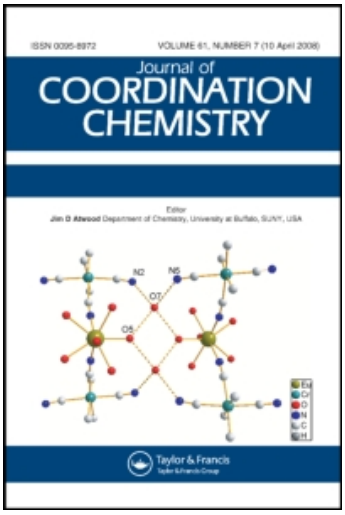

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Synthesis, characterization, and DNA binding of ruthenium(II) complexes with 2-benzo[b] furan-2-yl-1H-imidazo[4,5f][1,10]phenanthroline as an intercalative ligand

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Synthesis, characterization, and DNA binding of ruthenium(II) complexes with 2-benzo[b] furan-2-yl-1H-imidazo[4,5f][1,10]-phenanthroline as an intercalative ligand

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DNA-binding properties of a number of ruthenium complexes with different polypyridine ligands are reported. The new polypyridine ligand BFIP (=2-benzo[b] furan-2-yl-1H-imidazo[4,5-f][1,10]phenanthroline) and its ruthenium complexes $[Ru(bpy)_2BFIP]^{2+}$ (bpy = 2,2′-bipyridine), $[Ru(dmb)_2BFIP]^{2+}$ (dmb = 4,4′-dimethyl-2,2′-bipyridine), and $[Ru(phen)_2BFIP]^{2+}$ (phen = 1,10-phenanthroline) have been synthesized and characterized by elemental analysis, mass spectra, IR, UV-Vis, ^{1}H- and ^{13}C-NMR, and cyclic voltammetry. The DNA binding of these complexes to calf-thymus DNA (CT-DNA) was investigated by spectrophotometric, fluorescence, and viscosity measurements. The results suggest that ruthenium(II) complexes bind to CT-DNA through intercalation. Photocleavage of pBR 322 DNA by these complexes was also studied, and $[Ru(phen)_2BFIP]^{2+}$ was found to be a much better photocleavage agent than the other two complexes.

Keywords: Ruthenium(II) complexes; Polypyridyl ligand; DNA binding; Photocleavage

1. Introduction

Binding and cleavage of DNA with transition metal complexes have received attention during the past decade [1]. Interaction of transition metal complexes with DNA has been studied with the development of new tools for nanotechnology [2, 3]. Binding of small molecules to DNA is very important in the development of new therapeutic agents and DNA molecular probes [4–23]. Polypyridine ruthenium(II) complexes can bind to DNA by non-covalent interactions such as electrostatic binding, groove binding [24], intercalative binding, and partial intercalative binding [25, 26]. Application of such complexes requires that they bind to DNA through intercalation. Therefore, the vast majority of studies have focused on intercalation of transition metal complexes containing fully planar ligands [27–38]. Much study has been done on modifying intercalative ligands and the influence of ancillary ligands on DNA binding [26]. Arockiasamy *et al*. [39] studied the binding and photocleavage of $[Ru(bpy)_2HBT]^{2+}$, and $[Ru(phen)_2HBT]^{2+}$ (HBT = 11H,13H-4,5,9,10,12,14 hexaaza-benzo [b] triphenylene). As ruthenium(II) polypyridine complexes bind to DNA in three dimensions,

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ancillary ligands play an important role in governing the DNA binding. To understand clearly the effects of ancillary ligands, the selection of intercalative ligands is also very important. Appropriate intercalative ligands can help to distinguish the small differences of interaction with DNA of complexes containing ancillary ligands. There are many advantages of using ruthenium(II) polypyridine complexes in drug development: (1) the stable complexes with predictable structures can be prepared through reliable routs; (2) shape selectivity of the complexes can be improved by functionalization of the ligands; and (3) knowledge of biological effects of ruthenium complexes can be greatly developed. Our group [40–44] reported binding, and photocleavage studies of several mixed ligand complexes of ruthenium(II) and cobalt(III). Herein, we report the synthesis and characterization of 2-benzo[b] furan-2-yl-1H-imidazo [4,5-f] [1,10] phenanthroline [BFIP], a new polypyridine, and its Ru(II) complexes $[Ru(bpy)_2BFIP]^{2+}$ (**1**) (bpy = 2,2′-bipyridine), $[Ru(dmb)_2BFIP]^{2+}$ (**2**) (dmb = 4,4′-dimethyl-2,2′-bipyridine), and $[Ru(phen)_2BFIP]^{2+}$ (**3**) (phen = 1,10-phenanthroline). DNA binding of the complexes was explored by spectroscopic and viscosity measurements and their photocleavage behavior toward pBR-322 supercoiled plasmid DNA investigated. Understanding the binding of small molecules to DNA is useful in developing design principles to guide the synthesis of new drugs which can recognize a specific site or conformation of DNA, and to provide a good tool for biotechnology. The DNA-binding mechanism and behavior of the complexes are closely related to the size, shape, and planarity of the intercalative ligands.

