^+$, $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ and the inert species $Rubb_n$

[17]. In the present work, bb_n ligands were used to produce new flexibly-linked dinuclear complexes $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ in high yields via the previously reported route involving the use of $[Ru(terpy)Cl_3]$ as the precursor [18]. The reaction of Me_2bpy or phen with $[Ru(terpy)Cl_3]$ in ethanol/water (4:1) yielded the corresponding mononuclear complexes $[Ru(terpy)(Me_2bpy)Cl]^+$ and $[Ru(terpy)(phen)Cl]^+$, accompanied by the formation of the respective side products, $[Ru(terpy)(L)(H_2O)]^{2+,}$ which were separated from $[Ru(terpy)(L)Cl]^+$ using exclusion chromatography. Similar

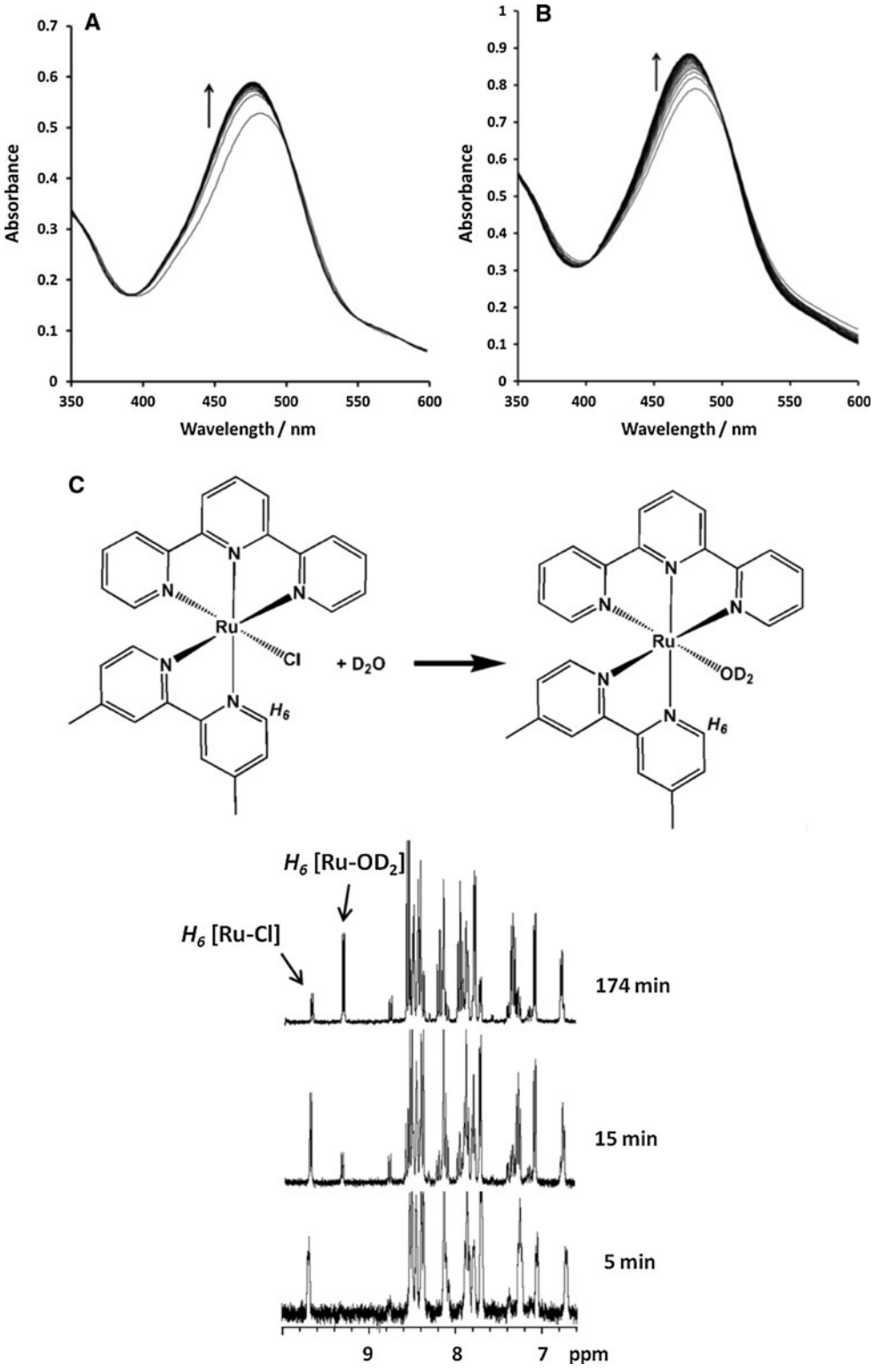

Fig. 3 The UV–Visible spectral changes of: **a** $[Ru(terpy)(Me_2bpy)Cl]Cl$ and **b** $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_7)]^+$ in buffer (Tris $HNO_3/NaNO_3$, pH 7.4) at 37 °C measured every 15 min; the *arrows* indicate the movement of the absorption as the time increases. **c** The ^1H NMR spectral changes for $[Ru(terpy)(Me_2bpy)Cl]^+$ in D_2O at 37 °C, showing the resonances at 9.30 and 9.75 ppm corresponding to the H6 Me_2bpy protons of $[Ru(terpy)(Me_2bpy)(D_2O)]^{2+}$ and $[Ru(terpy)(Me_2bpy)Cl]^+$, respectively

reactions using bb_n ligands afforded dinuclear $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ in much higher yields with almost no aqua complex impurities, presumably due to the greater inertness of these dinuclear complexes in an aqueous environment.

