Research Article

Synthesis, Characterization, Antibacterial Activity and Investigation of DNA Binding for Ru(II) Molecular "Light Switch" Complexes

S. Vidhisha[1], Kotha Laxma Reddy[1], Y. Praveen Kumar[1], M. Srijana[2], S. Satyanarayana[1]*
[1]Department of Chemistry, Osmania University,Hyderabad-500007,India.
[2]Department of Microbiology, Osmania University, Hyderabad- 500007. India.
***Corresponding author's E-mail:** ssnsirasani@gmail.com

Accepted on: 02-01-2014; **Finalized on:** 28-02-2014.

ABSTRACT

Three Ru(II) complexes $[Ru(bpy)_2DPPN]^{2+}$ (**1**) $[Ru(dmb)_2DPPN]^{2+}$ (**2**) and $[Ru(Phen)_2(DPPN)]^{2+}$ (**3**) [where bpy=2,2'-bipyridine; phen=1,10-phenanthroline; dmb=4,4'-dimethyl-2,2'-bipyridine and DPPN=**(**benzo[i]dipyrido-[3,2-a;2',3'-c]phenazine**)**] have been synthesized and characterized by IR, ^1H & ^{13}C NMR spectroscopy, mass spectrometry and elemental analysis. The interaction of these complexes with calf thymus DNA was investigated comparatively by UV–visible absorption and luminescence spectrophotometric titrations, steady-state emission quenching by $[Fe(CN)_6]^{4-}$, DNA thermal denaturation, viscosity measurements. In addition, in the presence of Co^{2+}, the emission of DNA-$[Ru(L)_2DPPN]^{2+}$ can be quenched. And when EDTA was added, the emission was recovered. The experimental results show that $[Ru(L)_2DPPN]^{2+}$ exhibited the "on-off-on" properties of molecular "light switch". Upon irradiation three Ru(II) complexes were found to promote the cleavage of plasmid PBR 322 DNA from super-coiled form I to nicked form II. It has been observed that $[Ru(Phen)_2(DPPN)]^{2+}$ shows maximum effect on gram negative (G-) and gram positive (G+) bacteria when compared with other two complexes.

Keywords: Ru(II) complexes, polypyridyl ligand, Molecular light switch, Calf-thymus DNA, photocleavage, intercalative mode, antibacterial studies.

INTRODUCTION

The interaction of Ruthenium(II) polypyridyl complexes with DNA has been a topic of major bioinorganic interest during the past decade. Indeed, interacting with DNA, these complexes have potential utilities as DNA structure probes,[1] DNA molecular light switches,[2] DNA colorimetric sensors,[3] anticancer drugs, and photocleavage agents.[4] How these small molecules bind to DNA will be potentially useful in the design of new drugs as well as sensitive spectroscopic and reactive probes including diagnostic reagents.[5,6] In general Ru(II) complexes can bind to DNA in a noncovalent interaction such as electrostatic binding, groove binding or intercalation. Many important applications of these complexes can bind to DNA in an intercalative mode. Therefore, much work has been done on modifying the intercalative ligand of these Ruthenium(II) complexes containing the ligand DPPZ [DPPZ= dipyrido ([3,2-a; 2',3'-c) phenazine].[7,8] However, much attention has been mainly focused on the symmetric aromatic ligands such as 1, 10–phenanthroline and its derivatives,[9-14] investigations of complexes with asymmetric ligands as DNA-binding reagents have been relatively few. During the last more than 15 years, $[Ru(phen)_2(dppz)]^{2+}$ and $[Ru(bpy)_2(dppz)]^{2+}$ well-known as molecular ''light switches'' of DNA have induced considerable interest, after the discoveries that they exhibit a negligible background emission in water but exhibit an intense luminescence in the presence of double strand DNA.[2,8] Moreover, their marked luminescence enhancement can owe to their ligand dppz binding to the DNA-base-pairs in intercalative mode,[15] because the intercalative ligand (dppz) of $[Ru(L)_2(dppz)]^{2+}$ (L= phen, bpy) can be protected by the DNA from its interaction with solvent water molecules, resulting in an enormous increase in quantum yield. It is the reason why these complexes possess an excellent molecular ''light switch'' performance.[16-18]

In our group, much efforts has been devoted to synthesizing some Ru(II) polypyridyl complexes and studying their interaction with DNA.[19-24] In order to obtain more insight into the DNA-binding properties of such Ru(II) complexes, a bidentate ligand DPPN and its Ru(II) complexes $[Ru(bpy)_2DPPN]^{2+}$ (**1**), $[Ru(dmb)_2DPPN]^{2+}$ (**2**) and $[Ru(phen)_2(DPPN)]^{2+}$ (**3**) have been synthesized and characterized. The DNA binding and photocleavage properties of Ru(II) complexes are also presented and discussed. The results show that $[Ru(L)_2DPPN]^{2+}$ (L= phen/bpy/dmb) possesses "molecular light switch" properties similar to those of $[Ru(bpy)_2(tpphz)]^{2+}$.[25,26] The effect of complexes and its free ligand on different cell wall based bacteria (gram positive and negative bacteria) were studied.

