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Synthesis, Characterization and DNA Binding Studies of [Ruthenium(II)$(bpy)_2L]^{2+}$ where L are Derivatives of imidazo[4,5-f]-1,10-phenanthrolines

(Sintesis, Pencirian dan Kajian Pengikatan DNA [Rutenium(II)$(bpy)_2L]^{2+}$ dengan L adalah Derivatif imidazo[4,5-f]-1,10-fenantrolina)

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ABSTRACT

Three novel ruthenium(II) complexes of the general formula $[Ru(II)(bpy)_2L]^{2+}$ were synthesized, where L = 1,10-phenanthroline derivatives of position 2 imidazole having 3,4-didecyloxy-phenyl (ddip), 3,4-ditetradecyloxy-phenyl (dtip) and 3,4-dihexadecyloxy-phenyl (dhip). All complexes were characterized by elemental analysis, ^{1}H-NMR and ESI-MS. Their photophysical properties have also been studied by UV-visible spectroscopy and fluorescence spectroscopy. The complexes exhibit Ru(II) metal centered emission at approximately 610 nm in acetonitrile solution at room temperature. DNA binding studies were carried out by UV-visible titration, luminescence titration and viscosity studies. The results indicated that $[Ru(bpy)_2(ddip)]^{2+}$ binds to CT-DNA by partial intercalation mode, while $[Ru(bpy)_2(dtip)]^{2+}$ and $[Ru(bpy)_2(dhip)]^{2+}$ bind intercalatively via extended ligands.

Keywords: DNA binding; polypyridyl ligand; ruthenium(II) complex; transition metal complexes

ABSTRAK

Tiga rutenium (II) kompleks baharu, $[Ru(II)(bpy)_2L]^{2+}$ dengan L = 1,10 derivatif-fenantrolina daripada imidazola mempunyai 3,4-didesiloksifenil (L10), 3,4-ditetradesiloksi-fenil (L14) dan 3,4-diheksadisiloksi-fenil (L16) pada kedudukan 2 telah disintesis. Semua kompleks telah dicirikan oleh analisis unsur, spekstroskopi resonans magnet nukleus dan ESI-MS. Sifat fotofizikal juga dikaji melalui spektroskopi UV dan spektroskopi pendarfluor. Kompleks rutenium(II) mempunyai pusat pelepasan kira-kira 610 nm dalam larutan asetonitril pada suhu bilik. Pengikatan kompleks ini dengan CT-DNA juga dikaji menggunakan pentitratan UV-Vis, pentitratan luminesens dan juga ukuran kelikatan. Hasil kajian menunjukkan bahawa $[Ru(bpy)_2L10]^{2+}$ mengikat kepada CT-DNA oleh interkalasi separa manakala ikatan interkalasi terjadi apabila $[Ru(bpy)_2L14]^{2+}$ dan $[Ru(bpy)_2L16]^{2+}$mengikat kepada DNA melalui ligan.

Kata kunci: Ikatan DNA; logam peralihan kompleks; polipiridil ligan; rutenium(II) kompleks

INTRODUCTION

For the past decade, transition metal complexes have shown astonishing prospects as distinctive therapeutic agents in various fields of chemistry and biology (Haq et al. 1995; Lawrence et al. 2006; Lincoln et al. 1996; Liu et al. 2006; Pellegrini & Aldrich-Wright 2003; Perrin et al. 1996; Smith et al. 2004). In particular, ruthenium(II) polypyridyl complexes have attracted wide attention attributable to the usefulness in many fields of applications such as catalysis (Kunz et al. 2012; Małecki et al. 2013), sensors (Gao et al. 2012; Yang et al. 2010), liquid crystals (Cardinaels et al. 2009) and DNA binding (Jing et al. 2004; Kumar et al. 2010; Li et al. 2011; Liu et al. 2010a, 2010b; Zhang et al. 2004). The studies on the binding of Ru(II) complexes with DNA have become prominent recently, investigating the binding mode either reversible or non-covalent. Numerous reports have been published on the DNA binding of metal complexes with bipyridyl ligands (Kumar et al. 2010; Narra et al. 2006; Xiong & Ji 1999) and significant effect on the spectral and DNA binding behaviour of the complexes were observed through varying the ancillary ligands of metal complexes (Liu et al. 2001; Xu et al. 2003).

The benefits of using Ru(II) polypyridyl complexes in drug development are such that dependable routes to prepare the complexes, enhancement of selectivity by functionalization of the ligands and promising candidates suitable for medicinal applications (Kumar et al. 2010). Besides that, Ru(II) complexes generally acquire a ligand which coordinate metal ions through two coordination sites, which is Ru(II) metal centre and other metal centre. The advantage of having another metal centre were useful in synthesizing metal molecular 'on-off' complexes with high emission for the use of sensors technologies (Liu et al. 2013).

