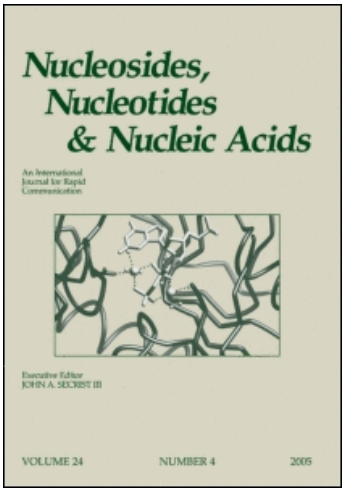

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DNA-BINDING AND PHOTOCLEAVAGE PROPERTIES OF RU(II) POLYPYRIDYL COMPLEXES WITH DNA AND THEIR TOXICITY STUDIES ON EUKARYOTIC MICROORGANISMS

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□ *Four Ru(II) polypyridyl complexes, $[Ru(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$, $[Ru(bpy)_2(7\text{-}CH_3\text{-}dppz)]^{2+}$, $[Ru(phen)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$, and $[Ru(phen)_2(7\text{-}CH_3\text{-}dppz)]^{2+}$ (bpy = 2,2′-bipyridine, phen = 1,10-phenanthroline), (7-Nitro-dppz = 7-Nitro dipyrido[3,2-a:2′-3′-c]phenazine, 7-CH_3-dppz = 7-Methyl dipyrido[3,2-a:2′-3′-c]phenazine), have been synthesized and characterized by IR, UV, elemental analysis, ^1H NMR, ^{13}C-NMR, and mass spectroscopy. The DNA-binding properties of the four complexes were investigated by spectroscopic and viscosity measurements. The results suggest that all four complexes bind to DNA via an intercalative mode. Under irradiation at 365 nm, all four complexes were found to promote the photocleavage of plasmid pBR 322 DNA. Toxicological effects of the selected complexes were performed on industrially important yeasts (eukaryotic microorganisms).*

Keywords Ru(II)complexes; polypyridyl ligand; calf-thymus DNA; photocleavage; intercalative mode; toxicology

1. INTRODUCTION

During the past decades, a number of transition metal complexes have been utilized in the design and development of synthetic restriction enzymes, chemotherapeutic drugs, DNA foot printing agents, and stereoselective probes of nucleic acids structure.[1–10] In particular, ruthenium(II) complexes with polypyridine ligands can bind DNA in a noncovalent intercalation fashion such as electrostatic binding, groove binding and intercalation. Many important applications of these complexes require that the complexes bind to DNA in an intercalative mode. Therefore, the

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vast majority of such studies have focused on modifying the intercalative ligand.[9,11] Thus, it is not surprising that the reported complexes contain mostly planar aromatic intercalative ligands. However, attention has mainly been focused on symmetric aromatic ligands such as 1,10-phenanthroline and its derivatives,[12–22] investigations of complexes with asymmetric ligands as DNA-binding reagents have been relatively few. In our group, much effort has been devoted to synthesizing new cobalt and ruthenium complexes.[23–26] Therefore, to evaluate more clearly and understand the factors that determine the DNA-binding mode of Ru(II) complexes with different shapes as well as electronic properties, the investigation of DNA binding behaviors is necessary. In this article, we report the synthesis and characterization of four ruthenium(II) complexes, $[Ru(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$, $[Ru(bpy)_2(7\text{-}CH_3\text{-}dppz)]^{2+}$, $[Ru(phen)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$, and $[Ru(phen)_2(7\text{-}CH_3\text{-}dppz)]^{2+}$. The DNA-binding properties of Ru(II) complexes were explored by spectroscopic methods and viscosity measurements. Their DNA-cleavage behavior toward pBR 322 DNA was also investigated. Previously Kazachenko et al.[27,28] established that some complex compounds of gold with glycine, histidine, tryptophan, and cysteine possess selective antimicrobial activity and low toxicity. In this work, the toxicological studies of the four selected complexes were examined with two yeasts of different origin and type.

2. EXPERIMENTAL

2.1. Materials

$RuCl_3 \cdot 3H_2O$, 1,10-phenanthroline monohydrate and 2,2′- bipyridine were purchased from Merk (Mumbai, India). Calf-thymus (CT) DNA, 4-nitro-ortho-phenylenediamine, 4-methyl-ortho-phenylenediamine, ammonium hexafluorophosphate, TBACl were obtained from Sigma (St. Louis, MO, USA). The supercoiled (CsCl purified) pBR-322 DNA (Bangalore Genei, Bangalore, India) was used as received. All reagents and solvents were purchased commercially and used without further purification unless otherwise noted. Deionized, double distilled water was used for preparing various buffers. Solutions of DNA in Tris HCl buffer (pH = 7.2), 50mM NaCl gave a ratio of UV absorbance at 260 and 280nm of 1.8–1.9, indicating that the DNA was sufficiently free from protein.[29] The concentration of calf-thymus DNA (CT DNA) was determined spectrophotometrically using the molar absorption coefficient $6600 M^{-1}cm^{-1}$(260 nm).[30] The compounds 1,10-phenanthroline-5,6-dione,[31] *cis*-$[Ru(bpy)_2Cl_2].2H_2O$, and *cis*-$[Ru(phen)_2Cl_2].2H_2O$[32] were prepared according to literature

