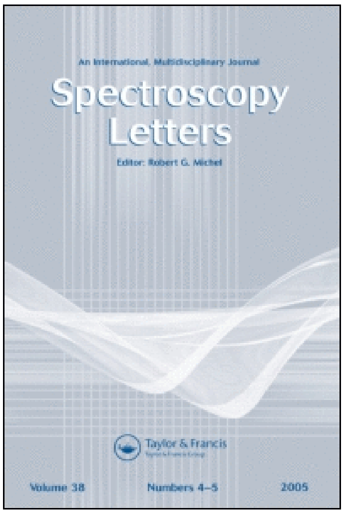

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Synthesis, DNA Binding, DNA Photocleavage and Antimicrobial Activity of $[Co(bpy)_2DMHBT]^{3+}$, $[Co(dmb)_2DMHBT]^{3+}$ and $[Co(phen)_2DMHBT]^{3+}$ Complexes

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Synthesis, DNA Binding, DNA Photocleavage and Antimicrobial Activity of $[Co(bpy)_2DMHBT]^{3+}$, $[Co(dmb)_2DMHBT]^{3+}$ and $[Co(phen)_2DMHBT]^{3+}$ Complexes

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ABSTRACT Cobalt(III) polypyridyl complexes of the type $[Co(bpy)_2DMHBT]^{3+}$, $[Co(dmb)_2DMHBT]^{3+}$ and $[Co(phen)_2DMHBT]^{3+}$ (where DMHBT = 11,13-dimethyl-13H-4,5,9,11,14-hexa aza-benzo[b] triphenylene-10,12-dione; bpy = 2,2′-bipyridine; dmb = 4,4′-dimethyl-2,2′-bipyridine; phen = 1,10-phenanthroline) have been synthesized and fully characterized by elemental analysis, IR, UV-Visible spectra, ^1H, ^{13}C-NMR and Mass spectra. The results of absorption and emission titrations as well as thermal temperature and Viscosity measurement studies reveal that $[Co(bpy)_2DMHBT]^{3+}$, $[Co(dmb)_2DMHBT]^{3+}$, and $[Co(phen)_2DMHBT]^{3+}$ are moderate to strong binders of Calf Thymus DNA. An experimental result indicates that the three complexes can intercalate in to DNA base pairs. Under the identical conditions the super-coiled pBR 322 DNA photo-cleavage activities of these three cobalt(III) complexes follow the order:$[Co(phen)_2DMHBT]^{3+} > [Co(bpy)_2DMHBT]^{3+} > [Co(dmb)_2DMHBT]^{3+}$. These complexes are also found to have good antimicrobial activity.

KEYWORDS biological activity, cobalt(III) complexes, DNA-binding, intercalation, photo-cleavage, polypyridyl ligands

INTRODUCTION

The interaction of transition metal polypyridyl complexes with DNA has received a great deal of attention during the past decade.[1–3] The photochemical properties of cobalt(III) complexes continue to receive considerable attention largely owing to their potential applications in various research areas, including solar energy conversion, luminescence sensing, biotechnology, electroluminescence displays, and photochemical molecular device.[4–6] In recent years, cobalt(III) polypyridyl complexes has also been employed in studies with DNA, with views to design and develop the synthetic restriction enzymes, new drug and DNA foot-printing agents.[7–11] Many complexes have been synthesized. These complexes can bind to DNA in non covalent

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modes such as electrostatic, intercalative, and groove binding.[12,13] In this respect cobalt(III) complexes have attracted a great deal of attention due to their unique spectroscopic and electrochemical nature. Varying the substitute(s) group or substituent position in the intercalative ligand can create some interesting differences in the space configuration and the electron density distribution of transition metal complexes, which will result in some differences in spectral properties and the DNA-binding behaviors of the complexes and will be helpful to clearly understand the binding mechanism of transition metal complexes to DNA.[14–21] Despite considerable literature on metal complex-DNA interactions, the binding of these complexes to DNA and their binding geometries have remained a subset of debate. In contrast investigations on the influence of the ancillary ligands of the cobalt(III) complexes are not many. Since octahedral polypyridyl cobalt(III) complexes bind to DNA in three dimensions, the ancillary ligands also play an important role in the DNA binding and mechanism. To obtain more insight into the relation between the structure of such complexes and their DNA binding abilities we introduced small modifications in the ligand and prepared cobalt(III) complexes. Recently our group has reported[22–25] binding and photo-cleavage studies of several mixed ligand complexes of Ru(II) and Co(III). We now choose to concentrate our work on cobalt(III) complexes of polypyridyls, which have DNA binding properties and also other interesting properties. The introduction of DMHBT ligand may provide an opportunity to search the photoprobes of DNA and therapeutic reagent. In the present study, the DNA-binding behaviour of $[Co(bpy)_2DMHBT]^{3+}$, $[Co(dmb)_2DMHBT]^{3+}$, and $[Co(phen)_2DMHBT]^{3+}$ complexes are explored by absorption, emission spectroscopy and viscosity measurements, their abilities to induce cleavage of super-coiled plasmid DNA. Further, all the three cobalt(III) complexes synthesized were screened for their antimicrobial activity.