Interaction of the complexes with mononucleotides

The mononuclear complexes $[Ru(terpy)(Me_2bpy)Cl]^+$and $[Ru(terpy)(phen)Cl]^+$, and the dinuclear species $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ aquate readily to form the respective $[Ru(terpy)(Me_2bpy)H_2O]^{2+}$, $[Ru(terpy)(phen)H_2O]^{2+}$ and $[\{Ru(terpy)(H_2O)\}_2(\mu\text{-}bb_n)]^{4+}$ species in water or buffer (pH 7.4) media at 37 °C, as detected by UV–Visible and ^{1}H NMR spectroscopy. The UV–Visible spectral changes show a shift in the metal-to-ligand charge transfer (MLCT) band to shorter wavelengths accompanied by an increase in the absorbance (Fig. 3a, b). A ^{1}H NMR kinetic study of $[Ru(terpy)(Me_2bpy)Cl]^+$ in D_2O at 37 °C shows the emergence of the proton resonance at 9.30 ppm and the gradual disappearance of the proton resonance at 9.75 ppm, which were assigned as the H6 (Me_2bpy) of the $[Ru(terpy)(Me_2bpy)(D_2O)]^{2+}$ and that of $[Ru(terpy)(Me_2bpy)Cl]^+$, respectively (Fig. 3c). Both the time-course UV–Visible and ^{1}H NMR data of the aquation of the mononuclear complexes are consistent with the previous result reported by Wasylenko et al. [14].

The reaction of $[Ru(terpy)(Me_2bpy)Cl]^+$ and $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ with guanosine 5′-monophosphate (5′-GMP), followed by UV–Visible spectroscopy, shows the decrease in the absorption intensity (hypochromism), in contrast to that for the similar experiment reported for the dinuclear ruthenium complexes $[\{Ru(bpy)_2Cl\}_2(L)]^{2+}$ (L = diaminoalkyl ligands) which showed a hyperchromism effect [19]. The formation of the adducts from the reaction of $[Ru(terpy)(Me_2bpy)Cl]^+$ and $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ with 5′-GMP is also accompanied by the movement of the absorption maxima to lower wavelengths with a noticeable shoulder at 420 nm (Fig. 4).

The binding sites for the interactions between $[Ru(terpy)(Me_2bpy)Cl]^+$, $[Ru(terpy)(phen)Cl]^+$, and $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ and 5′-GMP were determined by time-course ^{1}H NMR and ^{31}P NMR experiments. The new resonances at 9.30 and 8.90 ppm appear within 15 min of the digestion of