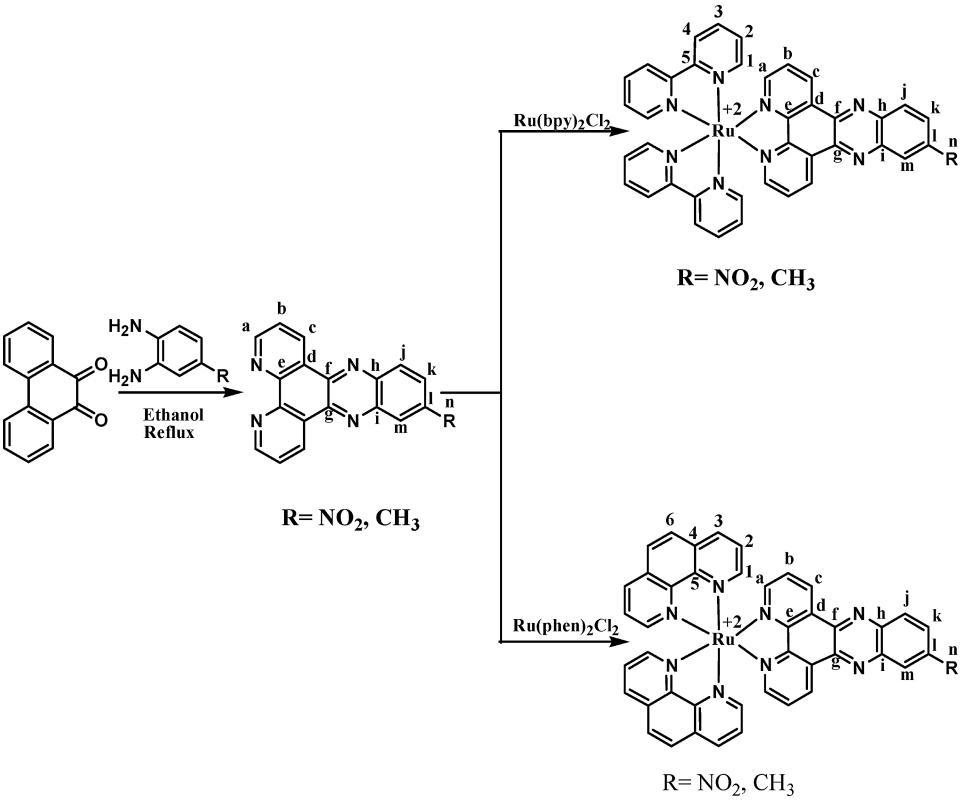

FIGURE 1 Synthetic routines of ligand and Ru (II) complexes.

procedures. Synthetic routes of ligands and their Ru(II) complexes are shown in Figure 1.

2.2. Physical Measurements

UV-visible spectra were recorded with an Elico biospectrophotometer, model BL198 (Elico, Hyderabad, India). IR spectra were recorded in KBr discs on a Perkin-Elmer FTIR-1605 spectrometer (Perkin Elmer, Waltham, MA, USA). ^{1}H NMR spectra were measured on a Varian XL-300 MHz spectrometer (Varian, Palo Alto, CA, USA) using DMSO d_6 as the solvent and TMS as an internal standard. Microanalysis (C, H, and N) was carried out on a Perkin-Elmer 240 elemental analyzer. Fluorescence spectra were recorded with a JASCO Model 7700 spectrofluorometer (JASCO, Tokyo, Japan) 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 $\pm$ 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.[33] The

flow time 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,[34] where η is viscosity of DNA in the presence of 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. For the gel electrophoresis experiments, supercoiled pBR-322 DNA (100 μM) was treated with Ru(II) complexes in 50 mM Tris-HCl, 18 mM NaCl buffer pH 7.8, and the solutions were then irradiated at room temperature with a UV lamp (365 nm,10W). The samples were analyzed by electrophoresis for 60 minutes at 75 V on a 1% agarose gel in Tris-acetic acid-EDTA buffer, pH 7.2. The gel was stained with 1μg /ml ethidium bromide and photographed under UV light.

Toxicity studies were performed on two eukaryotic microorganisms such as yeasts [Saccharomyces cerevisiae CFTRI 101 and Candida rugosa NCIM 3558] of different origin; these two are industrially important organisms and also reference strains for many microbiological assays at commercial level.[26] These microorganisms were grown as per the procedure given in clinical microbiology procedure.[35] Based on the preliminary experiments over a range of concentrations, toxicity was determined for all complexes at a level of 5 μM in the medium. Estimation of growth was done depending on an organism used over a time period of 5–6 days. 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.

2.3. Synthesis of Complexes

2.3.1. Synthesis of 7-Nitro dipyrido[3,2-a:2′-3′-c]phenazine (7-NO_2-dppz)

A solution of 1,10-phenanthroline-5,6-dione (0.210 g,1 mmol) and 4-nitro-1,2-phenylenediamine (0.153 g,1 mmol) in ethanol (20 ml) was heated at reflux for 4 hours. After cooling, the precipitate was collected by filtration, washed with cold ethanol, and vacuum-dried. (yields: 70%) $C_{18}H_9N_5O_2$; Calcd.(%); C:66.05; H:2.75; N:21.40; Found(%): C:66.24; H:2.63; N:21.36. IR(KBr): 1618(C=N), 1521(C=C) cm^{-1}; ESI-MS (DMSO) m/z; 328 (calcd.327); ^{1}H-NMR (DMSO-d_6,δ-ppm): 9.85 (d,H_a,2H); 9.30 (m,H_c,2H); 8.95 (m,H_j,1H); 8.70 (m,H_m,1H); 8.50 (m,H_k,1H); 7.80 (d,H_b,2H); ^{13}C[^{1}H]-NMR(DMSO-d_6, δppm): 118.0 (C_b, 2C), 124.0 (C_k, 1C), 126.7 (C_m, 1C), 128 (C_e, C_d, 4C), 131.0 (C_j, 1C), 132.2 (C_c, 2C), 134.5 (C_h,C_i, 2C), 141.2 (C_f,C_g,2C), 151.8(C_a, C_l,3C).

