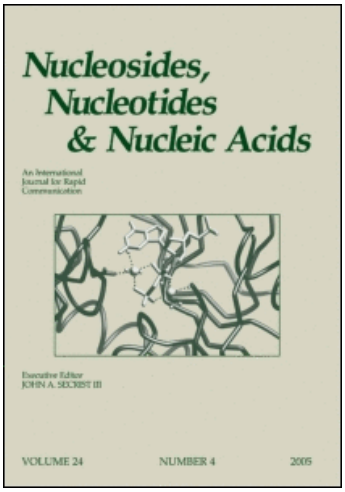

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Synthesis, Characterization, DNA-Binding, and DNA-Photocleavage Properties of $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, and $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ Complexes: (7-Nitro-dppz = 7-Nitro dipyrido[3,2-a:2'-3'-c]phenazine; bpy = 2,2'-bipyridine; dmb = 4,4'-dimethyl-2,2'-bipyridine; phen = 1,10-phenanthroline) and their Toxicity on Different Microorganisms

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SYNTHESIS, CHARACTERIZATION, DNA-BINDING, AND DNA-PHOTOCLEAVAGE PROPERTIES OF $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, AND $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ COMPLEXES: (7-NITRO-dppz = 7-NITRO DIPYRIDO[3,2-a:2′-3′-c]PHENAZINE; bpy = 2,2′-BIPYRIDINE; dmb = 4,4′-DIMETHYL-2,2′-BIPYRIDINE; PHEN = 1,10-PHENANTHROLINE) AND THEIR TOXICITY ON DIFFERENT MICROORGANISMS

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□ *The polypyridyl ligand 7-Nitro dipyrido[3,2-a:2′-3′-c]phenazine (7-Nitro-dppz) and its complexes* $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ (**1**) (bpy = 2,2′-bipyridine), $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ (**2**), (dmb = 4,4′-dimethyl-2,2′-bipyridine), and $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ (**3**) (phen = 1,10-phenanthroline) *were synthesized and characterized by UV/VIS, IR, elemental analysis,* ^1H *and* ^{13}C*-NMR, and mass spectra. The binding properties of the three complexes to CT-DNA were investigated by different spectroscopic methods and viscosity measurements and DNA cleavage assay. The experimental results suggest that these complexes bind to CT-DNA through an intercalative mode. Also, the three complexes promote the photocleavage of plasmid pBR-322 DNA under irradiation. Toxicological effects of the selected complexes were estimated with different microorganisms.*

Keywords Co(III)complexes; polypyridyl ligand; calf thymus DNA; photo cleavage; intercalative mode; toxicology

INTRODUCTION

Deoxyribonucleic acid plays an important role in the life process because it bears heritage information and instructs the replication and transcription of genetic information in living cells. DNA is a particularly good target for metal binding sites.[1–4] Such sites include the electron

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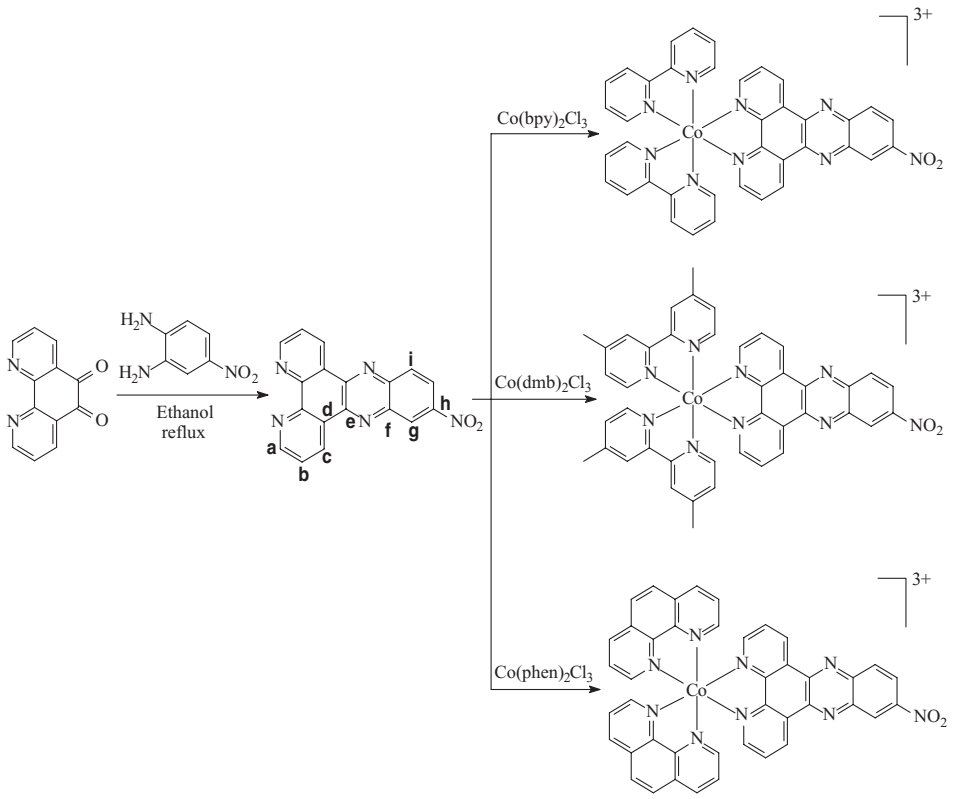
rich DNA bases or phosphate groups that are available for direct covalent coordination to metal center. There are noncovalent binding modes as well, such as hydrogen bonding and electrostatic binding to grooved regions of the DNA and intercalation of planar aromatic ligands into the stacked base pairs. The interaction of transition metal polypyridyl and mixed ligand complexes with DNA has been extensively studied during the past several years.[5–10] Especially, extensive work has been done on the DNA binding of metal complexes with bipyridyl ligands.[11–13] Clearly, further studies using various metals and ligands to evaluate and understand the effect and factors on DNA binding and cleavage mechanism are necessary. Recently our group has reported[14–16] DNA binding and photocleavage studies of several mixed ligand complexes of ruthenium (II) and cobalt (III). In this work, we present our results on the synthesis and studies on the interaction of DNA with Co(III) complexes, which possess the same interesting characteristics and DNA cleaving properties, but have not received as much attention as the Ru(II) systems.[17–21] Three Co(III) complexes, $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$(1), $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$(2), and $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$(3) were synthesized and characterized (Scheme 1). All three Co(III) complexes possess extended aromatic π-systems, like some bidentate ligands such as di pyrido[3,2-a;2′-3′-c]phenazine (dppz). This structural features offer the chance for them to be a candidate for DNA-binding reagents. Their binding properties to calf thymus DNA were studied; absorption spectroscopy, emission spectroscopy, DNA melting techniques, viscosity measurements, and their abilities to induce cleavage of pBR-322 DNA were also investigated. Previously Kazachenko et al. established that some complex compounds of gold with glycine, histidine, tryptophan, and cysteine possess selective antibacterial activity and low toxicity.[22,23] In this work the antimicrobial and toxicological studies of the three complexes were estimated with different microorganisms of different origin and type.