EXPERIMENTAL

Materials

$CoCl_2 \cdot 6H_2O$, 1,10-phenanthroline monohydrate and 2,2′-bipyridine were purchased from Merck (India). Calf-thymus (CT) DNA), 5,6-diamino-1,3-dimethyl uracil hydrate 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.[26] 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 absorbance at 260 and 280 nm of 1.8 to 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]

Synthesis and Characterization

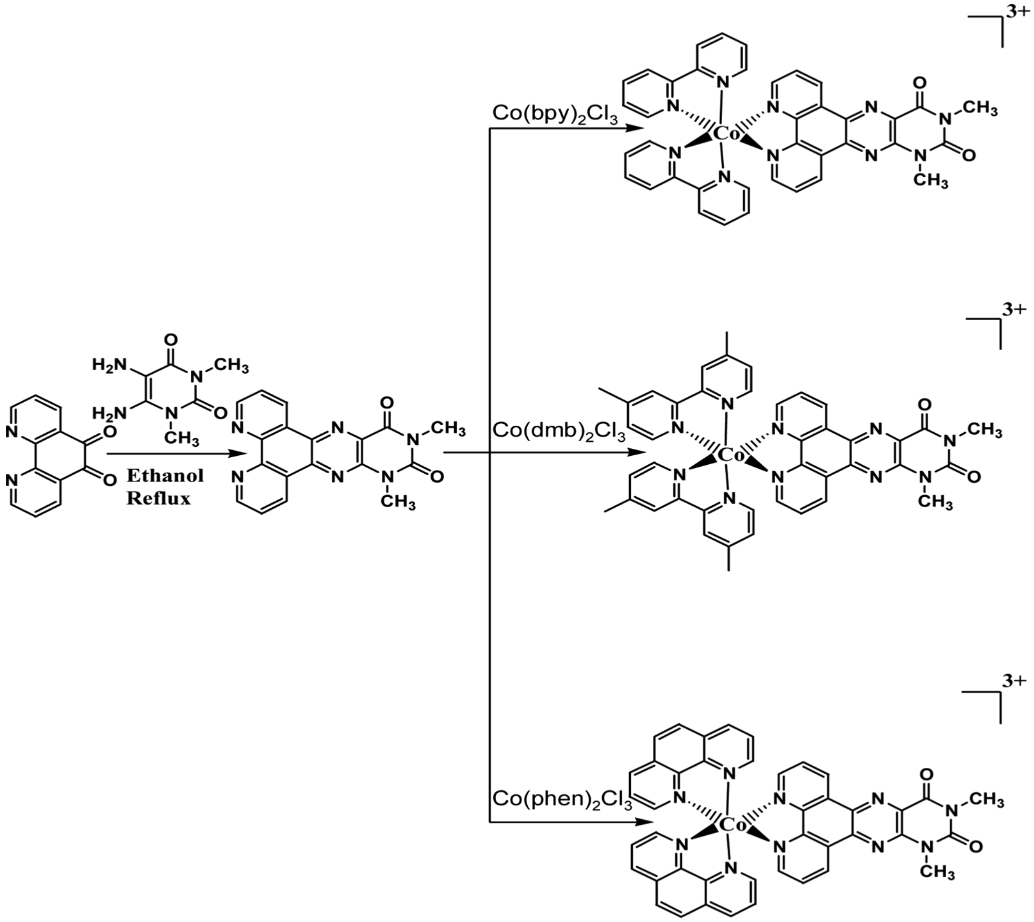
The compounds 1,10-phenanthroline-5,6-dione,[29] $[Co(bpy)_2Cl_2]Cl$, $[Co(dmb)_2Cl_2]Cl$[30] and $[Co(phen)_2Cl_2]Cl$[31] were prepared according to literature procedures. Synthetic routes of ligands and their cobalt(III) complexes are shown in Scheme 1.

A. Synthesis of Ligand – 11,13-dimethyl-13H-4,5,9,11,14-hexaaza-benzo[b]triphenylene-10,12-dione; (DMHBT)[32]

1,10-phenanthroline-5,6-dione (0.5 g, 2.38 mmol) was dissolved in 50 mL of hot ethanol. To this, ethanolic solution of 0.4 g (2.38 mmol) of 5,6-diamino-1,3-dimethyl uracil hydrate was added. This mixture was refluxed for 8 hrs at 80°C and the precipitate formed was filtered and dried with diethyl ether. It was recrystallalized from hot ethanol-acetonitrile (5:1) mixture. The purity of the product was ascertained through TLC. (yields; 68%) $C_{18}H_{12}N_6O_2$; Calcd. (%); C:62.79; H:3.49; N:24.42; Found (%): C:62.50; H:3.55; N:24.36; IR(KBr): 1662 (C=O), 1628 (C=N), 1431 (C=C), cm^{-1}; ESI-MS (DMSO) m/z; 345 (calcd. 344).

B. Synthesis of $[Co(bpy)_2(DMHBT](ClO_4)_3 \cdot 3H_2O$

To a 50 ml ethanolic solution of Cis-$[Co(bpy)_2Cl_2]Cl \cdot 3H_2O$ (0.531 g, 1.0 mmol) about 0.516 g (1.5 mmol) of DMHBT was added. The resulting



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

solution was refluxed for 1 hour and further stirred for 5 to 6 hrs 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_2 CO-Et_2O). (yields; 75%) Found (%): C:42.54, H:3.26; N:12.94; Calcd for $C_{38}H_{34}N_{10}Cl_3O_{17}Co$; C:42.71, H:3.18; N:13.11%. IR cm^{-1} (KBr): 1656 (C=O), 1621 (C=N), 1420 (C=C), 642 (Co–N(DMHBT)), 548 (Co–N(bpy)); ^{1}H-NMR (DMSO-d_6, δ-ppm): 9.9 (d,1H); 9.6 (d,1H); 9.2 (m,4H); 8.6 (d,1H); 8.4 (d,1H); 8.3 (d,2H); 8.0 (m,6H); 7.72 (d,2H); 7.32 (d,2H); 7.15 (d,2H); 2.8 (s,3H); 2.5 (s,3H); ^{13}C [^{1}H]-NMR (DMSO-d_6, δ-ppm, major peaks): 20, 126, 130.2, 148.4, 153.8, 155.