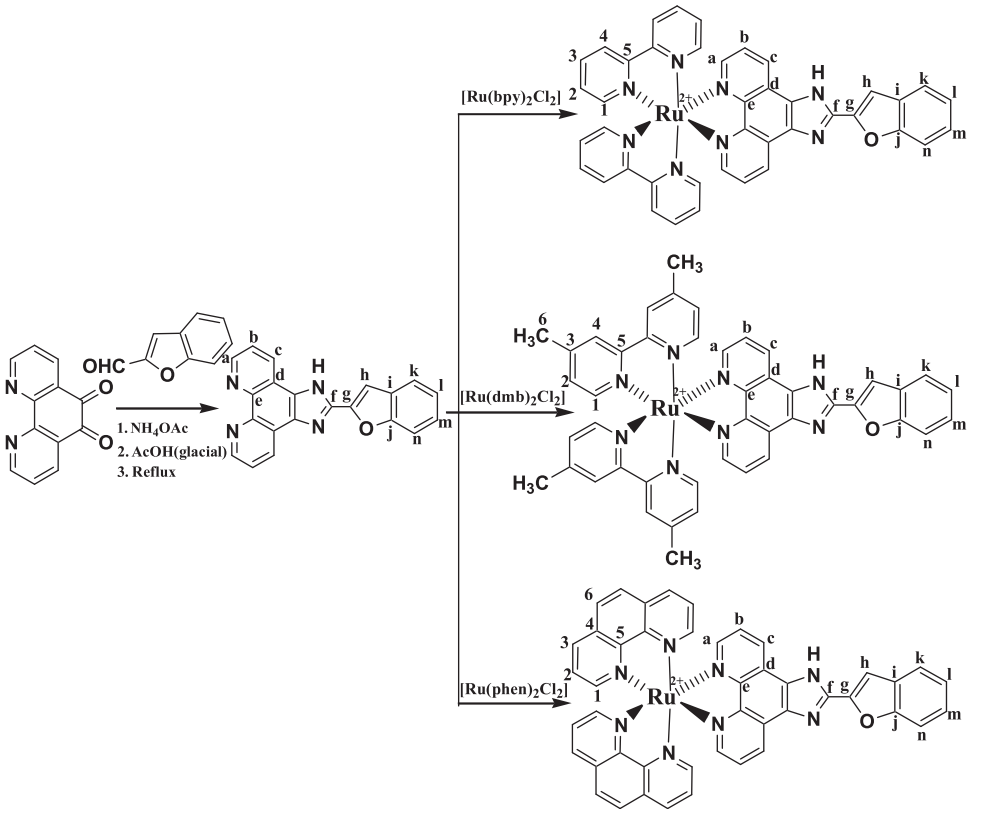
2. Experimental

2.1. *Materials*

$RuCl_3$, 1,10-phenanthroline monohydrate, and 2,2′-bipyridine were purchased from Merck (India). Calf-thymus (CT) DNA, tetrabutylammonium chloride (TBACl), benzo[b]furan-2-carboxaldehyde, tetrabutylammonium hexafluorophosphate ($TBAPF_6$), and 4,4′-dimethyl-2,2′-bipyridine were obtained from Sigma (St. Louis, MO, USA). The supercoiled (CsCl purified) pBR-322 DNA (Bangalore Genie, India) was used as received. All other common chemicals and solvents were procured from locally available sources. All solvents were purified before use with standard procedures [45]. Deionized, doubly distilled water was used for preparing various buffers. Solutions of DNA in Tris-HCl buffer (pH = 7.2), 50 mmol L^{-1} NaCl gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9 indicating that the DNA was sufficiently free of protein [46]. The concentration of CT-DNA was determined spectrophotometrically using the molar absorption coefficient 6600 $(mol\,L^{-1})^{-1}\,cm^{-1}$ (260 nm) [47].

2.2. *Synthesis and characterization*

The complexes 1,10-phenanthroline-5,6-dione [48], $[Ru(bpy)_2Cl_2]$, $[Ru(dmb)_2Cl_2]$, and $[Ru(phen)_2Cl_2]$ [49] were prepared according to procedures reported in the literature. The synthetic route for ligands and their Ru(II) complexes are shown in scheme 1.



Scheme 1. Synthetic routes of ligand and Ru(II) complexes.