2.3.2. Synthesis of 7-Methyl dipyrido[3,2-a:2′-3′-c]phenazine (7-CH_3-dppz)

A solution of 1,10-phenanthroline-5,6-dione (0.210 g,1 mmol) and 4-methyl-1,2-phenylenediamine (0.122 g,1 mmol) in ethanol (20 ml) was

heated at reflux for 4 hours. After cooling, the precipitate was collected by filtration, washed with cold ethanol, and vacuum-dried. (Yields: 70%) $C_{20}H_{14}N_4O$; Calcd.(%); C:73.61; H:4.32; N:17.17; Found(%): C:73.40; H:4.46; N:17.38. IR(KBr) : 1623 (C=N), 1420 (C=C) cm^{-1}; ESI-MS (DMSO) m/z; 327 (calcd.326); ^{1}H-NMR (DMSO-d_6,δ-ppm): 9.65 (d,H_a,2H); 8.50 (d,H_c,2H); 8.32 (d,H_b,2H) 8.03 (s,H_m,1H); 7.85 (d,H_k,1H); 7.02 (d,H_j,1H); 2.34 (s,H_n,3H); ^{13}C[^{1}H]-NMR(DMSO-d_6, δppm, major peaks): 22.30 (C_n, 1C), 120.0 (C_b, 2C), 126.0 (C_l, 1C), 127.70 (C_k, 3C), 129.0 (C_e,C_d, 4C), 132.0 (C_m,C_f,C_g, 3C), 133.50 (C_j, 1C), 136.50 (C_c, 2C), 143.20 (C_h,C_i, 2C), 152.60 (Ca, 2C).

2.3.3. Synthesis of [Ru(bpy)$_2$ (7-NO$_2$-dppz)] (PF$_6$)$_2$.2H$_2$O(1)

A mixture of *cis*-[Ru(bpy)$_2$Cl$_2$].2H$_2$O (0.5 mmol), 7-NO$_2$-dppz (0.5 mmol) was heated to reflux in 25 ml ethanol and 15 ml H$_2$O for 8 houors under nitrogen-atmosphere 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. Yields: 75%. Anal. Found (%): C, 42.34; H, 2.83; N, 11.91; Calcd for $C_{38}H_{29}N_9O_4P_2F_{12}Ru$: C, 42.77; H, 2.72; N, 11.81%. IR(KBr): 1625 (C=N), 1564(C=C), 557 (Ru-N) cm^{-1}; ^{1}H-NMR (DMSO-d_6,δ-ppm): 9.64 (d,H_a,2H); 9.26 (s,H_m,1H); 8.67 (m,H_1,4H); 8.77 (d,H_c,2H); 8.30 (m,H_j,1H); 8.22 (m,H_4,4H); 8.13 (m,H_3,4H); 8.03 (d,H_k,1H);7.82 (d,H_b,2H); 7.59 (d,H_2,4H); ^{13}C[^{1}H]-NMR(DMSO-d_6, δppm): 124.39 (C_2, 4C), 127.90 (C_b, 2C), 129.80 (C_4, 4C), 131.63 (C_k, 1C), 133.35 (C_m, 1C), 138.11 (C_e, 2C), 140.43 (C_d, 2C), 142.15 (C_j, 1C), 142.51 (C_c, 2C), 143.72 (C_3, 4C), 148.73 (C_i, 1C), 143.46 (C_h, 1C), 151.36 (C_g, 1C), 151.89 (C_f, 1C), 154.16 (C_l, 1C), 156.44 (C_a,C_1, 6C), 156.71 (C_5, 4C).

2.3.4. Synthesis of [Ru(bpy)$_2$ (7-CH$_3$-dppz)] (PF$_6$)$_2$.2H$_2$O(2)

This complex was obtained by a similar procedure to that described for complex 1, with a mixture of *cis*-[Ru(bpy)$_2$Cl$_2$].2H$_2$O (0.5mmol) and 7-CH$_3$-dppz (0.5mmol); yields: 65%. Anal. Found (%): C, 45.04; H, 3.15; N, 10.95; Calcd for $C_{39}H_{32}N_8O_2P_2F_{12}Ru$: C, 45.21; H, 3.09; N, 10.82%. IR (KBr): 1626(C=N), 1447(C=C), 557(Ru-N) cm^{-1}; ^{1}H-NMR (DMSO-d_6,δppm): 9.59 (d,H_a,2H); 8.86 (t, H_1,4H); 8.38 (d, H_j,1H); 8.22 (m, H_4,4H); 8.19(s, H_m,1H); 8.13 (d, H_j,2H); 8.00(m, H_4,4H); 7.82 (d, H_k,1H); 7.77 (m, H_b,2H); 7.61(m, H_2,4H) 2.72 (s,H_n,3H). ^{13}C[^{1}H]-NMR(DMSO-d_6, δppm, major peaks): 21.65 (C_n, 1C), 124.42 (C_2, 4C), 127.67 (C_b, 2C), 129.02 (C_4, 4C), 130.14 (C_m, 1C), 133.11 (C_j, 1C), 135.08 (C_e, 2C), 137.90 (C_d, 2C), 139.23 (C_k, 1C), 139.90 (C_c, 2C), 140.69 (C_3, 4C), 142.20 (C_l, 1C),

143.46 (C_h, 1C), 150.14 (C_i, 1C), 151.87 (C_f, 1C), 153.46 (C_g, 1C), 156.48 (C_a,C_1, 6C), 156.72 (C_5, 4C).