MARERIALS AND METHODS

Materials

$RuCl_3.3H_2O$, 1, 10-Phenanthroline monohydrate and 2, 2'-bipyridine were purchased from Merk (India). Calf Thymus DNA, 2,3-diamino naphthalene, 4,4'-dimethyl-2,2'-bipyridine, ammonium hexafluorophosphate, TBACl were obtained from sigma (St.Lowis, Mo,USA). The super coiled (CsCl purified) pBR-322 DNA (Bangalore Genei, India) was used as received. All reagents and solvents

were purchased commercially and used without further purification unless otherwise noted. Deionised, double distilled water was used for preparing various buffers. Solutions of DNA in Tris HCl buffer (pH=7.2), 50 mM 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.[27] The concentration of Calf-Thymus DNA (CT DNA) was determined spectrophotometrically using the molar absorption coefficient 6600 $M^{-1}cm^{-1}$(260 nm). [28]

MATERIALS AND METHODS

Physical measurements

UV-Visible spectra were recorded with an Elico Bio-spectra-photometer, model BL198. IR spectra were recorded in KBr discs on a Perkin-Elmer FT-IR-1605 spectrometer.^{1}H NMR spectra were measured on a Varian XL-300 MHz spectrometer using DMSO-d_6 as the solvent and TMS as an internal standard. Micro analysis (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 on Ostwald viscometer, immersed in thermostatted water-bath maintained at 30±0.1°C. DNA samples, approximately 200 base pairs of average length, were prepared by sonicating in order to minimize complexities arising from DNA flexibility. [29] The flow time was measured with a digital stop watch; each sample was measured thrice and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio,[30] where η is viscosity of DNA in the presence of the complex, and η_0 is the viscosity of DNA alone. The 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. The extent of cleavage of super coiled (SC) pBR322 DNA to its nicked circular (NC) form was determined by agarose gel electrophoresis in Tris– HCl buffer (50 mM, pH 7.2) containing NaCl (50 mM). In order to investigate the DNA photocleavage activity of the complexes **(1-3)** with different concentrations, it was irradiated with UV lamp (365 nm, 10W) under aerobic conditions for 30 minutes in buffered aqueous solutions in the presence of super coiled pBR322 plasmid DNA, which is a very sensitive tool for damage detection.[31] The samples were loaded on 1% agarose gel, and the run was carried out at 50 V for 4 hrs. After staining in ethidium bromide solution, the gel was washed with water and the DNA bands were detected under UV radiation with a UV transilluminator. The wavelength used for the photo-induced DNA cleavage experiments was 365 nm.

Effect (toxicity) of Ruthenium complexes (**1-3**) and ligand on the growth of gram positive (G+) *Bacillus subtilis* MTCC 1427 and gram negative (G-) *Pseudomonas putida* KT 2240 were determined in luria broth (LB) medium. The organisms were grown as per the procedure given in Clinical Microbiology Procedure Handbook. [32] Based on the preliminary Disc and Tube assay[32] for determination of Minimum inhibitory concentration (MIC), 6 µM concentration of Ruthenium complexes (**1-3**) and ligand was found to be the MIC for the selected bacteria and hence 5 µM concentration was selected for the toxicity studies. The microorganisms were grown for a time period of 24 hrs. At each time period, the cells were dispersed by vortexing the culture till a uniform suspension was obtained, and turbidity of aliquots was measured at 660 nm. Growth with no added complex served as control. All the experiments were conducted three times in duplicates and the results presented are the average values.

Synthesis and Characterization

The compounds 1,10-phenanthroline-5,6-dione,[33] Cis-[$Ru(bpy)_2Cl_2$].$2H_2O$, Cis-[$Ru(dmb)_2Cl_2$].$2H_2O$, and Cis-[$Ru(phen)_2Cl_2$].$2H_2O$ were prepared according to literature procedures.[34] Synthetic routes of ligands and their Ru(II) complexes are shown in Fig.1.

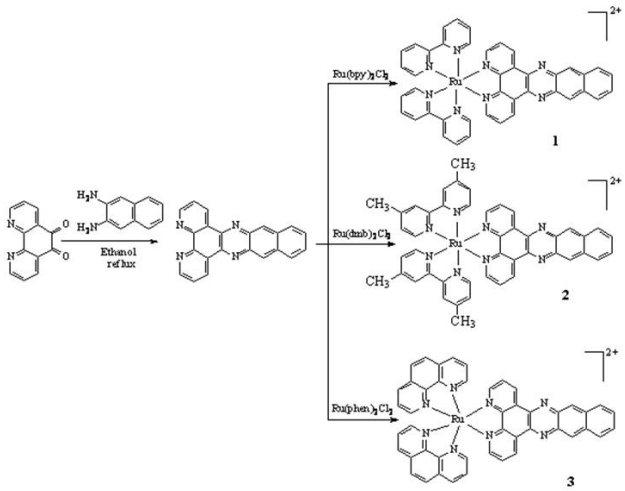


Figure 1: Synthetic route of ligand and Ru(II) complexes

Synthesis of benzo[i]dipyrido-[3,2-a;2',3'-c]phenazine (DPPN)

A solution of 1,10-phenanthroline-5,6-dione (0.210 g, 1 mmol) and 2,3-diamino naphthalene (0.158 g, 1 mmol) in ethanol (20 ml) was heated at reflux for 4 h. After cooling, the precipitate was collected by filtration, washed with cold ethanol and dried under vacuum. The product was recrystallized in chloroform to afford a Chocolate material. Yields; 70%; Color: Chacolate. $C_{22}H_{12}N_4$; Calcd.(%); C:79.50; H:3.64; N:16.86; Found(%): C:78.90; H:3.70; N:16.38. IR (KBr): 1616, 1515, 1411, 740 cm^{-1}; ESI-MS (in DMSO) m/z; 333 (calcd.332); ^{1}H-NMR (DMSO-d_6, δ ppm): 9.64 (2H, d); 9.22 (2H, m); 8.92 (2H, s); 8.19 (2H, d) ; 7.83 (2H , dd); 7.65(2H, d); ^{13}C[^{1}H]-NMR (DMSO-d_6, δ ppm, major peaks): 150.95, 144.52, 141.13, 140.10, 139.60, 138.10, 135, 134, 128.10, 127, 126.70, 125, 124.20, 123.8.