EXPERIMENTAL

Materials

$CoCl_2 \cdot 6H_2O$, 1, 10-phenanthroline monohydrate, and 2, 2′-bipyridine were purchased from Merck (India). calf thymus, 4-nitro-ortho-phenylenediamine and 4,4′-dimethyl-2,2′-bipyridine were obtained from Sigma (St. Louis, MO, USA). The super coiled (CsCl purified) pBR-322 DNA (Bangalore, Genei, India) was used as received. All other chemicals and solvents were procured from locally available sources. All the solvents were purified before use as per standard procedures.[24] Deionized, 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



SCHEME 1 Synthetic routines of ligand and Co (III) complexes.

absorbance at 260 and 280 nm of 1.8–1.9, indicating that the DNA was sufficiently free of protein.[25] The concentration of calf thymus DNA was determined spectrophotometrically using the molar absorption coefficient 6600 $M^{-1}cm^{-1}$(260 nm).[26]

Synthesis and Characterization

The compounds 1,10-phenanthroline-5,6-dione,[27] $[Co(bpy)_2Cl_2]Cl$, $[Co(dmb)_2Cl_2]Cl$,[28] and$[Co(phen)_2Cl_2]Cl$[29] were prepared according to literature procedures. Synthetic roots of ligands and their Co (III) complexes are shown in Scheme 1.

A. Synthesis of Ligand

7-Nitro dipyrido[3,2-a:2′-3′-c]phenazine; (7-nitro-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:2.83; 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-d6, δppm): All the protons resonate down field between 10 to 8.5 ppm corresponding to aromatic ring protons. 9.85 (2H,d); 8.70 (2H,m); 9.30 (2H,d); 7.80(1H,s); 8.95 (1H,d); 8.50 (1H,d); ^{13}C[^{1}H]-NMR(DMSO-d_6, δppm): The ^{13}C NMR of 7-nitro dppz gives a peak at 151.8 corresponding to carbon next to nitrogen(C_a) and 141.2 corresponding to carbon attached to nitro group(C_h), 134.5(C_e), 132.2(C_f), 131(C_i),128(C_c), 126.7(C_d), 124(C_g), 118(C_b).

B. Synthesis of $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)](ClO_4)_3\cdot 3H_2O$

To a 50 ml ethanolic solution of Cis-$[Co(bpy)_2Cl_2]Cl\cdot 3H_2O$ (0.531 g,1.0 mmol) was added about 0.490 g (1.5 mmol) of NDPPZ. The resulting solution was refluxed for 1 hour and further stirred for 5–6 hours under nitrogen. It was filtered and the complex was precipitated by upon addition of a saturated ethanolic solution of $NaClO_4$ to the filtrate. The complex was filtered and further dried under vacuum before recrystallization (Me_2CO-Et_2O). (yields; 75%) Found (%): C;43.54, H;2.86; N:12.14; Calcd for $C_{38}H_{31}N_9Cl_3O_{17}Co$; C;43.42, H;2.95; N:12%. IR(KBr): 1681(C = N), 624(Co–N(nitro-dppz)), 558(Co–N(bpy)) cm^{-1}; ^{1}H-NMR(DMSO-d_6,δ-ppm): 9.15 (4H,d); 8.75 (6H,m); 8.70 (4H,m); 8.40 (2H,d); 7.95 (4H,m); 7.90 (2H,m); 7.50 (1H,d); 8.72 (1H,s); 8.60 (1H, d). ^{13}C[^{1}H]-NMR(DMSO-d_6, δppm, major peaks): Upon coordination 7-nitro dppz to Co(III) the carbons next to nitrogen of phenanthroline ligand (C_a) shifted to down field and appeared at 155 and 152(bipyridine). Other peaks also shifted to down field and resonates at 151.6(C_h), 150(C_e), 148(C_f), 143.5(C_c), 138.1(C_i), 135.2(C_d), 130(C_g), 127(C_b).

C. Synthesis of $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)](ClO_4)_3\cdot 3H_2O$

This complex was obtained by a procedure similar to that described above, Cis-$[Co(dmb)_2Cl_2]Cl.3H_2O$ (0.587 g,1.0 mmol) in place of Cis-$[Co(bpy)_2Cl_2]Cl.3H_2O$. Yields; 65%. Found(%): C;45.33, H;3.64; N:11.19; Calcd for $C_{42}H_{39}N_9Cl_3O_{17}Co$; C;45.56, H;3.52; N:11.39%. IR (KBr): 1622, (C=N) 1420(C=C), 557(Co–N) cm^{-1}; ^{1}H-NMR (DMSO-d_6,δppm): 9.92 (2H,d); 9.05 (4H,d); 8.90 (4H,s); 8.24 (2H,m); 7.60 (4H,d); 7.92 (2H,d); 8.84 (1H,d); 8.38 (1H,d);9.30 (1H,s); 2.74 (12H, s). ^{13}C[^{1}H]-NMR(DMSO-d_6, δppm, major peaks): 156(C_a) of nitro-dppz, 155 (the carbon next to nitrogen of dimethyl bipyridine), 150(C_h), 132(C_i), 129.2(C_d), 127(C_g),125(C_b) and methyl carbon on bipyridine resonates at 21.3.

