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ORIGINAL ARTICLE

Synthesis, spectroscopic studies of new water-soluble Co(II) and Cu(II) macrocyclic complexes of 4,15-bis(2-hydroxyethyl)-2,4,6,13,15,17-hexaazatricyclodocosane: their interaction studies with calf thymus DNA and guanosine 5′ monophosphate

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Abstract New water soluble Co(II) **1**, Ni(II) **2** and Cu(II) **3** complexes of 4,15-bis(2-hydroxyethyl)-2,4,6,13,15,17-hexaazatricyclodocosane Co(II) were synthesized and characterized by various techniques, viz. elemental analysis, conductivity measurements, infrared, electronic, ESI-MS, ^{1}H and ^{13}C NMR spectroscopy. Molar conductance measurements in aqueous solution showed that complexes **1**, **2** and **3** are ionic in nature. On the basis of spectroscopic data, a square planar geometry was assigned to the complexes involving four N-atoms of the two cyclohexane moieties. Interaction studies of **1** and **3** with CT-DNA were carried using UV/Visible absorption spectroscopy, fluorescence spectrophotometry, cyclic voltammetry and viscosity measurements. Absorption spectral traces reveal 27.7 and 23.3% hyperchromism for complexes **1** and **3,** respectively indicative of strong binding to CT-DNA. These results were authenticated by fluorescence quenching experiments and viscosity measurements. The intrinsic binding constants K_b of **1** and **3** are 2.94×10^4 and 2.71×10^4 M^{-1}, respectively. Early transition metals show preference for O6 position while later ones copper and cobalt prefer N7 position of DNA base guanine. To validate this hypothesis, interaction studies of copper (II) and cobalt (II) complexes were carried out with 5′GMP, which revealed electrostatic interactions are more favored along with hydrogen bonding than coordinate covalent interaction to N7 position of guanine.

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Abbreviations

CT-DNA	Calf thymus DNA
CV	Cyclic voltammetry
Cy	Cyclohexane
EthBr	Ethidium bromide
5′GMP	5′Guanosine monophosphate
LF	Ligand field
TCNE	Tetracyanoethylene

Introduction

Metal complexes bearing macrocyclic architecture have been of great interest to many researchers in the areas of therapeutic and diagnostic medicine [1–5]. The molecular topology of the ligand framework assists these metal ions to achieve specificity for the target site DNA and enhances DNA binding affinity, which is a pre-requirement for their application as robust chemotherapeutic agent with reduced toxicity [6–8]. Many synthetic polyaza macrocyclic complexes with functional pendant arms have been synthesized and characterized owing to their unusual chemical and electronic properties [9]. Besides this, the functional groups of the arms assist in coordination by cooperative ring side-arm interaction while enhancing complex stability and improving pharmacological activity [10]. Shin-Geol Kang et al. have prepared and characterized tetraaza macrocyclic copper(II) and nickel(II) complexes bearing pendant arms [11]. Structural parameters can be varied which include metal ion, ring size and types and number of

substituents on the macrocyclic rings giving rise to diverse molecular topology. Relatively few studies have been focused on the potential of DNA-binding ability of the macrocyclic complexes. However, Kimura and colleagues [12] have described a series of macrocyclic complexes linked to intercalators and have shown that these interact with DNA in a sequence-selective manner. On the basis of structure-activity correlation, sequence-specific DNA binding properties can be invoked by varying the substituents on the macrocyclic skeleton. Macrocycles are known to exhibit anticancer activity and a few-namely bryostatin I have entered phase II human clinical trials for the treatment of melanoma, non-hodgkins lymphona and renal cancers [13]. Structural modification can also alter the DNA binding patterns, thereby affecting its anticancer activity.

The potential of metal complexes to act as therapeutic agents is already well established [14] and copper—a bioessential element which plays a key role in biological processes; its complexes are known to exhibit antitumour activity [15]. Besides this, it has also been demonstrated that copper accumulates in tumors due to selective permeability of cancer cell membranes to copper compounds [16]. Due to the efficient hydrolysis rates and kinetic inertness cobalt (III)-chelator complexes have been used for mechanistic studies of phosphodiester cleavage. This dual property of metal chelator system has been exploited to produce an efficient artificial nuclease system for sequence specific disruption of gene function [17].