Jing et al. (2004) reported that $[Ru(bpy)_2L]^{2+}$ group of compounds (L = 1,10-phenanthroline derivatives of imidazole) bind to DNA in intercalative mode. The present work reports the syntheses of 3 novel Ru(II) complexes with elongation of carbon chain at positions 3 and 4 of 1,10-phenanthroline imidazole derivatives $[Ru(bpy)_2(ddip)](PF_6)_2$ (ddip = 2-(3,4-didecyloxy)-

imidazo-[4,5-f][1,10]phenanthroline), $[Ru(bpy)_2(dtip)](PF_6)_2$ (dtip = 2-(3,4-ditetradecyloxy)-imidazo-[4,5-f][1,10]phenanthroline) and $[Ru(bpy)_2(dhip)](PF_6)_2$ (dhip = 2-(3,4-dihexadecyloxy)-imidazo-[4,5-f][1,10]phenanthroline). The complexes were fully characeterized using 2D ^{1}H-NMR , elemental analysis and ESI-MS.

Experimental Details

Chemicals

The starting materials, 1-bromotetradecane and 3,4-dihydroxybenzaldehyde (Merck); 1-bromodecane,1-bromohexadecane and 1-bromooctadecane (Aldrich); anhydrous magnesium sulphate, 1,10-phenanthroline monohydrate and sodium bromide (R & M Chemicals); anhydrous lithium chloride and sulphuric acid (Fisher); glacial acetic acid (AnalAR); ammonium acetate (Systerm); potassium hexafluorophosphate (Sigma); and ruthenium(III) chloride trihydrate (Precious Metals Online) were used as received. All solvents were obtained from commercial sources and used without further treatment. The compounds 1,10-phenanthroline-5,6-dione (Yamada et al. 1992) and *cis*-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$ (Sullivan et al. 1978) were synthesized according to the literature methods.

Spectroscopic Measurements

^{1}H NMR spectra and 2D-COSY spectra were measured on a JEOL ECA400 FT NMR or a JEOL ECX500 FT NMR spectrometer at room temperature with deuterated acetonitrile as solvent with tetramethylsilane (TMS) as the internal standard. Elemental analyses were carried out using a CHNS-930 (LECO) elemental analyser. UV-Visible spectra were recorded over the range of 200-600 nm on a Shimadzu H.UV.1650 PC UV-visible spectrophotometer using quartz cells of 10 mm path length and emission spectra were obtained on a Shimadzu RF-5301 PC spectrofluorophotometer. Electrospray ionization mass spectra (ESI-MS) were measured on a Finnigan TSQ7000 mass spectrometer, equipped with an ESI source.

Syntheses

Ligands

The ligands ddip, dtip and dhip were synthesized using a modified literature method (Cardinaels et al. 2009). 1,10-Phenanthroline-5,6-dione (100 mg, 0.48 mmol) was mixed with ammonium acetate (500 mg, 6.49 mmol) and then dissolved in glacial acetic acid (4 mL). During stirring, 3,4-dialkoxybenzaldehyde (0.48 mmol) in acetic acid (1.5 mL) was added to the mixture. The solution was heated at 90ºC for 4 h. The reaction mixture was cooled to room temperature, poured into 50 mL of water and neutralized to pH7 with ammonia. The precipitate was filtered off, washed with distilled water and dried. The crude product was purified by column chromatography on silica gel 60, eluting with a 7:3:2 chloroform:hexane:methanol mixture.

Complexes

The complexes $[Ru(bpy)_2(ddip)](PF_6)_2$, $[Ru(bpy)_2(dtip)](PF_6)_2$ and $[Ru(bpy)_2(dhip)](PF_6)_2$ were prepared as follows. *cis*-$Ru(bpy)_2Cl_2\cdot 2H_2O$ (0.162 mmol) was dissolved in 100 mL of a warm 7:3 ethanol:water mixture under nitrogen and the solution was stirred for 1 h at 80ºC. Then, the synthesized ligands (0.162 mmol) were added and the mixture was refluxed overnight under nitrogen after which the solution became red. Ethanol was removed under reduced pressure. The red-orange product was obtained by addition of solid KPF_6, filtered, washed successively with water (2×10 mL), the dried *in vacuo*. This crude product was purified by column chromatography on aluminium oxide 60, eluting with a 1:1 acetonitrile:toluene mixture.

C_1: Yield: 166 mg (79.0 %); ^{1}H-NMR (CD_3CN) δ: 9.00 (d, 2H, *J*= 8.0 Hz), 8.46 (dd, 4H, *J* = 8.0, 9.2 Hz), 8.05 (m, 2H), 7.93 (m, 4H), 7.82 (m, 4H), 7.71 (m, 2H), 7.55 (d, 2H, *J* = 6.9 Hz), 7.40 (m, 2H), 7.16 (m, 2H), 7.09 (d, 1H, *J* = 9.2), 4.04 (m, 4H), 1.27 (m, 32H), 0.84 (m, 6H); ESI-MS (CH_3CN): *m/z*1167.08 ($[M^+-PF_6]$), 511.48 ($[M^{2+}-PF_6]$); elemental analysis calculated for $C_{59}H_{68}F_{12}N_8O_2P_2Ru$: C, 54.00; H, 5.22; N, 8.54. Found: C, 56.15; H, 5.18; N, 7.82

