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ORIGINAL PAPER

Synthesis, Characterization, DNA Binding and Cleavage Studies of Mixed-Ligand Cu(II) Complexes of 2,6-bis(benzimidazol-2-yl)pyridine

M. Sunita · M. Padmaja · B. Anupama · C. Gyana Kumari

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Abstract Four novel copper(II) complexes of the composition [CuLX] where L = 2,6-bis(benzimidazole-2yl)pyridine, X = dipyridophenazine (L_1), 1,10-phenanthroline (L_2), hydroxyproline (L_3) and 2,6-pyridine dicarboxylic acid (L_4) were synthesized and characterized by using elemental analysis, FT-IR, UV–vis, ESI-MS, molar conductance and magnetic susceptibility measurements. The complexes [$CuLL_1$]$(NO_3)_2$ [**1**], [$CuLL_2$]$(NO_3)_2$ [**2**], [$CuLL_3$](NO_3) [**3**] and [$CuLL_4$] (NO_3) [**4**] are stable at room temperature. In DMSO the complexes [**1**] and [**2**] are 1:2 electrolytes, [**3**] and [**4**] are 1:1 electrolytes. Based on elemental and spectral studies five coordinated geometry is assigned to all the four complexes. The interaction of four copper ion complexes with calf thymus DNA were carried out by UV–vis titrations, fluorescence spectroscopy, thermal melting and viscosity measurements .The binding constant (K_b) of the above four metal complexes were determined as 5.43×10^4 M^{-1} 2.56×10^4 M^{-1}, 1.21×10^4 M^{-1} and 1.57×10^4 M^{-1} respectively. Quenching studies of the four complexes indicates that these complexes strongly bind to DNA, out of all complex **1** is binding more strongly. Viscosity measurements indicate the binding mode of complexes with CT DNA by intercalation through groove. Thermal melting studies also support intercalative binding. The nuclease activity of the above metal complexes shows that **1**, **2** and **3** complexes cleave DNA through redox chemistry.

Keywords 2, 6-bis (benzimidazole-2yl)pyridine · Copper complexes · DNA binding · Quenching studies · DNA cleavage

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Introduction

Compounds bearing benzimidazole moiety are reported to possess a number of interesting biological activities such as antitubercular [1], anticancer [2], antihelmintic [3], antiallergic [4], antioxidant [5], antihistaminic [6]. These also exhibit antibacterial, antifungal and antiviral activities [7]. The complexes of N, N-chelating ligands with Cu (II) are of continuing interest for their biological activity, since copper ion plays an important role in many biological processes [8]. Such complexes are also of importance for their structural, spectroscopic, and redox properties [9]. Their activity in combination with drugs has been the subject of a large number of research studies [10]. Copper (II) complexes with drugs are more active in the presence of nitrogen-donor heterocyclic ligands [9, 10]. Copper (II) complexes of 1, 10-phenanthroline are capable of cleaving DNA [11] and copper complexes of nitrogen-donor heterocyclic ligands have been widely used to improve nuclease activity [11, 12]. This is due to their high nucleolytic efficiency which able to break the DNA chain in the presence of reducing agents [13].

In last few decades, heterocyclic benzimidazole, their derivatives and transition metal complexes have received considerable attention in coordination chemistry because of their well-documented biological activities. It was found that such complexes showed larger antimicrobial activities than the free ligands. Several metal complexes which bind to DNA through different modes have been used as probes for DNA structure in solution, as agents for mediation of strand scission of duplex DNA and as chemotherapeutic agents [14, 15]. The success with these compounds stimulated the search for new biologically active derivatives. In the present investigation we are reporting the synthesis and characterization of four new Cu (II) complexes having planar-tridentate ligand 2,6-bis(benzimidazol-2-yl) pyridine

(L) and their DNA binding behavior have been obtained by spectroscopic methods and viscosity measurements. A study of DNA cleavage of cu (II) complexes is also reported.

Experimental

Materials and Chemicals

2, 6-pyridine dicarboxylic acid and *o*-phenylene diamine, 1, 10-phenanthroline, hydroxy prolene, Tris–HCl, Ethidium bromide (EB) and CT DNA were purchased from Sigma-Aldrich chemicals. All other chemicals and solvents were of analytical reagent grade and used without further purification. Millipore water was used for preparing buffer.

All the experiments involving with the interaction of the ligand and complexes with CT DNA were carried out in doubly distilled water buffer containing 5 mM Tris [Tris (hydroxymethyl)-aminomethane]. The solution of CT-DNA in the buffer gave ratios of UV absorbance of about 1.8–1.9:1 at 260 and 280 nm, indicating that the CT - DNA was sufficiently free of protein. The CT-DNA concentration per nucleotide was determined spectophotometrically by employing an extinction coefficient of 6,600 M^{-1} Cm^{-1} at 260 nm [16]. The ligands and complexes were dissolved in a solvent mixture of DMSO and Tris–HCl buffer at the concentration 1.0×10^{-5} M. The stock solution was stored at 4 °C and used within 4 days.

Physical Measurements

The percentage composition of C, H, and N of complexes and ligand L were determined using micro analytical methods on Perkin Elmer 240C (USA) elemental analyzer. FT-IR spectra of the ligand and its complexes were recorded by using KBr pellets in the range 4,000–400 cm-1 using Bruker FT-IR spectrometer. The UV-Visible spectra of the ligand and its metal complexes were carried out in DMSO using Elico SL159 spectrophotometer. ^{1}H NMR spectrum of the ligand was recorded at 200 MHz and 300 MHz on Varian Gemini Unity Spectrometer using TMS as internal standard. The mass spectra of the compounds were recorded by ESI technique on VG AUTOSPEC mass spectrometer. Magnetic measurements were carried out on a Gouy balance model 7550 using $Hg[Co(SCN)_4]$ as standard. Diamagnetic corrections were carried out by pascals constant. The conductivity measurements were carried out in DMSO (10^{-3} M) using Digisun Electronic Digital conductivity meter, 0.01 M KCl solution is used for calibration. Melting points of the ligand and decomposition temperature of complexes were determined on Polmon instrument (model No.MP-96). Emission spectra were recorded on a Hitachi RF-.2500 Spectro fluorimeter at room temperature. Thermal melting temperatures were recorded using Hitachi U-2800 double beam UV–vis spectrophotometer.