2.3.5. Synthesis of [Ru(phen)$_2$ (7-NO$_2$-dppz)] (PF$_6$)$_2$.2H$_2$O(3)

This complex was obtained by a similar procedure to that described for complex 1, with a mixture of *cis*-[Ru(phen)$_2$Cl$_2$].2H$_2$O (0.5 mmol) and 7-NO$_2$-dppz(0.5 mmol); yields: 75%. Anal. Found (%): C, 45.12; H, 2.72; N, 11.83; Calcd for $C_{42}H_{29}$ N_9 $O_4P_2F_{12}Ru$: C, 45.24; H, 2.60; N, 11.31%. IR (KBr): 1624(C=N), 1524(C=C), 557(Ru-N) cm^{-1}: ^{1}H-NMR (DMSO-d$_6$,δppm): 9.57 (d,H_a, 2H); 9.20 (s, H_m,1H); 8.80 (m, H_1,4H); 8.69 (d,H_k,1H), 8.40 (m, H_3,4H); 8.38–8.20 (m, H_c,H_4,6H); 8.08 (d, H_j,1H); 8.01–7.72 (m, H_2,H_b,6H). ^{13}C[^{1}H]-NMR(DMSO-d$_6$, δppm): 125.15 (C_2,C_b, 6C), 125.39 (C_k, 1C), 126.30 (C_m, 1C), 127.76 (C_6, 4C), 128.02 (C_4, 4C), 129.56 (C_e, 2C), 131.57 (C_d, 2C), 133.44 (C_j, 1C), 136.97 (C_3,C_c, 6C), 142.74 (C_5, 4C), 143.57(C_i, 1C), 147.17 (C_h, 1C), 148.66 (C_g, 1C), 151.43 (C_f, 1C), 152.60 (C_l, 1C), 153.25 (C_a, 2C), 154.69 (C_1, 4C).

2.3.6. Synthesis of [Ru(phen)$_2$ (7-CH$_3$-dppz)] (PF$_6$)$_2$.2H$_2$O(4)

This complex was obtained by a similar procedure to that described for complex 1, with a mixture of *cis*-[Ru(phen)$_2$Cl$_2$].2H$_2$O (0.5mmol) and 7-CH$_3$-dppz(0.5mmol); yields: 75%. Anal. Found (%): C, 47.08; H, 3.02; N, 10.63; Calcd for $C_{43}H_{32}$ N_8 $O_2P_2F_{12}Ru$: C, 47.64; H, 2.95; N, 10.34%. IR (KBr): 1627(C=N),1425(C=C),556(Ru-N) cm^{-1}: ^{1}H-NMR (DMSO-d$_6$,δppm): 9.51(d, H_a, 2H); 8.79(m, H_1,4H); 8.42–8.40 (m, H_3,4H); 8.37–8.19 (m,H_d,Hg,6H), 8.27–8.16 (m, H_m,H_j,2H); 8.06 -7.71 (m, H_2,H_b,H_k,7H); 2.69 (s,H_n,3H). ^{13}C[^{1}H]-NMR(DMSO-d$_6$, δppm): 21.64 (C_n, 1C), 126.29 (C_2,C_b, 6C), 127.40 (C_6, 4C), 127.62 (C_m, 1C), 128.02 (C_j, 1C), 128.86 (C_4, 4C), 130.43 (C_e, 2C), 133.13 (C_d, 2C), 134.23 (C_k, 1C), 135.04 (C_c, 2C), 136.90 (C_3, 4C), 139.86 (C_l, 1C), 141.98 (C_5, 4C), 143.98 (C_h, 1C), 147.17 (C_i, 1C), 150.43 (C_f, 1C), 152.60 (C_g, 1C), 153.23 (C_a, 2C), 153.76 (C_1, 4C).

The water-soluble halide salt was prepared from the hexafluorophosphate by precipitation in acetone solution using n- Bu_4NCl.

3. RESULTS AND DISCUSSION

3.1. Absorption Spectral Studies

Absorption titration can monitor the interaction of a metal complexes and DNA. In general, complex bound to DNA through intercalation usually results in hypochromism and red shift (bathocromism), due to the strong stacking interaction between aromatic chromophore of the complex and base pairs of the DNA. In Figure 2, the absorption spectra of the complex in

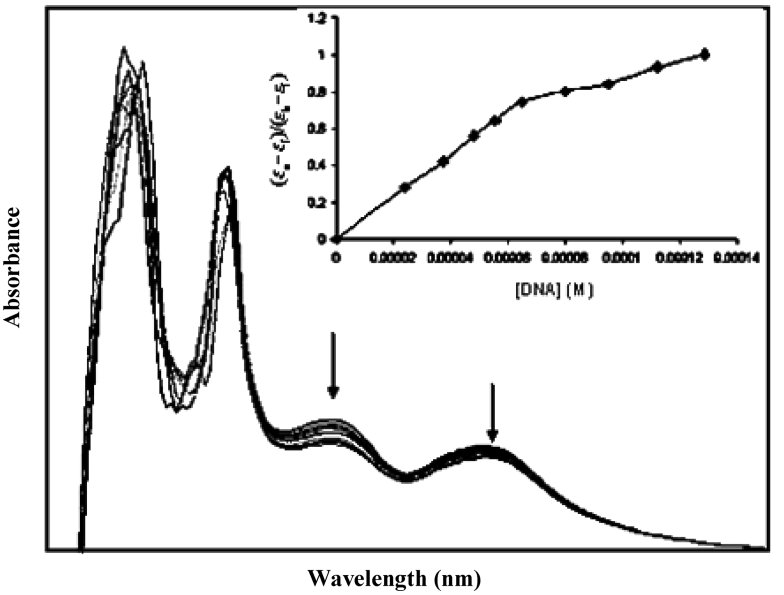

FIGURE 2 Absorption spectrum of complex $[Ru(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{+2}$(1), in Tris HCl buffer at 25°C in the presence of increasing amount of CT-DNA, [Ru] = 20μM, [DNA] = 0–120 μM.The arrows indicate the change in absorbance upon increasing the DNA concentration. Insert: Plot of $(\varepsilon_a-\varepsilon_f)/(\varepsilon_b-\varepsilon_f)$ versus [DNA].