D. Synthesis of $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)](ClO_4)_3\cdot 3H_2O$

This complex was obtained by a procedure similar to that described above, Cis-$[Co(phen)_2Cl_2]Cl.3H_2O$ (0.578 g,1.0 mmol) in place of

Cis-[Co(bpy)$_2$Cl$_2$]Cl.3H$_2$O. Yields; 65%. Found(%): C,45.83; H,2.65; N,11.56; Calcd for $C_{42}H_{31}N_9Cl_3O_{17}Co$; C,45.90; H,2.82; N,11.47%. IR (KBr): 1607(C=N), 1431(C=C), 654(Co-N(nitro-dppz), 467 (Co–N(phen)), cm^{-1}: ^{1}H-NMR (DMSO-d$_6$,δppm): 9.95 (2H,d); 9.20 (4H,d); 8.60 (4H,m); 8.45 (1H,d); 8.02 (8H,m); 8.00 (2H,m); 8.18 (2H,d); 9.38(1H,s); 8.80 (1H,d). ^{13}C[^{1}H]-NMR(DMSO-d$_6$, δppm, major peaks): 154(C$_a$) and 153.4 (the carbon next to nitrogen of phenanthroline), 148(C$_h$), 144(C$_e$), 141(C$_f$), 139.4(C$_c$), 136(C$_i$), 132(C$_d$), 130.5(C$_g$), 126(C$_b$).

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 $\pm$ 0.1°C. DNA samples approximately 200 base pairs in average length were prepared by sonication in order to minimize complexities arising from DNA flexibility.[30] Data were presented as $(\eta/\ \eta_0)^{1/3}$ versus concentration of [Co (III)]/[DNA], where η is viscosity of DNA in the presence of complex, and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions ($t > 100$ seconds) corrected for the flow time of buffer alone (t_0).[31] 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, super coiled pBR-322 DNA (100 μM) was treated with Co (III) 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. The samples were analyzed by electrophoresis for 2.5 hours at 40V on a 1% agarose gel in Tris- acetic acid-EDTA buffer, pH 7.2. The gel was stained with 1 μg /ml ethidium bromide and photographed under UV-light. Toxicity studies were performed on different type of micro organism of different origin, among them an industrially important yeast [*Saccharomyces cerevisiae* CFTRI 101]; soil borne *Pseudomonas sps;* commercially available pro biotic lactic acid bacteria, *Lactobacillus sporogenes* and soil borne pollutant degrading bacteria *Bacillus cereus* MTCC 8550 as the test organisms. These microorganisms were grown as per the given clinical microbiology procedure.[32] 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 organisms used over a time period, from 24 to 120 hours. 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.

RESULTS AND DISCUSSION

DNA Binding Studies

Characterization

All the compounds synthesized in this study have been characterized by elemental analysis, UV-Vis, IR, and ^{1}H-NMR spectroscopic methods. 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 361, 351, and 339 nm for compounds 1, 2, and 3 respectively are assigned to the metal-to-ligand charge transfer transition. The important stretching frequencies observed in the Infrared spectra, ^{1}H-NMR spectral data for the ligands and compounds synthesized in this study, show the expected peaks in the aromatic region.

Absorption Spectral Studies

The absorption spectra of complexes 1, 2, and 3 in the absence and presence of CT-DNA (at constant concentration of complexes) are given in Figure 1. As the concentration is increased, the MLCT transition bands of complexes 1, 2, and 3 at 361, 351, and 339 nm exhibit hypochromism of about 12.9, 8.7, and 14.5% as well as an insignificant bathochromism about 6, 5, and 8 respectively. These results are similar to those reported earlier for various metallo intercalators.[33,34] 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 three complexes, the intrinsic binding constants K_b of the three complexes with CT- DNA were obtained by monitoring the changes in absorbance at 353 nm for complex 1, at 356 nm for complex 2 and at 341 nm for complex 3 with increasing concentration of DNA using the following equation,[35] through a plot of [DNA] / $[\varepsilon_a - \varepsilon_f]$ Vs [DNA].

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

where [DNA] is the concentration per nucleotide, the apparent absorption co-efficients ε_a, ε_f and ε_b correspond to $A_{obsd\,/[Co(III)]}$, the extinction co-efficients for the free cobalt complex, extinction co-efficients of complex in presence of DNA and the extinction co-efficients for the cobalt complex in the fully bound form, respectively. In plots $[DNA]/(\varepsilon_a - \varepsilon_f)$Vs [DNA],K