DNA Binding and Cleavage Studies

Electronic Absorption Titration In order to affirm quantitatively the affinity of the compounds binding to DNA, the intrinsic binding constants K_b of the complexes **1 – 4** were obtained by the electronic absorption spectroscopic method. The complexes were dissolved in a mixture solvent of 1% DMSO and 99% Tris–HCl buffer (5 mM Tris–HCl; 50 mM NaCl, pH 7.1). Absorption titration experiments were performed in absence and presence of DNA. In these titration experiments fixed concentration of the complexes (10 μM) were titrated with increasing amounts of DNA over a range of 10 μM to 100 μM. The reference solution was the corresponding Tris–HCl buffer solution. While measuring the absorption spectra, equal amounts of DNA was added to both the compounds solution and the reference solution to eliminate the absorbance of DNA itself. Each sample solution was scanned in the range 210–400 nm. The binding constant for the interaction of complexes with DNA was obtained from absorption titration data. The binding constant of the complexes (K_b) were calculated by using the following Eq. (1)

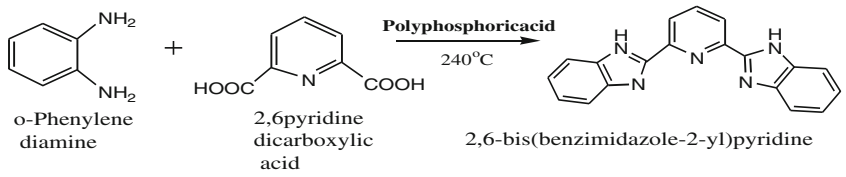
$$[\mathrm{DNA}]/(\varepsilon_A-\varepsilon_F)=[\mathrm{DNA}]/(\varepsilon_A-\varepsilon_F)+1/[K_b(\varepsilon_B-\varepsilon_F)] \quad (1)$$

Where ε_A, ε_F and ε_B correspond to A_{obs}/[compound], the extinction coefficient for free metal complex and the extinction coefficient for the metal complex in the fully bound form respectively. A plot of [DNA]/(ε_A- ε_F) Vs. [DNA], gives K_b as the ratio of the slope to the intercept.

Fluorescence Titration To have understanding of the interaction pattern of the complexes with DNA, fluorescence titration method was used. Fluorescence measurements were carried out by keeping the concentration of complexes constant (15 μM) and varied concentrations of CT DNA from 10 μM to 100 μM. An excitation wavelength of 335 nm was used.

Viscosity Measurements Viscosity measurements were carried out using an Ostwald viscometer maintained at a constant temperature at 28.0±0.1 °C in a thermostatic bath. Flow time was measured with a digital stopwatch and each sample was measured five times for which an average flow time was calculated. Data were presented as $(\eta / \eta^o)^{1/3}$ versus the ratio of the concentration of the complex and CT-DNA, where η is the viscosity of DNA in the presence of complex and η^o is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA

Scheme 1 Reaction scheme for the synthesis of ligand (L)



containing solution corrected for the flow time of buffer alone (t_o) $\eta = t - t_o$.

Thermal Denaturation Thermal studies were carried out with an Hitachi double beam spectrophotometer model U-2800, equipped with temperature-controlling programmer (0.1 °C). The absorbance at 260 nm was continuously monitored for solutions of CT-DNA (100 μM) in the absence and presence of the copper (II) complexes (10 μM). The temperature of the solution was increased by 1 °C min^{-1}.

DNA Cleavage A DMSO solution containing the metal complexes (15 μM) in a clean Eppendorf tube was treated with pUC 19 plasmid DNA (3.3 μl of 150 μg/ml) in Tris–HCl buffer (0.10 M, pH 8.0) containing NaCl (50 mM) in presence and absence of additives. The contents were incubated for 1 h at 37 °C and loaded on to a 1% agarose gel after mixing 5 μl of loading buffer (0.25% bromophenol blue). The electrophoresis was performed at a constant voltage (80 V) until the bromophenol blue had travelled through 75% of the gel. Subsequently, the gel was stained for 10 min by immersion in Ethidium bromide solution. The gel was then destained for 10 min by keeping it in sterile distilled water. The plasmid bands were visualized by viewing the gel under a transilluminator and photographed.

Synthesis of Ligands

Ligand (L) [2, 6-bis(benzimidazole-2yl)pyridine]

Pyridine 2,6 di carboxylic acid (3.35 g, 20 mM) was mixed with 20 ml 0f polyphosphoric acid and *o*-phenylene diamine (4.70 g, 44 mM) was mixed with 20 ml poly phosphoric acid separately, these two solutions were mixed together and heated at 240 °C on temperature controlled hot plate for 4 h. The colored solution was poured into 1 litre of vigorously stirred cold water. A bulky blue green precipitate formed was collected by filtration. It is stirred in hot 10% Na_2CO_3 solution (800 ml) for 1 h. The resulting solution is filtered and recrystallized from ethanol [17] (Scheme 1).