We describe herein, template synthesis of the new macrocyclic complexes 4,15-bis(2-hydroxyethyl)-2,4,6,13,15,17-hexaazatricyclodocosane derived from diaminocyclohexane, formaldehyde and 2-aminoethanol in presence of Co(II), Cu(II) and Ni(II) metal ions. Complexes bearing cyclohexylamine moiety—a non-planar amine ligand are known to affect the kinetics and cytotoxicity [18]. Ni(II) complex was synthesized only for NMR studies while binding studies with calf thymus DNA and guanosine 5′ monophosphate were carried out for Co(II) and Cu(II) complexes. These complexes exhibit efficient DNA binding activity and are soluble in water, which enhances their cellular uptake, fortifies their function as DNA binders for inhibition in cancers.

Experimental

Materials

Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$), copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), Tris-base, formaldehyde, ethanolamine (E. Merck) and 1, 2-diaminocyclohexane (Fluka) were used as received. Calf thymus DNA (CT-DNA) and guanosine 5′-monophosphate disodium salt (5′GMP) were purchased from Sigma chemical Co. and Fluka, respectively. All reagent grade compounds were used without further purification.

Methods and instrumentation

Carbon, hydrogen and nitrogen contents were determined using Carlo Erba Analyzer Model 1108. Molar conductances were measured at room temperature on a Digisun Electronic conductivity Bridge. Fourier-transform IR (FTIR) spectra were recorded on an Interspec 2020 FTIR spectrometer. UV–vis spectra were recorded on a UV-1700 Pharma Spec Shimadzu spectrophotometer in H_2O and the data were reported as λ_{max}/nm. The EPR spectrum of the copper complex was acquired on a Varian E 112 spectrometer using X-band frequency (9.1 GHz) at liquid nitrogen temperature in solid state. The ^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX-300 spectrometer operating at room temperature. Electrospray mass spectra were recorded on Micromass Quattro II triple quadrupol mass spectrometer. Molecular modeling of the complex was carried out by using CS Chem Draw 3D Pro 5.0.

DNA binding experiments

All the experiments involving interaction of the complex with CT-DNA were performed in twice distilled buffer containing tris (hydroxymethyl)-aminomethane (Tris, 0.01 M) and adjusted to pH 7.5 with hydrochloric acid. Solution of CT-DNA in buffer gave a ratio of UV absorbance at 260 and 280 nm of ca. 1.9:1, indicating that DNA was sufficiently free of protein [19]. The DNA concentration per nucleotide was determined by absorption spectroscopy with the molar absorption coefficient 6,600 M^{-1} cm^{-1} at 260 nm [20]. Solution of 5′GMP was prepared in double distilled water. NMR experiments with 5′GMP were carried out in D_2O on a Bruker Avance II 400 NMR spectrometer at 25°C.

Absorption spectral experiments

Absorption spectral titration experiments were performed at constant concentration of the complexes [0.133×10^{-3}M] while varying the CT-DNA concentration. The absorbance (A) of the most shifted band of investigated complexes was recorded after successive additions of CT-DNA. A reference cell contained DNA alone to nullify the absorbance due to the DNA at the measured wavelength. From the absorption titration data, the intrinsic binding constant (K_b) of the copper (II) complex with CT-DNA was determined using the equation,

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

where ε_a, ε_f and ε_b correspond to A_{obsd}/[Complex]), the extinction coefficient for free complex, and the extinction coefficient for the complexes in the fully bound form, respectively. A plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA], where [DNA] is the concentration of DNA in the base pairs, gives K_b as the ratio of slope to the intercept [21].

Luminescence experiments

Emission intensity measurements were carried out using Hitachi F-2500 fluorescence spectrophotometer at room temperature. Luminescence titration quenching experiments were conducted by adding aliquots of 6.6×10^{-6} –33.0×10^{-6} M solutions of the metal complexes and 1.2×10^{-5} M CT-DNA in Tris–HCl buffer. The Tris–HCl buffer was used as a blank to make preliminary adjustments. The *Stern-Volmer* equation [22]

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

where I_0 and I are the emission intensities in the absence and the presence of the complex, respectively. K_{sv} is a linear *Stern-Volmer* quenching constant and r is the ratio of total concentration of complex to that of CT-DNA.

Viscosity experiments

Viscosity measurements were carried out from observed flow time of CT-DNA containing solution ($t > 100$s) corrected for the flow time of buffer alone (t_0), using Ostwald's viscometer at 29 ± 0.01 °C. Flow time was measured with a digital stopwatch. Each sample was measured three times and an average flow time was calculated. Data were presented as (η/η_0) versus binding ratio ([complex]/[DNA]), [23] where η is a viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the $\eta = t - t_0$ [24].