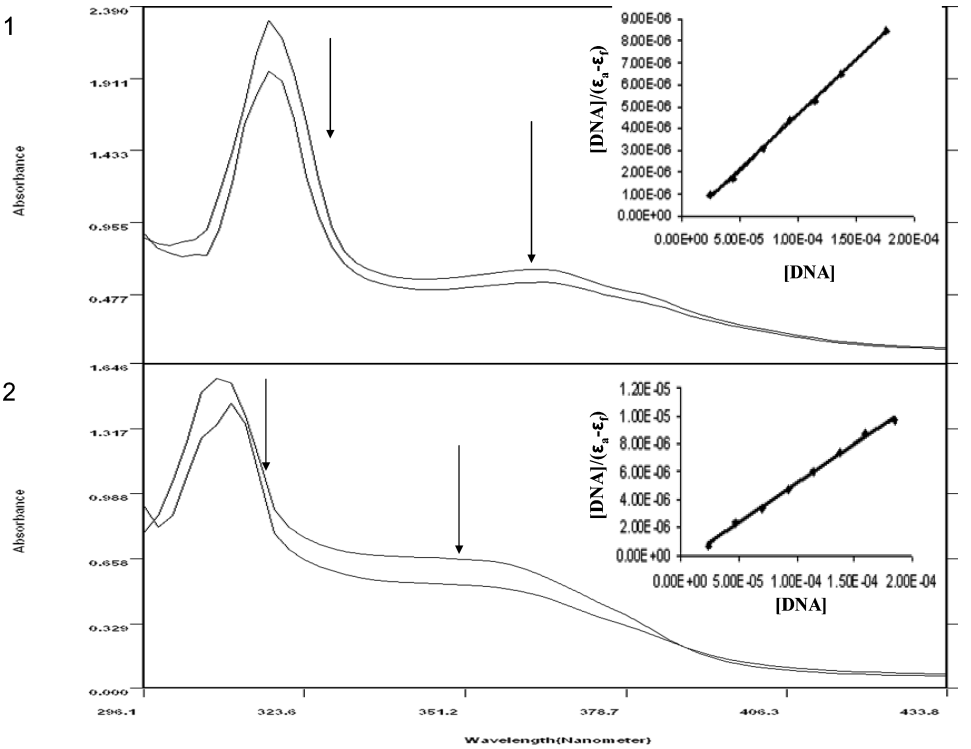


FIGURE 1 Absorption spectrum of complexes $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{+3}$ (1), $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ (2) and in Tris HCl buffer at 25°C in the presence of increasing amount of CT- DNA, [Co] = 10μM, [DNA] = 0–120 μM .The arrows indicate the change in absorbance upon increasing the DNA concentration. Insert: Plot of [DNA]/(ε_a-ε_f) versus [DNA] for titration of the Co(III) complexes.

is given by the ratio of slope to intercept. Intrinsic binding constants K_b of $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$(1), $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$(2) and $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$(3) were obtained about $1.4 \pm 0.2 \times 10^5$, $1.1 \pm 0.3 \times 10^5$, and $1.7 \pm 0.2 \times 10^5$ M^{-1}, respectively. Addition of increasing amounts of CT-DNA results in hypochromism and moderate bathochromic shift in the UV spectra of three $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, and $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$. According to the data presented in Table 1, it seems that the spectral perturbation of the three complexes upon addition of DNA follows: $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+} > [Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+} > [Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$. These spectral characteristics may suggest a mode of binding that involves a stacking interaction between the complex and the base pairs of DNA.

The difference in binding strength of complexes of 1 and 2, probably being caused by the different ancillary ligands. The four additional methyl groups in complex 2 relative to complex 1 exert some steric hindrances. Therefore complex 1 is probably more deeply intercalated and more tightly bound to adjacent DNA base pairs than complex 2. Similarly, the difference

TABLE 1 Results of absorption titration and thermal melting experiment

Complexes	T_M°C	Hypochromicity (%)	Absorption λ_{max} (nm)		$\Delta\lambda$
			Free	Bound	
CT DNA alone	61	—	—	—	—
$[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$	67	12.9	261	267	6
$[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$	64	8.7	253	258	5
$[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$	69	14.5	256	264	8

in binding strength of complexes 1 and 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

The complexes 1, 2, and 3 can emit luminescence in Tris buffer at ambient temperature with maxima at 422, 423, and 420 nm respectively. Binding of three complexes to DNA was found to increase the fluorescence intensity. Here the introduction of a nitro group of 7-Nitro-dppz ligand may be responsible for the negligible luminescence. The emission spectra of three complexes in the absence and presence of CT DNA are shown in Figure 2. In the presence of CT-DNA, the emission intensity increases 1.30 times for complex **1**, 1.17 times for complex **2** and 2.4 times 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. 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 Co(III) 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. 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 Figure 3. 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 $3 > 1 > 2$.

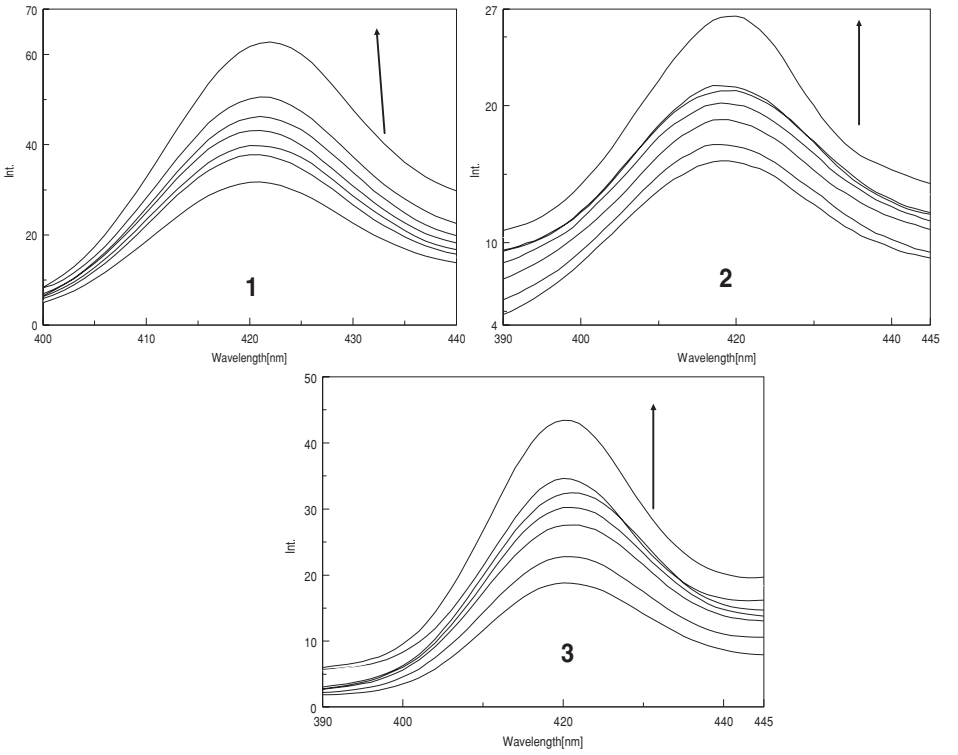


FIGURE 2 Emission spectra of complexes $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$(1), $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$(2) and $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$(3) in Tris HCl buffer at 25°C in the presence of CT-DNA, [Co] = 10μM, [DNA] = 0–120 μM. The arrow shows the intensity change upon increasing CT-DNA concentrations.