2.3. *Synthesis of ligand*

2.3.1. 2-Benzo[b] furan-2-yl-1H-imidazo[4,5-f][1,10]phenanthroline. BFIP was prepared from a solution of 1,10-phenanthroline-5,6-dione (0.260 g, 1.2 mmol), benzo[b]-furan-2-carboxaldehyde (0.262 g, 1.8 mmol), and ammonium acetate (1.9 g, 25 mmol) in 10 mL glacial acetic acid refluxed for 2 h. The deep red solution was cooled, diluted with water (25 mL), neutralized with ammonia, filtered, washed with H_2O and Me_2CO, and then dried. Yield: 82%. Anal. Calcd for (%) $C_{21}H_{12}N_4O$: C, 75.00; H, 3.57; and N, 16.66. Found (%): C, 74.50; H, 3.20; and N, 16.28. ESI–MS (in DMSO), *m*/*z*; 337 (Calcd 336); IR (KBr): 1601(C=N), 1434(C=C); ^{1}H-NMR (DMSO-d_6, 25°C, δ ppm): δ 9.12 (s, 1H), 9.01 (d, 2H), 8.92 (d, 2H), 8.03 (s,1H), 7.82 (m, 2H), 7.15 (d, 1H), and 6.92 (d, 1H); ^{13}C[^{1}H]-NMR (DMSO-d_6, δ ppm): 154.10 (2C, C_i, C_l), 147.00 (2C, C_a, $C_{a'}$), 146.70 (1C, C_h), 143.42 (2C, C_c, $C_{c'}$), 142.05 (2C, C_e, $C_{e'}$), 129.50 (2C, C_d, $C_{d'}$), 127.70 (1C, C_f), 125.40 (1C, C_g), 123.46 (1C, C_o), 123.00 (2C, C_n, C_k), 121.70 (3C, C_b, $C_{b'}$, C_m), 111.00 (1C, C_p), and 105.28 (1C, C_j).

2.4. *Synthesis of [Ru(bpy)$_2$(BFIP)](PF$_6$)$_2$·2H$_2$O*

$[Ru(bpy)_2BFIP]^{2+}$ was synthesized using a mixture of *cis*-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$ (0.104 g, 0.2 mmol), BFIP (0.0672 g, 0.2 mmol), and ethanol (70 mL) refluxed under nitrogen

for 2 h. Upon cooling, the resulting clear solution was filtered and the filtrate was treated with a saturated aqueous solution of ammonium hexafluorophosphate and a red precipitate was obtained. Yield: 73%. Anal. Calcd for (%) $C_{41}H_{32}F_{12}N_8O_3P_2Ru$: C, 45.81; H, 2.98; and N, 10.42. Found (%): C, 45.48; H, 2.79; and N, 10.22. ESI–MS (in DMSO), m/z; 1076 (Calcd 1075); IR (KBr): 1607 (C=N), 1443 (C=C), 759 (Ru–N (BFIP)), and 624 (Ru–N (bpy)) cm^{-1}: ^{1}H-NMR (DMSO-d_6, 25°C, δ ppm): δ 9.22–9.14 (d, 2H, H_a, $H_{a'}$), 8.94–8.84 (m, 4H, H_1, $H_{1'}$), 8.34 (s, 4H, H_4, $H_{4'}$), 8.30–8.23 (t, 2H, H_c, $H_{c'}$), 8.20–8.15 (d, 4H, H_3, $H_{3'}$), 8.15–8.10 (d, 1H, H_m), 8.05 (s, 1H, H_p), 8.02–7.94 (t, 2H, H_{b-}, $H_{b'}$), 7.94–7.80 (m, 1H, H_o), 7.70–7.60 (m, 4H, H_2, $H_{2'}$), 7.59–7.52 (t, 1H, H_n), and 7.50–7.42 (m, 1H, H_j); ^{13}C[^{1}H]-NMR (DMSO-d_6, δ ppm): 157.21 (4C, C_5, $C_{5'}$), 156.98 (2C, C_i, C_l), 155.06 (2C, C_a, $C_{a'}$), 152.00 (4C, C_1, $C_{1'}$), 151.80 (4C, C_3, $C_{3'}$), 150.60 (2C, C_c, $C_{c'}$), 146.00 (1C, C_h), 138.50 (3C, C_d, $C_{d'}$, C_f), 138.30 (2C, C_e, $C_{e'}$), 131.20 (1C, C_g), 128.40 (2C, C_n, C_k), 128.20 (4C, C_4, $C_{4'}$), 127.00 (1C, C_o), 125.00 (4C, C_2, $C_{2'}$), 123.00 (2C, C_b, $C_{b'}$), 122.00 (1C, C_m), 112.00 (1C, C_p), and 108.00 (1C, C_j).

2.5. *Synthesis of [Ru(dmb$_2$(BFIP)](PF$_6$)$_2$·2H$_2$O*

This complex was prepared by a procedure similar to that described above using *cis*-[Ru(dmb)$_2$Cl$_2$]·2H$_2$O (0.116 g, 0.2 mmol) in place of *cis*-[Ru(bpy)$_2$Cl$_2$]·2H$_2$O (spectral data given in Supplementary material).

2.6. *Synthesis of [Ru(phen$_2$(BFIP)](PF$_6$)$_2$·2H$_2$O*

This complex was synthesized by a procedure similar to that described above using *cis*-[Ru(phen)$_2$Cl$_2$·2H$_2$O (0.114 g, 0.2 mmol) in place of *cis*-[Ru(bpy)$_2$Cl$_2$]·2H$_2$O (spectral data given in Supplementary material).