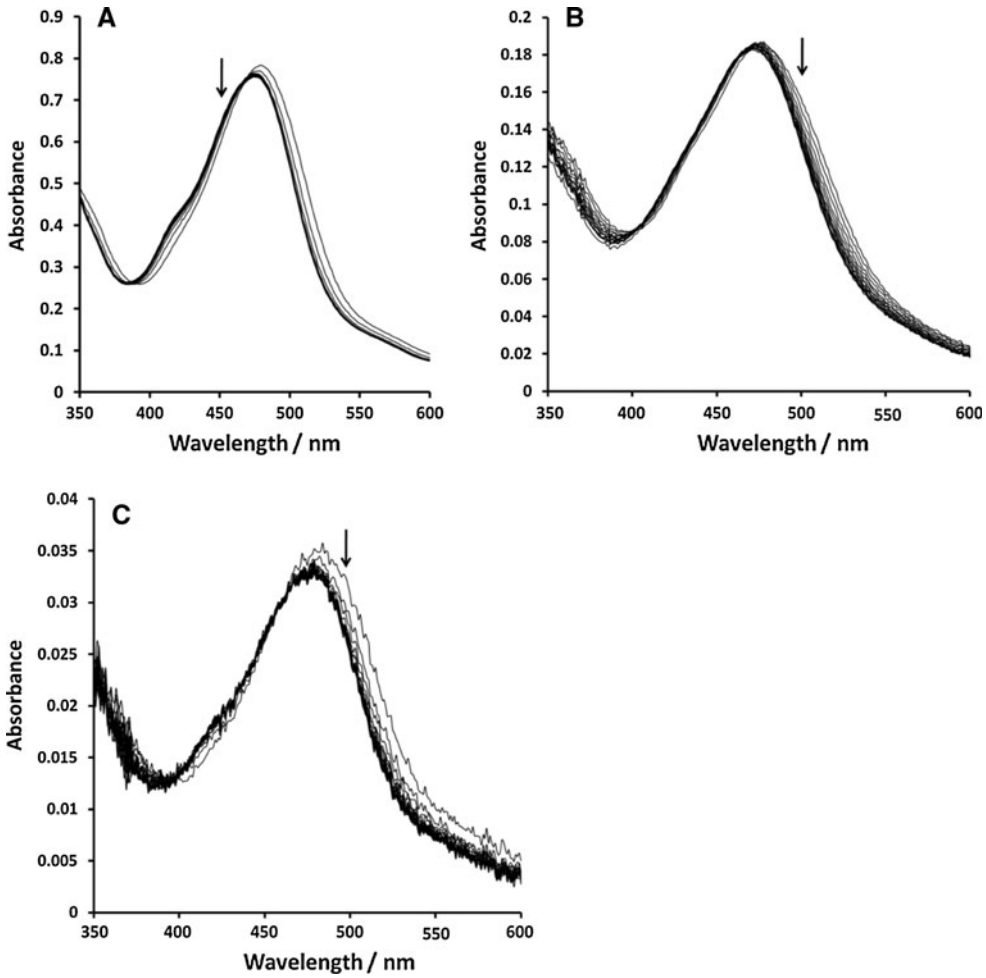

Fig. 4 The UV–Visible spectral changes of the reaction of: **a** $[Ru(terpy)(Me_2bpy)Cl]^+$, **b** $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_7)]^+$ and **c** $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_{14})]^{2+}$ with GMP in buffer (Tris $HNO_3/NaNO_3$, pH 7.4) at 37 °C measured every 15 min. The arrows indicate the movement of the absorption as the time increases. The gradual formation of the shoulder at 420 nm is apparent

$[Ru(terpy)(Me_2bpy)Cl]^+$ and 5′-GMP in D_2O at 37 °C. The resonance at 9.30 ppm is typical for the H6-Me_2bpy of $[Ru(terpy)(Me_2bpy)(H_2O)]^{2+}$ as previously noted in the aquation of $[Ru(terpy)(Me_2bpy)Cl]^+$, while the peak at 8.90 ppm is assigned to the H6-Me_2bpy belonging to the N7(guanine)-bound complex-GMP adduct. The binding through the N7 site is evident from the appearance of the new broad H8 peak at 8.60 ppm which is shifted to higher frequency compared to the H8 of the free GMP (8.20 ppm). The new resonance at 5.50 ppm also appeared corresponding to the new H1′ of the sugar phosphate of the formed adducts (Fig. 5A). A similar trend in the chemical shifts changes was also observed for the dinuclear $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_7)]^{2+}$ complex, although the spectrum was less clear due to the poor solubility of the complex in D_2O. For $[Ru(terpy)(phen)Cl]^+$, the formation of the new broad H8 peak was also observed at 8.60 ppm, although it coincides with the phen protons. The N7 binding site for these three complexes is consistent with the ^1H NMR spectral changes in the interaction of $[\{Ru(bpy)_2Cl\}_2(L)]^{2+}$ (L = diaminoalkyl ligands) and $[RuCl(\eta^6\text{-Bip})(en)]^+$ (Bip = biphenyl; en = ethylenediamine) with 5′-GMP reported by Nakabayashi

