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Biological Screening of Polypyridyl Chloro–Ruthenium(II) Complexes : Antimicrobial and DNA Interaction

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ABSTRACT

Polypyridyl ruthenium(II) complexes were synthesized and characterized. Binding mode of the complexes to DNA was evaluated from combine results of electronic absorption and viscosity measurement study. The results suggest that complexes **1**, **2** and **3** bind to DNA via classical intercalation, electrostatic interaction and partial intercalation, respectively. Complex **2** shows less affinity for DNA. Cleavage of pUC19 DNA by complexes was checked using gel electrophoresis. The data reveals that highest cleaving ability is of complex **1**.

Keywords : polypyridine; Ru(II) complex; DNA interaction; Antibacterial

Introduction

The interaction of polypyridyl ruthenium complexes with DNA has attracted considerable attention in recent decades for developing novel probes of DNA structure or new therapeutic agents.[1–3] Ruthenium(II) complexes bind with DNA in a non–covalent interaction fashion, such as electrostatic binding for cation. groove binding for large ligands, intercalative binding for planar ligands and partial intercalative binding for incompletely planar ligands.[4–6] Several ruthenium complexes were found to be potential anticancer substances with remarkable anticancer activity and lower toxicity than platinum compounds.[7]

In coordination chemistry, terpyridines are of special interest due to their ability to form stable complexes with many transition metal ions. Such complexes possess interesting photophysical, electrochemical and photochemical properties and they allow the construction of extended supramolecular architectures.[8] The influence of the ancillary ligands of the complexes has received little attention. Since the octahedral polypyridyl Ru^{II} complexes bind to DNA in three dimensions, the ancillary ligands can also play an important role in governing DNA–binding of complexes. At the same time, varying substitutive group or substituent position in the ancillary ligand can also create some interesting differences in the space configuration and the electron density distribution of Ru^{II} polypyridyl complexes, which will result in some differences in spectral properties and the DNA–binding behaviors of the complexes and will be helpful to understand the binding mechanism of Ru^{II} polypyridyl complexes to DNA.[9–13]

In a previous publication from our laboratory,[14] biological activities of copper complexes with gatifloxacin and various neutral bidentate ligands were studied. In this paper, we report the synthesis and characterization of $[Ru^{II}(4\text{-bptpy})(dmphen)Cl]ClO_4$, $[Ru^{II}(4\text{-fptpy})(dmphen)Cl]ClO_4$ and $[Ru^{II}(4\text{-mptpy})(dmphen)Cl]ClO_4$ complexes. Binding affinity of the complexes towards Herring Sperm DNA has been quantitatively determined by calculating binding constant (K_b), using absorption titration method. The binding

mode of the complexes with DNA has been determined using viscosity measurements. Cleavage ability of complexes towards pUC19 DNA has been also investigated by gel electrophoresis technique.

Results and Discussion

Thermogravimetric and Electronic absorption analysis

TGA data of the complexes shows no weight loss between the temperatures range 80 to 180 °C. So, there is an absence of coordinated or lattice water molecule. The electronic spectra of complexes consist of three well–defined bands in the range 250–500 nm, similar to that observed for [Ru(dpphen)(terpy)Cl]PF_6 complex reported by Yoshikawa et al.[15] The lowest energy absorption band (MLCT band) for complexes **1**, **2** and **3** appeared at 490.5, 488.5 and 492 nm, respectively (Table 1). Change in the substitution on terpyridine from 4-fptpy to 4-mptpy, red shift of the MLCT band is observed. The two higher energy absorption bands appeared in the range 282.5 to 285.5 and 308 to 310 nm. These bands can be assigned to the ligand centered transitions dmphen(π)→dmphen(π*) and terpy(π)→terpy(π*), respectively.[15]

Table 1: Electronic spectral data for the ruthenium(II) complexes

Complexes	λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	
	π → π*	MLCT
1	285.5 (46 900), 308 (48 900)	490.5 (20 050)
2	282.5 (66 400), 310 (59 650)	488.5 (24 400)
3	282.5 (43 600), 308 (56 550)	492.0 (22 350)

Infrared spectroscopy

The band appeared at ~722 cm^{-1} is due to C–H out of plan banding. The presence of perchlorate as a counter ion is confirmed by the very strong, broad band at ~1085 cm^{-1} and the strong, sharp band around 625 cm^{-1}.[16] In the spectra of complex **3**, band at 1259 cm^{-1} is due to the asymmetric stretching of aromatic ether. A weak, broad band around 3060 cm^{-1}, characteristic of aromatic C–H stretching as well as a sharp band at 2925 cm^{-1}, characteristic of C–H stretching of methyl. Sharp bands with medium intensity appeared around 1600 and 1495 cm^{-1}, characteristic of aromatic ring stretching. An intense, sharp band at ~760 cm^{-1}, characteristic of ring deformations and C–H out–of–plane deformations, appears as expected from a structure including aromatic rings. A weak, sharp band around 464–516 cm^{-1}, characteristic of Ru–N stretching mode. A Ru–Cl stretching mode would be expected in the region less than 400 cm^{-1}.[17]