Ligand (L_1) [Dipyridophenazine]

The ligand L_1 was synthesized by refluxing 1, 10–Phenanthroline-5, 6 dione and 1, 2-diamino benzene in ethanol for 4 h. Then the resulting solution gave precipitate on cooling. Which was collected by filtration and recrystallized from chloroform [18] (Scheme 2).

Synthesis of Complexes

$[CuLL_1](NO_3)_2$

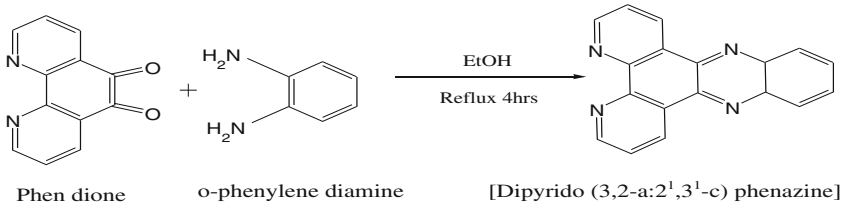
To a solution of Cu $(NO_3)_2.3H_2O$ (0.290 g, 1 mM) in MeOH (50 ml), a methanolic solution of dipyridophenazine (0.282 g, 1 mM) was added slowly, refluxed for 2 h. To this L (0.312 g, 1 mM) in methanol was added and refluxed at refluxing temperature for 3 h. The resulting green solution was slowly evaporated. After 5 days, an amorphous green precipitated was obtained; it was washed thoroughly with acetone, and then evaporated in vacuum over anhydrous $CaCl_2$. The color of the complex is brown and yield is 75%.

IR ν_{max} :3,406 cm^{-1}(ν_{N-H}), 1,527 cm^{-1} ($\nu_{C=C}$phen), 1,470 cm^{-1}($\nu_{C=N}$phen), 998 cm^{-1} ($\nu_{C=N}$Imd), 457 cm^{-1}(ν_{M-N}); Anal. Calc. for $C_{37}H_{22}N_{11}O_{6Cu}$ Cal: C 67.72, H 3.36, N 19.25; found: C 67.8, H 3.48, N 19.05; μ_{eff}: 2.84 BM; UV–vis (nm): 297, 415, 502, 642; ESI-MS (m/z): 655 $[CuLL_1]^+$

$[CuLL_2](NO_3)_2$

To a solution of $Cu(NO_3)_2.3H_2O$ (0.290 g, 1 mM) in MeOH (50 ml), a methanolic solution of 1, 10-phenanthroline (0.180 g, 1 mM) was added slowly, refluxed for 2 h. To this L (0.312 g, 1 mM) in methanol was added and refluxed at refluxing temperature for 3 h. The resulting green solution

Scheme 2 Reaction scheme for the synthesis of ligand (L_2)



was slowly evaporated. After 5 days, an amorphous green precipitated was obtained; it was washed thoroughly with acetone, then evaporated in vacuum over anhydrous $CaCl_2$. The color of the complex is green and yield is 69%.

IR ν_{max}: 3,407 cm^{-1}(ν_{N-H}), 1,518 cm^{-1} ($\nu_{C=C}$phen), 1,430 cm^{-1}($\nu_{C=N}$phen), 997 cm^{-1} ($\nu_{C=N}$Imd), 426 cm^{-1}(ν_{M-N}; Anal. Calc. for $C_{31}H_{20}N_9O_6Cu$ Cal: C 67.2, H 3.64, N 17.7; found: C 67.80, H 3.54, N 17.47; μ_{eff}: 1.73 BM; UV–vis (nm): 297, 402, 647; ESI-MS (m/z): 553 $[CuLL_2]^+$

[CuLL$_3$] (NO$_3$)

To a solution of $Cu(NO_3)_2.3H_2O$ (0.290 g, 1 mM) in MeOH (50 ml), a methanolic solution of hydroxyl prolene (0.192 g, 1 mM) was added slowly, refluxed for 2 h deprotonated by NaOH (1 mM). To this L (0.312 g, 1 mM) in methanol was added and refluxed at refluxing temperature for 3 h. The resulting green solution was slowly evaporated. After 5 days, an amorphous green precipitated was obtained, it was washed thoroughly with acetone, then evaporated in vacuum over anhydrous $CaCl_2$. The color of the complex is dark green and yield is 71%.

IR ν_{max}: 3,385 cm^{-1}(ν_{N-H}), 997 cm^{-1} ($\nu_{C=N}$Imd), 1,384 cm^{-}(νscoo$^-$), 1,628 cm^{-1} (νascoo$^-$), 426 cm^{-1}(ν_{M-N}), 536 cm^{-1}(ν_{M-O}); Anal. Calc. for $CuC_{24}H_{19}N_7O_6$ Cal: C 57.54, H 3.81, N 16.75; found: C 57.37, H 3.80, N 16.60; μ_{eff}: 1.686 BM; UV–vis (nm): 297, 392, 647; ESI-MS (m/z): 503 $[CuLL_3]^+$

[CuLL$_4$] (NO$_3$)

To a solution of $Cu(NO_3)_2.3H_2O$ (0.290 g, 1 mM) in MeOH (50 ml), a methanolic solution of 2,6-pyridine dicarboxylic acid (0.143 g, 1 mM) was added slowly, refluxed for 2 h, deprotonated by NaOH (1 mM). To this L (0.312 g, 1 mM) in methanol was added and refluxed at refluxing temperature for 3 h. The resulting green solution was slowly evaporated. After 5 days, an amorphous green precipitated was obtained; it was washed thoroughly with acetone, and then evaporated in vacuum over anhydrous $CaCl_2$. The color of the complex is green and yield is 73%.

