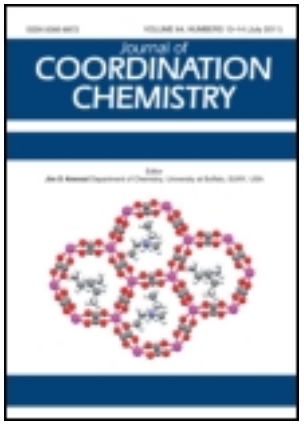

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Synthesis, DNA-binding, and photocleavage studies of ruthenium(II) complexes with an asymmetric ligand

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An asymmetric ligand (pdpiq = 2-(pyridine-2-yl)-6,7-diphenyl-1-H-imidazo[4,5-g]quinoxaline) and its ruthenium complexes with $[Ru(L)_2pdpiq]^{2+}$ (L = bpy (2,2′-bipyridine) or phen (1,10-phenanthroline)) have been synthesized and characterized by elemental analysis, ES-MS, and ^1H NMR. The DNA-binding behaviors of these complexes were studied by spectroscopic methods and viscosity measurements. The results indicate that the complexes can intercalate into DNA base pairs. When irradiated at 365 nm, the two complexes promote the cleavage of plasmid pBR322DNA. The mechanism of DNA cleavage is an oxidative process by generating singlet oxygen.

Keywords: Ru(II) complex; Asymmetric ligand; DNA-binding; Photocleavage

1. Introduction

There has been considerable interest in studies on the interactions of transition metal complexes with nucleic acid, ruthenium(II) polypyridyl complexes in particular. These Ru(II) complexes have attracted considerable attention due to their rich photophysical properties and potential applications in biology, such as the design and development of non-radioactive probes of nucleic acid structure, new therapeutic reagents, synthetic restriction enzymes, versatile catalysts for divergent organic reactions, DNA foot printing agents, and possible DNA cleaving agents [1–10]. In general, Ru(II) polypyridyl complexes interact with DNA through electrostatic binding, groove binding, or intercalation. Among these interactions, intercalation is the most important because many potential applications such as antitumor activity and molecular "light switch" [11–16] require that the complexes bind to DNA in an intercalative mode. $[Ru(bpy)_2(dppz)]^{2+}$ (bpy = 2,2′-bipyridine; dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine) is the most extensively investigated ruthenium(II) complex as a molecular "light switch" for DNA, because the complex shows negligible luminescence in aqueous solution at

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ambient temperature but displays strong luminescence after binding to DNA by intercalation [15]. Clarification of the trends in DNA-binding of Ru(II) polypyridyl complexes will facilitate understanding and control of interactions between the complexes and DNA, and thus mechanisms of DNA mutation and damage, as well as the design of new clinic anti-cancer drugs and complexes with biochemical activity [1–5].

In order to further understand the DNA-binding mechanism of Ru(II) complexes and obtain Ru(II) complexes with excellent bioactivity, many polypyridyl Ru(II) complexes were designed and studied for DNA-binding behavior, DNA cleavage, and spectral properties [17–28]. A number of reports have shown that varying the shape of the ligand can create different space configuration and electron density distribution of Ru(II) polypyridyl complexes, resulting in changes in DNA-binding properties. Studies of such changes will help to more clearly understand the DNA-binding mechanism of Ru(II) polypyridyl complexes. Therefore, further design and studies on different ligands are necessary to evaluate and understand the factors that determine DNA-binding mode. Designs of ligands for modulating DNA affinities of Ru(II) complexes are mainly classified into two categories: (i) structural change on the intercalative ligand, including symmetric (e.g., dppz, tpphz) [14–22] and asymmetric ones (e.g., dppt, ptdb, PZNI) (dppt = 3-(1,10-phenanthrolin-2-yl)-5,6-diphenyl-as-triazine; ptdb = 3-(pyridine-2-yl)-5,6-diphenyl-as-triazine; PZNI = 2-(pyrazin-2-yl)naphthoimidazole) [23–25]; (ii) changing the ancillary ligands [28–31]. Attention has primarily focused on symmetric aromatic ligands such as 1,10-phenanthroline and its derivatives [14–22]; the influence of asymmetric ligands on DNA-binding properties of Ru(II) complexes has attracted much less attention. Some of these complexes with asymmetric ligands exhibit interesting properties upon binding to DNA [23–25].

In order to obtain more insight into DNA-binding and photocleavage properties of ruthenium(II) complexes, herein, we report the synthesis and characterization of an asymmetric ligand (pdpiq = 2-(pyridine-2-yl)-6,7-diphenyl-1-H-imidazo [4,5-g]quinoxaline) and its ruthenium complexes, $[Ru(L)_2pdpiq]^{2+}$ (L = bpy or phen). DNA-binding and DNA-photocleavage properties of these Ru(II) complexes were carefully studied. We hope the results are of value in further understanding DNA-binding mechanisms.