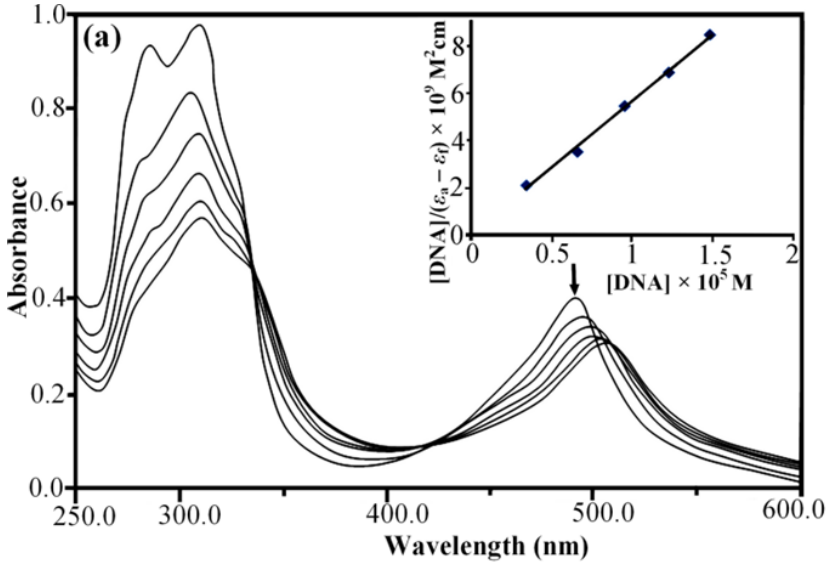
^{1}H NMR

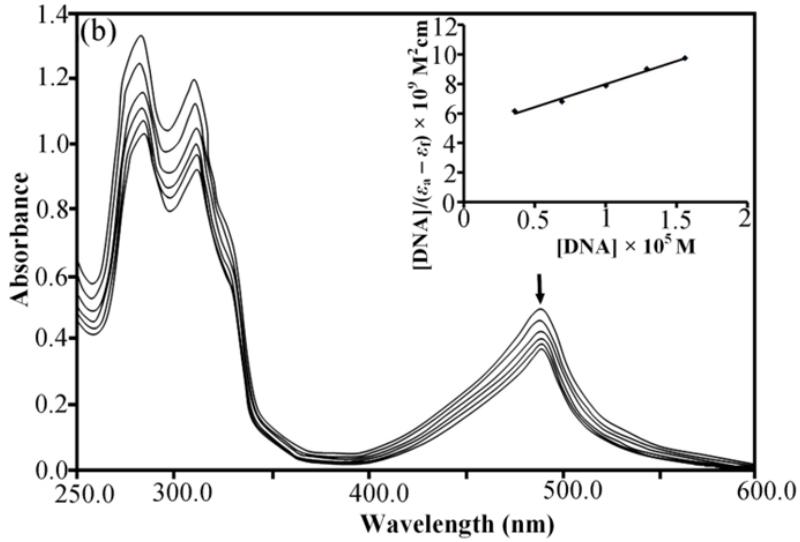
On coordination with ruthenium ion, chemical shift of the $T_{3',5'}$, $T_{6,6''}$ and $T_{4,4''}$ protons show large downfield due to metal–to–ligand π–back donation.[18] $T_{ph2,3,5,6}$ of complexes **2** and **3** show upfield shift. $T_{ph2,6}$ of complex **1** shows small upfield shift, while $T_{ph3,5}$ shows downfield shift. Small upfield shift of $T_{3,3''}$ protons, observed in all complexes. No considerable change observed in chemical shift of $T_{5,5''}$ protons. One methyl group of dmphen

appeared in downfield than other due to the ring–current anisotropic effect exerted by terpyridine, experienced through space.

Electronic absorption titration

DNA binding of polypyridyl Ru^{II} complexes with DNA can be quantitatively measure by monitoring changes in the electronic spectra at MLCT band. Complex binds to DNA through intercalation usually results in hypochromism and bathochromism. The extent of the hypochromism commonly parallels the intercalative binding strength.[19,20] Complexes interact with DNA through electrostatic interaction shows lower hypochromicity with no bathochromic shift.[18,21] The electronic spectral traces of the complexes in the absence and presence of Herring Sperm DNA are given in Fig. 1.