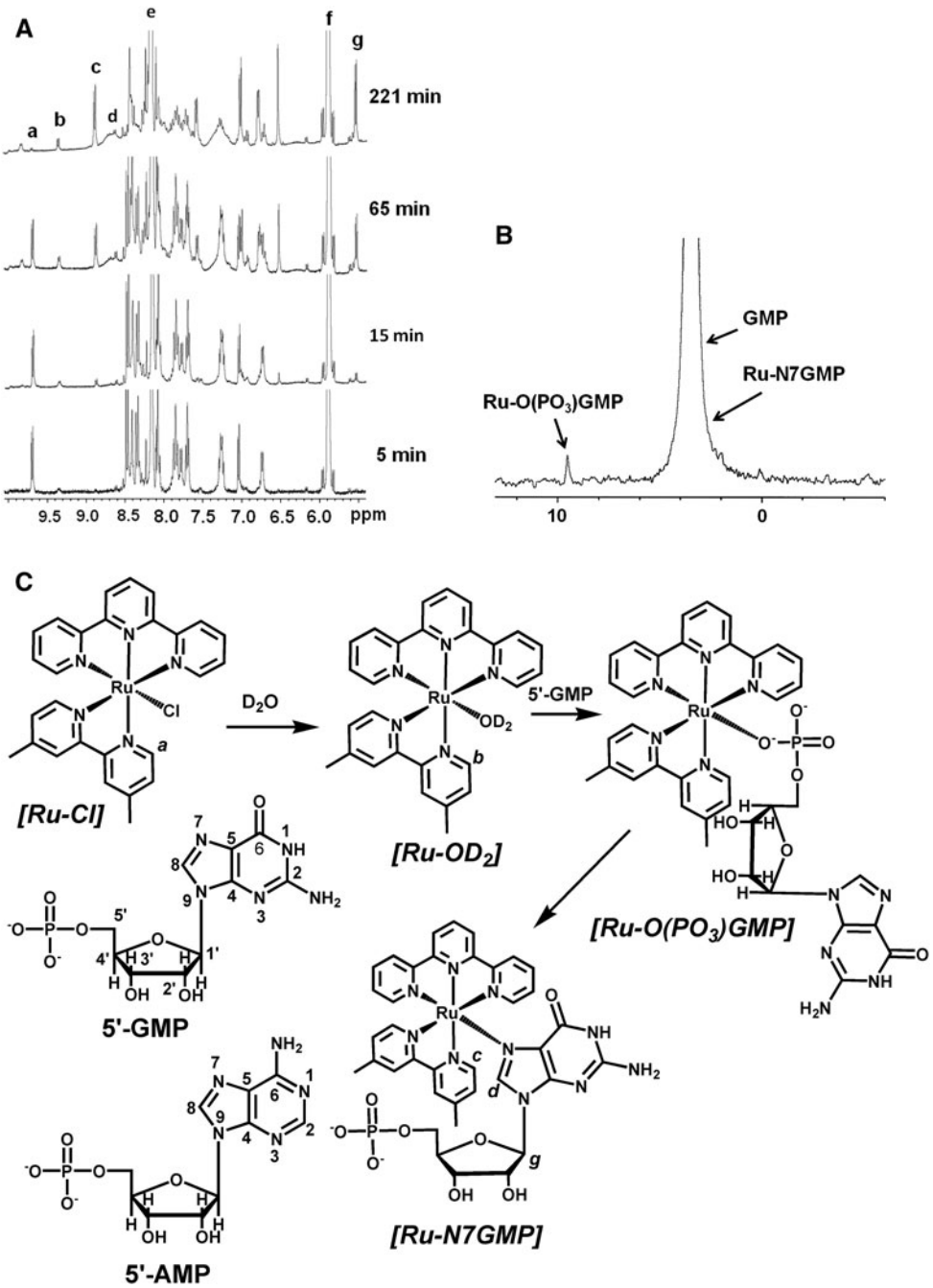

Fig. 5 **A** The time-course ^1H NMR experiment of the reaction between $[Ru(terpy)(Me_2bpy)Cl]^+$ and 5′-GMP in D_2O (1:3 ratio) at 37 °C, showing the gradual disappearance of H6-Me_2bpy of $[Ru(terpy)(Me_2bpy)Cl]^+$ (*a*); the appearance of H6-Me_2bpy of $[Ru(terpy)(Me_2bpy)D_2O]^{2+}$ (*b*); the appearances of H6-Me_2bpy (*c*), H8-guanine (*d*), and H1′ of the sugar phosphate of the $[Ru(terpy)(Me_2bpy)GMP]$ adduct (*g*); H8-guanine (*e*), and H1′ of the sugar phosphate of the free 5′-GMP (f). **B** The ^{31}P NMR spectrum of the $[Ru(terpy)(Me_2bpy)(5'\text{-}GMP)]$ reaction mixture, measured after 20 h of the reaction; the Ru–$O(PO_3)$GMP peak was detected at 9.50 ppm, while the Ru-N7GMP peak coincides with the free GMP peak. **C** The proposed reaction route to the formation of Ru-N7GMP; the diagram of 5′-GMP and 5′-AMP with the atomic numbering is given

et al. [19] and Sadler et al. [20], respectively. It is generally known that the N7 is the preferred binding site for coordination with a metal center because of its better nucleophilic properties and additional bonding (or non-bonding) effects influenced by other sites such as O=C6 and and C2–NH_2 which gives rise to the stabilized coordination system [21]. In addition to the coordination sites at the guanine moiety, Sadler et al. also reported that the organometallic complex $[RuCl(\eta^6\text{-Bip})(en)]^+$ also binds 5′-GMP through the oxygen atom of the phosphate group [20]. The Ru–O(PO_3)GMP adduct was identified as an intermediate in a ^{31}P NMR experiment before being converted to the final Ru–N7GMP product [20]. In the present work, a ^{31}P NMR experiment carried out for the $[Ru(terpy)(Me_2bpy)Cl]^+$-GMP reaction reveals a minor peak at 9.50 ppm corresponding to the Ru–O(PO_3)GMP intermediate product (Fig. 5B), consistent with that reported for the $[RuCl(\eta^6\text{-Bip})(en)]^+$-GMP reaction. This suggests that the reactions of $[Ru(terpy)(Me_2bpy)Cl]^+$, $[Ru(terpy)(phen)Cl]^+$, and $[\{Ru(terpy)Cl\}_2(\mu\text{-bb}_n)]^{2+}$ with 5′-GMP proceed via aquation, followed by the formation of Ru–O(PO_3)GMP which is unstable and is converted subsequently to Ru–N7GMP as described in Fig. 5C.