IR (ν_{max}): 3,417 cm^{-1}(ν_{N-H}),), 996 cm^{-1} ($\nu_{C=N}$Imd), 1,384 cm^{-}(νscoo$^-$), 1,616 cm^{-1} (νas(coo$^-$), 427 cm^{-1}(ν_{M-N}), 541 cm^{-1}(ν_{M-O}); Anal. Calc. for $CuC_{26}H_{26}N_7O_7$ Cal: C 57.94, H 2.73, N 15.3; found: C 57.37, H 3.12, N 15.76; μ_{eff}: 1.72 BM; UV–vis (nm): 232, 305, 361, 724; ESI-MS (m/z): 509 $[CuLL_4]^+$

Results and Discussion

Infrared Spectra and Type of Bonding

In the IR spectrum of L, the peaks observed in the range of 3,384–3,225 cm^{-1} are assigned to ν(NH) stretching vibrations, the peak observed in the range of 3,060 cm^{-1} is assigned to ν(C-H) stretching frequency, the band at 1,573 cm^{-1} is assigned to ν(C=N) of the imidazole moiety and a peak at 1,492 cm^{-1} is assigned to ν(C=C) stretching frequencies. In the IR spectra of complexes the ν(NH) is replaced by a new absorption in the region 3,385–3,417 cm^{-1}, confirming the coordination of the metal ions through N-atom of imidazole ring. The IR spectra of the complexes **3** and **4** shows an νas(COO$^-$) and νs(COO$^-$) stretching vibrations at 1,616 cm^{-1} and 1,428 cm^{-1} respectively. The large difference between these two indicates that the carboxylate groups are coordinated to metal ion in a monodentate fashion [19]. In the far IR spectra of the complexes **1** and **2** reveals ν(M-N) stretching vibrations in the region 569–593 $cm^{-1}$and in **3** and **4** the ν(M-O) and ν(M-N) stretching vibrations 420–461 cm^{-1} and 530–592 cm^{-1} respectively, confirm the formation of metal complexes in Table 1.

Electronic Spectra and Magnetic Moments

The magnetic momentum (μ_{eff}) of the four complexes (**1**–**4**) are 1.73BM, 1.84 BM, 1.78 BM and1.72 BM respectively at room temperature, which indicate that the complexes are monomeric.

The UV–vis spectra (in DMSO) for complexes **1**, **2, 3** and **4** shows a prominent broad band at 647 nm, 642 nm, 647 nm and 742 nm respectively ascribed to the d-d transition, which are followed by a strong bands in the UV region assigned to

Table 1 Major IR data of the ligand and the four complexes1–4(cm^{-1})

Compound	ν_{N-H}	ν_{C-H}	$\nu c_{=N}$	$\nu_{C=C}$	ν_{COO-}	$\nu c = o$	ν_{M-O}	ν_{M-N}
Ligand	3225–3384	3060	1523	1435	–	–	–	–
1	3406	3062	1576	1470	–	–	–	457
2	3407	3062	1581	1468	–	–	–	462
3	3385	3069	1591	1470	1591	1628	536	458
4	3417	3068	1588	1427	1588	1616	541	427

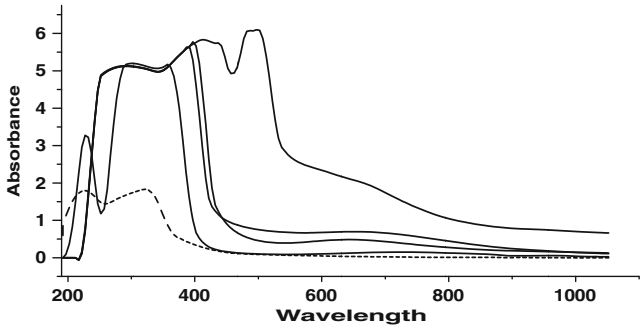


Fig. 1 UV–VIS spectra of bzimpy (—) and 1–4 complexes

ligand to metal charge transfer (LMCT) transitions these results in square pyramidal Cu (II) complexes [20] (Fig. 1).

^{1}H-NMR Spectra and Mass Spectra

In the ^{1}H-NMR spectra of the ligand (L), the peaks at 7.3–8.3 ppm are assigned to aromatic protons and 13.3 ppm are assigned to imidazole protons.

The ESI-Mass spectra of the complexes **1**, **2**, **3** and **4** give peaks at m/z: 657, 555, 504 and 510 respectively are assigned to $[M+H]^+$.

Molecular Modeling's

Molecular modeling's for the proposed structures of metal complexes were carried out by using MM2CS Chem Office version 11.0 molecular modeling program (Figs. 2 and 3).