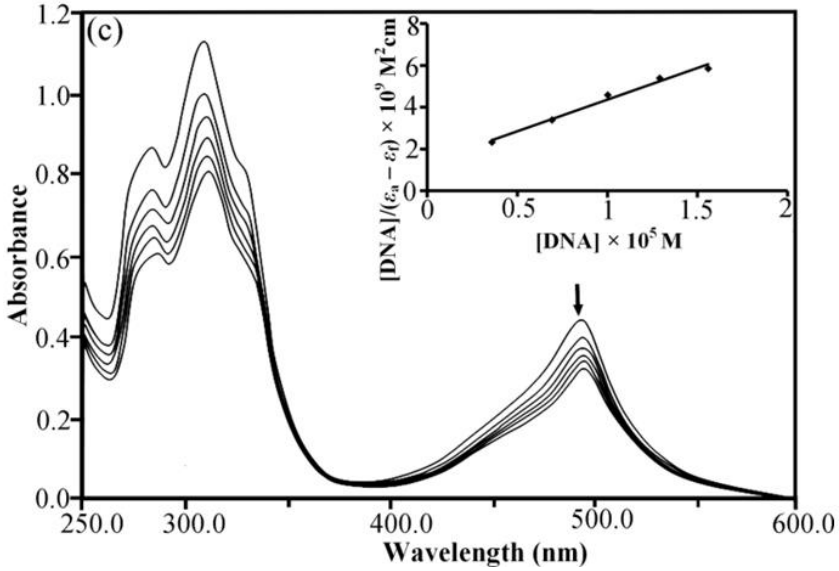


Fig. 1. Electronic absorption spectra of (a) [Ru^{II}(4-bptpy)(dmphen)Cl]ClO$_4$, (b) [Ru^{II}(4-fptpy)(dmphen)Cl]ClO$_4$ and (c) [Ru^{II}(4-mptpy)(dmphen)Cl]ClO$_4$ with increasing amount of DNA in phosphate buffer(Na_2HPO_4/NaH_2PO_4, pH 7.2). [complex] = 20 μM, [DNA] = 0–16.6 μM with incubation period of 15 min at 37 °C. Plots of [DNA]/($\varepsilon_a - \varepsilon_f$) vs. [DNA] for the titration of DNA with Ru^{II} complexes.

As the DNA concentration is increased, hypochromism is observed in the MLCT band of each complex, as shown in Table 2. For complex **1**, the MLCT absorption band shifts from 490.5 to 503.5 nm with 23.9% hypochromism. For complexes **2** and **3**, the MLCT transition bands exhibit red shifts of 0 and 2 nm and hypochromism of 5.6% and 13.3%, respectively. The bathochromic shift of complexes **1** and **3** suggest that they may bind to DNA via classical intercalative mode. Absence of red shift at MLCT band of complex **2** suggests that the complex may electrostatically interact with DNA. Highest hypochromisity was observed in complex **1** shows that it interact with DNA more strongly than others. More confirmation regarding binding mode of the complexes will be obtained from viscosity measurement. The intrinsic binding constants K_b of complexes **1**–**3** are 6.32×10^5, 6.57×10^3 and 1.85×10^4 M^{-1}, respectively (Table 2). The K_b value of complex **1** is comparable with the classical intercalator $[Ru(dmp)_2(HPIP)]^{2+}$.[11] Complexes with electron withdrawing group on ancillary ligand possess higher DNA binding affinity than electron donating group.[5] So, complex **1** has higher K_b value than complex **3**. In fact complex **2** also has electron withdrawing group, but has low binding constant. Electrostatic binding mode may be responsible for its lower affinity towards DNA.

Table 2: Electronic absorption data upon addition of herring sperm DNA

Complex	λ_{max} (nm)			Hypochromism H[A] (%)	Binding constant K_b (M^{-1})
	Free	Bound	Δλ		
1	490.5	503.5	13	23.9	6.32×10^5
2	488.5	488.5	0	5.6	6.57×10^3
3	492.0	494.0	2	13.3	1.85×10^4

[A] $H\% = 100 \times (A_{free} - A_{bound})/A_{free}$.

Viscosity measurement

From viscosity measurement study, we can confirm the binding mode of complexes. A classical intercalation results in lengthening in the DNA helix, as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity, while a partial and/or non–classical intercalation of compound may bend DNA helix, resulting in the decrease of its effective length and, thereby its viscosity.[13] When the compounds interact with DNA electrostatically, no effect on relative viscosity of DNA is observed.[18] The effect of increasing amount of EB and complexes on the relative viscosity of DNA is shown in Fig. 2. EB is well known classical intercalator. The relative viscosity of DNA solution increases on addition of increasing amount of complex **1**. So, complex **1** binds to DNA via classical intercalative mode. Complex $[Ru(bpy)_3]^{2+}$ has been known to bind with DNA in electrostatic mode, it exerts essentially no effect on DNA viscosity.[5] No change in relative viscosity is observed for complex **2,** which lead to conclusion that complex **2** interact with DNA electrostatically. Absorption titration also supports the same binding mode for complex **2** (0 nm red shift). In contrast, complex **3** decreases the relative viscosity of DNA as shown by the partial intercalators. Considering the results of spectroscopic and viscosity measurements, we may suggest that complex **1** binds to DNA via classical intercalative mode, complex **2** interacts electrostatically with DNA and complex **3** partially intercalate to DNA.

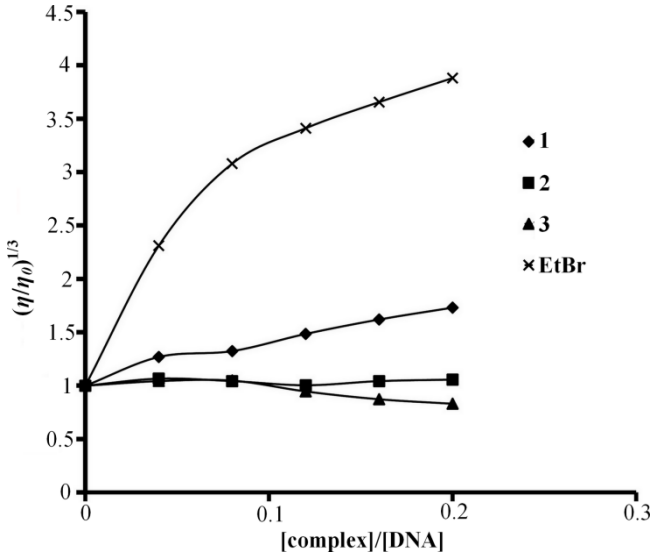


Fig. 2. Effect on relative viscosity of DNA under the influence of increasing amount of ethidium bromide and complexes at 27±0.1 °C in phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 7.2).