C_2: Yield: 153 mg (76.5 %); ^{1}H-NMR (CD_3CN) δ: 8.93 (d, 2H, *J*= 8.0 Hz), 8.47 (dd, 4H, *J* = 8.0, 8.0 Hz), 8.06 (m, 2H), 7.93 (m, 4H), 7.81 (m, 4H), 7.67 (m, 2H), 7.58 (d, 2H, *J* = 5.7 Hz), 7.41 (m, 2H), 7.19 (m, 2H), 7.01 (d, 1H, *J* = 8.0), 3.99 (m, 4H), 1.32 (m, 48H), 0.82 (m, 6H); ESI-MS (CH_3CN): *m/z*1279.17 ($[M^+-PF_6]$), 567.27 ($[M^{2+}-PF_6]$); elemental analysis calculated for $C_{67}H_{84}F_{12}N_8O_2P_2Ru$: C, 56.50; H, 5.94; N, 7.87. Found: C, 56.28; H, 5.59; N, 8.43

C_3: Yield: 151 mg (78.2 %); ^{1}H-NMR (CD_3CN) δ: 8.94 (d, 2H, *J*= 7.3 Hz), 8.45 (dd, 4H, *J* = 8.2, 8.2 Hz), 8.04 (m, 2H), 7.90 (m, 4H), 7.81 (m, 4H), 7.64 (m, 2H), 7.56 (d, 2H, *J* = 5.5 Hz), 7.40 (m, 2H), 7.17 (m, 2H), 7.03 (d, 1H, *J* = 9.2), 4.03 (m, 4H), 1.25 (m, 56H), 0.83 (m, 6H); ESI-MS (CH_3CN): *m/z*1335.30 ($[M^+-PF_6]$), 595.50 ($[M^{2+}-PF_6]$); elemental analysis calculated for $C_{71}H_{92}F_{12}N_8O_2P_2Ru$: C, 57.60; H, 6.26; N, 7.57. Found: C, 55.60; H, 6.86; N, 6.61.

DNA Binding

Calf thymus DNA was purchased from Merck as a solid sodium salt and was dissolved in a solution of Tris-HCl buffer (5 mM Tris-HCl, 25 mM NaCl, pH7.0) and purified according to published method (Chaires et al. 1982). The purity of DNA was determined by UV-Visible spectroscopy, with $A_{260}/A_{280} > 1.8$ indicating a protein free sample (Reichmann et al. 1954). DNA concentration was also determined by UV-Visible spectroscopy using $\varepsilon_{260} = 6600\ M^{-1}\ cm^{-1}$ (Marmur 1961).

UV-Vis titrations of the Ru(II) complexes (5 μM) in buffer were performed at 25.0ºC to determine the DNA binding affinities. Three mL of buffer were placed in both the reference and sample cuvettes. For the sample cuvette,

a volume of buffer was removed with a Gilson pipette and replaced with stock solution to form 5 μM stock solution of sample and maintained at 25.0°C. After equilibration, a spectrum was recorded between 200-600 nm. During the titration, a volume of buffer (1-5 μL) was removed from both cuvettes with a Gilson pipette and replaced with same volume of stock CT-DNA solution. The absorption spectra were recorded after equilibration. The titration process was repeated until the absorbance became constant, indicating saturation binding had occurred.

Luminescence titrations of the Ru(II) complexes (5 μM) in buffer were performed at 25.0°C to determine the DNA binding affinities. Three mL of buffer and the Ru(II) complex samples (5 μM) were loaded into a 1 cm path length optical quartz cuvette. After equilibration, a spectrum was recorded in the range of 500-850 nm using the excitation wavelength characteristic of the compound. During the titration, a volume of buffer (1-5 μL) was removed from the cuvette with a Gilson pipette and replaced with same volume of stock CT-DNA solution. The emission spectra were recorded after equilibration. The titration process was repeated until emission became constant, indicating saturation binding had occurred.

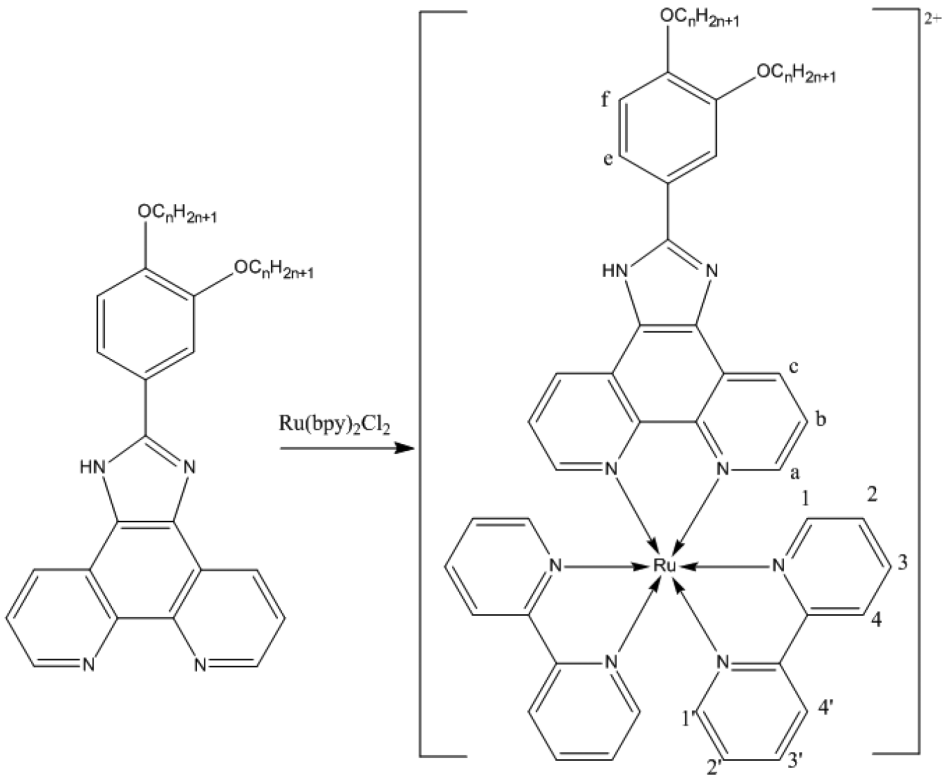
Viscosity measurements were carried out using a Cannon-Manning semi-micro viscometer (size 50) maintained at a constant temperature at 27.0±0.1°C in a thermostatic water bath. Flow time was measured with a digital watch and an average flow time was calculated from three readings for each sample. Data were presented as $(\eta/\eta^o)^{1/3}$ versus binding ratio (Cohen & Eisenberg 1969), where η is the viscosity of DNA in the presence of Ru(II) complex and η^o is the viscosity of DNA alone.