The final adducts proved to be stable and were successfully purified using exclusion chromatography. The ^1H NMR spectrum of the purified [Ru(terpy)(Me_2bpy)(5′-GMP)] adduct recorded at 37 °C reveals two sets of H1′ resonances at 5.75 (minor) and 5.47 ppm (major) of the sugar phosphate, with a broad GMP H8 signal which splits into two when the spectrum is measured at lower temperature (Fig. 6). The final product contains at least two different Ru-GMP adducts, one of which was identified as Ru–N7GMP, while the minor products may be formed with

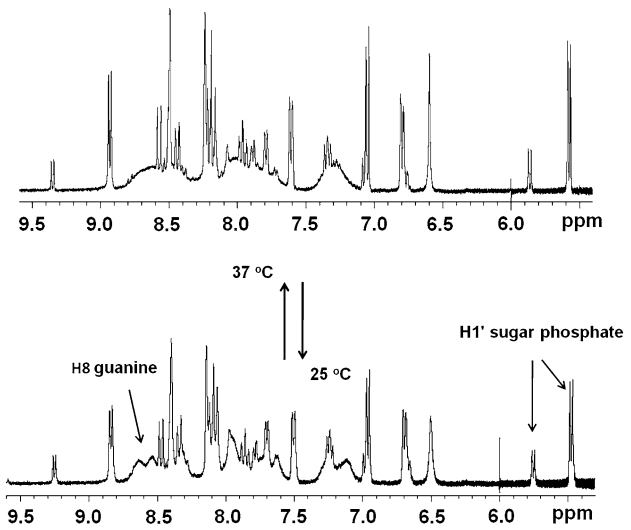

Fig. 6 The ^1H NMR spectra of the purified [Ru(terpy)(Me_2bpy)-(5′-GMP)] adduct measured at 25 and 37 °C showing the exchange of the H8 guanine protons

other sites such as N3 and C2–NH_2. There was no evidence of the presence of Ru–O(PO_3)GMP in the purified product as the ^{31}P NMR data shows the absence of the peak resonance at 9.50 ppm.

The time-course ^1H NMR experiment conducted for the interaction between $[Ru(terpy)(Me_2bpy)Cl]^+$ and adenosine 5′-monophsphate (5′-AMP) shows the absence of new H8 and H1′ signals. The complex does not react with 5′-AMP in D_2O media at 37 °C, although the replacement of the chloro ligand by D_2O takes place as indicated by the progressive appearance of the H6–Me_2bpy resonance of the $[Ru(terpy)(Me_2bpy)(D_2O)]^{2+}$ species. The UV–Visible spectral changes of the reaction of $[Ru(terpy)(Me_2bpy)Cl]^+$ with 5′-AMP and cytidine 5′-monophosphate (5′-CMP) reveal an aquation-only process, as shown from the increasing absorption over time and the absence of the shoulder at 420 nm. The preferred binding of labile ruthenium complexes to 5′-GMP over other mononucleotides has been reported for the dinuclear $[\{Ru(bpy)_2Cl\}_2(L)]^{2+}$ (L = diaminoalkyl ligands) [19] and mononuclear $[RuCl(\eta^6\text{-arene})(en)]^+$ complexes, [20] although the latter was found to have a binding ability to the phosphate and the N3 moieties of the AMP and TMP. Lippard et al. have reported that the DNA intrastrand cross-linking properties of Cisplatin is primarily driven by its ability to specifically target the N7 guanine [22]. A computational study revealed that the monoaqua species of Cisplatin forms a stable transitional state with guanine through a strong hydrogen bond between the ammine-hydrogen of Cisplatin and the O=C6 guanine. On the other hand, the interaction with adenine is less preferred because the hydrogen bond between the ammine ligand of the Cisplatin and the C6–H_2N is weak [23]. For the dinuclear complex $[\{Ru(bpy)_2Cl\}_2(L)]^{2+}$[16] (where L is the bridging diaminoalkyl ligand), the preferred N7 guanine binding was also predicted to result from a stable hydrogen bond between the ammine moiety of the bridging ligand and O=C6. The kinetic studies of a series of mononuclear $[RuCl(\eta^6\text{-arene})(en)]^+$ complexes suggested that the N7 binding is favored due to the enhanced arene-purine hydrophobic interactions [20]. In the present work, the N7 guanine binding of $[Ru(terpy)(Me_2bpy)Cl]^+$, $[Ru(terpy)(phen)Cl]^+$ and $[\{Ru(terpy)Cl\}_2(\mu\text{-bb}_n)]^{2+}$ presumably arises from the stable purine-terpy or purine-bpy/phen hydrophobic interaction, which is less favored in the Ru–N7 adenine system because of the more repulsive C6–NH_2 moiety pointing orthogonally towards the π clouds of the terpy or bpy ligands.