the absence and presence of CT-DNA are shown as specific examples, and complex **3** binds DNA more strongly than other three complexes. Based on the observations, we presume that there are some interactions between the complexes and the base pairs of DNA In order to compare quantitatively the binding strength of the four complexes, the intrinsic binding constants K_b of the three complexes with CT- DNA were obtained by monitoring the changes in absorbance at 428.5, 433, 426, and at 431nm for 1, 2, 3, and 4 complexes, respectively, with increasing concentration of DNA. The values of the intrinsic binding constant K_b illustrating the binding strength of the complexes with ct-DNA were determined from Equation (1) through a plot of $(\varepsilon_a - \varepsilon_f)/(\varepsilon_b — \varepsilon_f)$ versus [DNA],[36] where [DNA] is the concentration of DNA per nucleotide.

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

$$b = 1 + KC_t + K[DNA]/(2s) \quad (1b)$$

The apparent absorption coefficients ε_a, ε_f, and ε_b correspond to $A_{obsd}/[Ru]$, the extinction coefficient for the free ruthenium complex

and the extinction coefficient for the ruthenium complex in the fully bound form, respectively, Ct is the total Ru(II) complex concentration, [DNA] is the DNA concentration in nucleotides and s is the binding site size. Intrinsic binding constants K_b of $[Ru(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$, $[Ru(bpy)_2(7\text{-}CH_3\text{-}dppz)]^{2+}$, $[Ru(phen)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$, and $[Ru(phen)_2(7\text{-}CH_3\text{-}dppz)]^{2+}$were obtained about $2.92 \pm 0.2 \times 10^5$ (s = 0.69), $2.31 \pm 0.3 \times 10^5$ (s = 0.46), $3.56 \pm 0.3 \times 10^5$ (s = 1.26)and $3.13 \pm 0.1 \times 10^5$ (s = 0.98) M^{-1}, respectively. The values are smaller as expected than that of their parent complex $[Ru(phen)_2(dppz)]^{2+}$ ($K = 5.1 \times 10^6$ M^{-1}.[37] This may be attributed to the steric hindrances of the substituent group. As reported before, the DNA-binding affinity of complexes can be influenced by electronic effects of intercalative ligands.[38] These results shows that the electron withdrawing substituent ($-NO_2$) on the intercalative ligand can increase the DNA-binding affinity of the complex, whereas the electron-donating substituent ($-CH_3$) has the opposite effect, the reason being that the electron poor aromatic rings generally stack better than the electron rich ones. NO_2 is electron withdrawing and CH_3 is electron-donating; hence, CH_3 substituted ring is electron rich. The position of NO_2 on the ring may influence the electron density of the intercalative ligand. 6-NO_2 makes the DPPZ more electron deficient than 7-NO_2. $[Ru(phen)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$ binds to the DNA weakly compared to $[Ru(phen)_2(6\text{-}NO_2\text{-}dppz)]^{2+}$.[39]

3.2. Fluorescence Spectroscopic Studies

All four complexes exhibit fluorescence in Tris buffer at ambient temperature with maxima at 560 nm. As shown in Figure 3, upon addition of CT-DNA, the fluorescence emission intensities of complexes 1–4 increased by factors of 1.35, 1.42, 1.53, and 1.47, respectively, the extent of enhancement rises on going from complex 1 to complex 4, which is consistent with the above absorption spectral results. This implies that complexes 1–4 interact with DNA and are protected by 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 a decrease of the vibrational modes of relaxation. 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, therefore, positively charged free complex ions should be readily quenched by $[Fe(CN)_6]^{4-}$. The complex bound 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. The method essentially consists of titrating a given amount

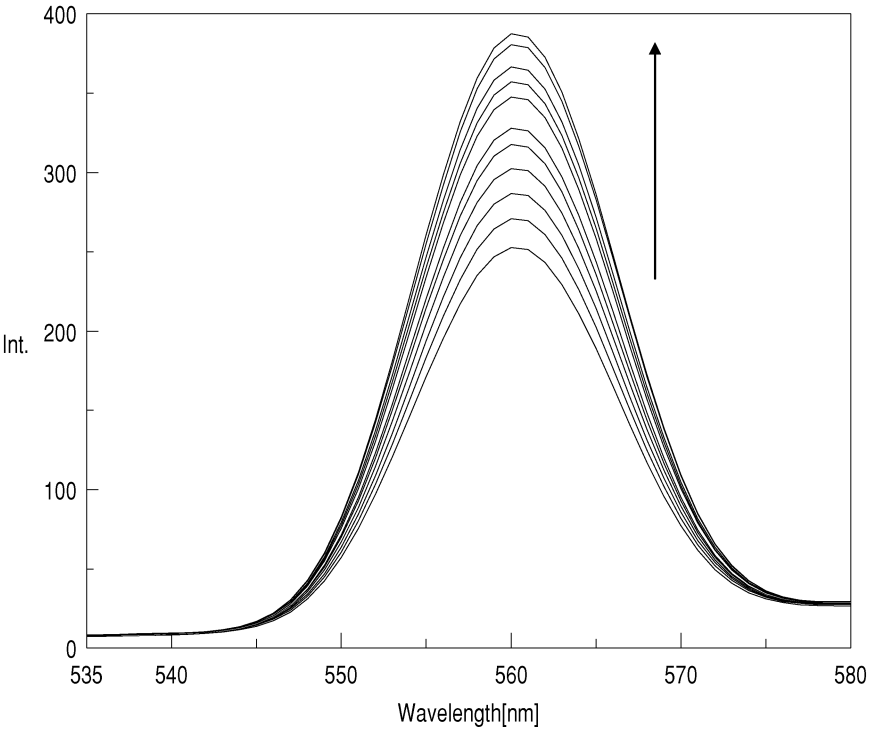

FIGURE 3 Emission spectra of complex $[Ru(phen)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$ (3) in Tris HCl buffer at 25°C in the presence of CT-DNA, [Ru] = 20 μM, [DNA] = 0–120 μM. The arrow shows the increase in intensity upon increasing CT-DNA concentrations.