C. Synthesis of [Co(dmb)$_2$(DMHBT)] $(ClO_4)_3 \cdot 3H_2O$

This complex was obtained by a procedure similar to the one described above, Cis-[Co(dmb)$_2$ Cl$_2$]Cl $\cdot$ $3H_2O$ (0.587 g,1.0 mmol) in place of Cis-[Co(bpy)$_2$Cl$_2$]Cl $\cdot$ $3H_2O$. Yields; 65%. Found (%): C:45.03, H:3.64; N:12.59; Calcd for $C_{42}H_{42}N_{10}$ $Cl_3O_{17}Co$; C:44.86, H:3.73; N:12.46%. IR cm^{-1} (KBr): 1664 (C=O), 1622 (C=N) 1428 (C=C), 625 (Co–N (DMHBT)), 540 (Co–N (bpy)) cm^{-1}; ^{1}H-NMR (DMSO-d_6, δ-ppm): 9.9 (d,1H); 9.7 (d,1H); 9.0 (m,4H); 8.26 (d,1H); 7.7 (d,1H); 7.6 (d,2H); 7.3 (m,6H); 7.2 (d,1H); 7.1 (d,1H); 2.7 (s,12H); 2.5 (s,6H); ^{13}C [^{1}H]-NMR (DMSO-d_6, δ-ppm, major peaks): 20, 23, 128, 134, 145, 154, 158.

D. Synthesis of [Co(phen)$_2$(DMHBT)] $(ClO_4)_3 \cdot 3H_2o$

This complex was obtained by a procedure similar to the one described above, Cis-[Co(phen)$_2$-Cl$_2$]Cl $\cdot$ $3H_2O$ (0.578 g,1.0 mmol) in place of Cis-[Co(bpy)$_2$Cl$_2$]Cl $\cdot$ $3H_2O$. Yields; 65%. Found (%): C:45.25; H:2.90; N:12.66; Calcd for $C_{42}H_{34}N_{10}$ $Cl_3O_{17}Co$; C:45.18; H:3.04; N:12.55%. IR cm^{-1} (KBr): 1668 (C=O), 1607 (C=N), 1431 (C=C), 654

(Co-N(DMHBT), 574 (Co–N(phen)), cm^{-1}: ^1H-NMR (DMSO-d_6, δ-ppm): 9.8 (d,1H); 9.2 (d,1H); 8.56 (m,4H); 7.9–8.1 (m,6H); 7.8 (d,2H); 7.72 (m,6H); 7.4 (s,1H); 7.2 (s,1H); 2.59 (s,6H); ^{13}C [^1H]-NMR (DMSO-d_6, δ-ppm, major peaks): 21, 126, 130, 138, 143, 150, 155.

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. ^1H 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) was 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 $30 \pm 0.1°C$. CT-DNA samples approximately 200 base pairs in average length were prepared by sonication in order to minimize complexities arising from DNA flexibility.[33] Data are presented as $(\eta/\eta_0)^{1/3}$ versus 1/R (where R = [DNA]/complex). Relative viscosity values were calculated from the observed flow time of DNA solution (t) and corrected for the flow time of buffer alone (t_0), using the expression $\eta = t - t_o/t_0$. Flow time was measured with a digital stop watch and each sample was measured three times and an average flow time was used.[34]

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 cobalt(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 (365 nm,10 W). The samples were analysed by electrophoresis for 2.5 h at 40 V on a 1% agarose gel in Tris- acetic acid-EDTA buffer, pH 7.2. The gel was stained with 1 μg/mL ethidium bromide and photographed under UV light.

The antimicrobial tests were performed by the standard disc diffusion method.[35] The complexes were screened for their antifungal activity against fungi viz. *Aspergillus niger*. These fungal species were isolated from the infected parts of the host plants on M test agar medium. The cultures of the fungi were purified by single spore isolation technique. A concentration of 1.5 mg/mL of each cobalt(III) complex compound in DMSO solution was prepared for testing against spore germination of each fungus. Filter paper discs of 5 mm in size were prepared using Whatman filter paper no. 1 (sterilized in an autoclave) and was saturated with 10 μL of the cobalt(III) complex compounds dissolved in DMSO solution or DMSO as negative control. The fungal culture plates were inoculated and incubated at 25 ± 2°C for 48 h. The plates were then observed and the diameters of the inhibition zones (in mm) were measured and tabulated. The results were also compared with a standard antifungal drug flucanazole at the same concentration.