2. Experimental

2.1. *Materials*

cis-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$, *cis*-$[Ru(phen)_2Cl_2]\cdot 2H_2O$ [32], methyl pyridine-2-carboximidate [33, 34], and 1,2-diamino-4,5-(p-toluenesulfamidobenzene) [35, 36] were prepared by literature methods. All DNA-binding experiments were carried out in buffer A (50 mmol L^{-1} NaCl, 5 mmol L^{-1} Tris–HCl, pH = 7.2). For DNA photocleavage experiments, samples were treated in buffer B (50 mmol L^{-1} Tris, 18 mmol L^{-1} NaCl, pH = 7.8). Calf thymus DNA (CT-DNA) was obtained from Sigma (St. Louis, MO, USA). Supercoiled pBR 322 DNA was purchased from MBI Fermentas. Solutions of CT-DNA in buffer A gave a ratio of UV-Vis absorbance of 1.8–1.9 : 1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein [37]. The concentration

of DNA was determined spectrophotometrically ($\varepsilon_{260} = 6600\,(\text{mol L}^{-1})^{-1}\,\text{cm}^{-1}$) [38]. Other materials were commercially available and used without purification.

2.2. *Physical measurement*

C, H, and N analyses were carried out with a Perkin-Elmer 240Q elemental analyzer. ^{1}H NMR spectra were recorded on a Bruker ARX-500 spectrometer with $(CD_3)_2SO$ as solvent at room temperature. Electrospray mass spectra were recorded on an LQC system (Finnigan MAT, USA) using CH_3CN as mobile phase. Fast atomic bombardment mass spectra (FAB-MS) were obtained on a VG ZAB-HS spectrometer. Absorption spectra were recorded with a Shimadzu UV-2450 spectrophotometer and emission spectra on a Hitachi F-2500 spectrofluorophotometer at room temperature.

2.3. *DNA-binding experiments*

2.3.1. Electronic absorption titration. Absorption spectral titrations of Ru(II) complexes in buffer A were carried out at room temperature to determine the DNA-binding affinities. Ruthenium-DNA solutions were allowed to incubate for 5 min before the absorption spectra were recorded. The intrinsic binding constants K of these two complexes to DNA were obtained by monitoring the changes of the ^{1}MLCT absorbance according to equation (1) [39],

$$(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f) = (b - (b^2 - 2K^2C_t[\text{DNA}]/s)^{1/2})/2KC_t, \tag{1a}$$

$$b = 1 + KC_t + K[\text{DNA}]/2s, \tag{1b}$$

where ε_a is the extinction coefficient observed for the ^{1}MLCT absorption band at a given DNA concentration, ε_f is the extinction coefficient of the complex in the absence of DNA, ε_b is the extinction coefficient of the complex fully bound to DNA. K is the equilibrium binding constant in $(\text{mol L}^{-1})^{-1}$, C_t is the total metal complex concentration, [DNA] is the concentration of DNA in mol L^{-1} (nucleotide), and s is the binding site size. The experimental absorption titration data were fitted to obtain the binding constants with a non-linear least-squares method.

2.3.2. Competitive binding experiment. The competitive binding experiments were conducted by adding increasing amounts of Ru(II) complex directly into the samples containing 5 μmol L^{-1} ethidium bromide (EB) and 100 μmol L^{-1} DNA in buffer A. Emission spectra were recorded in the region 500–700 nm and samples were excited at 340 nm. To further illustrate the DNA-binding strength of the two Ru(II) complexes, a competitive binding model was applied to calculate the apparent binding constants of Ru(II) complexes from EB competitive experiments using equation (2) [40],

$$K_{app} = K_{EB}([\text{EB}]_{50\%}/[\text{Ru}]_{50\%}), \tag{2}$$

where K_{app} is the apparent DNA-binding constant of the Ru(II) complex, K_{EB} is the DNA-binding constant of EB, and $[\text{EB}]_{50\%}$ and $[\text{Ru}]_{50\%}$ are the EB and Ru(II) complex concentrations at 50% fluorescence, respectively.

2.4. *Viscosity studies*

DNA viscosities were measured using an Ubbelohde viscometer maintained at $30.0 \pm 0.1^{\circ}C$ in a thermostatic bath. DNA samples for viscosity measurement were prepared by sonication in order to minimize complexities arising from DNA flexibility. Every sample was measured at least three times and an average flow time was calculated. The DNA viscosity was calculated according to $\eta_i = (t_i - t_0)/t_0$, where η_i is the corresponding value of DNA viscosity, t_i is the flow time of the solutions in the presence or absence of the complex, and t_0 is the flow time of buffer alone. Data are presented as $(\eta/\eta_0)^{1/3}$ *versus* binding ratio [41], where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone.

2.5. *DNA photocleavage experiment*

The DNA photocleavages by Ru(II) complexes were examined by gel electrophoresis experiments. Supercoiled pBR322 DNA (0.1 μg) was treated with Ru(II) complex in buffer B (50 mmol L^{-1} Tris, 18 mmol L^{-1} NaCl, pH = 7.8), and the solutions were incubated for 1 h in the dark, then irradiated at room temperature with a UV lamp (365 nm, 10 W). The samples were analyzed by electrophoresis for 2 h at 75 V on a 1% agarose gel in Tris-borate-EDTA (TBE) buffer C (89 mmol L^{-1} Tris-boric acid, 2 mmol L^{-1} EDTA, pH = 8.3). The gel was stained with 1 μg mL^{-1} EB and then photographed under UV light.