DNA Binding Studies

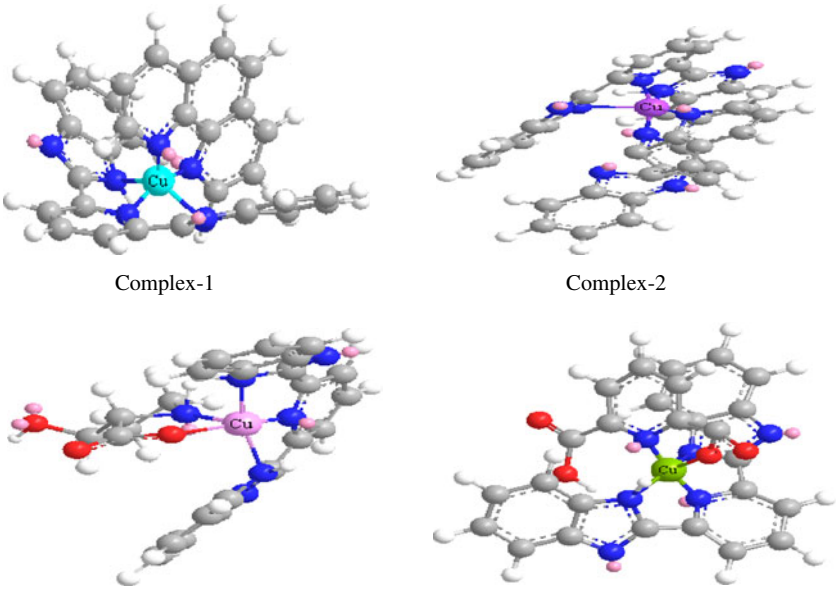
Electronic Absorption Titration

The interaction of the complexes with DNA was investigated using absorption spectra . The absorption spectra of complexes **1**, **2**, **3** and **4** in the absence and presence of CT-DNA (at a constant concentration of complexes) are given in Fig. 4. As the concentration of DNA was increased the LMCT transition bands of complexes **1**, **2**, **3** and **4** exhibited hypochromism. These spectral characteristics may suggest that there are some interactions between the complexes and CT DNA. In order to compare quantitatively, the binding strength of the four complexes, the intrinsic binding constants K_b of complexes with DNA were determined from the decay of the absorbance monitored for complexes. The intrinsic binding constant K_b of the complexes with CT-DNA was determined from the Eq. (1).

Intrinsic binding constants K_b of complexes **1**, **2**, **3** and **4** were obtained about 5.43×10^4 M,$^{-1}$ 2.56×10^4 M^{-1}, 1.211×10^4 M^{-1} and 1.57×10^4 M^{-1} from the decay of the absorbance respectively (Fig. 4). These values are comparable to those reported for$[Cr(Bzimpy)_2]^{+3}$,$[Ru(Bzimpy)_2]^{+2}$,$[Co(Bzimpy)_2]^{+2}$,$[Zn(Bzimpy)_2NO_3]NO_3$, $[Ru(Bzimpy)(bipy)(H_2O)]^{+3}$ and $[Ru(Bzimpy)(phen)(H_2O)]^{+3}$ are 1.21×10^4 M^{-1}, 1.8×10^4 M^{-1}, 1.6×10^5 M^{-1}, 3.58×10^4 $M^{-1}$and 2.87×10^4 M^{-1}. These values are lower than the classical intercalators (Ethidium bromide), whose binding constants is in the range of 10^6–10^7 M^{-1}. The binding constants of the complexes indicate that these four complexes bind to DNA.

In order to examine the binding mode of DNA with complexes, Ethidium bromide was employed in this

Fig. 2 Molecular modeling of proposed structures (MM2CS Chem Office version 11.0 molecular modeling program)



Complex-1 Complex-2

Complex-3 Complex-4

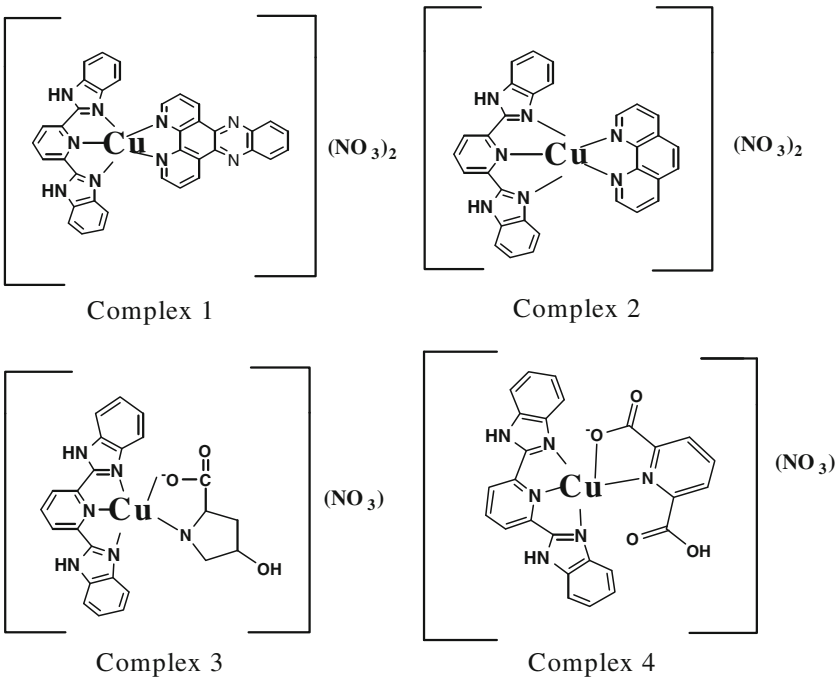


Fig. 3 Proposed geometries of four complexes (1–4)

experiment, as EB interact presumably with DNA as a typical indicator of intercalation. In the EB absorption titrations the maximal absorption of EB at 479.5 nm is decreased and shifted to 493 nm in the presence of DNA (curve-3). This was a characteristic of intercalation. The absorption titrations of the mixture solution of EB, complexes **1–4** and DNA were carried out, it was found that the absorption at 493 nm corresponding EB is increasing corresponding with EB and DNA (Fig. 5). It could result from two reasons: (1) EB bound to complexes strongly, resulting in the decrease of the amount of EB intercalated into DNA; (2) The binding mode of DNA with four complexes may be the