Synthesis of [$Ru(bpy)_2$ (DPPN)] ($(PF_6)_2$.$2H_2O$ (1)

A mixture of Cis-[$Ru(bpy)_2Cl_2$].$2H_2O$ (0.5 mmol), DPPN (0.5 mmol), EtOH (20 ml) and H_2O (10 ml) was stirred under

reflux in N_2-atmosphere for 4h to give a clear red solution. Upon cooling, the solution was treated with a saturated aqueous solution of NH_4PF_6 to give a red precipitate. The red solid was collected and washed with small amounts of water, ethanol and ether, dried under vacuum. The product was further purified by recrystallization from acetone-ether to afford a Saddle brown material. Yields: 75%. Color: Saddle brown. $C_{42}H_{32}F_{12}N_8O_2P_2Ru$; Calcd.(%); C:47.05; H:2.99; N:10.45; Found(%): C:47.30; H:2.78; N:10.13. IR(KBr): 1633, 1542, 1465, 1073, 844, 557, 490 cm^{-1}; ^{1}H-NMR(DMSO-d_6, δ ppm): 9.63 (2H, d); 9.24 (2H, s); 8.89 (4H, d); 8.45 (2H, d); 8.22 (4H, m); 8.15 (2H, m); 8.01 (4H, m); 7.81 (4H, m); 7.59 (2H, m); 7.41 (2H, d). ^{13}C[^{1}H]-NMR (DMSO-d_6, δ ppm, major peaks): 156.74, 156.46, 153.51, 152.17, 151.27, 150.91, 141.10, 138.20, 137.86, 134.70, 133.33, 130.57, 128.71, 127.93, 124.49.

Synthesis of [Ru(dmb)$_2$ (DPPN)] (PF_6)$_2$.2H_2O (2)

This complex was obtained by a procedure similar to that described above, with Cis-[Ru(dmb)$_2$$Cl_2$].2$H_2O$ (0.5 mmol) in place of Cis-[Ru(bpy)$_2$$Cl_2$].2$H_2O$. Yields; 65%. Color: Dark red. $C_{46}H_{40}F_{12}N_8O_2P_2Ru$; Calcd.(%); C:48.97; H:3.55; N:9.93 Found(%): C:48.37; H:3.39; N:9.78. IR (KBr): 1654, 1542, 1419, 1043, 845, 667, 556, 491 cm^{-1}; ^{1}H-NMR (DMSO-d_6,δppm): 9.60 (2H, d); 9.26 (2H, s); 8.77 (4H, d); 8.48 (2H, d); 8.21 (4H, s); 8.02 (2H, m); 7.81 (4H, d); 7.64 (2H, d); 7.45 (2H, t); 2.61 (12H, s). ^{13}C [^{1}H]-NMR (DMSO-d_6, δ ppm, major peaks): 160.14, 157.12, 156.32, 156, 153.18, 151.12, 149.63, 148.12, 141.10, 139.37, 137.80, 134, 130.48, 128.53, 128, 127, 20.74, 20.65.

Synthesis of [Ru(phen)$_2$ (DPPN)] (PF_6)$_2$.2H_2O (3)

This complex was obtained by a similar procedure to that described above, with Cis-[Ru(phen)$_2$$Cl_2$].2$H_2O$ (0.5 mmol) in place of Cis-[Ru(bpy)$_2$$Cl_2$].2$H_2O$. Yields; 75%. Color: Maroon. $C_{46}H_{32}F_{12}N_8O_2P_2Ru$; Calcd.(%); C:49.32; H:2.85 N:10.01 Found (%): C:48.96; H:2.93; N:10.23: IR (KBr): 1623,1544, 1420, 1074, 848, 556, 490 cm^{-1}: ^{1}H-NMR (DMSO-d_6, δ ppm): 9.67 (2H, d); 9.31 (2H, s); 8.86 (4H, m); 8.46-8.53 (6H, m); 8.40 (4H, s); 8.18 (2H, m); 7.81-7.98 (8H, m). ^{13}C[^{1}H]-NMR (DMSO-d_6, δ ppm, major peaks): 154, 153.28, 152.68, 151.32, 147.30, 141.06, 137.94, 136.95, 134.52, 133.41, 133.13, 130.45, 128.53, 128, 127.72, 126.25.

The water – soluble halide salts were prepared from the hexafluorophosphate salts by precipitation in acetone solution with tetra-n-butyl ammonium chloride (n-Bu_4NCl).