2.7. *Physical measurements*

UV-Vis spectra were recorded with an Elico Bio-spectrophotometer, model BL198. Infrared (IR) spectra were recorded in KBr discs on a Perkin Elmer FT-IR-1605 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured on a Varian XL-300 MHz spectrometer using DMSO-d_6 as the solvent and tetramethylsilane (TMS) as an internal standard with complete proton decoupling. Microanalyses (C, H, and N) were carried out on a Perkin Elmer 240 elemental analyzer. Fluorescence spectra were recorded with a JASCO Model 7700 spectrofluorometer for solutions having absorbance less than 0.2 at the excitation wavelength. Viscosity experiments were carried out on Ostwald viscometer, immersed in thermostated waterbath maintained at 30 ± 0.1°C. CT-DNA samples (approximately 200 base pairs in average length) were prepared by sonication in order to minimize complexities arising from DNA flexibility [50]. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* concentration of [Ru(II)]/[DNA], where η is viscosity of DNA in the presence of the complex and η_0 the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions ($t > 100$ s), corrected for the flow time of buffer alone (t_0) [51]. DNA melting experiments were carried out by controlling the temperature of the sample cell with a

Shimadzu circulating bath, while monitoring the absorbance at 260 nm. For gel electrophoresis experiments, supercoiled pBR-322 DNA (100 μmol L^{-1}) was treated with Ru(II) complexes in 50 mmol L^{-1} Tris-HCl, 18 mmol L^{-1} NaCl buffer pH 7.8, and the solutions were then irradiated at room temperature with a UV lamp (365 nm, 10 W). The samples were analyzed by electrophoresis for 2.5 h at 40 V on a 1% agarose gel in Tris–acetic acid–EDTA buffer, pH 7.2. The gel was stained with 1 μg mL^{-1} ethidium bromide (EtBr) and photographed under UV light.

Cyclic and differential pulse voltammetric measurements were performed on a PC-controlled CH instruments model CHI 620C electrochemical analyzer. Cyclic voltammetric experiments were performed on a 1 mmol L^{-1} ruthenium complexes solution in acetonitrile at a scan rate of 100 mV s^{-1} using tetrabutyl ammoniumperchlorate (TBAP, 0.1 mol L^{-1}) as the supporting electrolyte. The working electrode was glassy carbon, a standard calomel electrode (SCE) was the reference electrode, and platinum wire was an auxillary electrode. After a cyclic voltammogram (CV) had been recorded, ferrocene was added, and a second voltammogram was measured.

3. Results and discussion

3.1. *Spectral characterization*

The compounds synthesized in this study have been characterized by elemental analysis, UV-Vis, IR, ^{1}H- and ^{13}C-NMR, and cyclic voltammetry. Electronic absorption spectra of the complexes are characterized by metal-to-ligand charge transfer (MLCT) transition in the visible region. The low-energy bands (451.0 nm for **1**, 453.5 nm for **2** and 431.0 nm for **3**) are assigned to MLCT. The ligand and the PF_6 salts of **1**–**3** gave satisfactory elemental analyses. The ESI–MS spectrum of BFIP ligand shows a molecular ion peak at *m*/*z* 337 which is equivalent to its molecular weight (Calcd 336), and molecular ion peak at *m*/*z* 1076 is an envelope from ruthenium isotopes (Ru-99, 13%; Ru-101, 17%; Ru-102, 32%, and Ru-104, 19%), the observed biggest mass peak probably for Ru-102 (Calcd 1075) for **1**, 1132 (Calcd 1131) for **2**, and 1124 (Calcd 1123) for **3** (mass spectra of ligand, and complexes are given in Supplementary material). In IR spectra of **1**–**3**, bands at 1607 cm^{-1} (C=N), 1443 cm^{-1} (C=C) shifted to higher frequency, compared to free ligand indicate complexation. New bands at 759 cm^{-1} in (Ru–N (BFIP)), 624 cm^{-1} in (Ru–N (bpy)) support the complex formation. The IR spectrum of the PF_6 salt of each complex showed a strong band at 837–839 cm^{-1} from PF_6^-; this band was absent in the corresponding chloride salts. In the ^{1}H-NMR spectra of **1**–**3**, peaks due to various protons of bpy, phen, dmb, and BFIP shift in comparison with the corresponding free ligands suggesting complexation. All chemical shifts of carbons of Ru(II) complexes are shifted downfield. The ^{1}H- and ^{13}C-NMR spectra of the Ru(II) complexes were recorded in DMSO-d_6.

Oxidation of the complexes occurs at 1.25 V in a reversible process. The different ligands do not affect the Ru(II)/Ru(III) oxidation. The reduction is either reversible or quasireversible from ligands. Representative cyclic and differential pulse voltammograms of $[Ru(bpy)_2BFIP]^{2+}$ (**1**) are presented in "Supplementary material" and data are summarized in table 1.

Table 1. Redox potential data.[a]

Complex	Ox[b]	Red[c]
$[Ru(bpy)_2BFIP]^{2+}$	1.25	−1.43, −1.61
$[Ru(dmb)_2BFIP]^{2+}$	1.26	–
$[Ru(Phen)_2BFIP]^{2+}$	1.27, 1.50	−0.94, −1.47

[a]Solvent: CH_3CN. Working electrode: Glassy carbon, Reference electrode: SCE, Auxillary electrode: Platinum wire, 0.1 mol L^{-1} TBAP, Scan rate 100 mV s^{-1}.
[b]Reversible.
[c]Reversible or quasi reversible.