Quantitative determination of pUC19 DNA cleavage by agarose gel electrophoresis

The double–stranded plasmid pUC19 exists in a compact supercoiled (SC) conformation. If one strand breaks, the SC form of DNA will relax to produce an open circular (OC) form. If both strands are cleaved, a linear (L) form will be produced. SC form migrates faster; OC form migrates slowly while linear form migrates in between the SC form and the OC form.[22]

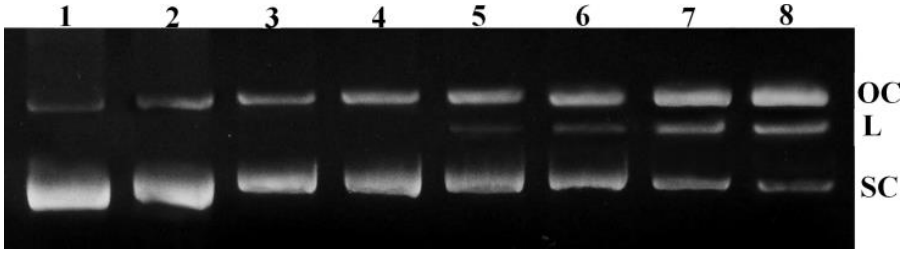


Fig. 3. Agarose gel (1%) of pUC19 (100 μg/mL) incubated for 2 h at 37 °C in TE buffer (pH 8) with increasing concentrations of the [Ru^{II}(4-bptpy)(dmphen)Cl]ClO_4. Lane 1, DNA control; lane 2, $RuCl_3$ (100 μM); lanes 3–8, [Ru^{II}(4-bptpy)(dmphen)Cl]ClO_4 complex: 25, 75, 125, 200, 300, and 400 μM, respectively.

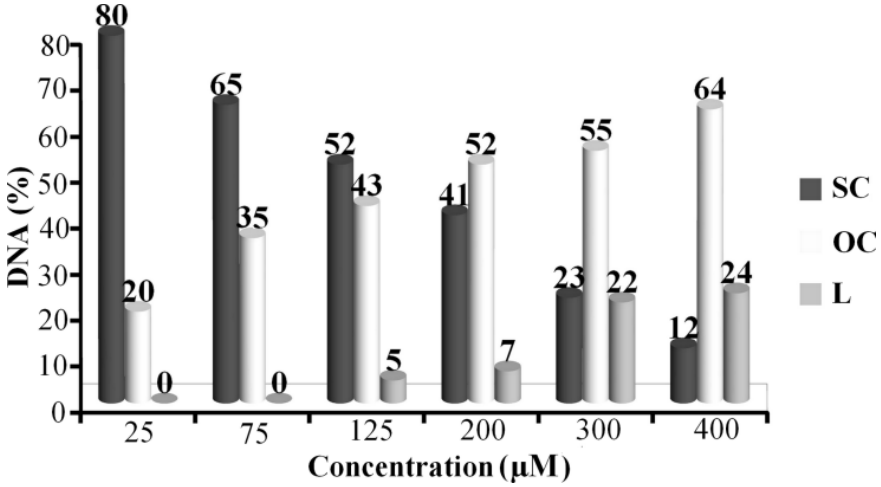


Fig. 4. The percentage of SC, OC and L forms of DNA produced by various concentration of [Ru^{II}(4-bptpy)(dmphen)Cl]ClO_4 complex.

DNA cleavage ability of complexes was quantified by measuring the transformation of the SC form into OC and L forms. The concentration dependant DNA cleavage induced by complex **1** with incubation time of 180 min is shown in Fig. 3. The percentage of different forms of DNA produced by various concentration of complex **1** is shown in Fig. 4. The data indicate that as the concentration of complex increases, percentage of OC and L forms increases. The linear form generates at ≥125 μM. The time dependence cleavage by complex **1** was studied at 200 μM concentration for 15–240 min (Fig. 5). As the incubation time increases, percentage of OC and L forms increases (Fig. 6). The linear form produced at 90 min. The SC form completely disappears at 240 min. So, cleavage ability of all complexes was checked at 200 μM concentration with incubation time of 240 min (Fig. 7). The amounts of linear DNA produced by complexes **1**, **2** and **3** were 28, 18 and 24%, respectively.

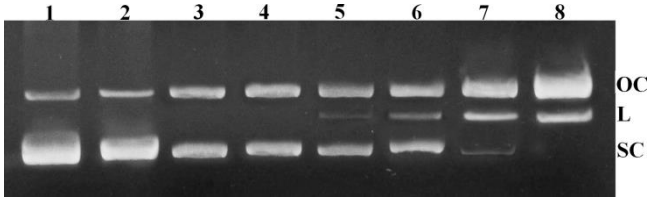


Fig. 5. Agarose gel (1%) of pUC19 (100 μg/mL) at 37 °C in TE buffer (pH 8) with 200 μM [Ru^{II}(4-bptpy)(dmphen)Cl]ClO_4 complex for increasing reaction time. Lane 1, DNA control; lanes 2–8: 15, 30, 60, 90, 120, 180 and 240 min, respectively.