Viscosity Studies

Viscosity measurements which were carried out further to clarify the interaction of metal complexes with CT-DNA. A hydrodynamic measurement such as viscosity is sensitive to length change and is regarded as the least ambiguous and most critical tests of a binding model. In classical intercalation, the DNA helix lengthens as base pairs are separated to accommodate the bound ligand leading to an increase of the viscosity of the DNA solution.[31,36] 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 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. The effects of the three complexes $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, and $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ on the viscosity of rod-like DNA are shown in Figure 4. As the concentration of complexes increases, the relative viscosity of DNA increases obviously, as a result the length of

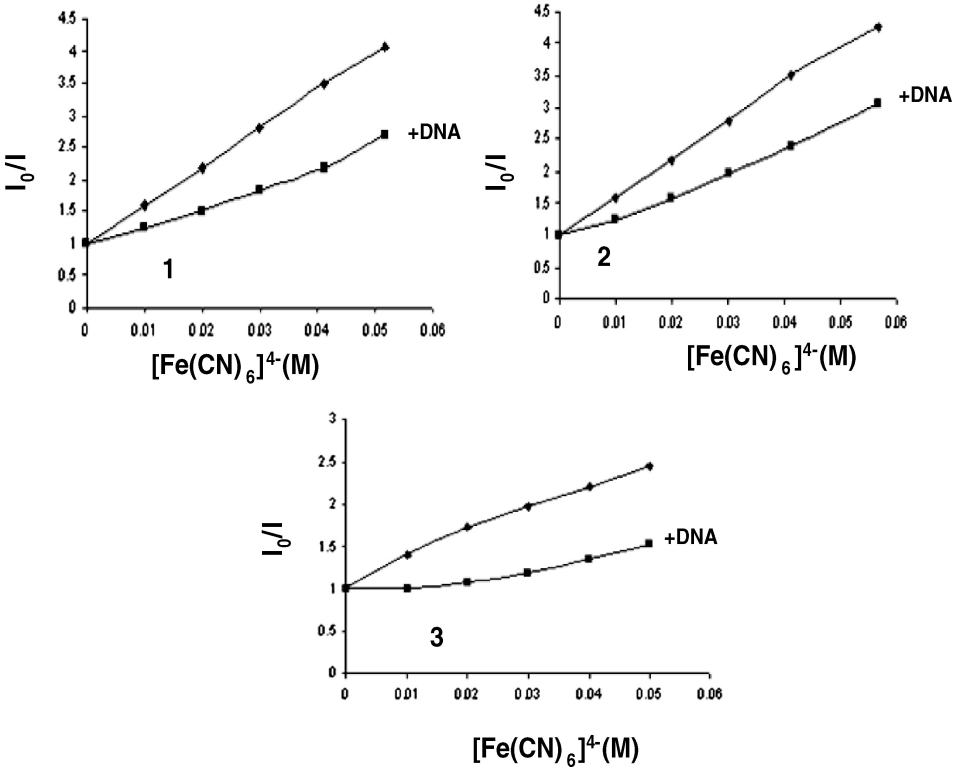


FIGURE 3 Emission quenching of Co(III) complexes 1–3 with $K_4[Fe(CN)_6]$ in the presence and absence of DNA. [Co] = 10μM, [DNA]/ [Co] = 40:1.

the duplex DNA increases following intercalation, which is similar to that of the classical intercalative complex $[Ru(phen)_2dppz]^{2+}$.[37] This result suggests an intercalative binding mode of three Co (III) complexes and also parallels the pronounced hypochromism, bathochromism and emission enhancement of three complexes in the presence of CT-DNA.

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.[38] All the three present complexes ([Co] = 10 μM) were incubated with CT-DNA (100 μM), heated to 85°C from ambient temperature and the OD at 260 nm was monitored.[39] The melting curves of CT DNA in the absence and presence of complexes $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, and $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ are presented in Figure 5. Here, a DNA melting experiment revealed that T_m of calf thymus DNA is 61 ±

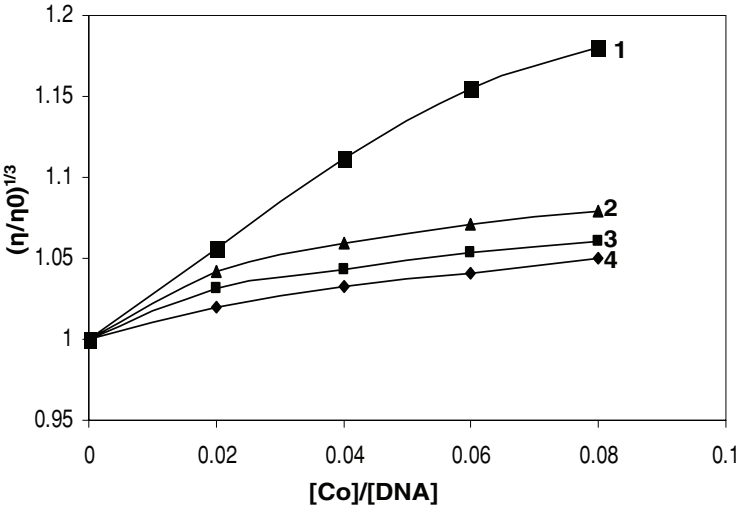


FIGURE 4 Effect of increasing amount of ethidium bromide (1) complexes $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ (2), $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ (3) and $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ (4) on relative viscosity of CT-DNA at 30± 0.1°C. The total concentration of DNA is 0.25 mM, [Co] = 10 μM.

0.2°C in the absence of the complexes. The observed melting temperature in the presence of the complexes were 67 ± 0.2°C, 64 ± 0.3°C, and 69 ± 0.2°C, for 1, 2, and 3 complexes, respectively, and gives strong support for intercalation into the helix. Binding of complexes does lead to an increase in ΔT_m of DNA is in order $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+} > [Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+} > [Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ (Table 1).