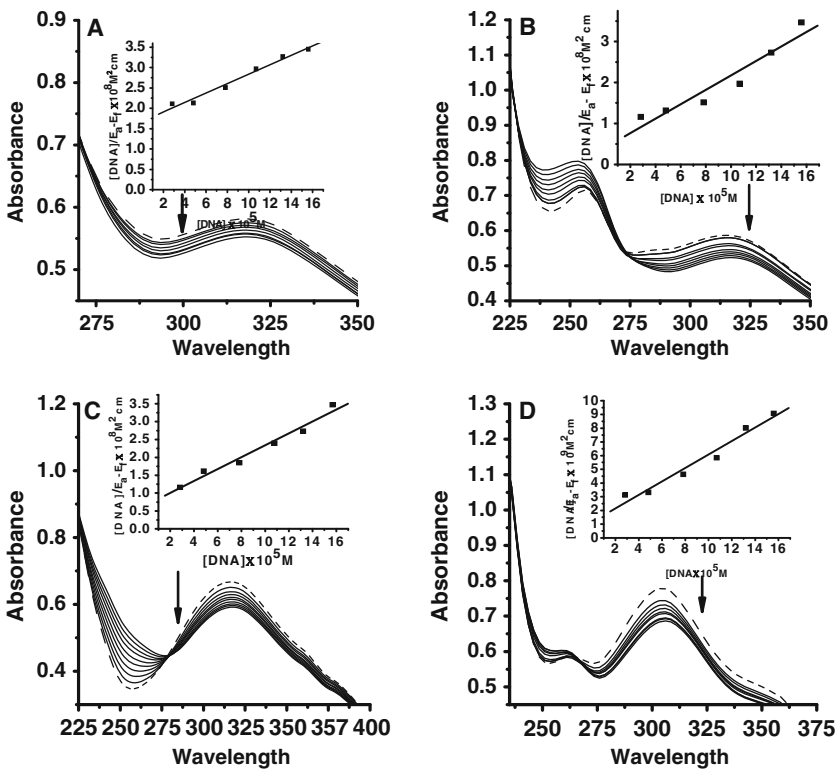


Fig. 4 Electronic spectra of four metal complexes (10 μM), (complex [1] **a**, [2] **b**, [3] **c**, [4] **d**) in the presence of increasing amounts of CT-DNA, [DNA]= 10–100 μM. ([DNA]=1.727× 10^{-4} M). *Arrow* shows the absorbance changes upon increasing DNA concentration. Inset: Linear plots for the calculation of intrinsic binding constant K_b

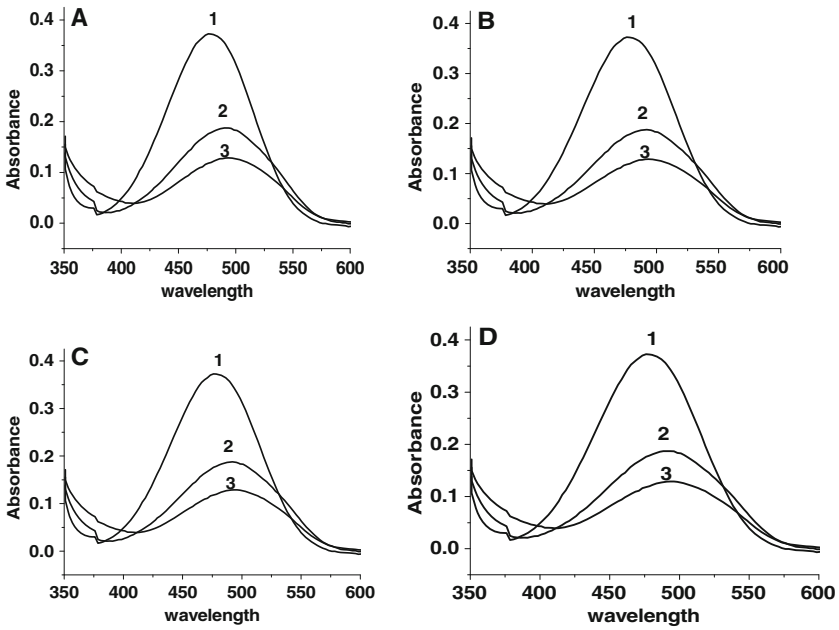


Fig. 5 The ultraviolet visible absorption spectra of 1×10^{-4} M EB (1); (1) + 2.5×10^{-4} M DNA ([DNA] =7.5×10^{-4} M). (3); (3) + 2.5×10^{-4} complex (complex [1] **a**, [2] **b**, [3] **c**, [4] **d**) in Tris–HCl buffer (5 mM Tris–HCl; 50 mM NaCl, pH 7.1) solution

minor groove binding, so there may be release of free EB from DNA-EB complex [21, 22]. However the former reason could be precluded because there was no new absorption peak appeared (Figs. 4 and 5).

Fluorescence Studies

Four Cu(II) complexes exhibit luminescence with a maxima at 424 nm (1), 433 nm (2), 438 nm (3) and 458 nm (4) in tris-buffer at ambient temperature. Addition of DNA leads to quenching of emission intensity of the complexes. On increasing the concentration of CT DNA, emission intensity of four complexes decreases (Fig. 6). The quenching of luminescence of four complexes by DNA was consistence with a photo- electron transfer from the guanine base of DNA to the excited MLCT state of metal complexes, as reported in the case of $[Co(Bzimpy)_2]$, $[Ru(Bzimpy)_2]^{+2}$, and $[Ru(TAP)_3]^{+2}$[23–25] (Fig. 6).