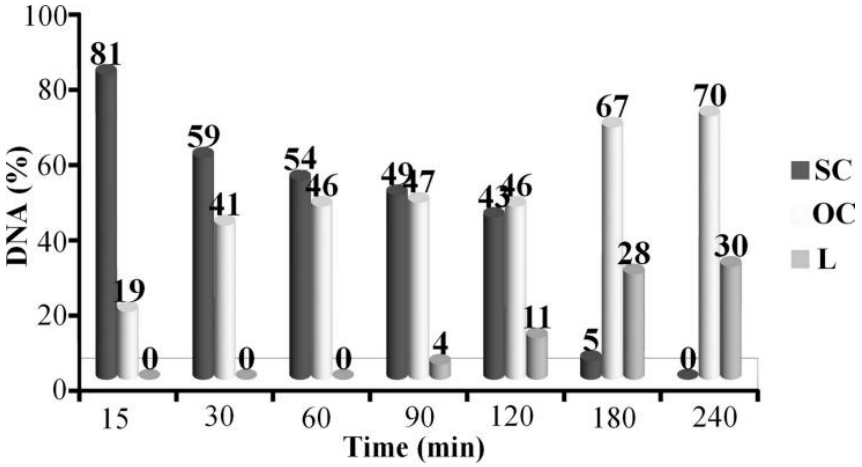


Fig. 6. The percentage of SC, OC and L forms of DNA produced by 200 μM of $[Ru^{II}(4\text{-bptpy})(dmphen)Cl]ClO_4$ complex at different time.

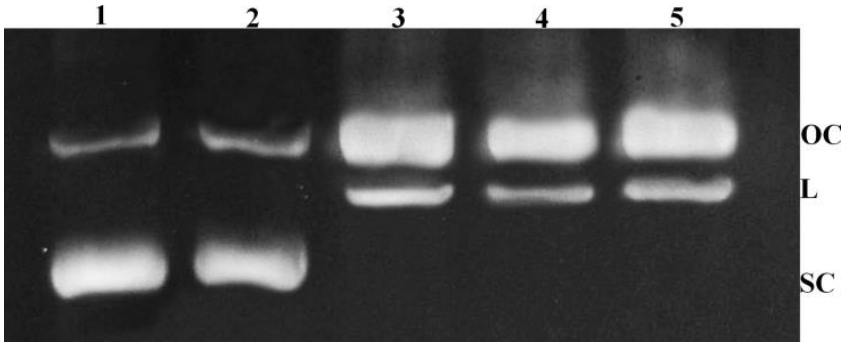


Fig. 7. Agarose gel (1%) of pUC19 (100 μg/mL) at 37 °C in TE buffer (pH 8) with 200 μM compounds incubated for 4 h. Lane 1, DNA control; lane 2, $RuCl_3$; lane 3, $[Ru^{II}(4\text{-bptpy})(dmphen)Cl]ClO_4$; lane 4, $[Ru^{II}(4\text{-fptpy})(dmphen)Cl]ClO_4$; lane 5, $[Ru^{II}(4\text{-mptpy})(dmphen)Cl]ClO_4$.

Conclusions

After studying the DNA interaction studies, we conclude that Ru^{II} complex with fluoro derivative of terpyridine shows lower binding and cleavage ability towards DNA. On the other hand complex with bromo derivative of terpyridine has higher DNA binding and cleavage ability. Complex **1** binds to DNA by classical intercalative mode. Complex **2** interacts with DNA electrostatically, while complex **3** partially intercalate to DNA. The difference in K_b value may be due to different binding mode of the complexes.

Experimental

Materials and instrumental details

2-Acetyl pyridine, 4-bromobenzaldehyde, 4-fluorobenzaldehyde and 4-methoxybenzaldehyde were purchased from Spectrochem (Mumbai, India). Ruthenium trichloride and sodium perchlorate were purchased from Chemport (Mumbai, India). Agarose, ethidium bromide, TAE (Tris–Acetyl–EDTA), bromophenol blue and xylene cyanol FF were purchased from Himedia, India. Herring sperm DNA was purchased from Sigma Chemical Co., India. 2,9-Dimethyl-1,10-phenanthroline was purchased from Loba chemie (India). Infrared

spectra were recorded on Fourier transform IR (FTIR) Shimadzu spectrophotometer as KBr pellets in the range 4000–400 cm^{-1}. The ^{1}H and ^{13}C NMR were recorded on a Bruker Avance (400 MHz). The fast atomic bombardment mass spectra (FABMS) were recorded on Jeol SX 102/Da–600 mass spectrophotometer/data system using Argon/Xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature. The electronic spectra were recorded on a UV–160A UV–Vis spectrophotometer, Shimadzu (Japan). TGA was carried out using a 5000/2960 SDTA, TA instrument (USA) operating at a heating rate of 10 °C per minute in the range of 20–800 °C in N_2. C, H and N elemental analyses were performed with a model 240 Perkin Elmer elemental analyzer.