The antibacterial activity of the complexes was studied against *Staphylococcus aureus* (MTCC 96) and *Escherichia coli* (MTCC 443) bacteria. Each of the cobalt(III) complex compounds was dissolved in DMSO at a concentration of 1 mg/mL. Paper discs of Whatman filter paper no. 1 were cut and sterilized in an autoclave. The paper discs were saturated with 10 μL of the cobalt(III) complex compounds dissolved in DMSO solution or DMSO as negative control and were placed aseptically in the petridishes containing M test agar media inoculated with *S. aureus* and *E. coli* bacteria separately. The petridishes were incubated at 37°C and the inhibition zones were recorded after 24 h of incubation. The results were also compared with standard antibacterial drug streptomycin at the same concentration. The filter paper discs of 4 mm size were prepared (Whatman filter paper No. 42).

RESULTS AND DISCUSSION

Absorption Spectral Studies

The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques. The absorption spectra of the complexes are shown in Fig. 1. They are characterized by intense π-π^* intraligand transition in the UV region below 300 nm (278, 271, and 281 nm) and metal to ligand charge transfer (MLCT) in the lower

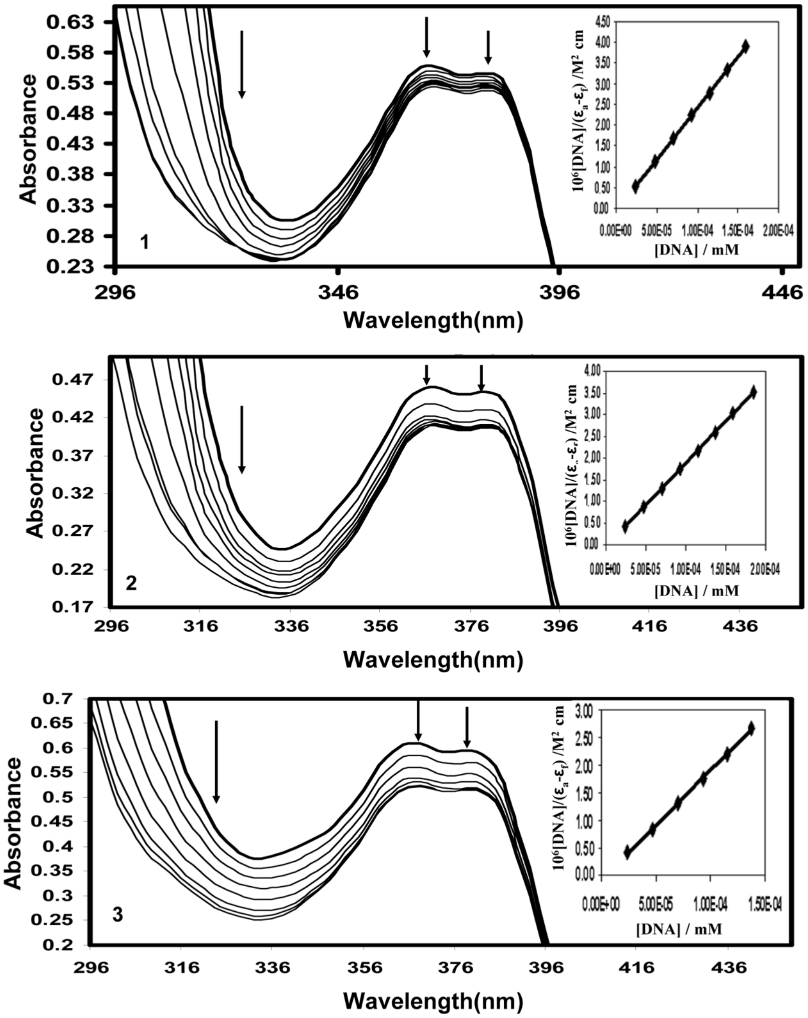


FIGURE 1 Absorption spectrum of complexes $[Co(bpy)_2(DMHBT)]^{3+}$ (1), $[Co(dmb)_2(DMHBT)]^{3+}$ (2) and $[Co(dmb)_2(DMHBT)]^{3+}$ (3) in Tris HCl buffer at 25°C in the presence of increasing amount of CT-DNA, [Co] = 10 μM, [DNA] = 0–120 μM. The arrows indicate the change in absorbance upon increasing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

energy region (368, 371, and 369 nm). The binding of mixed ligand cobalt(III) complexes to DNA helix has been studied through the changes in absorption and shift in the wavelength. Complexes binding with DNA through intercalation usually results in hypochromism and bathochromism (red shift) in the absorption spectra and the extent of spectral changes are closely related to the DNA-binding affinities of $[Co(bpy)_2DMHBT]^{3+}$, $[Co(dmb)_2DMHBT]^{3+}$, and $[Co(phen)_2DMHBT]^{3+}$ complexes. The spectral shifts for intercalation mode are usually greater than those in a groove-binding mode. In the presence of calf thymus DNA, the electronic absorption spectra for all these complexes exhibit hypochromism and bathochromism. The absorption spectra of complexes 1, 2, and 3 in the absence and presence of CT-DNA are given in Fig. 1. As the concentration increased, the MLCT transition bands of the complexes 1, 2, and 3 at 368, 371, and 369 nm exhibited hypochromism about 11.5, 9, and 13% as well as in significant bathochromism about 8, 5, and 10 nm, respectively. These results are similar to those reported earlier for various metallointercalators.[36,37] Based on the observations we assume that there are some intercalations between the complexes and the base pairs of DNA. In order to compare quantitatively the binding strength of these cobalt(III) complexes, the intrinsic binding constants, K_b of the three complexes with calf thymus DNA were obtained by monitoring the changes in absorbance at 368 nm for complex 1, at 371 nm for complex 2 and at 369 nm for complex 3 with increasing concentration of DNA using the following equation[38] through a plot of $[DNA]/(\varepsilon_a – \varepsilon_f)$ vs [DNA].