Photo Activated Cleavage of pBR-322 DNA by Co (III) Complexes

The cleavage of plasmid DNA can be monitored by agarose gel electrophoresis. In the dark the complexes does not promote DNA strand breaks. Figure 6 shows gel electrophoretic separation of pBR322 DNA after incubation with the complexes and irradiation at 365 nm. When the plasmid was irradiated in the presence of complexes, an efficient photo induced DNA-strand cleavage occurs. No DNA cleavage was observed for controls in which complexes were absent (lane 1). At low concentrations of the complexes, no significant cleavage of the plasmid DNA was observed. With increasing concentration of the complexes (1, 2, and 3; lanes 2–5), a significant nicking of the super coiled plasmid form took place. The amount of the super coiled form of pBR322 DNA diminishes gradually, whereas the movement of the nicked, slower moving open circular form[40] increases.

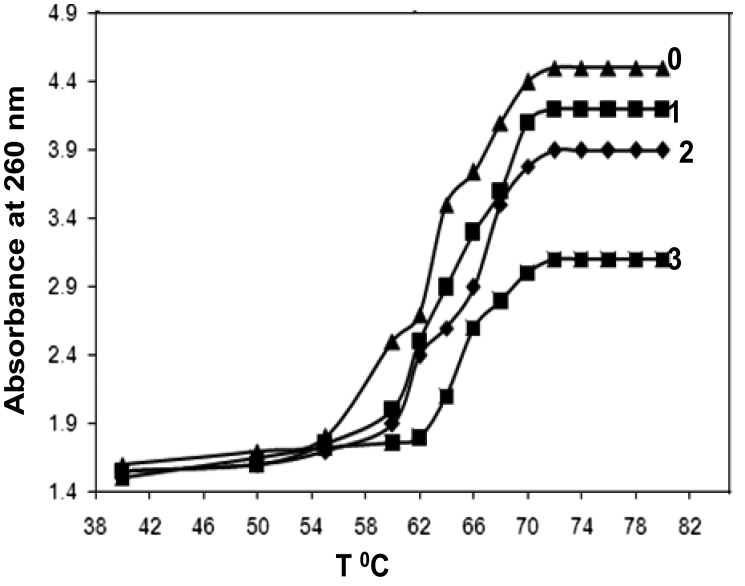


FIGURE 5 Melting temperature curves of DNA in the absence (0) and presence of complexes$[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ (1), $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ (2) and $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ (3).The total concentration of DNA is 100μM, [Co] = 10 μM.

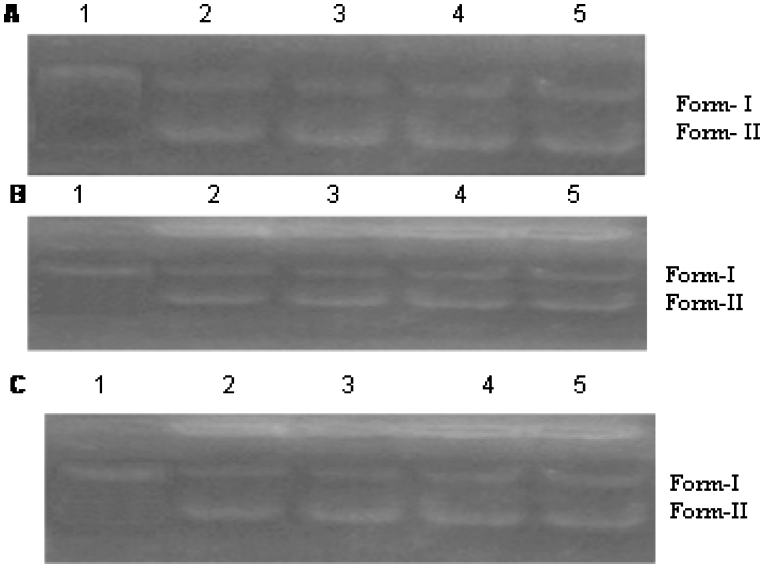


FIGURE 6 (A) Cleavage of pBR 322 DNA [10 μl of 100 μM stock] in the presence of $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$and light after 30 min irradiation at 365 nm. DNA alone (lane 1), the concentration of $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ was 20,40,60,80 μM (lane 2–5). (B) Cleavage of pBR 322 DNA [10 μl of 100 μM stock] in the presence of $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$and light after 30 min irradiation at 365 nm. DNA alone (lane 1), the concentration of $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$was 20,40,60,80 μM (lane 2–5). (C) Cleavage of pBR 322 DNA [10 μl of 100 μM stock] in the presence of $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$and light after 30 min irradiation at 365 nm. DNA alone (lane 1), the concentration of $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$was 20,40,60,80 μM (lane 2–5).

TABLE 2 Effect of cobalt complexes on different microorganisms [Saccharomyces cereviceae CFTRI 101 (2a); Psuedomonas sps (soil bacteria) (2b); Lactobacillus sporogenes (2c); *Bacillus cereus* MTCC 8550 (2d)] growth expressed on the bases of absorbance at 660 nm, growth of control (no complex) was taken as 100% for quantitative evaluation of toxicity of the complex

2a.				
	OD (Optical Density) of the microorganism growth observed at 660 nm			
Time (Hours)	Control	$[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$	$[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$	$[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$
24	0.4	0.395	0.3	0.38
48	0.8	0.61	0.426	0.64
72	1	0.73	0.577	0.755
96	1.01	0.88	0.586	0.776
120	1.01	0.89	0.592	0.781
2b.				
4	0.009	0	0.002	0
8	0.01	0.001	0.008	0
12	0.03	0.009	0.01	0.009
16	0.131	0.112	0.127	0.108
20	0.311	0.19	0.223	0.125
24	0.317	0.194	0.231	0.133
2c.				
4	0.04	0.001	0	0.001
8	0.066	0.041	0.038	0.054
12	0.165	0.139	0.091	0.143
16	0.411	0.293	0.269	0.303
20	0.52	0.461	0.433	0.466
24	0.529	0.48	0.461	0.481
2d.				
4	0.09	0.01	0	0
8	0.1	0.026	0.02	0.008
12	0.201	0.082	0.133	0.082
16	0.287	0.201	0.175	0.104
20	0.399	0.275	0.196	0.119
24	0.401	0.28	0.199	0.12

Toxicological Studies

A series of $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, and $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ complexes are evaluated with different microorganisms. Toxicological effects of the complexes 1, 2, and 3 are compared for their effects on growth of the selected micro organisms. The results shown in Tables 2a through 2d indicate that all three complexes

are growth inhibitory with toxicity being in the order 2 >3 > 1 with the yeast [*Saccharomyces cerevisiae* CFTRI 101]; whereas with the *Pseudomonas sp*, *Lactobacillus sporogenes* and *Bacillus cereus* MTCC 8550, the order of inhibition is 3 >1 >2; 2 >1 >3; 3 >2 > 1, 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.[41] This study on different microorganisms will lead to application and impacts of the synthetic complexes on environment and living systems.