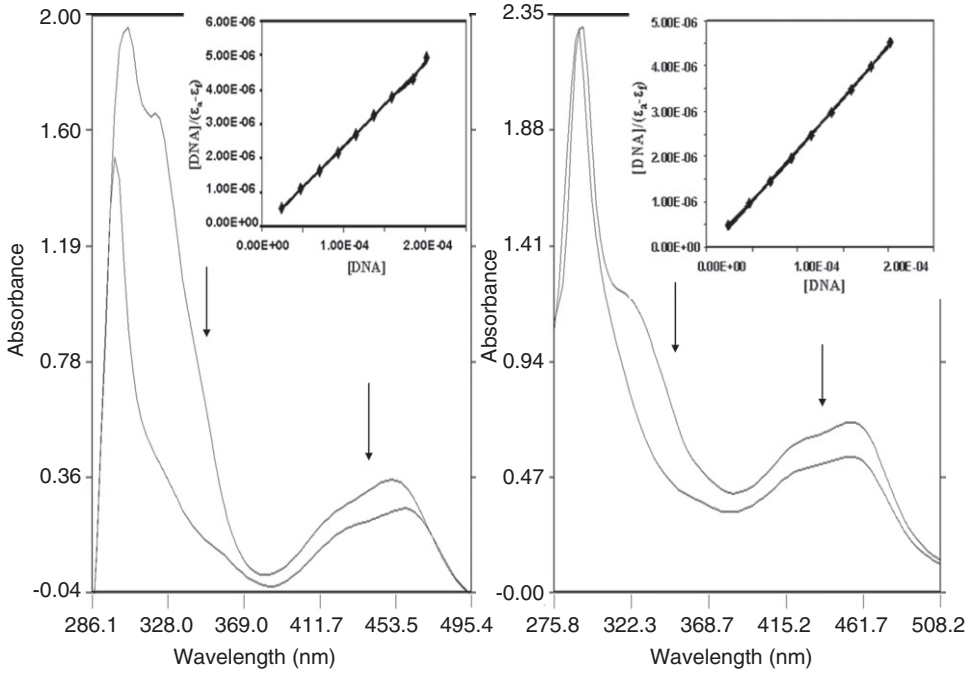


Figure 1. Absorption spectra of **1** and **2** in Tris-HCl buffer at 25°C in the presence of increasing amount of CT-DNA, [Ru] = 20 μmol L^{-1}, [DNA] = 0–200 μmol L^{-1}. The arrows indicate the change in absorbance upon increasing the DNA concentration. Inset: Plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ *vs.* [DNA].

3.2. *DNA-binding studies*

3.2.1. Electronic absorption titration. Electronic absorption spectroscopy is a useful technique in DNA-binding studies. Absorption spectra of $[Ru(bpy)_2BFIP]^{2+}$, $[Ru(dmb)_2BFIP]^{2+}$, and $[Ru(phen)_2BFIP]^{2+}$ in the absence and presence of CT-DNA at various DNA concentrations in figure 1 are characterized by distinct intense MLCT transitions in the visible region, attributed to $Ru(d\pi) \rightarrow bpy(\pi^*)$, $dmb(\pi^*)$, and $phen(\pi^*)$ and $Ru(d\pi) \rightarrow BFIP(\pi^*)$ transitions; absorptions below 300 nm (266 nm for **1**, 281 nm for **2**, and 258 nm for **3**) are attributed to intraligand (IL) $\pi \rightarrow \pi^*$ transitions, and MLCT at lower energy (451.0 nm for **1**, 453.5 nm for **2**, and 431.0 nm for **3**). As the

concentration of DNA increases, the hypochromism in the MLCT band increases and an obvious red shift is observed. Complexes binding with DNA through intercalation usually result in hypochromism and bathochromism (red shift) in absorption spectra with extent of spectral changes closely correlated to DNA-binding affinities. The spectral shifts for intercalation are usually greater than those in a groove-binding mode. As the concentration increases, the MLCT bands of **1**–**3** at 451.0, 453.5, and 431.0 nm exhibit hypochromism of 18%, 14%, and 22% as well as bathocromism of 7.5, 5, and 10 nm, respectively. These results are similar to those reported earlier for various metal intercalators [52, 53]. In order to quantitatively compare the affinity of the complexes for CT-DNA, indicated by absorption titrations, the binding constants K_b were measured by monitoring the change of absorbance in the MLCT band with increasing concentration of DNA (equation 1) [54] through a plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ *versus* [DNA].