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

Where [DNA] is the concentration per nucleotide, the apparent absorption coefficient ε_a, ε_f and ε_b, correspond to A_{obs}/Co(III) ε_a extinction co-efficients for the free Co(III) complex, ε_f extinction coefficient of the complex in presence of DNA and ε_b the extinction co-efficients for the Co(III) complex in the fully bound form, respectively. In plots [DNA]/($\varepsilon_a - \varepsilon_f$) vs [DNA], K_b is given by the ratio of slope to intercept. Intrinsic binding constants, K_b of $[Co(bpy)_2DMHBT]^{3+}$ (1), $[Co(dmb)_2$- $DMHBT]^{3+}$ (2) and $[Co(phen)_2DMHBT]^{3+}$ (3) were obtained about $6.3 \pm 0.1 \times 10^5$, $4.9 \pm 0.3 \times 10^5$ and $8.0 \pm 0.1 \times 10^5 M^{-1}$, respectively. The binding constants show the following order: $[Co(phen)_2DMHBT]^{3+} > [Co(bpy)_2DMHBT]^{3+} > [Co(dmb)_2DMHBT]^{3+}$. In general a planar extension of intercalative ligand would increase the strength of the intercalation of the complexes with DNA.

The difference in binding strength of complexes 1 and 2 is probably being caused by the different ancillary ligands. The four additional methyl groups in complex 2 relative to complex 1 exert some steric hindrance. Therefore complex 1 is probably more deeply interacted and more tightly bound to adjacent DNA base pairs than complex 2. Similarly the difference 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 increases leading to a greater binding affinity for complex 3 than 1.

Fluorescence Spectroscopic Studies

The interaction of the complex with DNA was studied using fluorescence spectroscopy. Support for the above intercalative binding mode also comes from the emission measurement of the complexes. The complexes $[Co(bpy)_2DMHBT]^{3+}$, $[Co(dmb)_2DMHBT]^{3+}$ and $[Co(phen)_2DMHBT]^{3+}$ can exhibit luminescence in tris buffer (pH 7.0) at room temperature with maxima at 558, 425, and 429 nm, respectively. Binding of three complexes to DNA was found to increase the fluorescence intensity. The emission spectra of three complexes in the absence and presence of CT-DNA are shown in Fig. 2. After binding to CT-DNA, the emission intensity of these three complexes increased sharply and reaches as high as 1.67 times (complex 1), 1.50 (complex 2) and 1.76 times (complex 3) larger than respective complexes alone, which is consistent with the above absorption spectra results. This implies that $[Co(bpy)_2DMHBT]^{3+}$, $[Co(dmb)_2DMHBT]^{3+}$ and $[Co(phen)_2DMHBT]^{3+}$ interact with CT-DNA efficiently. The hydrophobic environment inside the DNA helix reduces the accessibility of water to the complex and complex mobility is restricted at the binding site, leading to decrease of vibration mode of relaxation. Steady-state emission quenching experiments using $[Fe(CN)_6]^{4-}$ as quencher is also used to observe the binding of cobalt(III) complexes with CT-DNA. The Stern–Volmer quenching constant (K_{sv}) can be determined by the using Stern–Volmer equation[39]

$$I_0/I = 1 + K_{sv}[Q] \quad (2)$$

where I_0 and I are the intensities of the fluorophore in the absence and presence of quencher respectively, Q is the concentration of the quencher, and K_{sv} is a linear Stern–Volmer quenching constant. In the quenching plot (Fig. 3) of I_0/I vs [Q], K_{sv} is given by the slope The K_{sv} value for the complexes 1, 2, and 3 is 35, 30 and 45.

The ion $[Fe(CN)_6]^{4-}$ has been shown to be able to distinguish differentially bound cobalt(III) species, 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. This may be explained by repulsion of the highly negatively charged $[Fe(CN)_6]^{4-}$ and the DNA polyanion backbone which hinders access of $[Fe(CN)_6]^{4-}$ to the DNA-bound complexes.[40] The method essentially consists of titrating a given amount of DNA-metal complexes with increasing concentration of $[Fe(CN)_6]^{4-}$ and measuring the change in fluorescence intensity. The ferro-cyanide quenching curves for these three complexes in presence and absence of CT-DNA are shown in Fig. 3. The absorption and fluorescence spectroscopy studies determine the binding of complexes. Also, from quenching studies also it is clear that DNA binding ability of complexes follow the order: 3 > 1 > 2.