Results and Discussion

Synthesis and Characterization

Scheme 1 shows the reaction of imidazole derivative ligand with Ru(II) precursor to yield the complexes. The syntheses were carried out by reacting equimolar amounts of reagents in a mixture of hot ethanol and water (7:3) for 24 h in nitrogen atmosphere. The solution was then removed *in vacuo* and dropwise addition of a saturated solution of KPF_6 was added to get red-orange complexes. The resulting complexes were purified using column chromatography.

The synthesized complexes were characterized using ^{1}H-NMR spectroscopy and presented in Table 1. The Ru(II) complexes gave well-defined ^{1}H-NMR spectra (Figure 1). The spectra were very similar for 3 complexes and therefore only 1 spectrum is presented. The proton chemical shifts were confirmed with COSY (COSY= correlated spectroscopy) experiments and comparison with



SCHEME 1. Synthesis of C_1 - C_3

those of similar Ru(II) compounds (Chao et al. 2000; Wu et al. 1997). The fact that two pyridine rings of each bpy were not correspondent as a result of the shielding influence of bpy and the imdazo-[4,5-f]-1,10-phenanthroline lead to eight signals of by protons (Samy & Alexander 2011). The proton resonance of NH in the imidazole ring of the ligands was not observed, because of the quick exchange of proton between two nitrogens of the imidazole ring, characteristic of an active proton.

The Ru complexes were analyzed by elemental analysis and electrospray ionization mass spectrometry. The observed and calculated values for the complexes are shown in Table 2. In the ESI mass spectrum of complex, the loss of the 2 hexafluorophosphate ions is the dominant ionization process observed. The positive ion spectra showed two signals of $[M\text{-}PF_6]^+$, $[M]^{2+}$ and the determined molecular weights were consistent with expected values. The main peaks in the mass spectrum and their respective assignments are also shown in the same table. The main peak present is due to one $(PF_6)^-$ counterion detaching from the complex upon injection.

Ru(II) polypyridyl complexes are recognized and well-studied complexes with a d^6 octahedral strong crystal field configuration. The spectroscopic and photopyhsical data of C_1-C_3 are listed in Table 3. The absorption spectra of complexes $\mathbf{C_1}$-$\mathbf{C_3}$ are presented in Figure 2. All these complexes exhibit intense absorption bands in the UV region at 200-300 nm assignable to the spin-allowed ligand centered π-π* transitions of the ligand framework. The shoulder appearing around 330 nm is attributed to the imidazo[4,5-f]-1,10-phenanthroline-centered transition. The lowest energy bands at 460-465 nm is assigned to metal-to-ligand charge transfer (MLCT) transition. These bands are bathochromically shifted by comparison with that of $[Ru(bpy)_3]^{2+}$(452 nm) framework, in accord with the extension of the corresponding π framework in ligands (Tan et al. 2007).

The luminescence of complexes C_1-C_3 has been examined and the results presented in Figure 3. The spectra were obtained when photon flux absorbed by each system is controlled as equal, by giving a common absorbance at the excitation wavelength. The luminescence intensity of complexes in acetonitrile solution is around 610 nm.

DNA Binding Studies

UV-Visible Titration

The UV-visible spectra of $[Ru(bpy)_2(ddip)]^{2+}$, $[Ru(bpy)_2(dtip)]^{2+}$ and $[Ru(bpy)_2(dhip)]^{2+}$ with increased concentration of CT-DNA are illustrated in Figure 4. Upon titration with DNA, the complexes exhibited evident hypochromism with hypochromicity *H* values {$H\% = 100(A_{[DNA=0]} - A_{[DNA]=SAT})/A_{[DNA=0]}$} of 23, 12 and 52% in the MLCT transition, respectively. With an increase in concentration of DNA, all the absorption bands display