2.6. *Synthesis*

2.6.1. 5,6-(p-toluenesulfonamide)-2-(pyridine-2-yl)-1H-benzimidazole (1). A solution of methyl pyridine-2-carboximidate (*ca* 0.4 mmol) in CH_3OH was added to a solution of 1,2-diamino-4,5-(p-toluenesulfamidobenzene) 0.178 g (0.4 mmol) in 10 mL glacial acetic acid and the mixture was refluxed under argon for 4 h. The cooled solution was diluted with water and neutralized with concentrated aqueous ammonia. The khaki precipitate was collected and dried *in vacuo*. Yield: 0.195 g, 91.3%. Anal. (%): (Found: C, 58.37; H, 4.23; N, 12.92. Calcd for $C_{26}H_{23}N_5O_4S_2$: C, 58.52; H, 4.35; N, 13.13). FAB-MS: 534 $[M + 1]^+$.

2.6.2. 2-(pyridine-2-yl)-5,6-diamino-1H-benzimidazole (2). 5,6-(p-toluenesulfonamide)-2(pyridine-2-yl)-1H-benzimidazole (**1**) 0.533 g (1.0 mmol) and 4 mL concentrated sulfuric acid were stirred for 24 h at room temperature. The dark violet solution was then added dropwise to ice water. Treatment of the resulting solution with a saturated Na_2CO_3 solution gave a clear green solution; the solution was extracted with dichloromethane (3×100 mL). The combined extracts were dried over $MgSO_4$. Removal of the solvent at reduced pressure gave the product as a yellow solid. Yield: 0.089 g, 39.6%. Anal. (%): (Found: C, 63.11; H, 4.99; N, 30.82. Calcd for $C_{12}H_{11}N_5$: C, 63.99; H, 4.92; N, 31.09). FAB-MS: 226 $[M + 1]^+$.

2.6.3. 2-(pyridine-2-yl)-6,7-diphenyl-1-H-imidazo [4,5-g]quinoxaline (pdpiq) (3). A mixture of 2-(2′-pyridineyl)-5,6-diamino-1H-benzimidazole (**2**) 0.067 g (0.3 mmol) and

benzil was refluxed for 2 h in methanol (30 mL). After cooling to room temperature, the solution was poured into water, and the resulting yellow precipitate was collected and dried *in vacuo*. Yield: 0.094 g, 78.3%. Anal. (%): (Found: C, 77.87; H, 4.35; N, 17.36. Calcd for $C_{26}H_{17}N_5$: C, 78.18; H, 4.29; N, 17.53). ES-MS (CH_3CH_2OH): $m/z = 400.0$ ($[M + 1]^+$).

2.6.4. [Ru(bpy)$_2$pdpiq](ClO$_4$)$_2$ (3a). A mixture of pdpiq 0.120 g (*ca* 0.3 mmol), $[Ru(bpy)_2Cl_2] \cdot 2H_2O$ (0.156 g, 0.3 mmol), and ethylene glycol (10 mL) was refluxed under argon for 4 h. Upon cooling, the resulting clear red solution was diluted with water (*ca* 60 mL), then treated with a saturated aqueous solution of $NaClO_4$. The orange precipitate was collected and purified by column chromatography on neutral alumina with acetonitrile-toluene (2 : 1, v/v) as eluent. Yield: 0.179 g, 64.5%. Anal. (%): (Found: C, 54.38; H, 3.33; N, 12.32. Calcd for $C_{46}H_{33}N_9O_8RuCl_2$: C, 54.59; H, 3.29; N, 12.46). ES-MS (CH_3CN): $m/z = 811.3$ ($[M - 2ClO_4^- - H]^+$), 406.5 ($[M - 2ClO_4^-]^{2+}$). ^{1}H NMR (500 MHz, ppm, DMSO-d$_6$): 8.85 (dd, 3H, J = 8.0 Hz), 8.72 (d, 1H, J = 8.5 Hz), 8.58 (d, 1H, J = 7.5 Hz), 8.28 (s, 1H), 8.26 (7, 1H, J = 8.0 Hz), 8.16 (t, 1H, J = 8.0 Hz), 8.12 (t, 1H, J = 7.0 Hz), 8.09 (t, 1H, J = 7.5 Hz), 8.05 (d, 1H, J = 5.5 Hz), 8.02 (t, 1H, J = 8.0 Hz), 7.94 (d, 1H, J = 5.5 Hz), 7.90 (d, 1H, J = 5.5 Hz), 7.66 (m, 3H), 7.56 (t, 1H, J = 6.5 Hz), 7.52 (t, 1H, J = 5.5 Hz), 7.47 (t, 1H, J = 7.0 Hz), 7.45 (t, 1H, J = 6.5 Hz), 7.40 (d, 2H, J = 6.5 Hz), 7.34 (brs, 8H), 6.20 (s, 1H).