Viscosity Studies

Hydrodynamic method, such as sedimentation or viscosity, which is extremely sensitive to the change of length of DNA, may be the most effective means

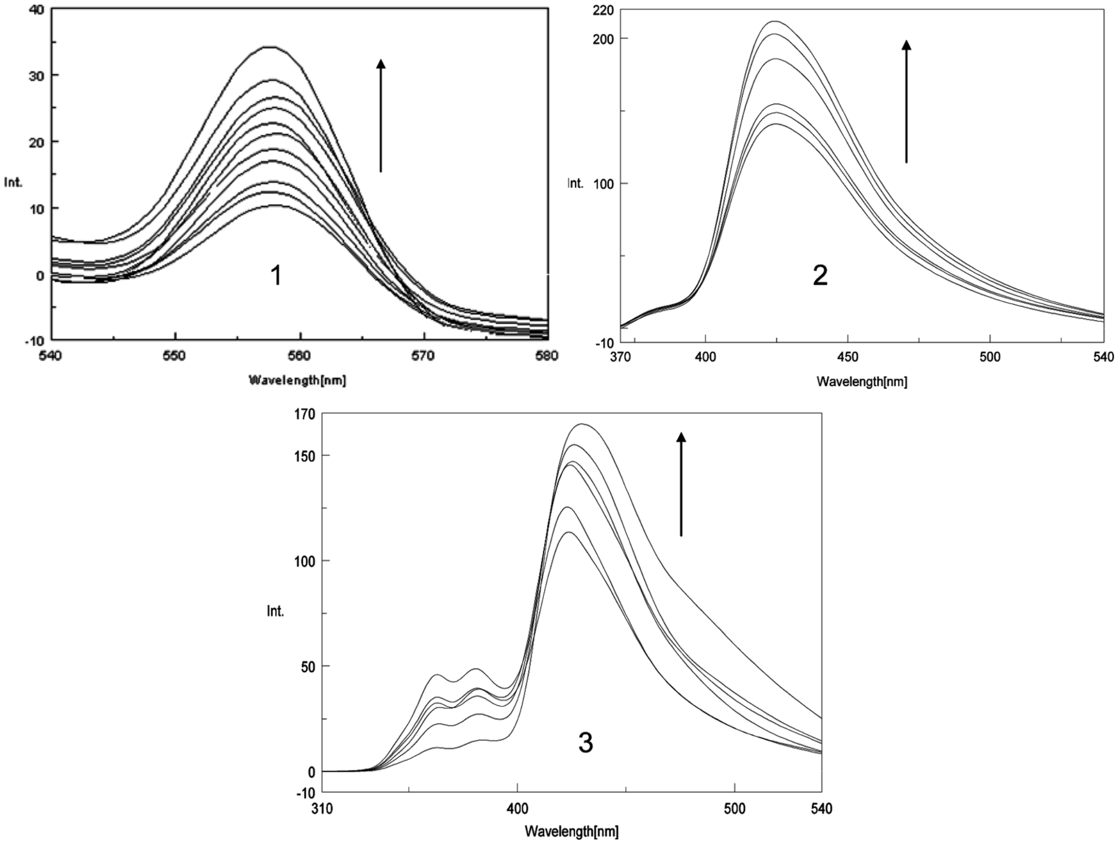


FIGURE 2 Emission spectra of complexes $[Co(bpy)_2(DMHBT)]^{3+}$ (1), $[Co(dmb)_2(DMHBT)]^{3+}$ (2) and $[Co(phen)_2(DMHBT)]^{3+}$ (3) in Tris HCl buffer at 25°C in the presence of CT-DNA, [Co] = 10 μM, [DNA] = 0–120 μM. The arrow shows the intensity change upon increasing CT-DNA concentrations. (Excitation wavelengths of complexes $[Co(bpy)_2(DMHBT)]^{3+}$, $[Co(dmb)_2(DMHBT)]^{3+}$, and $[Co(phen)_2(DMHBT)]^{3+}$ are 350, 352, and 375 nm, respectively).

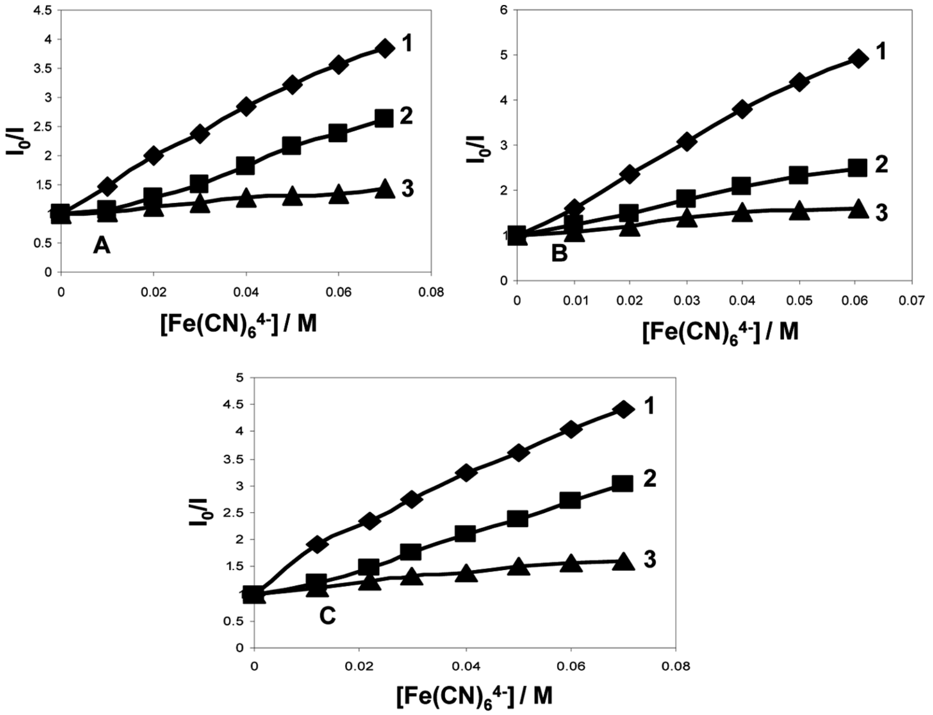


FIGURE 3 Emission quenching of complexes of $[Co(phen)_2(DMHBT)]^{3+}$ (A), $[Co(bpy)_2(DMHBT)]^{3+}$ (B), and $[Co(dmb)_2(DMHBT)]^{3+}$ (C) with $K_4[Fe(CN)_6]$ in the absence (1), presence (2) [Co] = 10 μM, and excess of DNA (3).