RESULTS AND DISCUSSION

Characterization

The ligand DPPN was prepared [35] by condensation of 2, 3-diamino naphthalene and phendione in ethanol in good yields. Their compositions were identified by elemental analyses, IR, ^{1}H, ^{13}C-NMR and mass spectra. The ESI-MS spectra of DPPN ligand shows a molecular ion peak at m/z; 333 which is equivalent to its molecular weight (calcd.332); The ^{1}H-NMR spectra of the DPPN gave 6 peaks in aromatic region between 9.64 ppm to 7.65 ppm with proper multiplicity. The ^{13}C-NMR of ligand DPPN gave peaks in the aromatic region. Their Ru(II) complexes were also synthesized and characterized by elemental analyses, IR, ^{1}H, ^{13}C-NMR. The ligand and the PF_6 salts of their mixed-ligand Ru(II) complexes **(1-3)** gave satisfactory elemental analyses. The important stretching frequencies observed in the Infrared spectra are given in the experimental section. In the infrared spectra of Ru(II) complexes have bands at 1633 (C=N), 1542 (C=C) shifted to a higher frequency when compared to free ligand indicating complexation. New band at 557 cm^{-1} in (Ru-N (DPPN)) support complex formation. The infrared spectrum of the PF_6 salt of each complex showed a strong band in the 844 - 848 cm^{-1} region ascribable to the counter anion, and this band was absent in the corresponding chloride salts. Ru(II) complexes display resolvable ^{1}H-NMR spectra in DMSO-d_6. ^{1}H-NMR spectral data for the ligand and complexes synthesized in this study show the expected peaks in aromatic region. In the ^{1}H-NMR spectra of the Ru(II) complexes, the peaks due to various protons of bpy, dmb, phen and DPPN ligand are seen to be shifted to downfield upon complexation compared to free ligands, suggesting complexation. In ^{13}C-NMR spectra of the Ru(II) complexes, upon coordination of DPPN to Ru(II) all peaks shifted to downfield and resonate in the aromatic region. Electronic absorption spectra of the complexes are characterized by metal to ligand charge transfer (MLCT) transition in the visible region .The low energy bands at 406 and 403 nm for compounds 1, 2 and 3 respectively are assigned to the metal –to – ligand charge transfer (MLCT) transition. The bands below 300 nm are attributed to intraligand (IL) π-π* transitions by comparison with the spectra of $[Ru(bpy)_3]^{2+}$ [36] and the lowest energy bands are assigned to metal to ligand charge transfer (MLCT) transition. [37]

DNA binding studies

Electronic absorption spectra and binding constants

The electronic spectra are the most common way to investigate the interaction of complexes with DNA. A Complex bound to DNA through intercalation usually results in hypochromism and bathochromism (red shift), due to the intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The extent of the hypochromism in the visible MLCT band is commonly consistent with the strength of intercalative interaction. [37]

The absorption spectra of complexes 1, 2 and 3 in the absence and presence of CT-DNA at constant concentration of complexes ([Ru]=20 μM) are given in Fig.2. By increasing the concentration of DNA, the hypochromism increases. These results probably reflect the binding affinity of the complexes to DNA. In order to quantitatively compare the binding strength of the three complexes, the intrinsic binding constants K_b of the three complexes with CT- DNA were obtained by monitoring

the changes in absorbance at 406nm for complex1, complex 2 and at 403nm for complex 3 with increasing concentration of DNA using the following equation,[38] through a plot of [DNA] / [ε_a-ε_f] Vs [DNA].

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

Where [DNA] is the concentration per nucleotide, the apparent absorption co-efficient ε_a, ε_f and ε_b correspond to $A_{obsd\ /\ [Ru(II)]}$, the extinction co-efficients for the free ruthenium complex is ε_a, extinction coefficient of the complex in presence of DNA is ε_f and the extinction co-efficient for the ruthenium complex in the fully bound form is ε_b. In plots [DNA]/(ε_a-ε_f)Vs [DNA], K_b is given by the ratio of slope to intercept. Intrinsic binding constants K_b of $[Ru(bpy)_2DPPN]^{2+}$, $[Ru(dmb)_2DPPN]^{2+}$, and $[Ru(phen)_2(DPPN)]^{2+}$ were obtained about 2±0.2 x 10^5, 1.3±0.2 x 10^5 and 2.4±0.1 x 10^5 M^{-1}, respectively. The values that are close to those of some complexes, such as K_b=5.3 ± 0.1 × 10^5 M^{-1} observed for $[Ru(bpy)_2(BDPPZ)]^{2+}$,[39] but smaller than that observed for $[Ru(bpy)_2(DPPZ)]^{2+}$ (>10^6).[2] The results indicate that the binding magnitudes of the complexes with CT-DNA lie in the following order: $[Ru(dmb)_2DPPN]^{2+}$ < $[Ru(bpy)_2DPPN]^{2+}$ < $[Ru(phen)_2(DPPN)]^{2+}$. Obviously, these spectral characteristics of the large K_b values observed suggest that these three complexes most likely intercalatively bind to DNA, involving a strong stacking interaction between the aromatic chromophore and the base pairs of DNA.