of DNA alone and DNA-metal complexes while 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 Figure 4. The absorption and fluorescence spectroscopy studies determine the binding of complexes. From the quenching studies it is also clear that the DNA binding ability of complex 4 > 3 > 2 > 1.

The K_b value of the complex could also be obtained by fitting the fractional changes in emission intensities, $(I_a–I_f)/(I_b–I_f)$, as a function of DNA concentrations according to the Bard-Torp-Murphy Equations (2) and (3).[40]

$$(I_a - I_f)/(I_b - I_f) = (b - (b^2 - 2K_b^2 C_t[DNA]/s)^{1/2})/(2K_b C_t) \quad (2)$$

$$b = 1 + K_b C_t + K_b[DNA]/2s \quad (3)$$

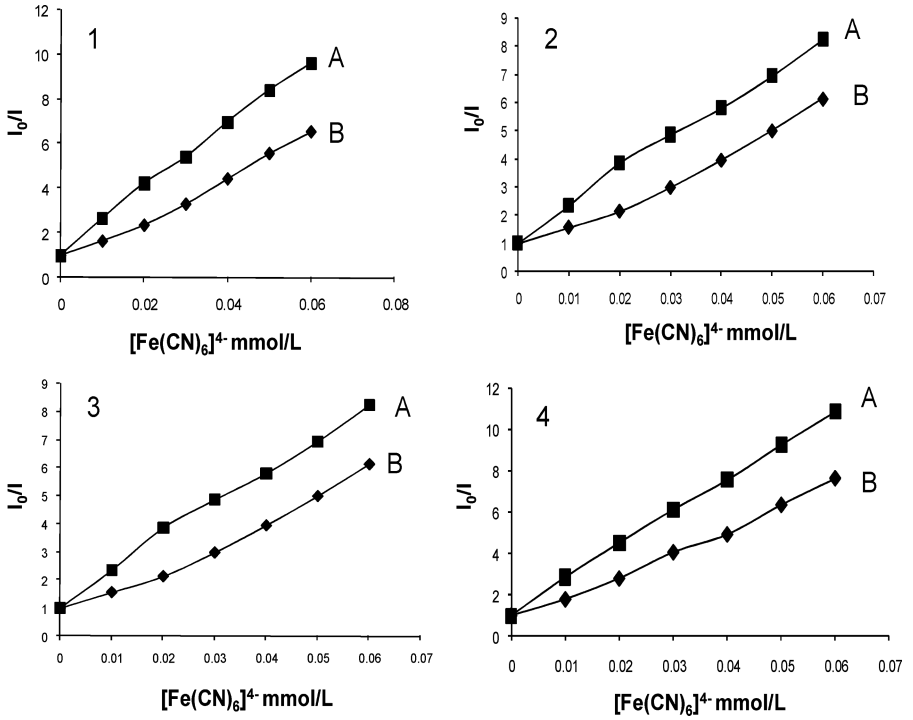

FIGURE 4 Emission quenching of Ru(II) complexes 1–4 with $K_4[Fe(CN)_6]$ in the presence (B) and absence (A) of DNA. [Ru] = 20 μM, [DNA]/ [Ru] = 40:1.

where I_a, I_f, and I_b are the fluorescent intensity at a given DNA concentration, the free complex in solution and the complex fully bound to DNA, respectively. K_b is the equilibrium binding constant, C_t is the total metal complex concentration, [DNA] is the DNA concentration in nucleotides and s is the binding site size. Intrinsic binding constants K_b of $[Ru(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$, $[Ru(bpy)_2(7\text{-}CH_3\text{-}dppz)]^{2+}$, $[Ru(phen)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$, and $[Ru(phen)_2(7\text{-}CH_3\text{-}dppz)]^{2+}$ were obtained about 2.62 $\pm$ 0.2 $\times$ 10^5, 2.01 $\pm$ 0.3 $\times$ 10^5, 3.16 $\pm$ 0.3 $\times$ 10^5 and 2.83 $\pm$ 0.1 $\times$ 10^5 M^{-1}, respectively. These results are in complete agreement with the values derived from UV-vis spectral titration data. Further investigation is clearly needed to establish the exact nature of the complex binding to DNA.