Quenching Studies

Ethidium bromide (EB) emits intense fluorescence light in the presence of DNA, due to strong intercalation between the adjacent DNA base pairs. It was previously reported that

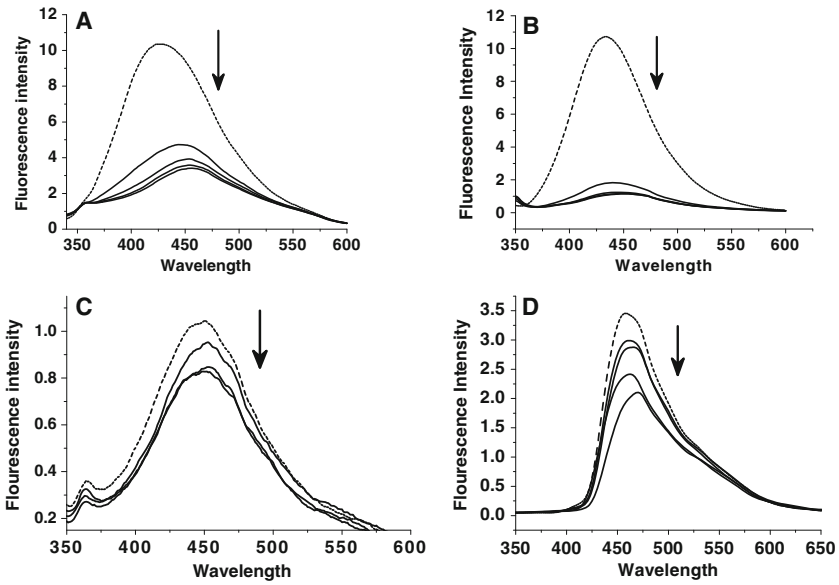


Fig. 6 Emission spectra of complexes (complex [1] **a**, [2] **b**, [3] **c**, [4] **d**) with the increasing amounts of DNA ([DNA]=1.727×10^{-4} M)

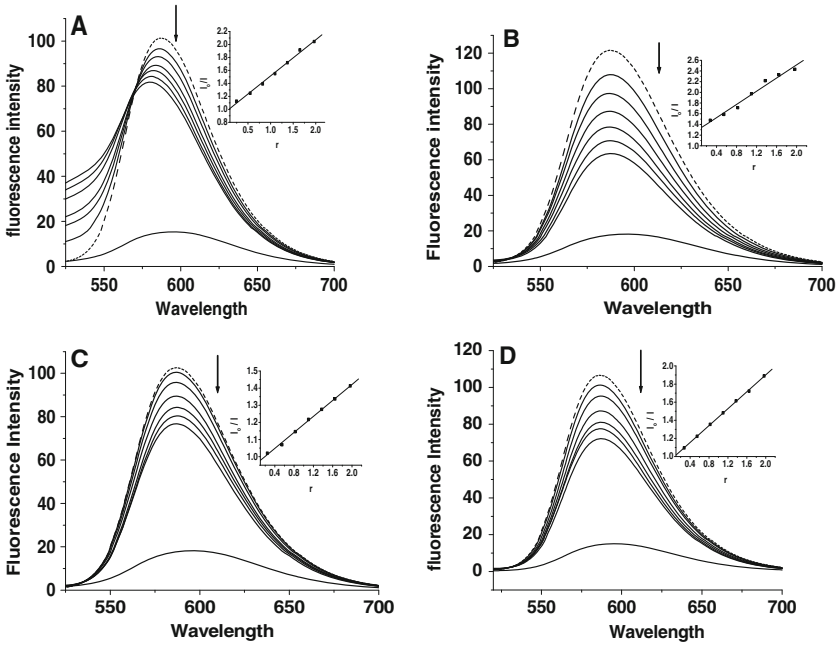


Fig. 7 Emission spectra of EB bound to DNA ([DNA]=2.33×10^{-5} M). in the absence and presence of complexes (10 μM) (complex [1] **a**, [2] **b**, [3] **c**, [4] **d**); Inset: Stern-Volmer quenching curves

the enhanced fluorescence can be quenched by the addition of a second molecule [26]. The extent of fluorescence of EB bound to DNA is used to determine the extent of binding between the second molecule and DNA.

The emission spectra of EB bound to DNA in the absence and in the presence of Cu (II) complexes are given in (Fig. 7). The addition of the complexes **1**, **2**, **3** and **4** to DNA pretreated with EB causes obvious reduction in emission intensity, indicating that the complexes compete with EB in binding to DNA.

The classical Stern—Volmer equation [27] is,

$$I_o/I = 1 + Kr$$

Where I_o and I are the fluorescence intensities in the absence and the presence of complex, respectively, K is a linear Stern-Volmer quenching constant dependent on the ration of r_{EB} (the ratio of the bound concentration of EB to the concentration of DNA) and r is the ratio of total concentration of complex to that of DNA.

The fluorescence quenching curves of EB bound to DNA by **1**, **2**, **3** and **4** are shown (Fig. 7 inset). The quenching plots illustrate that the quenching of EB bound to DNA by the complexes **1**, **2**, **3** and **4** are in good agreement with the linear Stern—Volmer equation, which proves that the four complexes bind to DNA. In the linear fit plot of I_o/I versus [complex]/[DNA], K is given by the ratio of the slope to the intercept. The K values for **1**, **2**, **3** and **4** are 0.462, 0.350, 0.175 and 0.235 respectively. Based on the K values the order of binding strength of metal complexes to CT DNA is **1 > 2 > 4 > 3** (Fig. 7).

Viscosity Measurements

For further establishment of the interactions between the complexes and DNA, viscosity measurements were carried out. Optical photo physical probes provide necessary, but not sufficient clues to support a binding model. The hydrodynamic measurements that are sensitive to length change (viscosity and sedimentation) are regarded as the least ambiguous and the most critical test of a binding in solution, in the absence of crystallographic structural data [28]. A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding ligands, which leading to the increase of DNA viscosity. In contrast a partial and/or non-classical intercalation ligands could bend (or kink), the DNA helix reducing its effective length and, concomitantly, its viscosity [29]. In the presence of complexes **1**, **2**, **3** and **4** complexes with DNA viscosity has been found to increase (Fig. 8). The increase in viscosity suggests that the complexes could bind to DNA by the intercalation binding mode (Fig. 8).