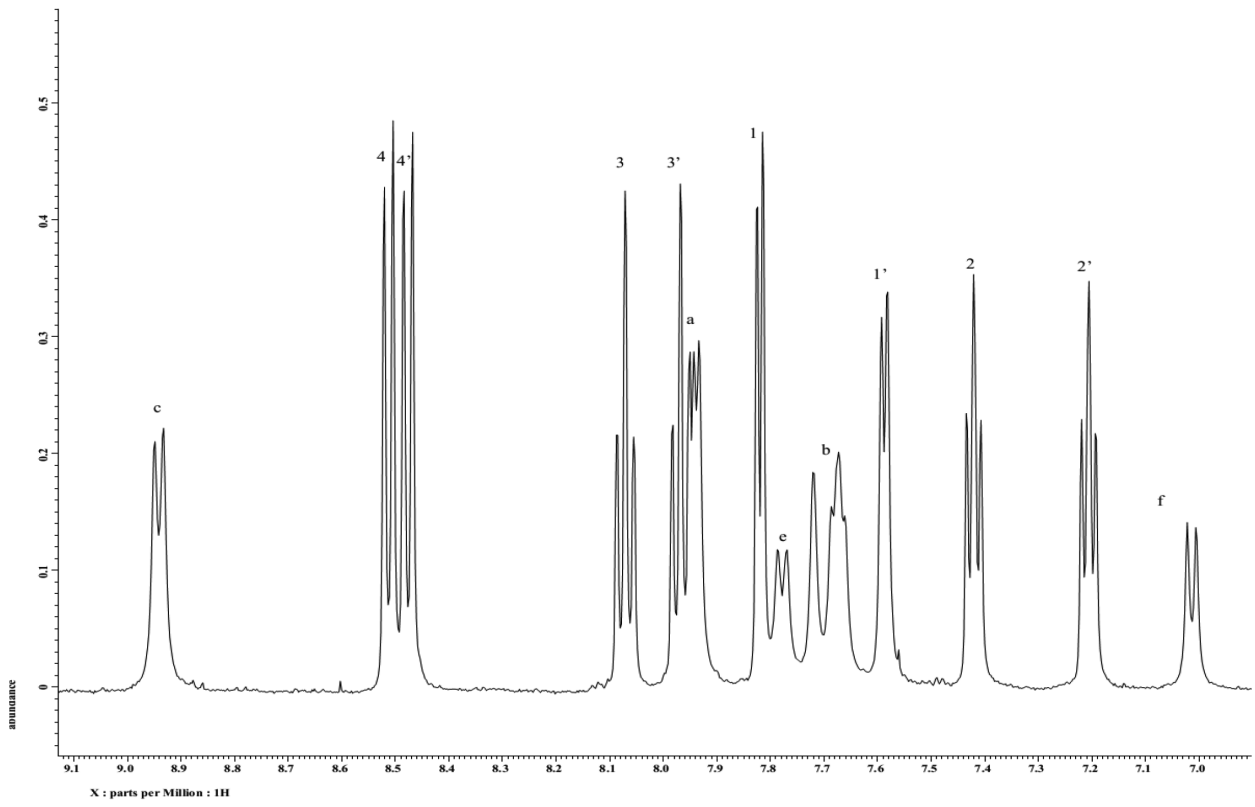


FIGURE 1. ^{1}H-NMR spectrum (500 MHz, CD_3CN) and peak assignments of C_2 in the aromatic region between δ = 7.0 and δ = 9.1 ppm [(CD_3CN solvent, TMS reference]

TABLE 1. ^{1}H-NMR spectral data for complexes C_1-C_3. Coupling constants in italic

	$\mathbf{C_1}$	$\mathbf{C_2}$	$\mathbf{C_3}$
H_c	9.00 d *J*= 8.0	8.93 d *J*= 8.0	8.94 d *J*= 7.3
H_4,H_4'	8.46 dd *J* = 8.0, 9.2	8.47 dd *J* = 8.0, 8.0	8.45 dd *J* = 8.2, 8.2
H_3	8.05 M	8.06 M	8.04 m
H_3', H_a	7.93 M	7.93 M	7.90 m
H_1, H_e	7.82 M	7.81 M	7.81 m
H_b	7.71 M	7.67 M	7.64 m
H_1'	7.55 d *J* = 6.9	7.58 d *J* = 5.7	7.56 d *J* = 5.5
H_2	7.40 M	7.41 M	7.40 m
H_2'	7.16 M	7.19 M	7.17 M
H_f	7.09 d *J* = 9.2	7.01 d *J* = 8.0	7.03 d *J* = 9.2

TABLE 2. Analytical and ESI-MS Spectral Data for the complexes C_1-C_3

Complex	Molecular formula (M.W)	% C Found (Calcd)	% H Found (Calcd)	% N Found (Calcd)	ESI-MS m/z
C_1	$C_{59}H_{68}F_{12}N_8O_2P_2Ru$ (1312.22)	56.15 (54.00)	5.18 (5.22)	7.82 (8.54)	1167.08 $[Ru(bpy)_2\mathbf{L_1}](PF_6)^+$
C_2	$C_{67}H_{84}F_{12}N_8O_2P_2Ru$ (1424.43)	56.28 (56.50)	5.59 (5.94)	8.43 (7.87)	1279.17 $[Ru(bpy)_2\mathbf{L_2}](PF_6)^+$
C_3	$C_{71}H_{92}F_{12}N_8O_2P_2Ru$ (1480.54)	55.60 (57.60)	6.86 (6.26)	6.61 (7.57)	1335.30 $[Ru(bpy)_2\mathbf{L_3}](PF_6)^+$