3.3. Viscosity Studies

Viscosity measurements were carried out to clarify the mode of interaction of metal complexes with CT-DNA. A hydrodynamic measurement such as viscosity is sensitive to change in length and is regarded as the least ambiguous and most critical of tests of a binding model. In classical intercalation, the DNA helix lengthens as base pairs are separated to

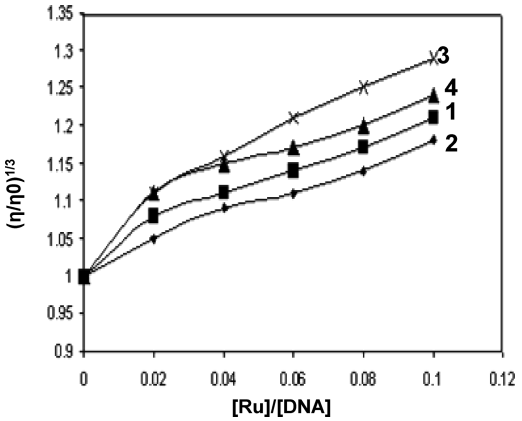

FIGURE 5 Effect of increasing amount of complexes $[Ru(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$(1), $[Ru(bpy)_2(7\text{-}CH_3\text{-}dppz)]^{2+}$(2), $[Ru(phen)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$ (3), and $[Ru(phen)_2(7\text{-}CH_3\text{-}dppz)]^{2+}$ (4) on relative viscosity of CT-DNA at 30 ± 0.1°C. The total concentration of DNA is 0.25 mM, [Ru] = 20 μM.

accommodate the bound ligand leading to an increase in the viscosity of the DNA solution.[41,42] For example, under appropriate conditions, intercalation of drugs like ethidium bromide (EtBr) causes a significant increase in the overall DNA length. On the other hand, partial and/or nonclassical intercalation of the ligand may bind (or kink) the DNA helix, resulting in a decrease in its effective length and concomitantly, its viscosity. The effects of the three complexes on the viscosity of rod-like DNA are shown in Figure 5. As the concentration of complexes increases, the relative viscosity of DNA increases proportionately; as a result, the length of the duplex DNA increases following intercalation, which is similar to that of the proven DNA intercalator $[Ru(phen)_2dppz]^{2+}$.[43] The result show that the four Ru(II) complexes interact with CT-DNA through intercalation.

3.4. DNA Melting Studies

Thermal behaviors of DNA in the presence of complexes can give insight into their conformational changes when temperature is raised, and offer information about the interaction strength of complexes with DNA. It is well known that when the temperature in the solution increases, the double-stranded DNA gradually dissociates to single strands, and generates a hypochromic effect on the absorption spectra of DNA bases (λ_{max} = 260 nm). The melting temperature T_m, which is defined as the temperature where half of the total base pairs are unbonded, is determined from the thermal denaturation curves of DNA. According to the literature,[44–46] the intercalation of natural or synthesized organics and metallointercalators generally results in a considerable increase in melting temperature (T_m).

Here, a DNA melting experiment revealed that T_m of calf thymus DNA is 61 ± 0.2°C in the absence of the complexes. The observed melting temperature in the presence of the complexes were 67 ± 0.2°C, 65 ± 0.2°C, 71 ± 0.2°C and 69 ± 0.2°C, for 1, 2, 3, and 4 complexes, respectively, and gives strong support for intercalation into the helix. The absorption spectral results also indicate that complex 3 exhibits larger DNA binding affinity than complex 1, 2, and 4.

3.5. Photo Activated Cleavage of pBR-322 DNA by Ru(II) Complexes

The DNA photocleavage ability was studied at room temperature, pH 7.8 in Tris-EDTA buffer.[22] The cleavage of plasmid DNA can be monitored by agarose gel electrophoresis. Since the intact supercoil form (form I) migrates relatively fast, if scission occurs on one strand (nicking), the supercoil will relax to generate a slower-moving open circular form (form II). If both strands are cleaved, a linear form (form III) migrates between form I and form II.[47] Figure 6 shows gel electrophoretic separation of pBR322 DNA after incubation with Ru(II) complexes and irradiation at 365 nm. No DNA cleavage was observed for controls in which complexes were absent lane 1 (for complexes 1, 2, 3, and 4)), or incubation of the plasmid DNA with the Ru(II) complexes in the dark (data not presented). With increasing concentration of the Ru(II) complexes 1, 2, 3, and 4 (lanes 2–5), the amount of Form I of pBR322 DNA diminishes gradually, whereas Form

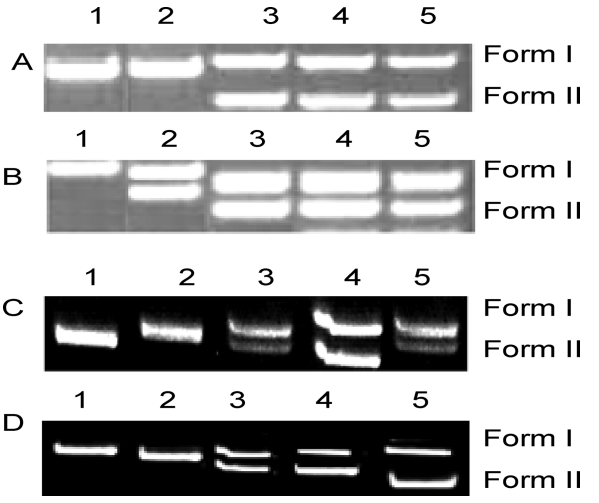

FIGURE 6 Photocleavage of pBR 322 DNA [10 μl of 100 μM stock], in absence and in the presence of complexes 1 (A), 2 (B), 3 (C), and 4 (D) light after 60 minutes of irradiation at 365 nm. Lane 1 control plasmid DNA (untreated pBR 322), lanes 2–5 addition of complexes, in amounts of 5, 10, 20, 30 μM.