Cytotoxicity studies

The in vitro cytotoxicities of $[Ru(terpy)(Me_2bpy)Cl]^+$ and $[\{Ru(terpy)Cl\}_2(\mu\text{-bb}_n)]^{2+}$ (n = 7, 10, 12, 14) were determined against the murine leukemia L1210 cell line,

Table 1 The IC_{50} of the complexes in the L1210 murine leukemia cancer cell line, defined as the concentration (μM) of the complex required to inhibit cell growth by 50%

Complex	IC_{50}
$[Ru(terpy)(Me_2bpy)Cl]Cl$	50
Δ-$[Ru(phen)_2(Me_2bpy)]Cl_2$ [10]	>200
$[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_7)]Cl_2$	9.9
$\Delta\Delta$-$Rubb_7$ [24]	82
$[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_{10})]Cl_2$	7.2
$\Delta\Delta$-$Rubb_{10}$ [24]	42
$[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_{12})]Cl_2$	7.2
$\Delta\Delta$-$Rubb_{12}$ [24]	18
$[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_{14})]Cl_2$	4.2
$\Delta\Delta$-$Rubb_{16}$ [24]	5

and compared to the values previously reported for the corresponding inert ruthenium complexes $Rubb_n$. As shown in Table 1, the inert complex $[Ru(phen)_2(Me_2bpy)]^{2+}$ is inactive, but the inclusion of one labile chloro ligand, $[Ru(terpy)(Me_2bpy)Cl]^{+}$, results in a moderately cytotoxic complex. Of greater significance, the dinuclear ruthenium complexes containing one chloro group on each metal center displayed good activity, with $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_{14})]^{2+}$ having an IC_{50} of 4 μM. For the dinuclear complexes, the inclusion of a labile ligand on each ruthenium greatly increased the cytotoxicity, with the effect most noticeable for $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_7)]^{2+}$ and the corresponding inert complex. While the cytotoxicity of the inert complexes was strongly affected by the lipophilicity, [10–12, 24] the activity of the ruthenium complexes containing labile ligands was only marginally affected by lipophilicity.

The cytotoxicity results obtained for the dinuclear complexes $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ indicate the potential of this class of compound as anti-cancer agents. The cytoxicity for the n = 14 complex is similar to the prototype dinuclear platinum complex *trans*-$[\{PtCl(NH_3)_2\}_2(\mu\text{-}NH_2(CH_2)_6NH_2)]^{2+}$(called BBR3005, IC_{50} = 3 μM against L1210 cells) developed by Farrell and co-workers [25]. The mechanism of anti-cancer activity for the $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ complexes is yet to be established. The corresponding inert ruthenium complexes selectively accumulate in the mitochondria of the L1210 cells and induce cell death via apoptosis [12]. However, the dinuclear platinum complexes developed by Farrell and co-workers are cytotoxic due to their covalent binding to DNA, and in a manner that is dissimilar to that of cisplatin [25]. The results of the reaction of the $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ complexes with GMP indicates that the ruthenium complexes are capable of covalently binding to DNA, and with similar reaction times to BBR3005 ($t_{1/2}$ for DNA binding for BBR3005 is 200–300 min, compared to 80–100 min for $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$.[1] Furthermore, like BBR3005, $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ complexes can also form long-range inter- and intra-strand DNA adducts. Although the study of the anti-cancer properties of the $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_n)]^{2+}$ complexes is only in the preliminary stages, it is interesting to consider the potential differences between these complexes and the corresponding dinuclear platinum complexes like BRR3005. It has been established that the pre-covalent binding association of the platinum complexes affects the type of covalent adduct that is finally formed. Similarly, should it be established that the dinuclear ruthenium complexes described in this study exert their biological activity through covalent binding to DNA, it might be possible to use the greater diversity in shape and size afforded by the octahedral geometry to control the site of covalent adduct formation on DNA. For example, the corresponding inert complexes have shown a binding preference for non-duplex DNA structures, such as single base bulges [10, 26].

Experimental

Physical measurements

^1H NMR and ^{31}P NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer (JCU) and a Varian Unityplus 400 MHz spectrometer (UNSW@ADFA) using D_2O {99.9%, Cambridge Isotope Laboratories(CIL)}, $CDCl_3$ (99.8%, CIL), or CD_3CN (>99.8%, Aldrich). For the time-course NMR experiments, all the measurements were carried out in pure D_2O at 37 °C. The typical ratio between the complex and the mononucleotides was 1:3. The spectra were measured every 15 min, except when prolonged data acquisition was needed to obtain better quality spectra. The time-course UV–Visible spectroscopy was performed using a Varian Cary 50 Bio UV–Visible Spectrophotometer, equipped with a temperature-controlled cell compartment. The measurements were carried out in a 1 cm quartz cell at 37 °C. Both the complexes (40 μM) and the mononucleotides (0.4 mM) solutions were prepared in 5 mM Tris-HNO_3/50 mM $NaNO_3$ (pH 7.4). Due to the limited solubility in the buffer, $[Ru(terpy)(Me_2bpy)Cl]^{+}$ and

[1] The estimated $t_{1/2}$ values for the $[Ru(terpy)(Me_2bpy)Cl]Cl$-GMP, $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_7)]$-GMP and $[\{Ru(terpy)Cl\}_2(\mu\text{-}bb_{12})]$-GMP reactions were 25, 80 and 92 mins, respectively, calculated on the basis of the changes in the absorbance over time at 500 nm. With the excess amount of the mononucleotides used, it was assumed that there was only a small change in the concentration of the mononucleotides such that the reaction followed pseudo-first-order kinetics. The detailed investigation of the reaction mechanism and kinetics is currently underway.