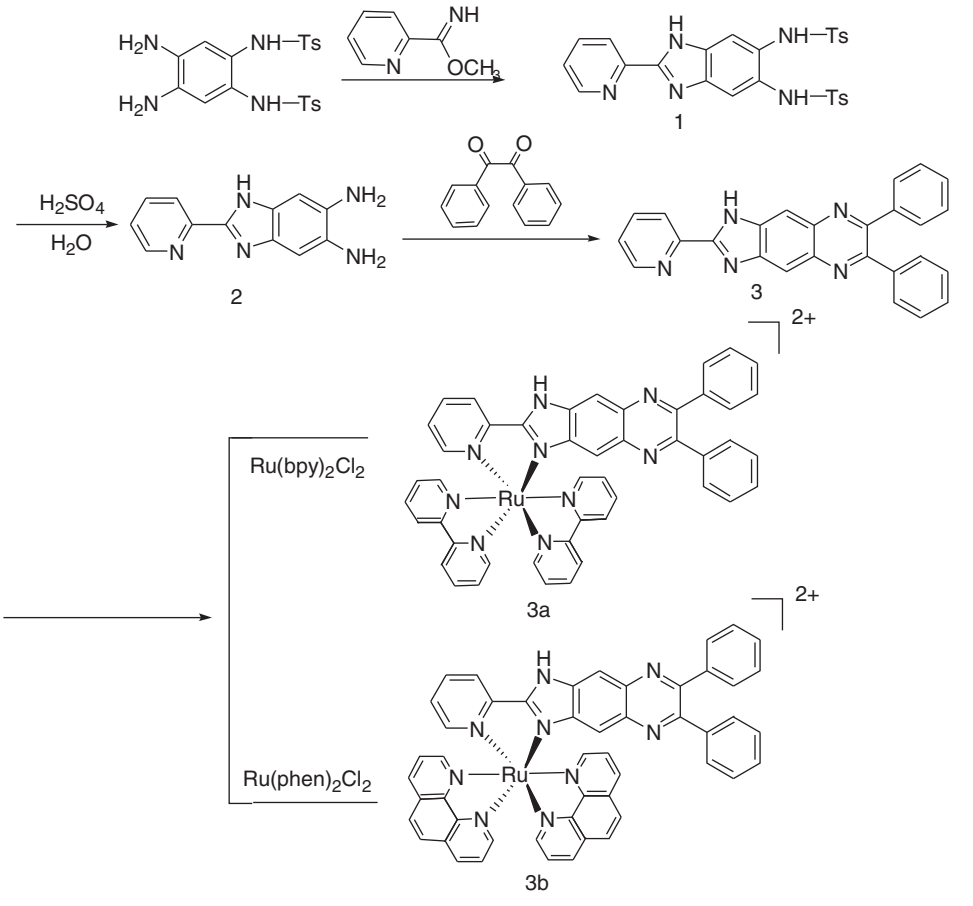
2.6.5. [Ru(phen)$_2$pdpiq](ClO$_4$)$_2$ (3b). This red complex was obtained by a similar procedure as described for complex **3a**, with the only difference being that $[Ru(phen)_2]Cl_2 \cdot 2H_2O$ (0.170 mg, 0.3 mmol) was used instead of $[Ru(bpy)_2]Cl_2 \cdot 2H_2O$. Yield: 0.180 g, 61.4%. Anal. (%): (Found: C, 56.41; H, 3.22; N, 11.81, Calcd for $C_{50}H_{33}N_9O_8RuCl_2$: C, 56.65; H, 3.14; N, 11.90). ES-MS (CH_3CN): $m/z = 860.2$ ($[M - 2ClO_4^- - H]^+$), 430.5 ($[M - 2ClO_4^-]^{2+}$). ^{1}H NMR (500 MHz, ppm, DMSO-d$_6$): 8.82 (d, 1H, J = 8.0 Hz), 8.75 (d, 1H, J = 8.0 Hz), 8.72 (d, 1H, J = 8.0 Hz), 8.69 (d, 1H, J = 8.0 Hz), 8.60 (d, 1H, J = 7.5 Hz), 8.41 (d, 1H, J = 5.0 Hz), 8.38 (m, 6H), 8.14 (d, 1H, J = 5.0 Hz), 8.09 (t, 2H, J = 8.0 Hz), 7.93 (t, 1H, J = 8.0 Hz), 7.85 (t, 2H, J = 5.5 Hz, J = 8.5 Hz), 7.74 (t, 1H, J = 5.5 Hz), 7.62 (m, 1H), 7.31 (brm, 11H), 5.92 (s, 1H).

Each of the above ClO_4 salts was dissolved in the minimum amount of acetone and a saturated solution of tetrabutylammonium chloride (TBACl) in acetone solution was added dropwise until precipitation was complete. The water-soluble chloride salts were filtered off, washed thoroughly with acetone, and vacuum dried (yield ~91% in each case).

3. Results and discussion

3.1. *Synthesis and characterization*

The outline of the synthesis of the ligand and its complexes is presented in scheme 1. Compound **1** was obtained on the basis of the method for imidazole ring preparation established by Schaefer and Peters [33, 34, 42]. The diamino compound **2** was prepared by detosylation of the corresponding tosylated **1**. The synthesis of pdpiq **3** was performed by the condensation of benzil with the precursor diamine **2**.