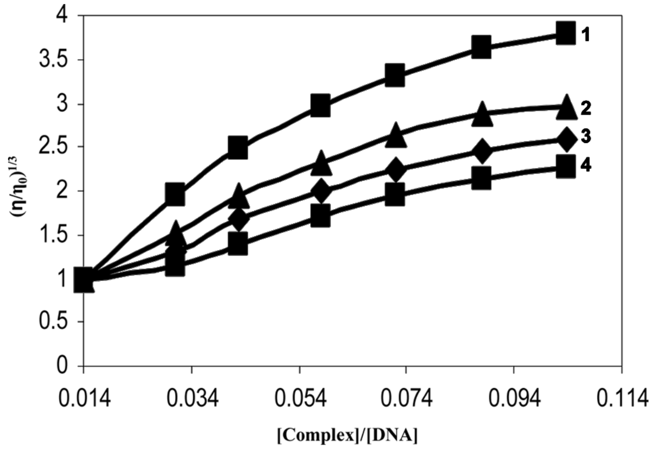


FIGURE 4 Effect of increasing amount of ethidium bromide (1) complexes of $[Co(phen)_2(DMHBT)]^{3+}$ (2), $[Co(bpy)_2(DMHBT)]^{3+}$ (3), and $[Co(dmb)_2(DMHBT)]^{3+}$ (4) on relative viscosity of CT-DNA at 30 ± 0.1°C. The total concentration of DNA is 0.25 mM, [Co] = 10 μM.

to study the binding mode of complexes to DNA in the absence of X-ray crystallography or NMR structural data.[41,42] To further confirm the interaction mode of the cobalt(III) complexes with DNA, a viscosity study was carried out (Fig. 4). The viscosity measurement is based on the flow rate of a DNA solution through a capillary viscometer. The specific viscosity contribution (η) due to the DNA in the presence of a binding agent was obtained. The results indicate that the absence and presence of metal complexes have a marked effect on the viscosity of the DNA. The viscosity studies yield a significant result for intercalation.[34,42–45] 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.[41] For example, under appropriate conditions, intercalation of dye like ethidium bromide (EtBr) causes a significant increase in the over all DNA length. On the other hand, partial and/or nonclassical intercalation of the ligand may bend or kink the DNA helix, resulting in a decrease in its effective length and concomitantly, its viscosity. The increase in viscosity of DNA indicates the intercalative binding mode of the complex.[44] The result show that all the three cobalt(III) complexes interact with CT-DNA through intercalation.

DNA Melting Studies

Since DNA melting experiments are useful in establishing the extent of intercalation,[46] 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.[47] Binding of complexes does lead to an increase in ΔTm of DNA, in the order $[Co(phen)_2\ DMHBT]^{3+} > [Co(bpy)_2DMHBT]^{3+} > [Co(dmb)_2DMHBT]^{3+}$ (Table 1).

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

There has been considerable interest in DNA nucleolytic cleavage reactions that are activated by metal ions.[48,49] The cleavage reaction on plasmid DNA can be monitored by agarose gel electrophoresis. When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoiled form (Form I). If scission occurs on one strand (nicking), the supercoil will relax to generate a slower moving open circular form (Form II).[50] If both strands are cleaved, a linear form (Form III) that migrates between Form I and Form II will be generated. Fig. 5 shows gel electrophoresis separation of pBR 322 DNA after incubation with three cobalt(III) complexes and irradiation at 365 nm. Fig. 5 shows the conversion of Form I and Form II after 60 min irradiation in the presence of varying concentrations of $[Co(bpy)_2DMHBT]^{3+}$ (1), $[Co(dmb)_2DMHBT]^{3+}$ (2) and $[Co(phen)_2DMHBT]^{3+}$ (3), Form II increases gradually, while Form I diminishes gradually. Under

TABLE 1 Results of Absorption Titration and Thermal Melting Experiment

			Absorption λ_{max} (nm)		
Complexes	T_M °C	Hypochromicity (%)	Free	Bound	$\Delta\lambda$
CT DNA alone	62	–	–	–	–
$[Co(bpy)_2DMHBT]^{3+}$	68	11.5	368	376	8
$[Co(dmb)_2DMHBT]^{3+}$	65	9	371	376	5
$[Co(phen)_2DMHBT]^{3+}$	71	13	368.5	378.5	10

FIGURE 5 Cleavage of pBR 322 DNA [10 μL of 100 μM stock] in the presence of [Co(bpy)$_2$(DMHBT)]$^{3+}$ and light after 60 min irradiation at 365 nm. DNA alone (lane 0), the concentration of [Co(bpy)$_2$(DMHBT)]$^{3+}$ was 20, 40, 60, 80 μM (lane 1–4). Cleavage of pBR 322 DNA [10 μL of 100 μM stock] in the presence of [Co(dmb)$_2$(DMHBT)]$^{3+}$ and light after 60 min irradiation at 365 nm, the concentration of [Co(dmb)$_2$(DMHBT)]$^{3+}$ was 20, 40, 60, 80 μM (lane 5–8). Cleavage of pBR 322 DNA [10 μL of 100 μM stock] in the presence of Co(phen)$_2$(DMHBT)]$^{3+}$ and light after 60 min irradiation at 365 nm, the concentration of Co(phen)$_2$(DMHBT)]$^{3+}$ was 20, 40, 60, 80 μM (lane 9–12).