$[\{Ru(terpy)(Cl)\}_2(\mu\text{-}bb_{12})]^{2+}$ solutions were prepared in distilled water at lower concentration (10 μM) prior to mixing them with the mononucleotides.

Materials and methods

4,4′-Dimethyl 2,2′-bipyridine (Me_2bpy), 1,10-phenanthroline (phen), terpyridine (terpy), guanosine 5′-monophosphate disodium salt (5′-GMP), adenosine 5′-monophosphate disodium salt (5′-AMP), cytidine 5′-monophosphate disodium salt (5′-CMP), ammonium hexafluorophosphate (NH_4PF_6), potassium hexafluorophosphate (KPF_6) and Amberlite® IRA-400 (chloride form) anion-exchange resin were purchased from Aldrich and used as supplied. Sephadex® LH-20 was obtained from Amersham Pharmacia Biotech. The syntheses of ligands bb_n (n = 7, 10, 12 and 14) were performed according the reported procedures [10, 27]. The precursor $[Ru(terpy)Cl_3]$ was synthesized according to the method reported earlier [18].

Synthesis of complexes

The synthesis of mononuclear [Ru(terpy)(L)Cl]Cl (L = phen and Me_2bpy) and dinuclear $[\{Ru(terpy)(Cl)\}_2(\mu\text{-}bb_n)]Cl_2$ (n = 7, 10, 12 and 14) complexes were performed according to the previously reported methods with a modified purification procedure [18]. A typical procedure was as follows. Solid $[Ru(terpy)Cl_3]$ (0.20 g, 0.45 mmol) and appropriate ligands (0.45 mmol for Me_2bpy and phen and 0.23 mmol for bb_n) were refluxed in $EtOH/H_2O$ (4:1; 40 ml) for 4 h. After cooling, the solvent mixture was evaporated to approximately half of the original volume and excess NH_4PF_6 was added causing the precipitation of the dark brown-purple material, which was filtered and washed with cold ethanol followed by diethyl ether. The crude product was dissolved in acetone and loaded onto a Sephadex LH-20 exclusion column, and separated using acetone as the eluent. The pure mononuclear $[Ru(terpy)(Me_2bpy)Cl](PF_6)$, $[Ru(terpy)(phen)Cl](PF_6)$ and dinuclear $[\{Ru(terpy)(Cl)\}_2(\mu\text{-}bb_n)](PF_6)_2$ complexes were isolated as dark purple materials. For mononuclear complexes, the PF_6^- forms were converted to the chloride salts by dissolving the solid in a minimum amount of acetone which was then added to a saturated solution of tetraethylammonium chloride in acetone followed by stirring for 30 min. The precipitates were filtered and washed with cold acetone and diethyl ether and dried under vacuum to afford [Ru(terpy)(L)Cl]Cl. For dinuclear complexes, the chloride salts were obtained by stirring the solid in an aqueous solution using Amberlite® IRA-400 (chloride form) anion-exchange resin. The resin was removed by filtration, and the dark purple solution was freeze-dried to obtain a fluffy dark red purple powder of $[\{Ru(terpy)(Cl)\}_2(\mu\text{-}bb_n)]Cl_2$. Typical yields after conversion: 30–60%. A separation of any possible geometric isomers of $[\{Ru(terpy)(Cl)\}_2(\mu\text{-}bb_n)]^{2+}$ was not attempted. *$[Ru(terpy)(Me_2bpy)Cl]PF_6 \cdot H_2O$*: Anal. Found C, 45.6; H, 3.62: N, 9.1%. Calcd. for $C_{27}H_{25}N_5F_6OPRu$: C, 45.2; H, 3.51; N, 9.8% ^{1}H NMR (300 MHz, CD_3CN) δ 10.05 (1H, d, $J = 6.0$ Hz); 8.51 (d, $J = 8.4$ Hz); 8.41 (d $J = 7.8$ Hz); 8.21 (s); 8.10 (t); 7.91 (t); 7.72 (d, $J = 5.4$ Hz); 7.31 (t); 7.12 (d, $J = 6.0$); 6.82 (d, $J = 5.1$ Hz); 2.79 (s, $-CH_3$); 2.36 (s, $-CH_3$). *$[\{Ru(terpy)(Cl)\}_2(\mu\text{-}bb_7)](PF_6)_2 \cdot 3H_2O$*: Anal. Found C, 46.8; H, 3.63: N, 8.7%. Calcd. for $C_{59}H_{60}N_{10}F_{12}O_3P_2Ru_2$: C, 46.6; H, 3.98; N, 9.2% ^{1}H NMR (300 MHz, CD_3CN) δ 10.04 (s); 8.61 (s); 8.49 (m); 8.40 (d, $J = 7.2$ Hz); 8.31 (d, $J = 7.2$ Hz); 8.20 (d, $J = 11.7$ Hz); 8.10 (t); 7.90 (t); 7.81 (s); 7.70 (s); 7.55 (s); 7.30 (s); 7.12 (t); 6.81 (d, 5.1 Hz); 3.04 (t); 2.84 (t); 2.78 (s); 2.77–2.55 (m); 1.76 (s); 1.53 (s); 1.37–1.22 (m). *$[\{Ru(terpy)(Cl)\}_2(\mu\text{-}bb_{10})](PF_6)_2 \bullet 3acetone$*: Anal. Found C, 50.7; H, 4.49: N, 8.4%. Calcd. for $C_{71}H_{78}N_{10}F_{12}O_3P_2Ru_2$: C, 50.7; H, 4.67; N, 8.3% ^{1}H NMR (300 MHz, CD_3CN) δ 10.04 (s); 8.64 (s); 8.51 (d, $J = 8.1$ Hz); 8.40 (d, $J = 7.8$ Hz); 8.33 (m); 8.22 (m); 8.10 (t); 7.91 (t); 7.81 (s); 7.71 (s); 7.60 (s); 7.30 (s); 7.12 (t); 6.80 (s); 3.04 (t); 2.85 (t); 2.78 (s); 2.60 (s); 1.75 (s); 1.50 (s); 1.41–1.22 (m). *$[\{Ru(terpy)(Cl)\}_2(\mu\text{-}bb_{12})](PF_6)_2 \bullet 5acetone$*: Anal. Found C, 52.5; H, 4.69: N, 8.5%. Calcd. for $C_{79}H_{94}N_{10}F_{12}O_5P_2Ru_2$: C, 52.0; H, 5.19; N, 7.8% ^{1}H NMR (300 MHz, CD_3CN) δ 10.04 (s); 8.65 (s); 8.50 (m); 8.47–8.34 (m); 8.20 (d, $J = 12.0$ Hz); 8.09 (t); 7.90 (t); 7.82 (s); 7.71 (s); 7.30 (s); 7.12 (t); 6.81 (d, 5.7 Hz); 3.04 (t); 2.89 (t); 2.78 (s); 2.63-2.55 (m); 1.76 (s); 1.53 (s); 1.37–1.22 (m). *$[\{Ru(terpy)(Cl)\}_2(\mu\text{-}bb_{14})](PF_6)_2 \bullet 3acetone$*: Anal. Found C, 52.1; H, 4.88: N, 8.5%. Calcd. for $C_{75}H_{86}N_{10}F_{12}O_3P_2Ru_2$: C, 51.8; H, 4.99; N, 8.1% ^{1}H NMR (300 MHz, CD_3CN) δ 10.04 (s); 8.61 (s); 8.49 (m); 8.40 (d, $J = 7.2$ Hz); 8.31 (d, $J = 7.2$ Hz); 8.20 (d, $J = 11.7$ Hz); 8.10 (t); 7.90 (t); 7.81 (s); 7.70 (s); 7.55 (s); 7.30 (s); 7.12 (t); 6.81 (d, 5.1 Hz); 3.04 (t); 2.84 (t); 2.78 (s); 2.77–2.55 (m); 1.76 (s); 1.53 (s); 1.37–1.22 (m).