Scheme 1. The synthetic routes of the ligand, $[Ru(bpy)_2(pdpiq)]^{2+}$ and $Ru(phen)_2(pdpiq)]^{2+}$.

The corresponding ruthenium(II) complexes **3a** and **3b** were prepared by direct reaction of ligand with the appropriate mol ratios of the precursor complexes in ethylene glycol. All these complexes were purified by column chromatography and characterized by elemental analysis, ES-MS, and ^{1}H NMR. In the ES-MS spectra for the two complexes **3a** and **3b**, only the signals of $[M - 2ClO_4^- - H]^+$ and $[M - 2ClO_4^-]^{2+}$ were observed. The measured molecular weights were consistent with expected values.

The two Ru(II) complexes $[Ru(bpy)_2pdpiq]^{2+}$ and $Ru(phen)_2pdpiq]^{2+}$ gave well-defined ^{1}H NMR spectra (figure S1). The proton chemical shifts were assigned *via* comparison with those of similar Ru(II) complexes with asymmetric ligand [23–25, 43–47]. The chemical shifts of all the protons in aromatic region are presented in section 2.

3.2. *Electronic absorption titration*

Small molecules bound to DNA by intercalation are associated with hypochromism and red shift (bathochromism), because of the strong π–π stacking interaction between

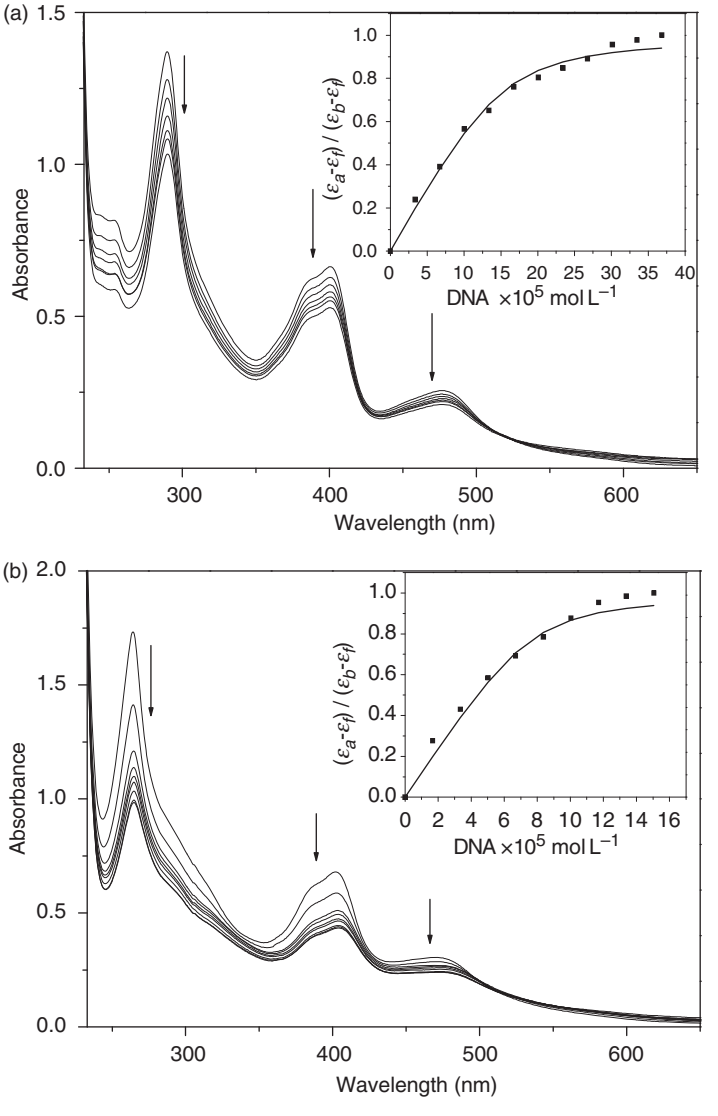


Figure 1. Absorption spectra of **3a** (a) and **3b** (b) in buffer A upon the addition of CT-DNA, [Ru] = 20 μmol L^{-1}, [DNA] = 0–360 μmol L^{-1}. Arrow shows the absorbance changing upon the increase in DNA concentration. Inset: plots of $(\varepsilon_a-\varepsilon_f)/(\varepsilon_b-\varepsilon_f)$ *vs.* [DNA] for the titration of DNA to Ru(II) complexes.

aromatic chromophore and the base pairs of DNA. The extent of the hypochromism in the visible ^{1}MLCT band depends on the strength of intercalative interaction [15]. Figure 1 shows absorption spectra of **3a** and **3b** in the absence and presence of CT-DNA (at a constant concentration of complexes, [Ru] = 20 μmol L^{-1}).

Absorption spectra of the two complexes in buffer A exhibited three well-resolved absorptions from 200 to 650 nm. The lowest energy bands at 478 nm for **3a** and 472 nm for **3b** are attributed to the metal-to-ligand charge transfer (MLCT) transition, the bands at 400 nm for **3a** and 402 nm for **3b** are assigned to π–π^* transitions, and the

bands at 289 nm for **3a** and 264 nm for **3b** are assigned to bpy-centered π–π^* transitions and phen-centered π–π^* transitions in comparison with $[Ru(bpy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$, respectively. Increasing the amount of DNA for **3a** decreases the ^{1}MLCT transitions as much as 17.6% at 478 nm at a ratio of [DNA]/[Ru] = 18.2. For **3b**, upon addition of DNA, the MLCT band at 472 nm exhibits hypochromism of about 21.4% at a ratio of [DNA]/[Ru] = 7.4.