Cyclic voltammetric experiments

Cyclic voltammetric studies were performed on a CH Instrument Electrochemical analyzer in a single compartmental cell with 5 mM Tris–HCl/50 mM KCl buffer as supporting electrolyte. A three-electrode configuration was used comprising of a Pt wire as auxiliary electrode, platinum micro cylinder as working electrode and Ag/AgCl as the reference electrode. Electrochemical measurements were made under a dinitrogen atmosphere. The formal potentials, E^0 (or voltammetric $E_{1/2}$) were taken as the average of the anodic (E_{pa}) and cathodic peak potentials (E_{pc}) obtained from cyclic voltammetry.

Syntheses

*Synthesis of 4,15-bis(2-hydroxyethyl)-2,4,6,13,15, 17-hexaazatricyclodocosane cobalt(II) dichloride $C_{20}H_{42}N_6O_2Cl_2Co$ (**1**)*

To a stirred methanol solution (50 mL) of $CoCl_2 \cdot 6H_2O$ (5.94 g, 25 mmol) were slowly added 1,2-diaminocyclohexane (6.08 mL, 50 mmol) formaldehyde (2.8 mL, 100 mmol) and ethanolamine (3.07 mL, 50 mmol). The resulting mixture was refluxed for ca. 24 h until a dark orange colored solution appeared. The volume of the solution was reduced to 15 mL on a rotary evaporator and then kept in refrigerator overnight. The dark orange crystals were separated and washed thoroughly with hexane and then dried in vacuo. (**1**) (62%). M.p. 270 °C, Λ_M (1×10^{-3} M, H_2O): 192.00 Ω^{-1} cm^2 mol^{-1} (1:2 electrolyte). UV/VIS (25 °C, 1×10^{-3}M (ε, M^{-1} cm^{-1}), H_2O): 224, 340, 490 (115) IR (KBr): 3,233 ν (OH), 3,110 ν (N–H), 2,931 $\nu(CH_{2Cy})$, 2,380 $\nu(CH_2)$, 1,441 ν (C–N), 1,049 ν (C–O), 550 ν (Co–N). ESI-MS (m/z); 321 [$C_{12}H_{24}N_4ClCo + 3H^+$] Anal. calc. for $C_{20}H_{42}N_6O_2Cl_2Co \cdot$ C 45.43, H 8.01, N 15.90; found: C 45.39, H 8.05, N 15.88.

*Synthesis of 4,15-bis(2-hydroxyethyl)-2,4,6,13,15, 17-hexaazatricyclodocosane nickel(II) dichloride $C_{20}H_{42}N_6O_2Cl_2Ni$ (**2**)*

This complex was prepared by using $NiCl_2 \cdot 6H_2O$ (5.92 g, 25 mmol) according to the method outlined for complex **1**. Colour, Brown. (62%). (**2**) M.p.290 °C (dec). Λ_M (1×10^{-3} M, H_2O): 148.7 Ω^{-1} cm^2 mol^{-1} (1:2 electrolyte). UV/Vis (25 °C, 1×10^{-3}M (ε, M^{-1} cm^{-1}) H_2O): 240, 340, 510 (105). IR (KBr) 3,200 ν (OH), 3,117 ν (N–H), 2,870 $\nu(CH_{2Cy})$, 2,372 $\nu(CH_2)$, 1,448 ν (C–N), 1,043 ν (C–O), 534 ν (Ni–N). ^{1}H NMR (300 MHz, D_2O, 25 °C): 4.79 (O–H), 3.67 (CH_2OH), 3.36 (CH_2–N), 2.56–2.86 (CH–NH*cy*), 1.44–1.74 (CH_{2Cy}) ^{13}C NMR (75 MHz, D_2O, 25 °C): 19.00, 20.29, 23.94, 26.70, 39.13 (CH_{2Cy}): 58.58–58.85 (CH_2OH), 54.64 (N–C–C–O), 68.62 (N–C–N–C–N). ESI-MS (m/z); 231 [M–2Cl$^-$ + 2H$^+$]$^{2+}$ Anal. calc. for $C_{20}H_{42}N_6O_2Cl_2Ni \cdot$ C 45.45, H 8.01, N 15.91; found: C 45.41, H 8.04, N 15.88.