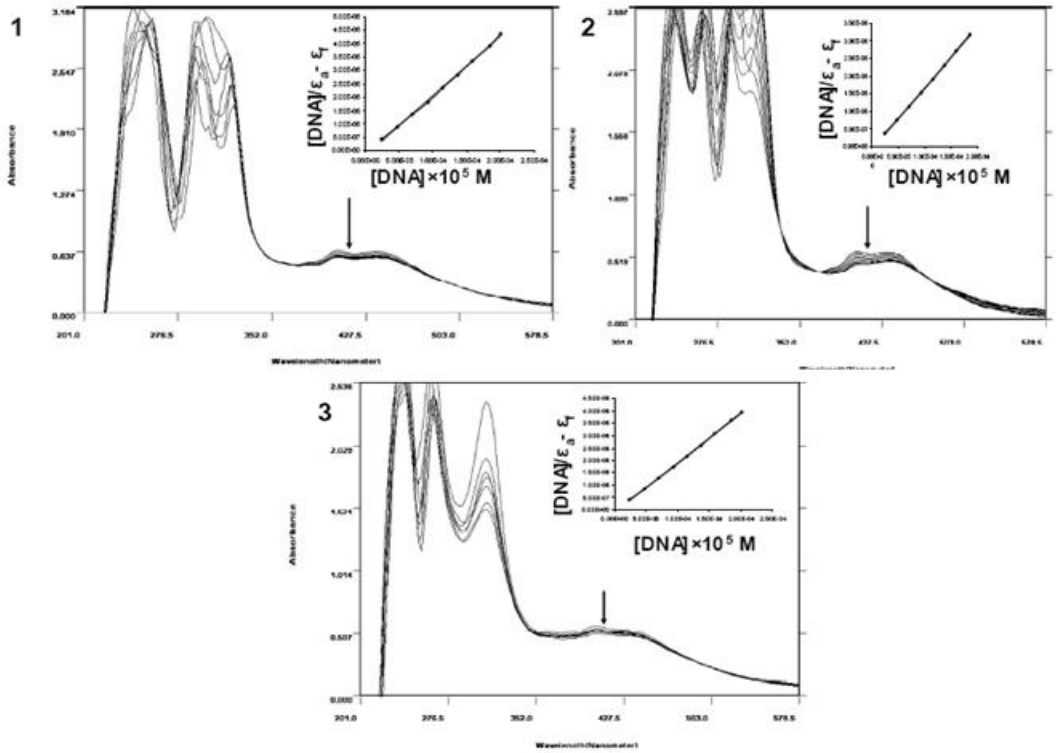


Figure 2: Absorption spectra of complexes $[Ru(bpy)_2DPPN]^{2+}$ **(1)** $[Ru(dmb)_2DPPN]^{2+}$ **(2)** and $[Ru(phen)_2(DPPN)]^{2+}$ (**3**) in Tris HCl buffer at 25 °C in the presence of increasing amount of CT- DNA, [Ru]=20 μM, [DNA]=0-120 μM .The arrows indicate the change in absorbance upon increasing the DNA concentration. Insert: Plot of [DNA]/(ε_a-ε_f) vs [DNA] for titration of the Ru(II) complexes.

The difference in binding strength of complexes of 1 & 2 is probably being caused by the different ancillary ligands. The four additional methyl groups in complex 2 when compared to complex 1 exert some steric hindrances. Therefore complex 1 probably interacts more deeply and is more tightly bound to adjacent DNA base pairs than complex 2. Similarly the difference in binding strength of complexes 1 & 3 is due to the difference in the ancillary ligands. On going from bpy to phen, the planarity area and hydrophobicity increase leading to a greater binding affinity for complex 3 than 1.

Fluorescence Spectroscopic Studies

To further clarify the interaction of these Ru(II) complexes with DNA, the emission spectra of complexes 1, 2, and 3 have been measured in the absence and presence of CT DNA (Fig.3). The complexes 1, 2 and 3 can emit luminescence in Tris buffer at ambient temperature with maxima at 560nm. Binding of three complexes to DNA was found to increase the fluorescence intensity. Upon addition of CT-DNA, the emission intensity increases steadily and reaches 1.20 times larger than that of in the absence of DNA for complex **1**, 1.18 times larger than that of in the absence of DNA for complex **2** and 1.47 times larger than that of in the absence of DNA for complex **3**, respectively. The extent of enhancement increases on going from complex 1 to complex 3, which is consistent with the above absorption spectral results.

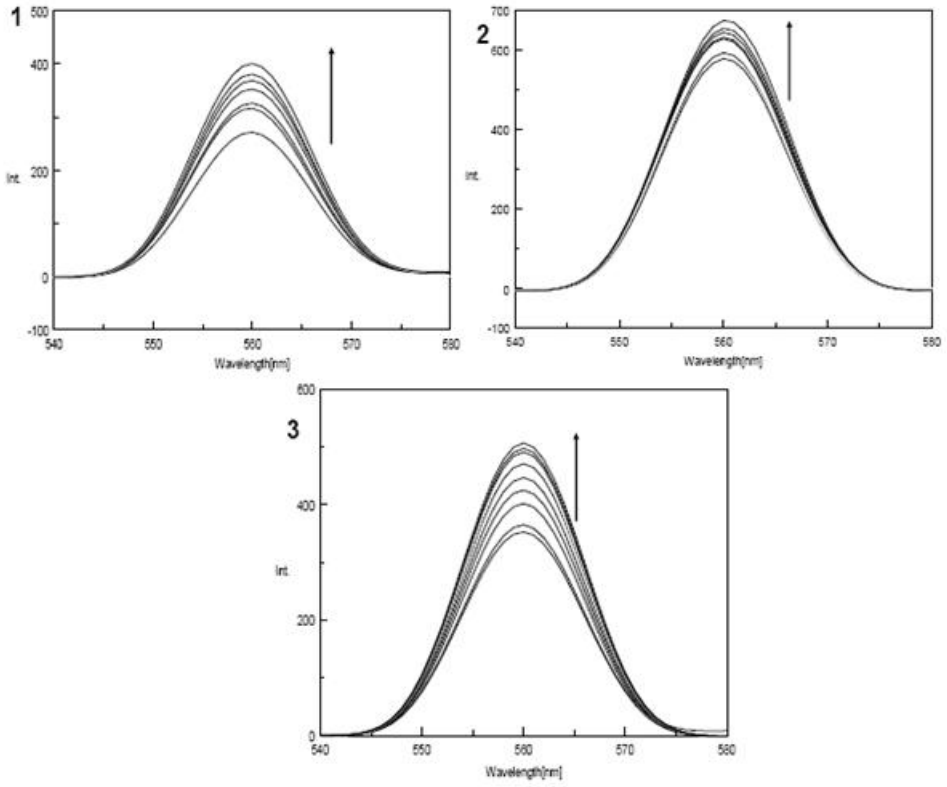


Figure 3: Emission spectra of complexes $[Ru(bpy)_2DPPN]^{2+}$ (1) $[Ru(dmb)_2DPPN]^{2+}$ (2) and $[Ru(phen)_2(DPPN)]^{2+}$ (3) in Tris HCl buffer at 25°C in the presence of CT-DNA, [Ru]=20 μM, [DNA]=0-120 μM. The arrow shows the intensity change upon increasing CT-DNA concentrations.