In order to compare quantitatively the DNA-binding affinities of the complexes, the intrinsic binding constants K of these two complexes to DNA were determined by monitoring the changes of the ^{1}MLCT absorbance at 478 nm for **3a** and at 472 nm for **3b** according to equation (1) [39]. The intrinsic binding constants K of **3a** and **3b** were $2.8 \pm 0.1 \times 10^5\,(\text{mol L}^{-1})^{-1}$ ($s = 3.79$, figure 1a) and $7.6 \pm 0.5 \times 10^5\,(\text{mol L}^{-1})^{-1}$ ($s = 1.93$, figure 1b), respectively. Complex **3b** exhibits a stronger DNA-binding affinity than **3a** due to the different plane area and hydrophobicity of the ancillary ligands. These values are compared to that of Ru(II) complexes with the asymmetric ligand, $[Ru(dmb)_2(pdta)]^{2+}$ ($2.37 \times 10^5\,(\text{mol L}^{-1})^{-1}$) [47], $[Ru(bpy)_2PZNI]^{2+}$ ($3.42 \times 10^4\,(\text{mol L}^{-1})^{-1}$) [23], and $[Ru(phen)_2PZNI]^{2+}$ ($5.86 \times 10^4\,(\text{mol L}^{-1})^{-1}$) [23], but smaller than that of the classical intercalator, $[Ru(phen)_2dppz]^{2+}$ ($5.1 \times 10^6\,(\text{mol L}^{-1})^{-1}$) [48]. From the results, we deduce that the two complexes bind to DNA with high affinities. Further studies are needed to elucidate the DNA-binding mode of the complexes.

3.3. *Competitive binding experiments*

The two Ru(II) complexes do not emit distinct fluorescence in buffer A even in the presence of DNA. Therefore, the DNA-binding properties of these complexes and DNA cannot be directly studied in emission spectra. The competitive binding experiments were carried out using a molecular fluorophore EB as a probe. EB emits strong fluorescence in the presence of DNA due to its strong intercalation between adjacent DNA base pairs. If a complex replaces EB from DNA-bound EB, the fluorescence of the solutions would be efficiently quenched as free EB shows no apparent emission intensity in buffer A because of solvent quenching. Figure 2 shows fluorescence quenching spectra of DNA-bound EB by Ru(II) complexes. Upon addition of Ru(II) complexes, sharp decreases in EB emission intensities were observed, indicating that the two Ru(II) complexes could displace EB from DNA. The competitive binding experiment results suggest that the two Ru(II) complexes interact with DNA through intercalation. In the plot of percentage of quenching fluorescence, $(I_0 - I)/I_0$ *versus* [Ru]/[EB], we see that 50% EB molecules were displaced from adjacent DNA base pairs at a concentration ratio of [Ru]/[EB] = 5.34 for **3a** and 2.52 for **3b**, as shown in figure 2. By taking the DNA-binding constant of $1.4 \times 10^6\,(\text{mol L}^{-1})^{-1}$ for EB [49, 50], the apparent DNA-binding constants K_{app} values of the two complexes were calculated according to equation (2) [39] as $2.62 \times 10^5\,(\text{mol L}^{-1})^{-1}$ for **3a** and $5.56 \times 10^5\,(\text{mol L}^{-1})^{-1}$ for **3b**, respectively, in agreement with the K_b values derived from the absorption spectral studies.

3.4. *Viscosity properties*

The DNA viscosity measurement is useful to determine whether a complex intercalates into DNA, which is sensitive to length change of DNA (i.e., viscosity and