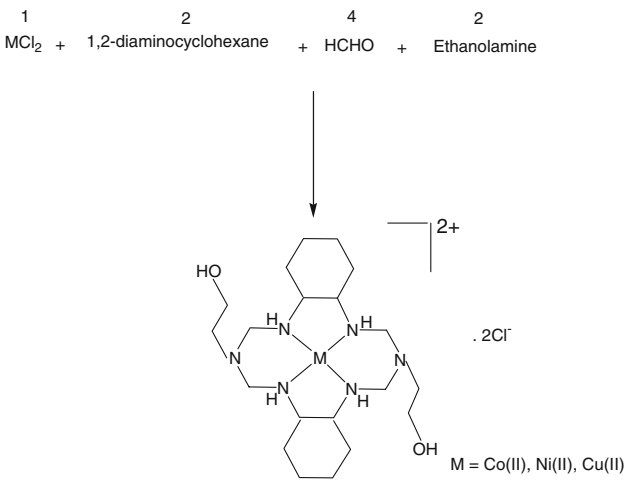
*Synthesis of 4,15-bis(2-hydroxyethyl)-2,4,6,13,15, 17-hexaazatricyclodocosane copper(II) dichloride $C_{20}H_{42}N_6O_2Cl_2Cu$ (**3**)*

This complex was prepared by adopting the procedure given for **1** and **2** by using $CuCl_2 \cdot 2H_2O$ (4.26 g, 25 mmol). Colour, dark blue. (62%) (**3**) M.p. 240 °C. Λ_M (1×10^{-3} M, H_2O):152.5 Ω^{-1} cm^2 mol^{-1} (1:2 electrolyte). UV/VIS (25 °C, 1×10^{-3} M (ε, M^{-1} cm^{-1}) H_2O,): 240, 570 (122). IR (KBr) 3,210 ν (OH), 3,130 ν (N–H),

2,898 $\nu(CH_{2Cy})$, 2,367 $\nu(CH_2)$, 1,452 ν (C–N), 1,048 ν (C–O), 542 ν (Cu–N). ESI-MS (m/z); 496 ($[M–Cl]^+$). Anal. Calc. for $C_{20}H_{42}N_6O_2Cl_2Cu \cdot$ C 45.04, H 7.94, N 15.77; found: C 45.07, H 7.91, N 15.73.

Results and discussion

The one step template reaction of 1,2-diaminocyclohexane, formaldehyde and ethanolamine in presence of metal chloride salts yielded the macrocyclic complexes of 4,15-bis(2-hydroxyethyl)-2,4,6,13,15,17-hexaazatricyclodocosane Cobalt(II), Nickel(II) and Copper(II) in which 2-hydroxyethyl groups are appended as shown in Scheme 1. These complexes are extremely stable in solid state; readily dissolve in H_2O, MeOH, EtOH, DMF and DMSO. Molar conductivity data for 10^{-3} M solutions in H_2O observed in the range $\sim$150–200 $\Omega^{-1}cm^2\ mol^{-1}$ are in accordance with those expected for 1:2 electrolytes, implying the non-participation of chloride anions in coordination to the metal ion. The stretching mode ν (O–H) in the IR spectra of all the complexes confirm to the existence of free hydroxyl groups. Thus, the potentially hexadentate ligand behaves as a tetradentate ligand with the central metal ion assuming a square planar coordination geometry as also authenticated by the UV/VIS absorption spectroscopy. The molecular model of the complex **1** as depicted in Fig. 1 indicates that there is no strain on any bond and angle. The interaction studies of complex **1** and **3** with CT-DNA and guanosine 5′monophosphate in aqueous solution were examined by various techniques while complex **2** was synthesized only for NMR studies. Molecular architecture of the macrocyclic complexes which, contain –NH– and –OH groups of appended arms with two cyclohexane rings, provides a rationale to explore their DNA binding propensity. An additional feature is their solubility in water—a highly significant physical property of pharmacological relevance, as all biochemical reactions are based on small molecules that dissolve in an aqueous phase [25].