TABLE 3. Absorption and emission properties of $\mathbf{C_1}$-$\mathbf{C_3}$

	Absorption	Emission
Complex	λ_{max} / nm (ε / $M^{-1}cm^{-1}$)	λ_{max} / nm
$\mathbf{C_1}$	460 (15000) 288 (83857)	610
$\mathbf{C_2}$	460 (17857) 287 (95200)	610
$\mathbf{C_3}$	460 (20557) 287 (106542)	608

clear hypochromism although no large red shifts were observed. The titration data were unable to be fit using McGhee-von Hippel model. As a result, the intrinsic binding constants, K_b, were determined from the ratio of slope to the intercept according to the following equation (Wolfe et al. 1987):

$$[DNA] / (|\varepsilon_a - \varepsilon_f|) = [DNA] / (|\varepsilon_b - \varepsilon_f|) + 1 / K_b (|\varepsilon_b - \varepsilon_f|),$$

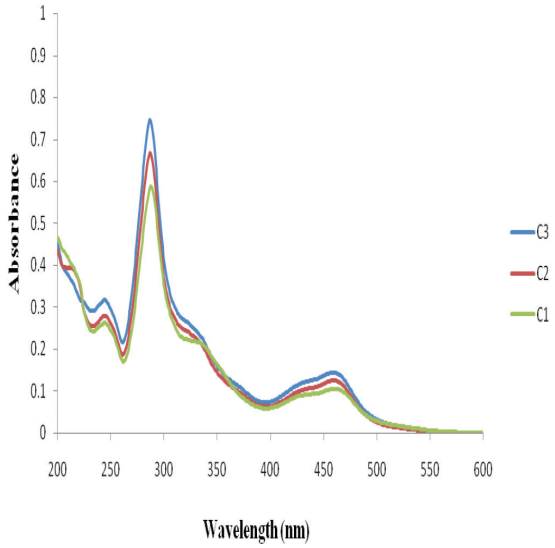


FIGURE 2. Absorption spectra of C_1, C_2, and C_3 in acetonitrile solution

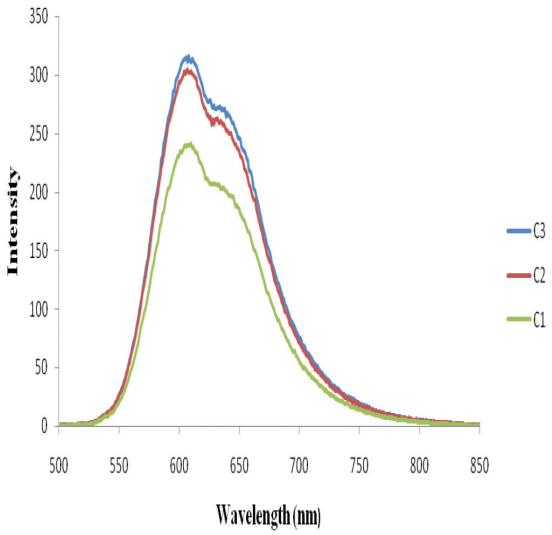


FIGURE 3. Luminescence spectra of C_1, C_2, and C_3 in acetonitrile solution, where the excitation wavelength was fixed at 460 nm

where ε_a, ε_f and ε_b are the apparent, free and bound ligand extinctions, respectively. A representative plot of [DNA] / $\Delta\varepsilon$ versus [DNA] for $[Ru(bpy)_2(ddip)]^{2+}$ is shown in Figure 5. The binding constants obtained for $\mathbf{C_1}$, $\mathbf{C_2}$ and $\mathbf{C_3}$ are 2.4×10^5 M^{-1}, 5.0×10^5 M^{-1} and 5.0×10^5 M^{-1}, respectively. The results are comparable to that observed for the parent complex $[Ru(bpy)_2(pip)]^{2+}$ (Zheng et al. 2004). However, the K_b values are much lower than $K_b > 10^6$ M^{-1} for (dppz)-based DNA intercalators (Friedman et al. 1990). Among the complexes examined, $[Ru(bpy)_2(ddip)]^{2+}$ showed the least binding strength to double helical DNA. This is mainly because the extended aromatic structures increase the action between complexes and DNA. Furthermore, it can be seen that increasing the chain length of the main ligand did increase the binding constant of the complexes. The above results indicate that the chain length of the main ligand directly affect the DNA-binding affinity.

LUMINESCENCE TITRATION

The emission spectras were obtained using excitation wavelength, λ = 460 nm. The first titration was done using the highest stock concentration of DNA (1 mM). The emission intensities were unchanged for $\mathbf{C_1}$, $\mathbf{C_2}$ and $\mathbf{C_3}$. This phenomenon has been reported by Morgan et al. (1991) which observed no emission change upon titration of CT-DNA into the solution of $[Ru(bpy)_2qpy]^{2+}$. In order to study the binding properties, the CT-DNA solution was diluted into stock concentration of 4 µM. As an example, the emission spectra of $\mathbf{C_1}$ were shown (Figure 6). The emission intensities were decreased by a factor of 0.65, 0.22 and 0.70 after adding CT-DNA solution. From the graph (Figure 7), $\mathbf{C_3}$ has the strongest binding when compared with the other two complexes. The binding of $\mathbf{C_2}$ is not strong as compared to the other two Ru(II) complexes synthesized. It can be seen that binding between complexes with low concentration of DNA is possible. However, the binding constant was not able to obtain due to limited titration data. The ligand may not accommodate symmetrically within the major groove of DNA due to length of ligand that caused change in emission results.