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K(\varepsilon_b - \varepsilon_f) \quad (1)$$

where [DNA] is the concentration per nucleotide, the apparent absorption coefficient ε_a, ε_f, and ε_b correspond to A_{obs}/Ru(II). The extinction coefficients are for the Ru(II) complex, extinction coefficient of the complex in the presence of DNA and the extinction coefficient for the Ru(II) complex in the fully bound form, respectively. In plots of $[DNA]/(\varepsilon_a - \varepsilon_f)$ *versus* [DNA], K_b is given by the ratio of slope to intercept. Intrinsic binding constants, K_b of **1**, **2**, and **3** were $4.6 \pm 0.1 \times 10^4$, $3.2 \pm 0.3 \times 10^4$, and $5.4 \pm 0.1 \times 10^4\,mol\,L^{-1}$, respectively, smaller than those of classical intercalators, such as $[Ru(bpy)_2(dppz)]^{2+}$ ($>10^6$) [55], $[Ru(bpy)_2(ppd)]^{2+}$, $K = 1.3 \times 10^6$) [56], $[Ru(bpy)_2BDPPZ]^{2+}$ (5.3×10^4), $[Ru(dmb)_2BDPPZ]^{2+}$ (3.7×10^4), and $[Ru(phen)_2BDPPZ]^{2+}$ (6.0×10^4) [40], and comparable close to those of $[Ru(bpy)_2(MCMIP)]^{2+}$ (3.92×10^4), $[Ru(phen)_2(MCMIP)]^{2+}$ (4.8×10^4) [23], $[Ru(dmb)_2(MCMIP)]^{2+}$ (2.25×10^4), $[Ru(dmp)_2(MCMIP)]^{2+}$ (5.42×10^4) [57], $[Ru(dmb)_2(dtni)]^{2+}$ (2.63×10^4), $[Ru(dmb)_2(dtni)]^{2+}$ (8.65×10^4) [58], $[Ru(dmb)_2(ITAP)]^{2+}$ (4.5×10^4) [59], $[Ru(dmb)_2(ipdp)]^{2+}$ (1.18×10^4), and $[Ru(dmb)_2(ipdp)]^{2+}$ (7.18×10^3) [60]. The binding constants show the following order: $[Ru(phen)_2BFIP]^{2+} > [Ru(bpy)_2BFIP]^{2+} > [Ru(dmb)_2BFIP]^{2+}$.

The difference in binding strength of **1** and **2** is probably caused by the different ancillary ligands. The four additional methyl groups in **2** relative to **1** exert steric hindrance. Therefore, **1** can more tightly bind to adjacent DNA base pairs than **2**. Similarly, the difference in binding strength of **1** and **3** is due to the difference in ancillary ligands. On going from bpy to phen, the planarity area and hydrophobicity increase leading to greater binding affinity for **3** than **1**.

3.2.2. Fluorescence spectroscopic studies. Luminescence spectroscopy is a sensitive way toanalyze drug–DNA interaction. The emission spectra of **1**–**3** in the absence and presence of CT-DNA are shown in figure 2. In the absence of DNA and under 413 nm excitation, **1**–**3** emit relatively moderate luminescence in Tris buffer at room temperature with emission maxima at 558, 600, and 559 nm, respectively. The luminescent properties of the complexes were perturbed when DNA was titrated into the solution; binding of **1**–**3** to DNA increased the fluorescence intensity. Upon addition of CT-DNA, emission is enhanced 1.54 times for **1**, 1.26 times for **2**, and 1.79 times for **3** than complex without DNA, consistent with the absorption spectral results.

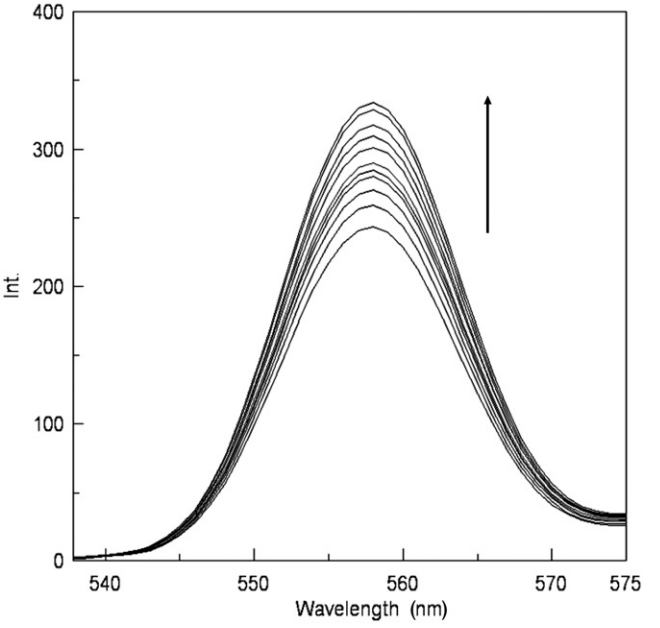


Figure 2. Emission spectra of $[Ru(phen)_2BFIP]^{2+}$ in Tris-HCl buffer at 25°C upon addition of CT-DNA, $[Ru] = 20\,\mu mol\,L^{-1}$, $[DNA] = 0–120\,\mu mol\,L^{-1}$. The arrow shows the increase in intensity on increasing CT-DNA concentrations.