the comparable experimental conditions, complexes 1 and 3 exhibit more effective DNA cleavage activity than complex 2. Further studies are currently underway to clarify the cleavage mechanism. To identify the nature of the reactive species responsible for photoactivated cleavage of plasmid DNA, we have further investigated with the potentially $^1O_2$ inhibiting agent histidine. Figure 6 shows the photocleavage of pBR-322 DNA in the presence of complex alone and complex+histidine. Indeed, plasmid DNA cleavage by complexes (A, B, and C) was inhibited in the presence of histidine, (lane 2 and 3), which indicated that $^1O_2$ acts as a competing cleavage agent. In presence of histidine Form II is not observed.

Antimicrobial Activity

The antifungal activity data (Table 2) indicate that the complexes show an appreciable activity against *Aspergillus niger* at 1.5 mg/mL concentration. DMSO control has shown a negligible activity as compared with the metal complexes. The experimental results of the compounds were compared against DMSO as the control and are expressed as inhibition zone diameter (in mm) versus control. The complexes are more effective against *Aspergillus niger* [Co(phen)$_2$DMHBT]$^{3+}$ shows the highest activity (21 mm) against *Aspergillus niger* at the concentration of 1.5 mg/mL among all the metal complexes. The same metal complex exhibited greater antifungal activity against *Aspergillus niger* as compared with the standard drug flucanazole. The [Co(bpy)$_2$ DMHBT]$^{3+}$ and [Co(dmb)$_2$DMHBT]$^{3+}$ complexes show less activity against these fungi than the standard drug, flucanazole.

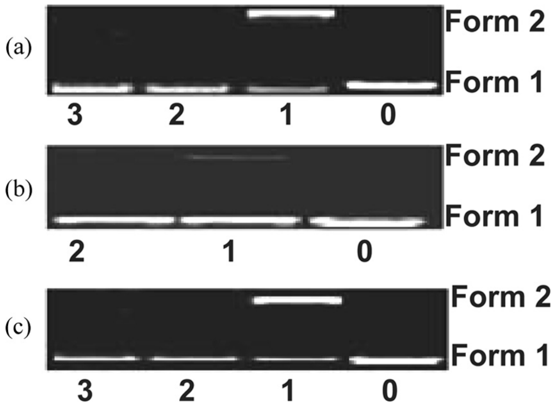


FIGURE 6 Photoactivated cleavage of pBR 322 DNA in the presence of [Co(bpy)$_2$(DMHBT)]$^{3+}$ (A), [Co(dmb)$_2$(DMHBT)]$^{3+}$ (B) and [Co(phen)$_2$(DMHBT)]$^{3+}$ (C) and after 60 min irradiation at 365 nm. The concentrations of each complex were 20 μM (lane 1), and the concentration of the histidine inhibitor was 1.0, 2.0 mM.

The antibacterial activity data (Table 2) indicate that the complexes show a high activity against both *Staphylococcus aureus* (gram-positive bacteria) and *E. coli* (gram-negative bacteria) at 1 mg/ml concentration. DMSO control has shown a negligible activity as compared with the metal complexes. The experimental results of the compounds were compared against DMSO as the control and are expressed as inhibition zone diameter (in mm) versus control. The complexes are more effective against *Staphylococcus aureus* (gram-positive bacteria) than *Escherichia coli* (gram-negative bacteria).

TABLE 2 Antimicrobial Activity of the Co(III) Complexes

Inhibition zone diameter (in mm)

	Bacterial species		Fungal species
Complex	*S. aureus*	*E. coli*	*A. niger*
[Co(bpy)$_2$DMHBT]$^{3+}$	15	13	14
[Co(dmb)$_2$DMHBT]$^{3+}$	13	11	13
[Co(phen)$_2$DMHBT]$^{3+}$	17	15	18
Fluconazole	–	–	12–15
Streptomycin	13–17	13–17	–

$[Co(phen)_2DMHBT]^{3+}$ shows the highest activity (21 mm) against *Staphylococcus aureus* (gram-positive) at the concentration of 1 mg/mL among all the metal complexes. The same complex also shows an activity of 15 mm inhibition against *Escherichia coli* (gram-negative). The same metal complex exhibited greater antibacterial activity against *Staphylococcus aureus* as compared to the standard drug streptomycin. The $[Co(bpy)_2 DMHBT]^{3+}$ and $[Co(dmb)_2DMHBT]^{3+}$ complexes show less activity against these bacteria than does the standard drug, streptomycin. It is clearly evident from our results that all the three metal complexes possess antifungal and antibacterial activity.

CONCLUSION

In summary, three Co(III) complexes of $[Co(bpy)_2(DMHBT)]^{3+}$ (1), $[Co(dmb)_2(DMHBT)]^{3+}$ (2) and $[Co(dmb)_2(DMHBT)]^{3+}$ 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 DMHBT ligand. When irradiated at 365 nm three cobalt(III) complexes are efficient photocleavers of the plasmid pBR-322 DNA. Complex 3 is found to show slightly more activity than the standard drugs against bacterial species.

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