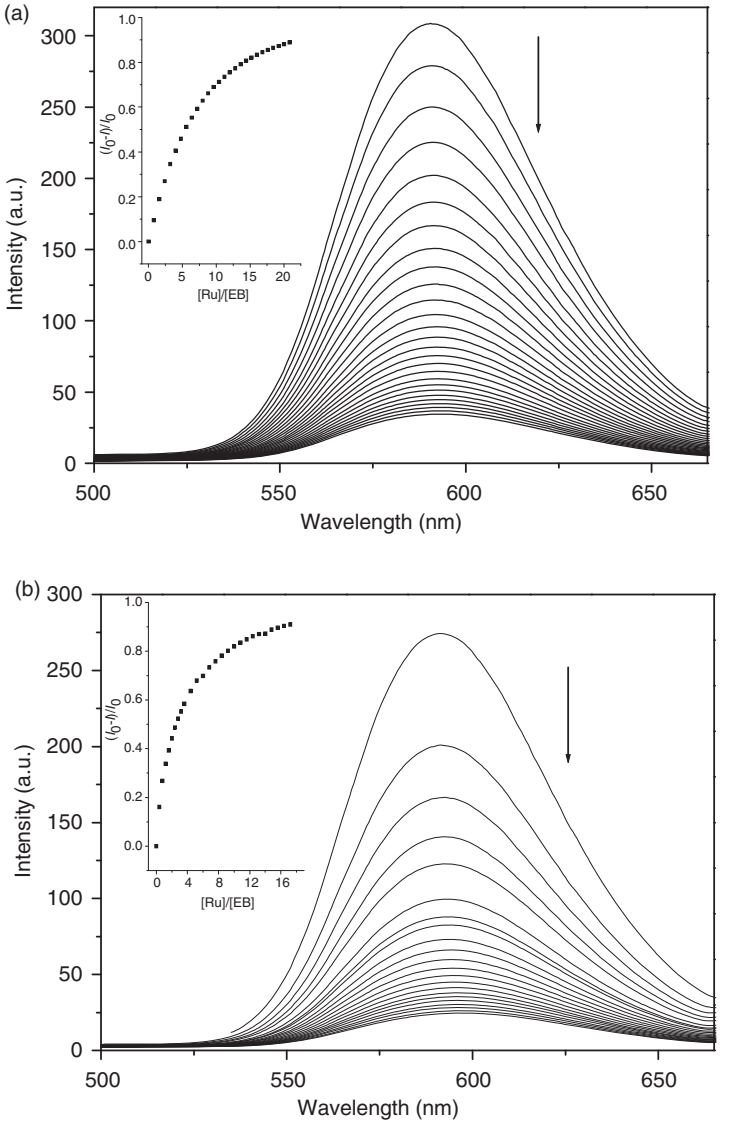


Figure 2. Fluorescence quenching spectra of EB bound to DNA by **3a** (a) and **3b** (b), $[DNA] = 100\,\mu mol\,L^{-1}$, $[EB] = 5\,\mu mol\,L^{-1}$. Arrow shows the intensity change upon increasing Ru(II) complex concentration. Inset: plots of relative integrated fluorescence intensity *vs.* [Ru]/[EB].

sedimentation) and regarded as the least ambiguous and most critical tests for a classical intercalation model in solution in the absence of crystallographic structural data [42, 43]. When a complex intercalates into DNA, the DNA helix lengthens as base pairs are separated to accommodate the bound ligand, which results in increase of DNA viscosity. In contrast, a partial, non-classical intercalation of ligand could bend (or kink) the DNA helix, reducing its length and, concomitantly, its viscosity [51, 52]; electrostatic binding has little effect on DNA viscosity.

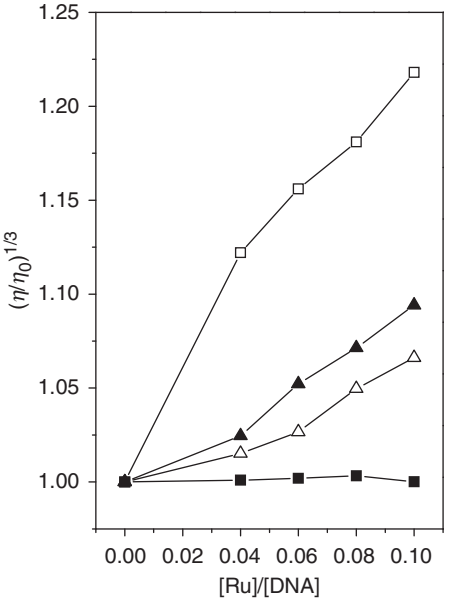


Figure 3. Effects of the increase in amounts of EB (□), **3a** (△), **3b** (▲), and $[Ru(bpy)_3]^{2+}$ (■) on the relative viscosity of CT-DNA at 30 (±0.1)°C, respectively. The total concentration of DNA is 0.25 mmol L^{-1}.

Figure 3 shows the changes in DNA viscosity upon addition of EB, **3a**, **3b**, and $[Ru(bpy)_3]^{2+}$. EB, as a typical intercalator, increases the relative DNA viscosity; $[Ru(bpy)_3]^{2+}$, which bind to DNA in an electrostatic binding mode, has little effect on DNA viscosity. On increasing amounts of **3a** and **3b**, the relative viscosities of CT-DNA increases steadily, but smaller than those of DNA bound with EB. The increased viscosity, which may depend on the DNA-binding mode and affinity, follows the order **EB** > **3b** > **3a** > $[Ru(bpy)_3]^{2+}$. In a study by Chao *et al.* [24] on DNA-binding of Ru(II) complexes containing asymmetric tridentate ligand similar to our ligand, the two phenyl rings in the asymmetric dppt are rotated away from the 1,2,4-triazine ring with large dihedral angles, causing severe steric constraints when the complex interacts with DNA, resulting in a partial, non-classical intercalation. However, some similar examples of Ru(II) complexes with the bidentate ligand containing two rotated phenyl rings, $[Ru(bpy)_2(dptatp)]^{2+}$ [53] and $[Ru(bpy)_2(ptdb)]^{2+}$ [25] (dptatp = 2,3-diphenyl-1,4,8,9-tetraazatriphenylene), have been reported by Zheng *et al.*, suggesting that $[Ru(bpy)_2(dptatp)]^{2+}$ and $[Ru(bpy)_2(ptdb)]^{2+}$ interact with DNA through intercalation despite steric constraints caused by the two phenyl rings. The different results may be due to the difference between the structure of the complexes and ligands. Considering these reported results and our experimental results (absorption titration, competitive binding and viscosity experiments), we deduce that **3a** and **3b** intercalate between the base pairs of DNA, consistent with the spectroscopic results.