This implies that **1**–**3** interact with CT-DNA, efficiently. The hydrophobic environment inside the DNA helix reduces the accessibility of water to the complex and complex mobility is restricted at the binding site, leading to decrease of vibrational modes of relaxation. Differential luminescence quenching was also utilized in monitoring DNA binding. A negatively charged quencher is expected to be repelled by the negatively charged phosphate backbone, and therefore a DNA-bound cationic molecule should be readily quenched [61]. Steady-state emission quenching experiments using $[Fe(CN)_6]^{4-}$ as quencher are also used to observe the binding of Ru(II) complexes with CT-DNA. The $[Fe(CN)_6]^{4-}$ has been shown to distinguish differentially bound Ru(II) species, and positively charged free complex ions should be readily quenched by $[Fe(CN)_6]^{4-}$. The method essentially consists of titrating a given amount of DNA–metal complexes with increasing the concentration of $[Fe(CN)_6]^{4-}$ and measuring the changes in fluorescence intensity. The ferro-cyanide quenching curves for these three complexes in the presence and absence of CT-DNA are shown in figure 3. From quenching studies, it is clear that DNA-binding ability of complexes follow the order: **3** > **1** > **2**.

The Stern–Volmer quenching constant (K_{sv}) for $[Fe(CN)_6]^{4-}$ as quencher can be determined by using Stern–Volmer equation [62].

$$I_0/I = 1 + K_{sv}[Q]$$

where I_0 and I are intensities of the fluorophore in the absence and presence of quencher, respectively, Q the concentration of the quencher, and K_{sv} a linear Stern–Volmer quenching constant. In the quenching plot (figure 3) of I_0/I *versus* $[Q]$,

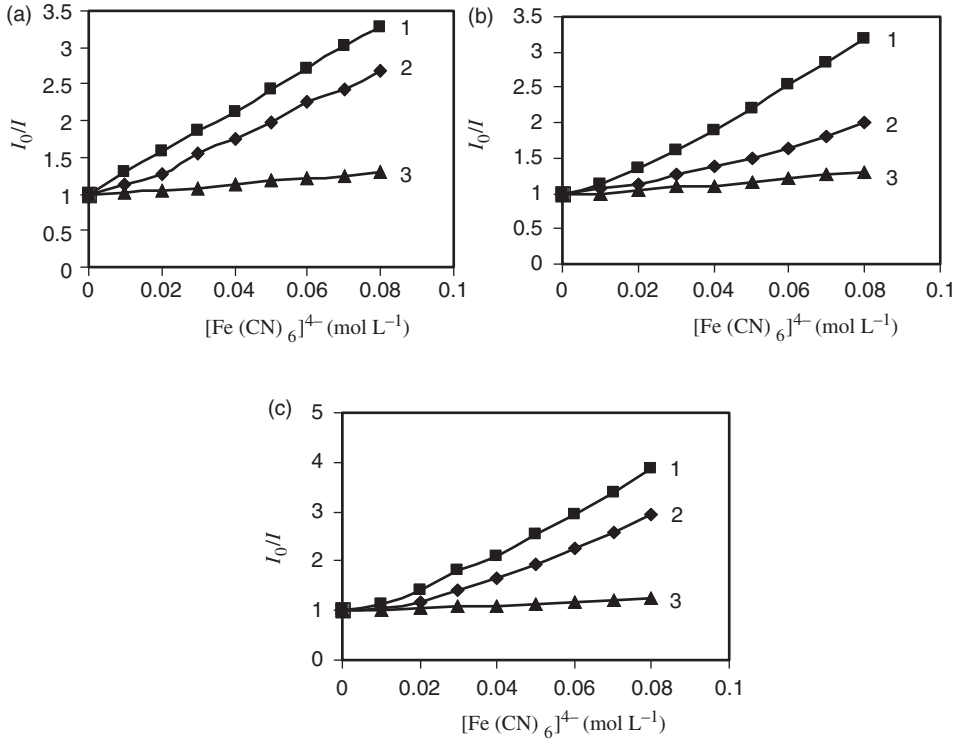


Figure 3. Emission quenching of complexes **1** (A), **2** (B), and **3** (C) with $K_4[Fe(CN)_6]$ in the absence (1), presence of DNA (2), [Ru] = 20 μmol L^{-1}, and excess DNA (200 μmol L^{-1}) (3).

K_{sv} is given by the slope. Figure 3 shows Stern–Volmer plots for the free complex in solution and in the presence of increasing amounts of DNA. All the complexes show linear Stern–Volmer plots with K_{sv} values for the complexes in absence of DNA 29, 26, and 33 for **1**–**3**, respectively.

The K_{sv} value for the complexes in presence of DNA is 22, 11, and 22 for **1**–**3**, respectively. In the presence of DNA, K_{sv} value is smaller and at high concentration of DNA (1 : 200; Ru^{2+} : DNA) essentially of zero slope, indicating that the bound species is inaccessible to quencher.