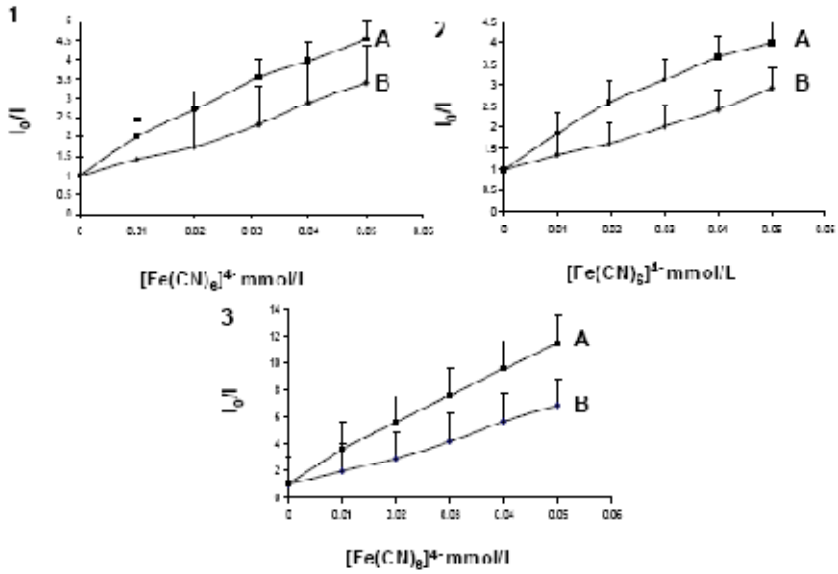


Figure 4: Emission quenching of Ru(II) complexes 1-3 with $K_4[Fe(CN)_6]$ in the presence (B) and absence (A) of DNA. [Ru]=20 μM, [DNA]/ [Ru] = 40:1

Quenching studies

This observation is further supported by the emission quenching experiments using $[Fe(CN)_6]^{4-}$ as quencher. The ion $[Fe(CN)_6]^{4-}$ has been shown to be able to distinguish differentially bound Ru(II) species, positively charged free complex ions should be readily quenched by $[Fe(CN)_6]^{4-}$. The complex binding to DNA can be protected from the quencher, because highly negatively charged $[Fe(CN)_6]^{4-}$ would be repelled by the negative DNA phosphate backbone, hindering quenching of the emission of the bound complex.[40, 41] 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 change in fluorescence intensity. The Ferro-cyanide quenching curves for these three complexes in the presence and absence of CT DNA are shown in Fig.4. The absorption and fluorescence spectroscopy studies

determine the binding of complexes. From the quenching studies also it is clear that the binding ability of the complexes to DNA is in the order 3>1>2.

Recovered luminescence of $[Ru(L)_2dppn]^{2+}$ in the presence of Co^{2+} by EDTA

Interestingly, as shown in (Figs. 5) while adding EDTA into the buffer system containing $[Ru(L)_2dppn]^{2+}$ with Co^{2+} ion, the emission intensity of the complex is recover again. The phenomenon implies that the chelation of $[Ru(bpy)_2dppn]^{2+}$ with Co^{2+} is weakened owing to the strong coordination of Co^{2+} to EDTA, as a result, $[Ru(bpy)_2dppn]^{2+}$ becomes free again as shown in (Figs. 5).[42] It is therefore interesting to investigate that and EDTA. (Fig. 5) shows the decrease in the luminescence intensity of DNA-bound $[Ru(bpy)_2dppn]^{2+}$ due to the interactions of Co^{2+} with DNA. While further adding EDTA into the buffer system containing DNA-bound $[Ru(bpy)_2dppn]^{2+}$ with Co^{2+} ion, the emission intensity is recovered based on the strong coordination of Co^{2+} to EDTA. For example, the presence of 0.03 mM Co^{2+} could decrease the luminescence intensity by 10.5%, and the addition of the equimolar EDTA (0.03 mM) could result in the recovery of the luminescence up to 9.5 %. The value is more than 100%, possibly owing to the enhancement of EDTA on the luminescence of DNA-bound $[Ru(bpy)2dppn]^{2+}$.[43, 44] The luminescent change of DNA-bound $[Ru(bpy)_2dppn]^{2+}$ in the presence of Co^{2+} and EDTA reveals the modulation of Co^{2+} and EDTA to luminescence intensities of DNA-bound $[Ru(bpy)_2dppn]^{2+}$.

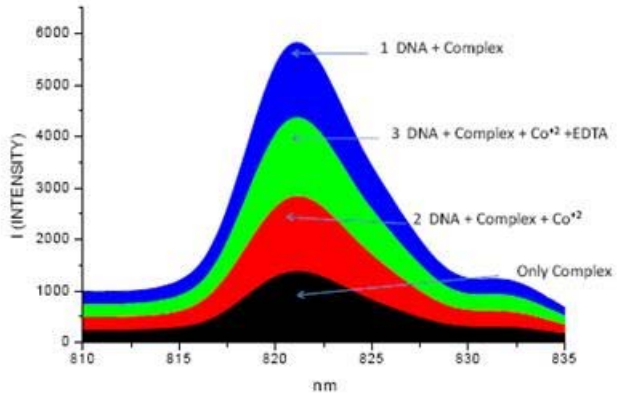


Figure 5: DNA light switch experiments showing the luminescence changes upon addition of Co^{2+}, EDTA to $[Ru(phen)_2\ dppn]^{2+}$ + DNA.