CONCLUSION

In summary, three Co(III) complexes of $[Co(bpy)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, $[Co(dmb)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$, and $[Co(phen)_2(7\text{-}NO_2\text{-}dppz)]^{3+}$ 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 ligand. Upon comparison with ethidiumbromide the results reveal that our complexes are not as good intercalators as ethidiumbromide. Toxicological studies show that the three complexes have effect over eukaryotic and also prokaryotic microorganisms. The effect varies on different microorganisms depending on their constitution of the system. This study will give a pavement for further studies of the complexes effect and its applications on various living systems.

REFERENCES

1. Pyle, A.M.; Long, E.C.; Barton, J.K. Shape-selective targeting of DNA by phenanthrenequinone diiminerhodium(III) photocleaving agents. *J. Am. Chem. Soc.* **1989**, 111, 4520–4522.
2. Sitlani, A.; Long, E.C.; Pyle, A.M.; Barton, J.K. DNA photocleavage by phenanthrenequinone diimine complexes of rhodium(III): shape-selective recognition and reaction. *J. Am. Chem. Soc.* **1992**, 114, 2303–2312.
3. Jin, L.; Yang, P. Synthesis and DNA binding studies of CoIII mixed-ligand complex containing dipyrido [3,2-a: 2′,3′-c]phenazine and phen. *Polyhedron.* **1997**, 16, 3395.
4. Song, Y.-M.; Lu, X.-l.; Yang, M.-l.; Zheng, X-R. Study on the interaction of platinum(IV), gold(III) and silver(I) ions with DNA. *Transition Met. Chem.* **2005**, 30, 499.
5. Zhang, Q.-L.; Liu, J.-G.; Chao, H.; Xue, G.-Q.; Ji, L.-N. DNA-binding and photocleavage studies of cobalt(III) polypyridyl complexes: [Co(phen)2IP]3+ and [Co(phen)2PIP]3+. *J. Inorg. Biochem.* **2001**, 83, 49–55.
6. Liu, J.-G.; Zhang, Q.-L.; Ji, L.-N. Synthesis, characterization and interaction of mixed polypyridyl ruthenium(II) complexes with calf thymus DNA. *Transition Met. Chem.* **2001**, 26, 733.
7. Jiang, C.-W.; Chao, H.; Li, H.; Ji, L.-N. Syntheses, characterization and DNA-binding studies of ruthenium(II) terpyridine complexes: [Ru(tpy)(PHBI)]2+ and [Ru(tpy)(PHNI)]2+. *J. Inorg. Biochem.* **2003**, 93, 247.
8. Vaidyanathan, V.G.; Nair, B.U. Synthesis, characterization and electrochemical studies of mixed ligand complexes of ruthenium(II) with DNA. *J. Chem. Soc., Dalton Trans.* **2005**, 2842.
9. Chao, H.; Mei, W.-J.; Huang, Q.-W.; Ji, L.-N. DNA binding studies of ruthenium(II) complexes containing assymetric tridentate ligands. *J. Inorg. Biochem.* **2002**, 92, 165–170.