General Experimental Procedure for the Preparation of Terpyridines: preparation of 4′-(4-bromophenyl)-2,2′:6′,2′′-terpyridine (4-bptpy)

2-Acetylpyridine (2.42 g, 20.0 mmol) was added to an ethanolic solution of 4-bromobenzaldehyde (1.85 g, 10.0 mmol) in EtOH (70 mL). KOH pellets (1.4 g, 26 mmol) and aqueous NH_3 (30 mL, 25%, 0.425 mol) were added to the solution and was then stirred at room temperature for 8 h. An off–white solid formed which was collected by filtration and washed with H_2O (3 × 10 mL) and EtOH (2 × 5 mL). Recrystallization from $CHCl_3$–MeOH gave white crystalline solid. Yield 1.84 g, 47.54%, mp: 125 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 8.591–8.721 (m, 6H), 7.984 (t, 2H, $H_{4,4''}$), 7.802 (d, 2H, $H_{ph3,5}$), 7.710 (d, 2H, $H_{ph2,6}$), 7.486 (d, 2H, $H_{5,5''}$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 156.15 ($C_{2',6'}$), 155.27 ($C_{2,2''}$), 149.67 ($C_{6,6''}$), 148.53 ($C_{4'}$), 137.77 (C_{ph1}), 137.08 ($C_{4,4''}$), 132.66 ($C_{3,3''}$), 129.35 ($C_{ph2,6}$), 124.89 ($C_{5,5''}$), 123.48 (C_{ph4}), 121.34 ($C_{ph3,5}$), 118.14 ($C_{3',5'}$). Anal. Calc. for $C_{21}H_{14}N_3Br$: C 64.96, H 3.63, N 10.82. Found: C 64.76, H 3.83, N 10.67%.

4′-(4-fluorophenyl)-2,2′:6′,2′′-terpyridine (4-fptpy)

This ligand was prepared by the same method describe above, but using 4-fluorobenzaldehyde instead of 4-bromobenzaldehyde. Yield 1.32 g, 40.49%, mp: 182 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 8.691–8.743 (m, 6H), 7.902–7.918 (m, 4H, $H_{ph2,3,5,6}$), 7.391 (t, 2H, $H_{4,4''}$), 7.215 (t, 2H, $H_{5,5''}$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 162.29 (C_{ph4}), 155.96 ($C_{2',6'}$), 155.73 ($C_{2,2''}$), 149.40 ($C_{4'}$), 148.94 ($C_{6,6''}$), 137.18 ($C_{4,4''}$), 129.22 ($C_{3,3''}$), 129.12 ($C_{ph2,6}$), 123.97 ($C_{5,5''}$), 121.52 (C_{ph1}), 118.91 ($C_{3',5'}$), 115.82 ($C_{ph3,5}$). Anal. Calc. for $C_{21}H_{14}N_3F$: C 77.05, H 4.31, N 12.84. Found: C 77.24, H 4.09, N 12.71%.

4′-(4-methoxyphenyl)-2,2′:6′,2′′-terpyridine (4-mptpy)

This ligand was prepared by the same method describe above but using 4-methoxybenzaldehyde instead of 4-bromobenzaldehyde. Yield 1.16 g, 34.31%, mp: 158 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 8.76–8.795 (m, 4H, $H_{3,3',5',3''}$), 8.71 (d, 2H, $H_{6,6''}$), 7.913–7.940 (m, 4H), 7.396 (d, 2H, $H_{ph3,5}$), 7.055 (dd, 2H, $H_{5,5''}$), 3.90 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 160.57 (C_{ph4}), 156.15 ($C_{2',6'}$), 155.59 ($C_{2,2''}$), 149.81 ($C_{4'}$), 148.88 ($C_{6,6''}$), 137.08 ($C_{4,4''}$), 130.63 (C_{ph1}), 128.55 ($C_{ph2,6}$), 123.81 ($C_{5,5''}$), 121.48 ($C_{3,3''}$), 118.41 ($C_{3',5'}$), 114.35 ($C_{ph3,5}$), 55.38 (OCH_3). Anal. Calc. for $C_{22}H_{17}N_3O$: C 77.86, H 5.05, N 12.38. Found: C 77.65, H 4.78, N 12.53%.

General Experimental Procedure for the Preparation of Ru^{II} complexes: preparation of [Ru^{II}(4-bptpy)(dmphen)Cl]ClO_4 (**1**)