Viscosity studies

To further clarify the interaction between the complexes and DNA, viscosity measurements were carried out. Photophysical probes generally provide necessary, but insufficient clues to support an intercalation binding model. Hydrodynamic measurements which are sensitive to length increases (i.e. viscosity, sedimentation etc.) are regarded as the least ambiguous and the most critical tests of a binding model in solution in the absence of crystallographic structural data. [45] A classical intercalation model results in lengthening of the DNA helix as base pairs are separated in order to accommodate the binding ligand, leading to an increase in DNA viscosity. On the other hand, partial and/or non- classical intercalation of the ligand may bend (or kink) the DNA helix, resulting in a decrease in its effective length and concomitantly, its viscosity.[46] The effects of the complexes on the viscosity of rod –like DNA are shown in Fig.6. Upon increasing the amount of complex, the relative viscosity of DNA increases steadily, this suggests that all the complexes bind with CT-DNA intercalatively. However, a much smaller increase is observed for $[Ru(dmb)_2DPPN]^{2+}$ among the complexes examined. The results imply that $[Ru(dmb)_2DPPN]^{2+}$ may intercalate less deeply into the DNA base pairs due to steric hindrance exerted by the methyl groups located at the 4-and 4'-positions of the dmb ligand. This result suggests an intercalative binding mode of three Ru(II) complexes and also parallels the pronounced hypochromism, bathochromism and emission enhancement of three complexes in the presence of CT-DNA.

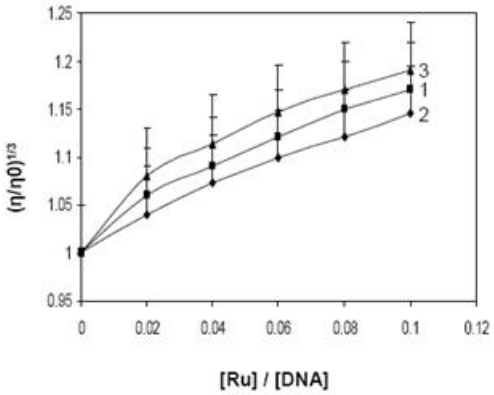


Figure 6: Effect of increasing amount of complexes $[Ru(bpy)_2DPPN]^{2+}$ (1) $[Ru(dmb)_2DPPN]^{2+}$ (2) and $[Ru(phen)_2DPPN]^{2+}$ (3), on relative viscosity of CT-DNA at 30± 0.1 ^{o} C. The total concentration of DNA is 0.25 mM, [Ru] = 20 µM.

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 double standard DNA; the DNA melting experiments are useful in establishing the extent of intercalation.[47] All the three present complexes ([Ru] = 20 µM) were incubated with CT-DNA (100 µM), heated to 85°C from ambient temperature and the OD at 260 nm was monitored.[48] Here, a DNA melting experiment revealed that T_m of calf thymus DNA is 61 ± 0.2 ^{0}C in the absence of the complexes. The observed melting temperature in the presence of the complexes was 69± 0.2 ^{0}C, 67 ± 0.2 ^{0}C and 72 ± 0.2 ^{0}C, for **1**, **2** and **3** complexes respectively. Binding of complexes does lead to an increase in ΔTm of DNA is in the order $[Ru(phen)_2(DPPN)]^{2+}$>$[Ru(bpy)_2DPPN]^{2+}$>$[Ru(dmb)_2DPPN]^{2+}$.

Photo activated cleavage of pBR-322 DNA by Ru(II) complexes

The photosensitized cleavage of plasmid DNA can be monitored by agarose gel electrophoresis. When the plasmid was irradiated in the presence of complexes, an efficient photo induced DNA-strand cleavage occurs.[49] In the dark, the complexes do not promote DNA strand breaks. The intact super-coil form (Form I) migrates relatively fast, if scission occurs on one strand (nicking), the super-coil will relax to generate a slower-moving open circular form (Form II).[50, 51] Fig.7. Shows gel electrophoretic separation of pBR322 DNA after incubation with the complexes and irradiation at 365 nm. [49] Photo-sensitized DNA (pBR 322) cleavage experiments were carried out with different concentrations (20, 40, 60, 80 µM) of complexes; all the lanes except for lane 1 (DNA alone) show cleavage activity of DNA. The selected Ruthenium complexes (**1, 2** and **3**) (lane **2-5**) exhibited fairly good cleavage activity.

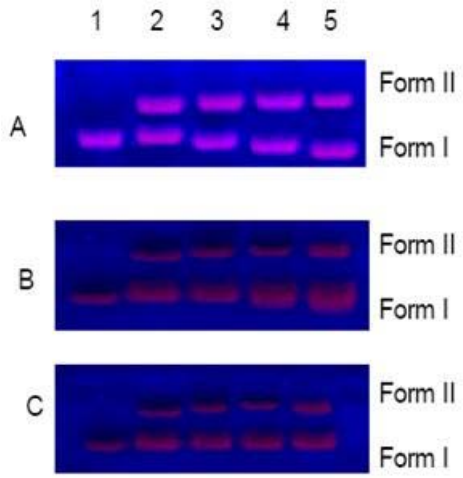
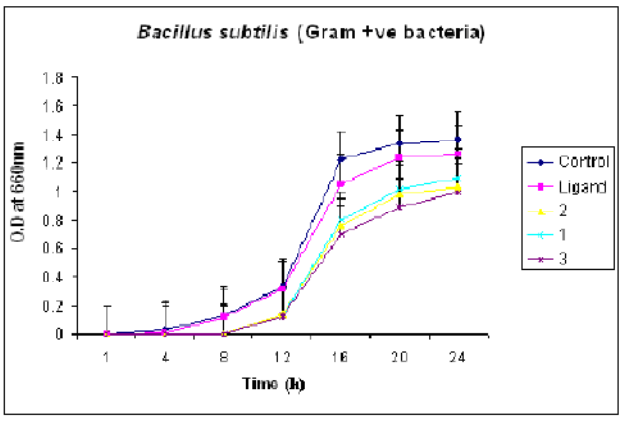


Figure 7: (A) Photoactivated Cleavage of pBR 322 DNA [10 µl of 100 µM stock] in the presence of $[Ru(bpy)_2(DPPN)]^{2+}$ (A), $[Ru(dmb)_2(DPPN)]^{2+}$ (B) and $[Ru(phen)_2(DPPN)]^{2+}$ (C) and light after 30 min irradiation at 365 nm. DNA alone (lane 1), the concentrations of each complex was 20, 40, 60, 80 µM (lane 2-5).