VISCOSITY

Crystals of $\mathbf{C_1}$ - $\mathbf{C_3}$ were not successfully grown using various solvents after many attempts. Therefore, viscosity measurements were carried out in the absence of crystallographic structural data (Satyanarayana et al. 1992). The increase of DNA length in base pairs separation at intercalation sites will thus increase the viscosity of DNA solution, which are properties of intercalation binding mode. By contrast, complexes that binds exclusively in the DNA grooves by partial and/or nonclassical intercalation typically cause less pronounced (positive or negative) or no change in DNA viscosity (Kelly et al. 1985).

From the graph (Figure 8), the relative viscosity of DNA increases upon increasing the concentration of $\mathbf{C_2}$ and $\mathbf{C_3}$, leading to classical intercalation binding mode. In contrast, $\mathbf{C_1}$ essentially exerts no effect on DNA viscosity at low binding ratios (r<0.04), but upon further binding of the complex to DNA, the DNA produces about 16% decreases in viscosity. Such behaviour is consistent with bending or kinking of the helix upon binding Ru(II) complex to DNA. A similar behaviour in relative viscosity has been observed on the addition of $[Ru(bpy)_2(HNAIP)]^{2+}$, which was proposed to be bound to DNA by partial intercalation (Liu et al. 1999). The results then suggested that $\mathbf{C_1}$ could bind DNA in partial intercalation mode.

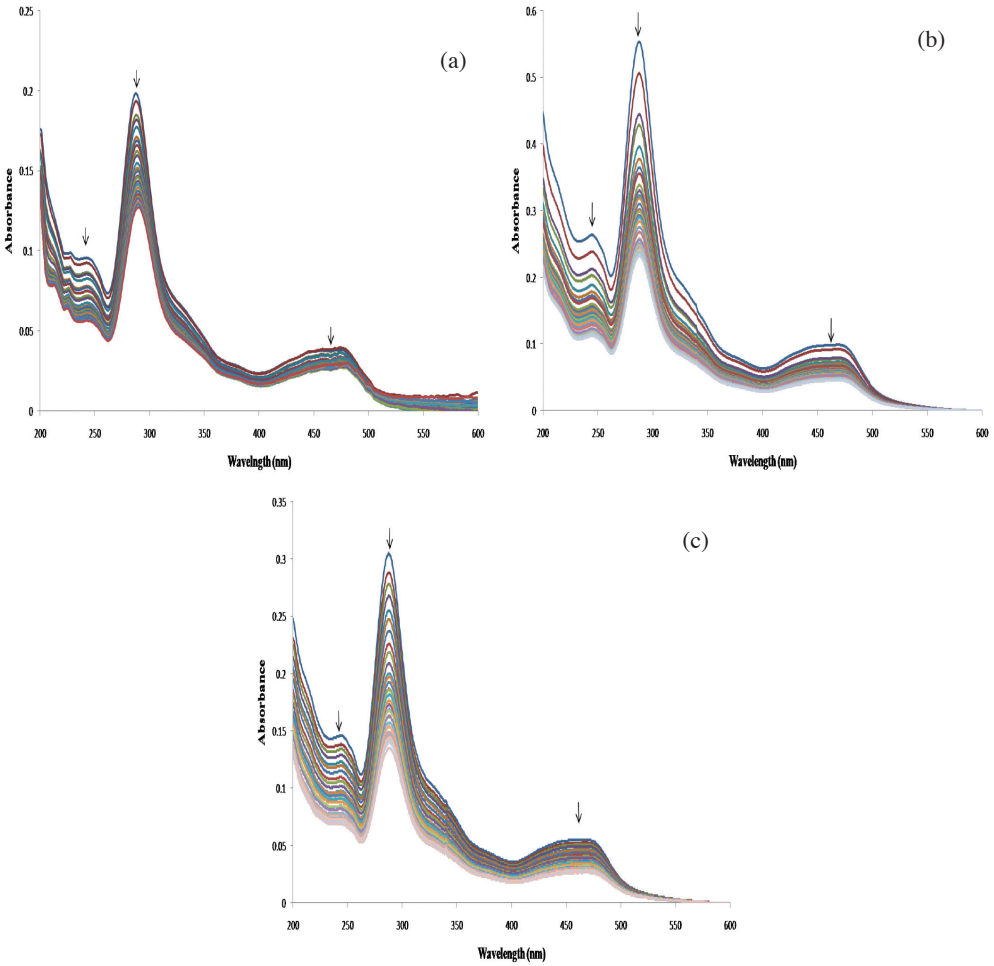


FIGURE 4. Absorption spectra of C_1 (a), C_2 (b) and C_3 (c) in the presence of increasing amounts of DNA. The arrow shows the absorbance changes on increasing DNA concentration