10. Jiao, K.; Wang, Q.-X.; Sun, W.; Jian, F.F. Synthesis, characterization and DNA-binding properties of a new cobalt(II) complex: Co(bbt)2Cl2. *J. Inorg. Biochem.* **2005**, 99, 1369.
11. Zhang, Q.-L., Liu, J.-G., Liu, J., Xue, G.-Q., Li, H., Liu, J.-Z., Zhou, H., Qu, L.-H., Ji, L.-N. DNA-binding and photocleavage studies of cobalt(III) mixed-polypyridyl complexes containing 2-(2-chloro-5- nitrophenyl)imidazo [4,5-f][1,10]phenanthroline. *Inorg. Biochem.* **2001**, 85, 291–296.
12. Torshizi, H.M.; Ghadimy, S.; Akbarzadeh, N. Synthesis, characterization, DNA binding and cytotoxic studies of platinum(II) and palladium(II) complexes of the 2,2′-bipyridine and an anion of 1,1-cyclobutanedicarboxylic acid. *Chem. Pharm. Bull.* **2001**, 49, 1517.
13. Yang, Z.-S.; Wang, Y.-L.; Zhao, G.-C. The interaction of copper-bipyridyl complex with DNA and cleavage to DNA. *Anal. Sci.* **2004**, 20, 1127.
14. Penumaka Nagababu, Satyanarayana, S. DNA binding and cleavage properties of certain ethylenediamine cobalt(III) complexes of modified 1,10-phenanthrolines. *Polyhedron* **2007**, 26, 1686.
15. Penumaka Nagababu, D.; Aravind Kumar, K.L.; Reddy, K.; Ashwini Kumar, Md.; Mustafa, B.; Shilpa, M.; Satyanarayana, S. DNA binding and photocleavage studies of cobalt(III) ethylenediamine pyridine complexes: [Co(en)2(py)2]3+ and [Co(en)2(mepy)2]3+. *Metal-Based Drugs* **2008**, 1–8.
16. Pallavi, P.; Nagababu, P.; Satyanarayana, S. Biomimetic model of coenzyme B12: Aquabis(ethane-1,2-diamine-N,N)ethylcobalt(III)—Its kinetic and binding studies with imidazoles and amino acids and interactions with CT DNA. *Helvetica Chemica Acta* **2007**, 90, 627.
17. Arounaguiri, S.; Maiya, B.G. Dipyridophenazine complexes of cobalt(III) and Nickel(II): DNA-binding and photocleavage studies. *Inorg. Chem.* **1996**, 35, 4267.
18. Sastri, C.V.; Eswaramoorthy, D.; Giribabu, L.; Maiya, B.G. DNA interactions of new mixed-ligand complexes of cobalt(III) and nickel(II) that incorporate modified phenanthroline ligands. *J. Inorg. Biochem.* **2003**, 94, 138.
19. Zhang, Q.-L.; Liu, J.-G.; Liu, J.Z.; Li, H.; Yang, Y.; Xu, H.; Chao, H.; Ji, L.-N. Effect of intramolecular hydrogen-bond on the DNA-binding and photocleavage properties of Polypyridyl cobalt(III) complexes. *Inorg. Chim. Acta* **2002**, 339, 34–40.
20. Cheng, C.C.; Kuo, Y.N.; Chuang, K.S.; Luo, C.F.; Wang, W.J. A new coii complex as a bulge-specific probe for DNA. *Angew. Chem., Int. Ed.* **1999**, 38, 1255.
21. Vaidyanathan, V.G.; Nair, B.U. Photooxidation of DNA by a cobalt(II) tridentate complex. *J. Inorg. Biochem.* **2003**, 94, 121.
22. Kazachenko, A.S.; Legler, A.V.; Per'yanova, O.V.; Vstavskaya, Yu. A. Synthesis and antimicrobial activity of silver complexes with histidine and tryptophan. *Pharmaceutical Chemistry Journal* **2000**, 34(5), 34–35.
23. *RF Patent No.* 203,695,1995.
24. Perrin, D.; Annarego, W.L.F.; Perrin, D.R. *Purification of Laboratory Chemicals 2nd Edition*, Pergamon Press, New York, 1980.
25. Marmur, J. A Procedure for the Isolation of deoxyribo nucleic acid from microorganisms. *J. Mol. Biol.***1961**, 3, 208.
26. Rreichmann, M.E.; Rice, S.A.; Thomas, C.A.; Doty, P. A further examination of the molecular weight and size of desoxypentose nucleic acid. *J. Am. Chem. Soc.* **1954**, 76(11), 3047–3053.
27. Yamada, M.; Tanaka, Y.; Yoshimoto, Y.; Kuroda, S.; Shimo, I. Synthesis and properties of diamino-substituted dipyrido [3,2-a: 2′,3′-c]phenazine. *Bull. Chem. Soc. Jpn.***1995**, 65, 1006.
28. Vleek, A.A. Preparation of Co(dipy)2×2+ complexes (X- = chloride, bromide, iodide, nitrite) by controlled oxidative processes. *Inorg.Chem.* **1967**, 6, 1425.
29. Ablore, A.V. 1,10-phen complexes of Co(III). *Russ.J. Inorg.Chem.* **1961**, 6, 157.
30. Chaires, J.B.; Dattaguptha, N.; Crother, D.M. Studies on interaction of anthracycline antibiotics and deoxyribonucleic acid: equilibrium binding studies on the interaction of daunomycin with deoxyribonucleic acid. *Biochemistry* **1982**, 21(17), 3933–3940.
31. Satyanarayana, S.; Dabrowiak, J.C.; Chaires, J.B. Tris(phenanthroline)ruthenium(II) enantiomer interactions with DNA: Mode and specificity of binding. *Biochemistry* **1993**, 32(10), 2573–2584.
32. Isenberg, H.D. (ed.). *Clinical Microbiology Procedures Handbook*, vol. 1 and 2. American Society for Microbiology, Washington, D.C. 1992.
33. Moucheron, C.; Mesmaeker, A.K.D.; Choua, C. Photophysics of Ru(phen)2(PHEHAT)2+: A novel "light switch" for dna and photo-oxidant for mononucleotides. *Inorg. Chem.* **1997**, 36(4), 584–592.
34. Pyle, A.M.; Barton, J.K. "Probing Nucleic Acids with Transition Metal Complexes". S.J. Lippard (Ed.), *Progress in Inorganic Chemistry: Bioinorganic Chemistry*, Wiley, New York, 1990, Vol. 38, 413–475.

35. Wolfe, A.; Shimer, G.H.; Mechan, T. Polycyclic aromatic hydrocarbons physically intercalate into duplex regions of denatured DNA. *Biochemistry* **1987**, 26(20), 6392–6396.
36. Satyanarayana, S.; Dabrowiak, J.C.; Chaires, J.B. Neither .DELTA.- nor .LAMBDA.-tris(phenanthroline)ruthenium(II) binds to DNA by classical intercalation. *Biochemistry* **1992**, 31(39), 9319–9324.
37. Haq, I.; Lincoln, P.; Suh, D.; Norden, B.; Chowdhry B.Z.; Chaires, J.B. Interaction of .LTA.-DE and LAMBDA.-[Ru(phen)2DPPZ]2+ with DNA: A calorimetric and equilibrium binding study. *J. Am. Chem. Soc.* **1995**, 117(17), 4788–4796.
38. Kelly, T.M.; Tossi, A.B.; Mc Connell, D.J.; Ohuigin, C. A study of the interactions of some polypyridylruthenium(II) complexes with DNA using fluorescence spectroscopy, topoisomerisation and thermal denaturation. *Nucleic Acids Res.* **1985**, 13, 6017.
39. Tselepi-Kalouli, E.; Katsaros, N. The interaction of [Ru(NH3)5Cl]2+ and [Ru(NH3)6]3+ ions with DNA. *J. Inorg. Biochem.* **1989**, 37, 271.
40. Barton, J.K.; Raphael, A.L. Photoactivated stereospecific cleavage of double-helical DNA by cobalt(III) complexes. *J. Am. Chem. Soc.* **1984**, 106(8), 2466–2468.
41. Ogunniran, K.O.; Ajanaku, K.O.; James, O.O.; Ajani, O.O.; Adekoya, J.A.; Nwinyi, O.C. Synthesis, characterization, antimicrobial activity and toxicology study of some metal complexes of mixed Antibiotics. *African Journal of Pure and Applied Chemistry* **2008**, 2(7), 069–074.