Effect of Ruthenium complexes on gram negative (G-) and gram positive (G +) bacteria.

The effect of the Ruthenium complexes and its ligand against Gram negative (G–) bacteria-*Pseudomonas putida* KT 2240 and Gram positive (G+) bacteria – *Bacillus subtilis* MTCC 1427 in LB medium were studied. The results shown in the Fig.8 indicate that the complexes are inhibiting growth of the bacteria being in the order of; **3>1>2>** ligand on Gram negative bacteria (G-); whereas with the gram positive bacteria (G+) the order of inhibition is; **3>2>1>** ligand respectively. The absence of any lag in the onset of growth effect suggests that the complexes are taken up rapidly and affect intracellular metabolism and regulation, the same was described by Penumaka et al. [52] The results of growth parameters are presented in figure 8. Microbiological studies confirmed a strong inhibitory activity of ruthenium complexes against G– bacteria. Based on these results, it is supposed that the ruthenium based complexes has a role in inhibition of selected G+ and G – bacteria.



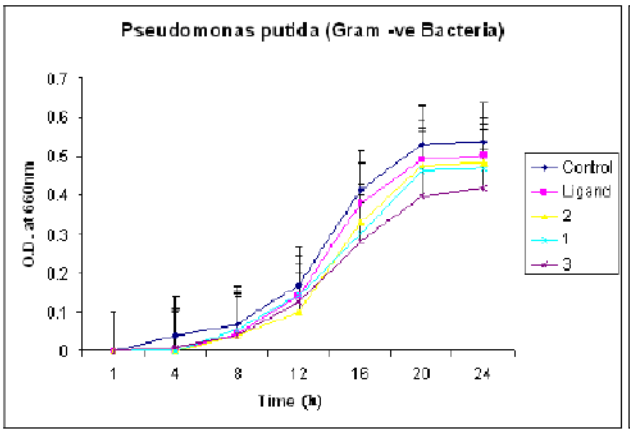


Figure 8: Effect of ligand-DPPN and complexes 1-3 ($[Ru(bpy)_2(DPPN)]^{2+}$ (5 µM) (**1**), $[Ru(dmb)_2(DPPN)]^{2+}$ (5 µM) (**2**) and $[Ru(phen)_2(DPPN)]^{2+}$ (5 µM) (**3**)) on Gram positive (G+) bacteria – *Bacillus subtilis* MTCC 1427 and Gram negative (G–) bacteria – *Pseudomonas putida* KT 2240 growth expressed on the basis of absorbance at 660 nm growth of control (no complex) was taken as 100% for quantitative evaluation of complex. Experimental details see text.

In this study $[Ru(phen)_2(DPPN)]^{2+}$ complex showed better inhibition on the selected bacteria. Yasbin et al., [53] reported the mutagenic capacity of ruthenium complexes. The compound $[Ru(dmb)_2DPPN]^{2+}$ showed better inhibition on *Bacillus subtilis* MTCC 1427 and $[Ru(bpy)_2DPPN]^{2+}$ has better inhibitory effect on the growth of *Pseudomonas putida* KT 2240. Overall the results suggest that Ruthenium-ligand co-ordination complexes inhibited the growth of *Bacillus subtilis* MTCC 1427 and *Pseudomonas putida* KT 2240.

A relatively new line of investigation focuses on ruthenium and its complexes chemistry as an alternative metallopharmaceutical approach to platinum and ruthenium chemistry may also allow for photodynamic approaches to therapy.[54] Recently structures of the analogous ruthenium (III) complexes $[RuCl_3(N\text{-}N)L]$ and their cytostatic activity have been described. A large

variety of potential ruthenium and its polypyridyl complexes drugs have been synthesized with ligands such as amines, imines, DMSO, polypyridyl compounds and arenas (. The diversity of the active structures suggests that different mechanisms of action may be involved for different types of ruthenium complex. [53] Ruthenium anticancer chemistry has already yielded many promising results. Several compounds have been described which display an activity comparable to that of cisplastin and in some cases it is even better. Two Ruthenium dimethyl sulfoxide (DMSO) compounds are also currently in clinical trials. [54]

CONCLUSION

In summary, three Ru(II) complexes of $[Ru(phen)_2(DPPN)]^{2+}$, $[Ru(bpy)_2DPPN]^{2+}$ and $[Ru(dmb)_2DPPN]^{2+}$ were synthesized and characterized. Their DNA-binding and photocleavage properties were also investigated. Spectroscopic studies and viscosity experiments illustrated that the three complexes can intercalate into DNA base pairs via DPPN ligand. The photo sensitized cleavage and antibacterial studies of metal-ligand coordination complexes of ruthenium were studied. It is believed that the results gathered from this work may represent, at the very least, good starting points for further medicinal chemistry programs aiming to discover antibacterial drug candidates based on ruthenium structures.

Acknowledgment: We are grateful to UGC New Delhi for financial support and CFRD Osmania University.

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Source of Support: Nil, **Conflict of Interest:** None.