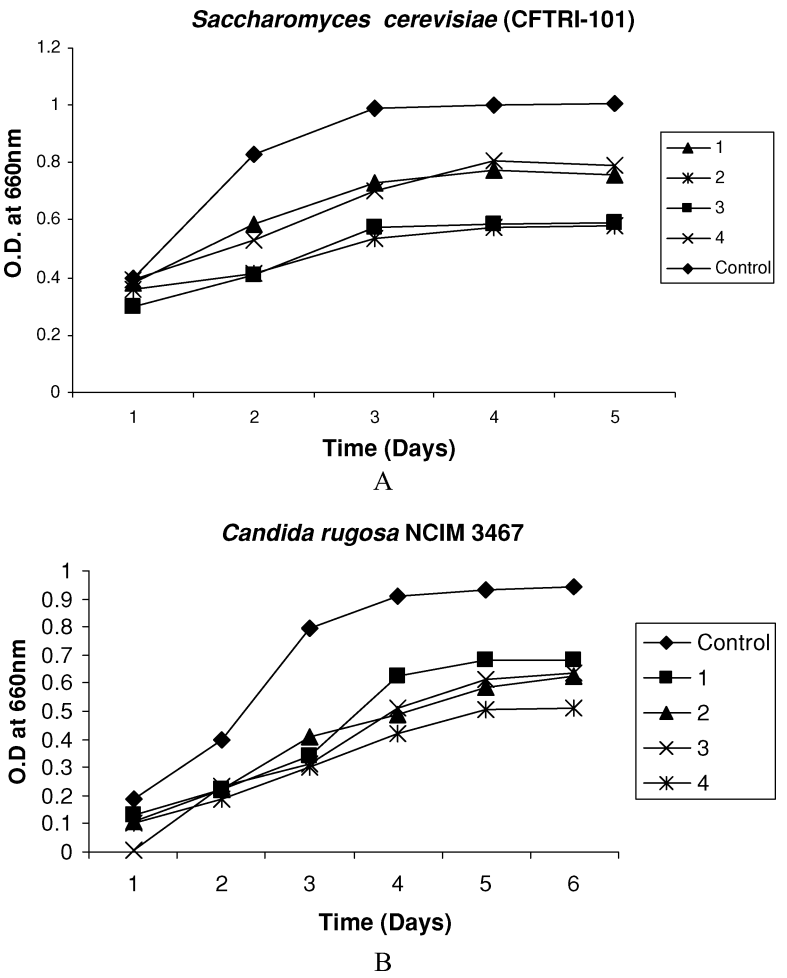

FIGURE 7 Effect of complexes 1–4 ($[Ru(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$(1), $[Ru(bpy)_2(7\text{-}CH_3\text{-}dppz)]^{2+}$(2), $[Ru(phen)_2(7\text{-}NO_2\text{-}dppz)]^{2+}$ (3), and $[Ru(phen)_2(7\text{-}CH_3\text{-}dppz)]^{2+}$ (4)) on *Saccharomyces cereviceae* CFTRI-101 (A) and *Candida rugosa* NCIM 3558 (B) growth expressed on the basses of absorbance at 660 nm growth of control (no complex) was and taken as 100% for quantitative evaluation of complex.

II increases. Under comparable experimental conditions, all complexes exhibit more effective photocleavage of DNA. Further studies are currently underway to clarify the cleavage mechanism.

There were reports on DNA-endonucleolytic cleavage reactions that are activated by Ruthenium (II) complexes.[49,50] Gao et al. and Sigman et al. also carried photoactivated cleavage of PBR322 in the presence of $[Ru\ (bpy)_2\ (ppd)]^{2+}$ and also noted the effect of hydroxyl radical (OH^-) scavengers such as mannitol and DMSO at even high concentrations. Tan et al reported that $[Ru\ (phen)_2\ (mdpz)]^{+2}$ has been found to promote the

photocleavage of pBR 322DNA under irradiation and singler oxygen ($^1O_2$) is suggested to be the reactive species responsible for the cleavage.

3.6. Toxicological Studies

A series of [[Ru(bpy)$_2$(7-NO$_2$-dppz)]$^{2+}$, [Ru(bpy)$_2$(7-CH$_3$-dppz)]$^{2+}$, [Ru(phen)$_2$(7-NO$_2$-dppz)]$^{2+,}$ and [Ru(phen)$_2$(7-CH$_3$-dppz)]$^{2+}$ complexes are synthesized and evaluated with two eukaryotic microorganisms (yeasts). Toxicological effects of the complexes 1, 2, 3, and 4 are compared for their effects on growth of the selected microorganisms. Figure 7 indicate that all four complexes are growth inhibitory with toxicity being in the order 4 > 2 > 3 > 1 and 2 > 3 > 1 > 4 with the yeasts *Candida rugosa* NCIM 3558 and *Saccharomyces cerevisiae* CFTRI 101 respectively; the absence of any lag in the onset of growth effects suggests that the complexes are taken up rapidly and affect intracellular metabolism and regulation.[26,48] This study on eukaryotic microorganisms like yeasts will lead to application and impacts of the synthetic complexes on environment and eukaryotic systems.

4. CONCLUSION

In summary, four Ru(II) complexes of [Ru(bpy)$_2$(7-NO$_2$-dppz)]$^{2+}$, [Ru(bpy)$_2$(7-CH$_3$-dppz)]$^{2+}$, [Ru(phen)$_2$(7-NO$_2$-dppz)]$^{2+,}$ and [Ru(phen)$_2$(7-CH$_3$-dppz)]$^{2+}$ have been synthesized and characterized. Their DNA-binding and photocleavage properties were also investigated. Spectroscopic studies and viscosity experiments supported that the three complexes can intercalate into DNA base pairs via 7-NO$_2$-dppz and 7-CH$_3$-dppz ligand. Toxicological studies show that the three complexes have effect over eukaryotic and also prokaryotic microorganisms. The effect of synthetic complexes varies depending on their constitution of the eukaryotic system. Taken as a whole, the results described in this study should be valuable in further understanding the selectivity and efficiency of DNA recognized and cleaved by the metal complexes of dppz based systems; in addition, this type of an approach will open new vistas to know the affects of synthetic metal complexes on the eukaryotes

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