$[Ru^{II}(4\text{-bptpy})(dmphen)Cl]ClO_4$ (**1**) was synthesized by taking $[Ru^{III}(4\text{-bptpy})Cl_3]$ (262 mg, 0.44 mmol), 2,9-dimethyl-1,10-phenanthroline (104 mg, 0.5 mmol), excess LiCl (122 mg, 2.94 mmol) and NEt_3 (0.9 mL) in 45 mL of ethanol and the mixture was refluxed for 2 h under a dinitrogen atmosphere (Scheme 1). The initial dark brown color of the solution gradually changed to a deep purple. The solvent was then removed under reduced pressure. The dry mass was dissolved in a minimum volume of acetonitrile, and an excess saturated aqueous solution of $NaClO_4$ was added to it. The precipitate was filtered off and washed with cold ethanol followed by ice-cold water. The product was dried in vacuum and purified using a silica column. The complex was eluted by 2:1 CH_2Cl_2/CH_3CN. Yield: 0.231 g, 63%, mol. wt. 832.39. IR (KBr): ν 3063 w,br; 2922 sh; 1596 m,sh; 1498 m,sh; 1086 s,sh; 754 s,sh; 627 vs,sh; 516 w,sh cm^{-1}. ^1H NMR [dimethyl sulfoxide-d_6 (DMSO–d_6), 400 MHz] δ/ppm 9.512 (s, 2H, $T_{3',5'}$, where T = Terpyridine), 9.116 (d, 2H, $T_{6,6''}$), 8.441 (d, 2H, P_4, where P = phenanthroline), 8.428 (d, 2H, $T_{3,3''}$), 8.111 (d, 2H, $T_{ph3,5}$), 8.084 (t, 2H, $T_{4,4''}$), 7.866 (s, 2H, $P_{5,6}$), 7.698 (d, 1H, P_7), 7.621 (d, 1H, P_3), 7.583 (d, 1H, P_8), 7.561 (d, 2H, $T_{ph2,6}$), 7.292 (t, 2H, $T_{5,5''}$), 3.36 (s, 3H, CH_3), 2.78 (s, 3H, CH_3). Anal. Calc. for $C_{35}H_{26}N_5O_4BrCl_2Ru$: C 50.50, H 3.15, N 8.41. Found: C 50.35, H 3.27, N 8.28%. FABMS: m/z = 209 $[dmphen + H]^+$, 699 $[M – ClO_4 – Cl]^+$, 734 $[M – ClO_4]^+$, 735 $[M – ClO_4 + H]^+$, 833 $[M]^+$,.

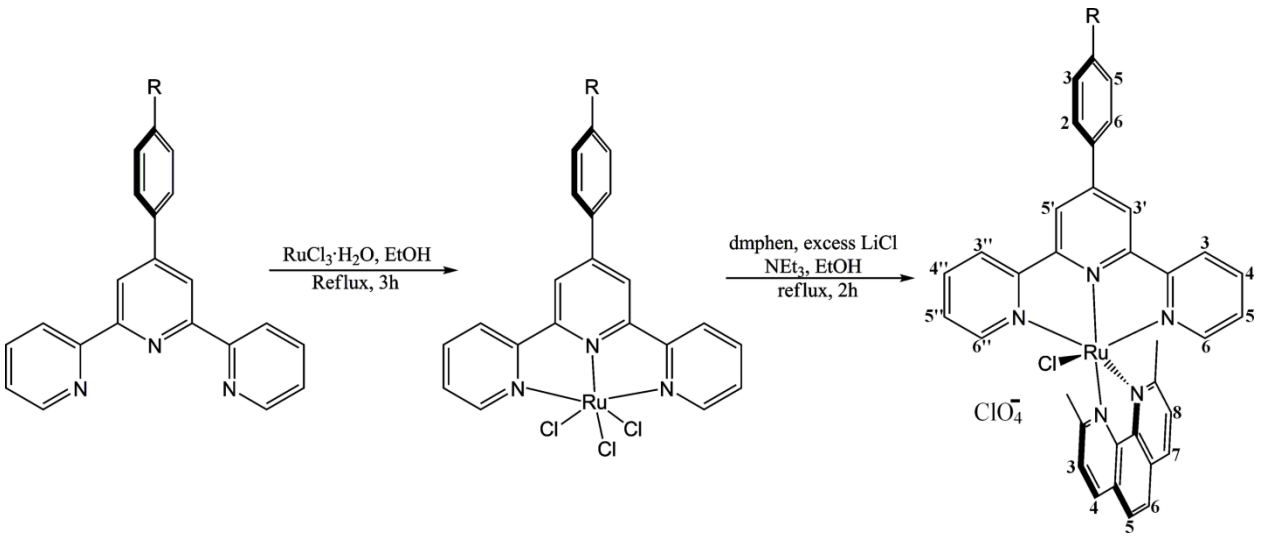
$[Ru^{II}(4\text{-fptpy})(dmphen)Cl]ClO_4$ (**2**)

This complex was synthesized in a manner identical to that described for $[Ru^{II}(4\text{-bptpy})(dmphen)Cl]ClO_4$, with $[Ru^{III}(4\text{-fptpy})Cl_3]$ (235 mg, 0.44 mmol) in place of $[Ru^{III}(4\text{-bptpy})Cl_3]$. Yield: 0.265 g, 78%, mol. wt. 771.59. IR (KBr): ν 3058 w,br; 2923 sh; 1599 m,sh; 1496 m,sh; 1088 s,sh; 757 s,sh; 626 vs,sh; 483 w,sh cm^{-1}. ^1H NMR (DMSO–d_6, 400 MHz) δ/ppm 9.489 (s, 2H, $T_{3',5'}$), 9.111 (d, 2H, $T_{6,6''}$), 8.524 (q, 2H, $T_{3,3''}$), 8.445 (d, 2H, P_4), 8.086 (t, 2H, $T_{4,4''}$), 7.861 (s, 2H, $P_{5,6}$), 7.672 (d, 1H, P_7), 7.655 (t, 2H, $T_{ph3,5}$), 7.623 (d, 1H, P_3), 7.586 (d, 1H, P_8), 7.563 (d, 2H, $T_{ph2,6}$), 7.295 (t, 2H, $T_{5,5''}$), 3.363 (s, 3H, CH_3), 2.786 (s, 3H, CH_3). Anal. Calc. for $C_{35}H_{26}N_5O_4FCl_2Ru$: C 54.48, H 3.40, N 9.08. Found: C 54.63, H 3.23, N 9.24%. FABMS: m/z = 209 $[dmphen + H]^+$, 637 $[M – ClO_4 – Cl]^+$, 672 $[M – ClO_4]^+$, 673 $[M – ClO_4 + H]^+$, 771 $[M]^+$.