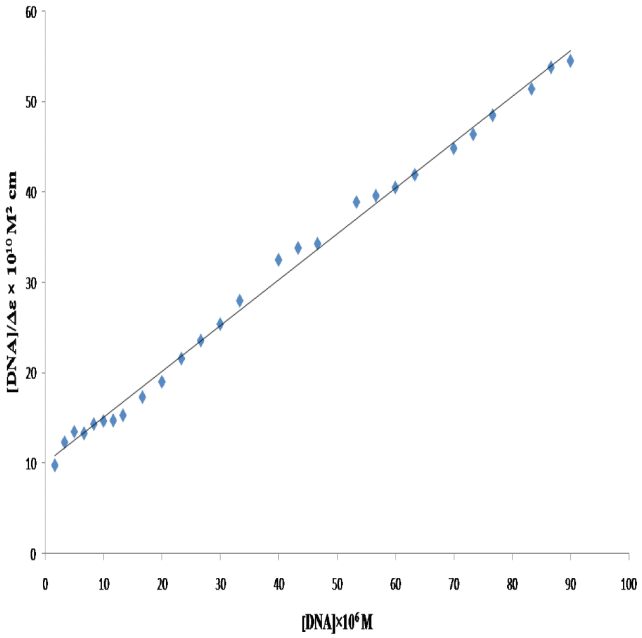


FIGURE 5. Plot of [DNA]/Δε vs. [DNA] for the absorption titration of DNA with $[Ru(bpy)_2(ddip)]^{2+}$

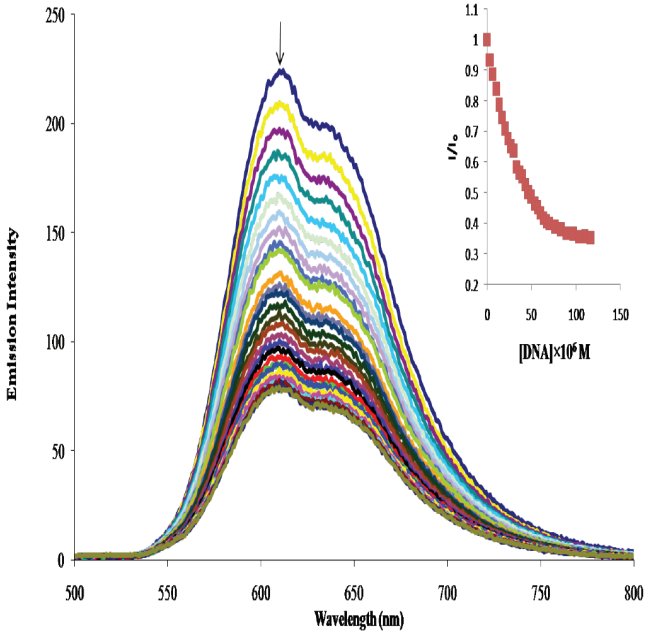


FIGURE 6. Changes in emission spectra (λ_{ex} = 460 nm) of C_1 (5 μM) with increasing concentrations of CT-DNA in 5mM Tris-HCl buffer (pH=7, 25mM NaCl). Arrow shows the emission intensity changes upon increasing DNA concentrations

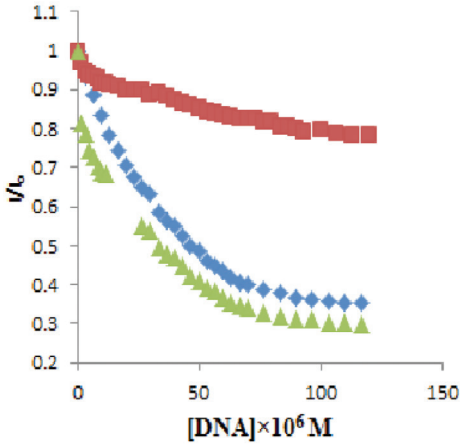


FIGURE 7. Plots of relative emission intensity (I/I_o) vs. [DNA] for C_1 (♦), C_2 (■) and C_3 (▲)

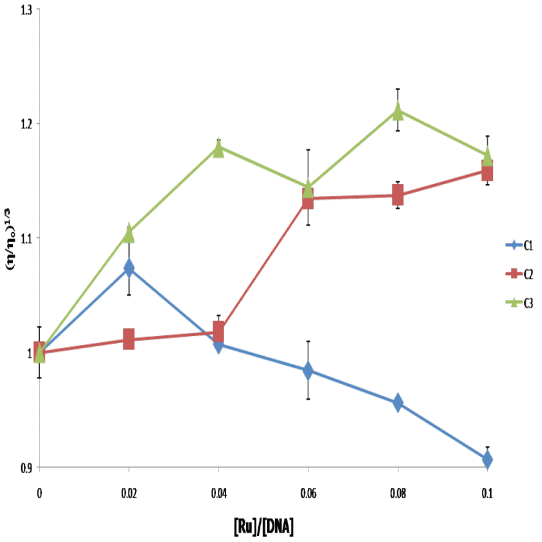


FIGURE 8. Effect of increasing amounts of C_1 (♦), C_2 (■) and C_3 (▲) on the relative viscosities of CT-DNA

CONCLUSION

In summary, three new Ru(II) complexes have been synthesized and characterized. Complete ^{1}H-NMR assignments have been obtained by the aid of two-dimensional techniques. Photophysical study have also been carried out and the results showed that the complexes are highly luminescent when irradiated at 460 nm. The results showed that $[Ru(bpy)_2(ddip)]^{2+}$ binds to CT-DNA with a partial intercalative mode, while $[Ru(bpy)_2(dtip)]^{2+}$ and $[Ru(bpy)_2(dhip)]^{2+}$ intercalatively bind *via* extended planar ligands.

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