Synthesis of metal complex-GMP adducts

The mononuclear complexes-GMP adducts were obtained by reacting solid [Ru(terpy)(L)Cl]Cl (0.08 mmol) and disodium guanosine monophosphate (0.24 mmol) in degassed water (5 ml) at 37–40 °C under an argon atmosphere for 12 h. The reaction mixture was loaded onto a Sephadex LH-20 exclusion column and eluted with methanol. The first major brown band was collected and reloaded onto a new column to completely remove the starting complex and the free GMP. The solvent was evaporated to dryness to give the complex-GMP adducts in 20–50% yield. Adducts of the dinuclear complex $[\{Ru(terpy)(Cl)\}_2(\mu\text{-}bb_7)]Cl_2$ with GMP were obtained in an analogous manner using a degassed mixture of methanol/water (1:1).

Cytotoxicity

Cytotoxicity was determined using cell proliferation assays with the L1210 murine leukemia cell line. These studies were carried out at the Peter MacCallum Cancer Centre (Melbourne, Australia) using metal complexes described above, provided as the chloride salts. The murine leukemia line L1210 was grown in RPMI 1640 medium supplemented with 10% fetal bovine serum. The cells were maintained in a humidified incubator with 5% CO_2 in air at 37 °C and were tested routinely for mycoplasma. The metal complex was dissolved in warm ultrapure water, diluted to the required concentrations and incubated with cells in duplicate for 48 h. Cells were then counted using a Coulter Counter (Beckman) and the percent inhibition of cell proliferation was determined for each drug dose. The IC_{50} reported represents the drug dose that inhibits cell proliferation by 50% [28].

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