$[Ru^{II}(4\text{-mptpy})(dmphen)Cl]ClO_4$ (**3**)

This complex was synthesized in a manner identical to that described for $[Ru^{II}(4\text{-bptpy})(dmphen)Cl]ClO_4$, with $[Ru^{III}(4\text{-mptpy})Cl_3]$ (241 mg, 0.44 mmol) in place of $[Ru^{III}(4\text{-bptpy})Cl_3]$. Yield: 0.241 g, 70%, mol. wt. 783.62. IR (KBr): ν 3070 w,br; 2929 sh; 1603 m,sh; 1495 m,sh; 1259 s; 1082 s,sh; 764 s,sh; 625 vs,sh; 464 w,sh cm^{-1}. ^1H NMR (DMSO–d_6, 400 MHz) δ/ppm 9.44 (s, 2H, $T_{3',5'}$), 9.11 (d, 2H, $T_{6,6''}$), 8.435 (d, 2H, P_4), 8.446 (d, 2H, $T_{3,3''}$), 8.067 (t, 2H, $T_{4,4''}$), 7.863 (s, 2H, $P_{5,6}$), 7.693 (d, 1H, P_7), 7.618 (d, 1H, P_3), 7.572 (d, 1H, P_8), 7.55 (d, 2H, $T_{ph2,6}$), 7.326 (d, 2H, $T_{ph3,5}$), 7.281 (t, 2H, $T_{5,5''}$), 3.968 (s, 3H, OCH_3), 3.354 (s, 3H, CH_3), 2.776 (s, 3H, CH_3). Anal. Calc. for $C_{36}H_{29}N_5O_5Cl_2Ru$: C 55.18, H 3.73, N 8.94. Found: C 55.37, H 3.61, N 8.76%. FABMS: m/z = 209 $[dmphen + H]^+$, 649 $[M – ClO_4 – Cl]^+$, 684 $[M – ClO_4]^+$, 685 $[M – ClO_4 + H]^+$, 783 $[M]^+$.

Caution: Perchlorate salts of metal complexes with organic ligands are potentially explosive, and only small amounts of the material should be prepared and handled with great care.



Scheme 1: The synthesis of the Ru^{II} complexes (**1**, R = Br; **2**, R = F; **3**, R = OCH_3).

Absorption titration

The absorption titrations of Ru^{II} complexes in the buffer were performed by using a fixed complex concentration to which increments of the nucleic acid stock solution were added. Concentration of complex solutions employed was of 20 μM. Influence of DNA on MLCT band of Ru^{II} complexes were measured via UV–Vis absorbance spectra.[23–26] After addition of equivalent amount of DNA to reference cell, it was incubated for 10 min at room temperature, followed by absorbance measurement. DNA–mediated hypochromism (decrease in absorbance) or hyperchromism (increase in absorbance) for test compounds were calculated. The intrinsic binding constant K_b, was determine by making it subject in following equation.[27]

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

where, [DNA] is the concentration of DNA in base pairs, the apparent absorption coefficient ε_a, ε_f and ε_b correspond to $A_{obs.}$/[Ru], the extinction coefficient for the free complex and the extinction coefficient for the free complex in the fully bound form, respectively. In plots [DNA]/($\varepsilon_a - \varepsilon_f$) vs [DNA], K_b is given by the ratio of slope to the y intercept.

Viscosity study

Viscosity measurement was carried out using a Cannon–Ubbelohde viscometer maintained at a constant temperature of 27.0 (±0.1) °C in a thermostatic jacket. DNA samples with an approximate average length of 200 base pairs were prepared by sonication in order to minimize complexities arising from DNA flexibility.[28] Flow time was measured with a digital stopwatch with an accuracy of 0.01 second. Each sample was measured three times with a precision of 0.1 second and an average flow time was calculated. Data were represented graphically as $(\eta/\eta_0)^{1/3}$ versus concentration ratio ([Complex]/[DNA]),[29] where viscosity of DNA in the presence of complex η and η_0 is 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), $\eta = t - t_0$.

Quantitative determination of pUC19 DNA cleavage by agarose gel electrophoresis

Cleavage of plasmid pUC19 DNA by Ru^{II} complexes was measured by the conversion of supercoiled pUC19 plasmid DNA to open circular and linear. Gel electrophoresis of pUC19 DNA was carried out in TAE buffer (0.04 M Tris–Acetate, pH 8, 0.001 M EDTA). 15 µL of reaction mixture contains 100 µg/mL plasmid DNA and complex. Reactions mixture was incubate at 37 ^{o}C. All reactions were quenched by addition of 3 µL loading buffer (0.25% bromophenol blue, 40% sucrose, 0.25% xylene cyanole, and 200 mM EDTA). The aliquots were loaded directly on to 1% agarose gel and electrophoresed at 50 V in 1X TAE buffer. Gel was stained with 0.5 µg/mL ethidium bromide and was photographed on a UV illuminator. After electrophoresis, the proportion of DNA in each fraction was estimated quantitatively from the intensity of the bands using AlphaDigiDoc™ RT. Version V.4.1.0 PC–Image software.

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