In addition, due to the more hydrophobic ability of phen, **3b** can intercalate deeper into DNA base pairs and thus show stronger DNA-binding affinity than **3a**.

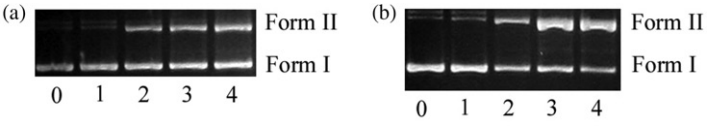


Figure 4. Photoactivated cleavage of pBR322 DNA in the presence of Ru(II) complexes after 2 h irradiation at 365 nm. Lane 0, DNA alone; Lanes 1–4: **3a** (a) and **3b** (b) at 10, 20, 40, and 80 μmol L^{-1}.

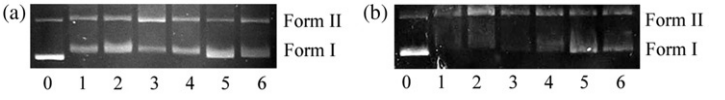


Figure 5. Agarose gel showing cleavage of pBR322 DNA incubated with **3a** (a), **3b** (b) and different inhibitors after 2 h irradiation at 365 nm, [Ru] = 80 μmol L^{-1}. Lane 0: DNA alone, lane 1: DNA + Ru, lanes 2–6: DNA + Ru + 1 mol L^{-1} DMSO, 100 mmol L^{-1} mannitol, 1000 U mL^{-1} SOD, 25 mmol L^{-1} NaN_3, 1.2 mmol L^{-1} histidine.

3.5. *Photocleavage of pBR 322 DNA by Ru(II) complexes*

There has been interest in transition metal complexes which cleave nucleic acids. Ru(II) complexes with polypyridyl ligands have well-behaved redox-active and rich photochemical properties, making them good candidates for DNA photocleavers. Upon irradiation, most generate singlet oxygen, thus inducing single-strand or double-strand cleavage of DNA [54].

The photocleavage reactions of the present complexes on supercoiled pBR322 DNA were studied by gel electrophoresis in TBE buffer (pH = 8.3). When circular plasmid DNA is subjected to gel electrophoresis, relatively fast migration will be observed for the intact supercoil form (Form I). If scission occurs on one strand (nicked circulars), the supercoil will relax to generate a slower moving nicked circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Forms I and II will be generated [55].

Figure 4 shows the photoactivated cleavage of pBR322DNA in the presence of different concentrations of **3a** and **3b** upon irradiation at 365 nm. No obvious DNA cleavage was observed for controls in the absence of complex (lane 0). On increasing the concentration of complexes, the amount of Form I of pBR322 DNA is decreased and that of Form II (nicked circular DNA) is increased. Both complexes can induce single-strand scissions in supercoiled DNA. However, under the same experimental conditions, when the concentration reached 80 μmol L^{-1}, **3b** promotes the conversion of DNA from Form I to Form II. These results show that **3a** and **3b** can cleave DNA upon irradiation and **3a** exhibits a higher efficiency in DNA-photocleavage than **3b**.

In order to determine the reactive species responsible for the DNA photocleavage of the two Ru(II) complexes, mechanism experiments were performed in the presence of hydroxyl radical (OH•) scavengers [41, 43, 56] (DMSO and mannitol), singlet oxygen ($^1O_2$) scavengers [57] (NaN_3 and histidine), and a superoxide anion radical ($O_2^{\bullet-}$) scavenger (SOD). As shown in figure 5, NaN_3 and histidine inhibited the DNA cleavage activity of the two complexes, suggesting that singlet oxygen ($^1O_2$) is the cleaving agent. In the presence of other scavengers DMSO, mannitol or SOD, little inhibition

was observed. These results indicated that superoxide anion radical ($O_2^{\bullet -}$) and hydroxyl radical (OH•) were not involved in DNA cleavage by **3a** and **3b** under irradiation and the mechanism of DNA cleavage is an oxidative process by generating singlet oxygen. Similar cases are found in other Ru(II) complexes [58, 59].

4. Conclusion

An asymmetric ligand **3** and its Ru(II) complexes $[Ru(bpy)_2pdpiq)]^{2+}$ (**3a**) and $[Ru(phen)_2pdpiq]^{2+}$ (**3b**) have been synthesized and characterized. The DNA-binding and photocleavage properties of the two complexes have been investigated by absorption spectroscopy, competitive binding, viscosity, and agarose gel electrophoresis. Both complexes bind to DNA in an intercalative mode. Also, the two complexes are efficient DNA-photocleavers upon irradiation at 365 nm and **3a** exhibits stronger DNA-photocleavage efficiency than **3b**. The mechanism experiments indicate that singlet oxygen may play an important role in the DNA photocleavage of the two Ru(II) complexes.

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