3.2.3. Viscosity studies. Viscosity, which is sensitive to the change of length of DNA, may be the most effective means to study binding of complexes to DNA in the absence of X-ray crystallography or NMR structural data [51, 63]. A classical intercalation model results in lengthening of the DNA helix and also leads to increase of DNA viscosity; partial and/or non-classical intercalation would bend the DNA helix, reduce its effective length and concomitantly, its viscosity [64]. For example, under appropriate conditions, intercalation of EtBr causes a significant increase in the overall DNA length. The effects of the three complexes on the viscosity of rod-like DNA are shown in figure 4. As the concentration of complex increases, the relative viscosity of DNA increases as the length of the duplex DNA increases following intercalation [65].

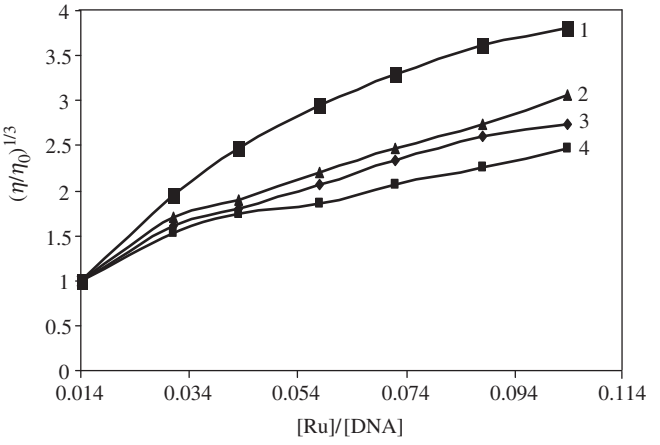


Figure 4. Effect of increasing amount of ethidium bromide (1), $[Ru(phen)_2BFIP]^{2+}$ (2), $[Ru(bpy)_2BFIP]^{2+}$ (3), and $[Ru(dmb)_2BFIP]^{2+}$ (4) on relative viscosity of CT-DNA at $30 \pm 0.1°C$. The total concentration of DNA is $0.25\,mmol\,L^{-1}$, $[Ru] = 20\,\mu mol\,L^{-1}$.

Though the intercalating ligand is the same in all three complexes, there is small difference in the viscosity due to the difference in the ancillary ligands. The presence of viscosity further suggests that the Ru(II) complexes show an intercalative binding mode to CT-DNA.

3.2.4. Photoactivated cleavage of pBR 322 DNA by Ru(II) complexes. A number of metal polypyridine complexes have been studied in relation to their DNA photocleavage behavior [25, 33, 37, 66–68]. Cleavage reactions on plasmid DNA can be monitored by agarose gel electrophoresis. When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact super coil form (Form I). If scission occurs on one strand (nicking), the super coil will relax to generate a slower-moving open circular form (Form II); if both strands are cleaved, a linear form (Form III) that migrates between Form I and Form II will be generated [69]. "Supplementary material" shows the gel electrophoresis separation of pBR-322 DNA after incubation with **1**–**3** and irradiation at 365 nm. No DNA cleavage was observed for the control in which metal complex was absent (lane 0); with increasing concentration of complex, the amount of Form I of plasmid DNA diminishes gradually, whereas Form II increases, under comparable experimental conditions; **3** exhibits more DNA cleavage activity than **1** and **2**. To identify the nature of the reactive species responsible for photoactivated cleavage of plasmid DNA, we have further investigated with the potentially $^1O_2$-inhibiting agent histidine. The photocleavage of pBR-322 DNA in the presence of complex alone and with histidine are shown in "Supplementary material." Indeed, plasmid DNA cleavage by **1**–**3** was inhibited in the presence of histidine which indicated that $^1O_2$ acts as a competing cleavage agent. In the presence of histidine, Form II is not observed.

3.2.5. DNA melting studies. As intercalation of the complexes into DNA base pairs causes stabilization of base stacking and hence raises the melting temperature of the

Table 2. Absorption titration and thermal melting experiments.

Complexes	T_M (°C)	Hypochromicity (%)	Absorption λ_{max} (nm)		$\Delta\lambda$ (nm)
			Free	Bound	
CT-DNA alone	62	–	–	–	–
$[Ru(bpy)_2BFIP]^{2+}$	66	18	266	273.5	7.5
$[Ru(dmb)_2BFIP]^{2+}$	64	14	281	286	5
$[Ru(phen)_2BFIP]^{2+}$	71	22	216	226	10

double standard DNA, DNA melting experiments are useful in establishing the extent of intercalation. All three complexes were incubated with CT-DNA, heated to 85°C from ambient temperature, and the optical density (OD) at 260 nm was monitored. Binding of complexes increases T_M of DNA in the order **3** > **1** > **2** (table 2).

4. Conclusion

Three Ru(II) complexes, $[Ru(bpy)_2BFIP]^{2+}$ (**1**), $[Ru(dmb)_2BFIP]^2$ (**2**), and $[Ru(phen)_2BFIP]^{2+}$ (**3**), have been synthesized and characterized and their DNA-binding and photocleavage properties investigated. Spectroscopic studies and viscosity experiments showed that the complexes intercalate into DNA base pairs *via* BFIP ligand. When irradiated at 365 nm, the Ru(II) complexes are efficient photocleavers of plasmid pBR-322 DNA.

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