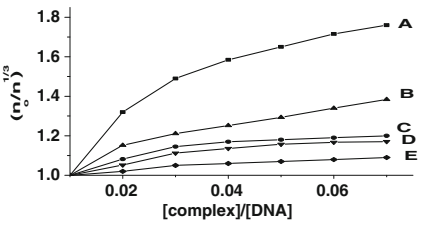


Fig. 8 Effects of increasing amount of EB (**a**) and complexes **1** (**b**), **2** (**c**), **3** (**e**) and **4** (**d**) on the relative viscosity of CT-DNA at 29 °C±0.1, [DNA]=15 μM

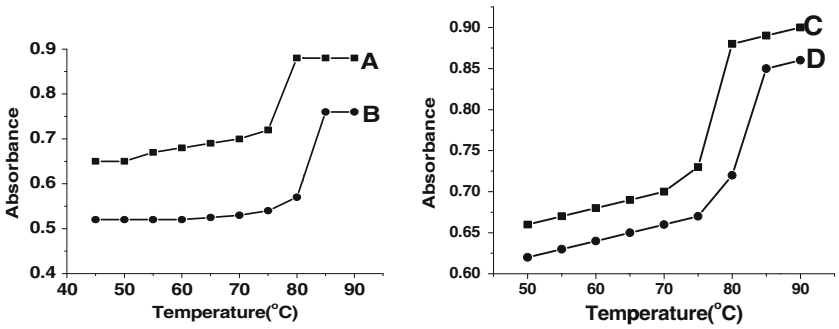


Fig. 9 Plots of absorbance versus temperature for the melting of CT DNA: **a** only DNA, **b** DNA + complex-1, **c** only DNA, **d** DNA + complex-2 ([DNA]= 1.727×10^{-4} M)

DNA Melting Studies

As intercalation of the complexes into DNA base pairs causes stabilization of base stacking thereby raising the melting temperature of the double-stranded DNA. The DNA melting experiment is useful in establishing the extent of intercalation [30]. The complexes were incubated with CT DNA and their temperature raised from 25 to 90 °C and the absorbance at 260 nm was monitored. Conductivity and pH measurements were also carried out prior and after heating the complexes to 85 °C for 1 h. The complexes show ΔTm values of 5 °*C* which is characteristic of intercalative binding mode (Fig. 9).

DNA Cleavage Studies

Gel electrophoresis experiments using pUC19 DNA were performed with complexes in the presence and absence of H_2O_2 as an oxidant. The nuclease activity was greatly enhanced by the incorporation of metal ion in the respective copolymer; it is evident from Fig. 9, which shows that the complexes **1**, **2, 3** and **4** cleave DNA more efficiently in the presence of oxidant, which may be due to the formation of hydroxyl free radicals. The production of hydroxyl free radical is due to the reaction between the metal complex and oxidant (Surendra Babu et al., 2007). These hydroxyl radicals participate in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of the sugar phosphate backbone (Surendra Babu et al., 2007). The more pronounced nuclease activity in the metal complexes in the presence of oxidant may be due to the increased production of hydroxyl radicals. The cleavage efficiency was measured by determining the ability of the complex to convert the super coiled DNA to nicked (open circular) form or sheared form. As it is evident from Fig. 9, there is a considerable increase in the intensity of bands for open circular form in the case of complexes **1**, **2, 3** and **4**. This suggests that samples have nicking activity (Fig. 10).

Conclusions

Four novel complexes of copper metal ion **1–4** were synthesized, characterized by elemental, conductance and various spectral techniques. Five coordinated geometry is assigned to the four complexes. DNA binding of the complexes was investigated by UV–vis, fluorescence, thermal melting and viscosity measurements. These studies confirm that the complexes **1** – **4** bind to CT-DNA. The binding constants of complexes **1** – **4** have been determined by absorption titrations and spectrofluorimetric method. The binding constant (K_b) of **1** – **4** were determined as 5.43×10^4 M^{-1}, 2.56×10^4 M^{-1}, 1.211×10^4 M^{-1} and 1.57×10^4 M^{-1}, respectively. Quenching studies of the four complexes indicated that these complexes strongly bind to DNA, out of all complex **1** binds more strongly. Viscosity measurements indicated the binding mode of complexes with CT DNA by intercalation through groove. Thermal melting studies also support intercalative binding. The nuclease activity of the above metal complexes shows that **1**, **2, 3** and **4** complexes cleave DNA through redox chemistry.

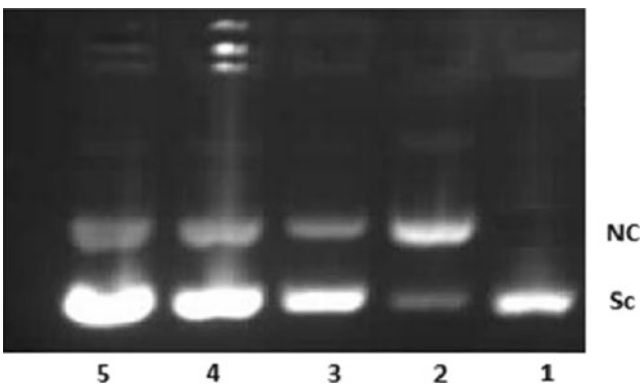


Fig. 10 Changes in the Agarose gel electrophoretic pattern of pUC19 plasmid DNA, induced by H_2O_2 and metal complexes: DNA alone (1); DNA + complex1 + H_2O_2 (2); DNA + complex2 + H_2O_2 (3); DNA + complex3 + H_2O_2 (4); DNA + complex4 + H_2O_2 (5)

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