Scheme 1 General synthetic route

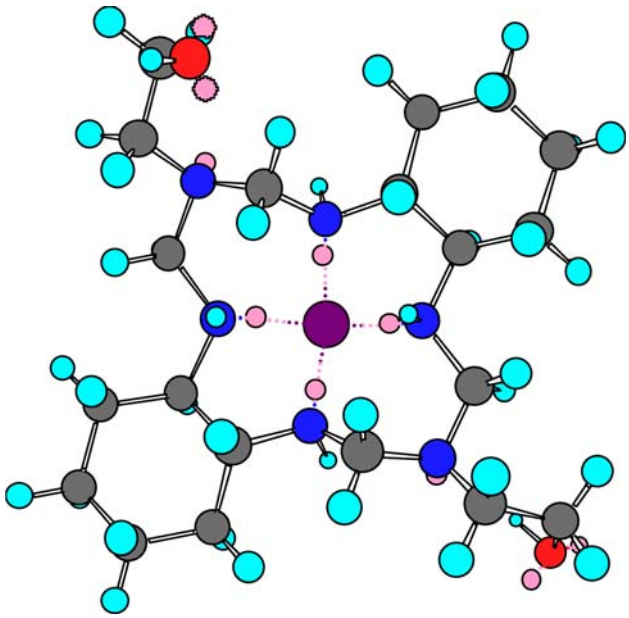


Fig. 1 Molecular model of complex **1**

IR spectra

The IR spectra of the complexes **1–3** exhibit an intense band in 3,100–3,130 cm^{-1} region due to ν(N–H) [26] of the coordinated secondary amino group, which was further confirmed by the appearance of bands in the 534–550 cm^{-1} range corresponding to ν(M–N) vibrations [27]. Absorption bands due to $\nu(CH_2)$ vibrations of the cyclohexyl ring and the ethanolamine moiety were observed at 2,860–2,931 cm^{-1} and 2,367–2,380 cm^{-1} region, respectively while a moderate peak at $\sim$1,600 cm^{-1} was assigned to δ(N–H) vibrations [28, 29]. The strong characteristic bands at 1,441–1,452 cm^{-1} and 1,043–1,049 cm^{-1} were associated with the C–N and C–O vibrations of the ethanolamine fragment. The stretching mode of the O–H group observed at ca. 3,200 cm^{-1} suggests that the oxygen atom does not interact with the metal ion [30]. Together with these observations, the absence of the free $–NH_2$ group stretching modes at $\sim$3,300–3,400 cm^{-1} confirms the condensation and the subsequent cyclization resulting in the formation of new macrocyclic complexes.

NMR spectra

The ^1H and ^{13}C–NMR spectra of the diamagnetic Ni(II) complex were obtained in D_2O solution. The ^1H–NMR

spectrum revealed an intense signal at 4.79 ppm assigned to the presence of free –OH protons of the ethanolamine pendant arm as observed for the analogous ethanolamine metal complexes [31]. Signature due to the two CH_2 groups of the ethylene chain appeared at ~3.67 ppm (–CH_2OH) and at 3.36 ppm (–CH_2N), respectively [32, 33]. The cyclohexane ring protons were observed in the 2.56–2.86 ppm and 1.44–1.74 ppm range as doublets [34].

The ^{13}C–NMR spectrum confirms the ^{1}H–NMR data. The ^{13}C–NMR spectrum features various resonances due to the cyclohexyl ring carbons at 19.00, 20.29, 23.94, 26.70, 39.13 ppm [35]. A doublet appearing at 58.58–58.85 ppm has been assigned to the CH_2OH carbons of ethanolamine moiety [36]. Additional signals at 54.64 ppm and at 68.62 ppm were observed due to N–C–C–O carbons and N–C–N–C–N carbons of the macrocyclic framework, respectively [37].

Electronic spectra

The electronic absorption spectra of the complexes **1**–**3** were characterized by strong ligand absorptions at around 224–240 nm. The visible region of the Cu(II) complex exhibited a very broad and intense band centered at 570 nm, which was assigned to $^{2}B_{1g} \rightarrow {}^{1}A_{1g}$ transition [38], a characteristic of CuN_4 chromophore with the copper ion in the square planar environment. The electronic spectrum of the Co(II) complex displays two main absorption bands; an intense band at 340 nm attributed to the charge transfer (CT) transitions; and a broad band at 490 nm ascribed to the d–d transition indicative of the square planar Co(II) ion [39]. Similarly, the Ni(II) complex exhibits an intense charge transfer band at 340 nm. The d–d band due to the Ni(II) ion of the complex **2** was not fully resolved, and seemed to be obscured under the charge transfer band, which was tailed to near IR region. However, a discernible shoulder appeared at 510 nm, which may be assigned to $^{1}A_{1g}(F) \rightarrow {}^{1}B_{1g}(G)$ transition, typical of a low spin d^8 electronic configuration of square planar Ni(II) ion [40].

EPR spectrum

The solid state X-band EPR spectrum of the Cu(II) complex was recorded at 77K (liq-N_2 temperature) using TCNE as a field marker (2.00277). The spectrum exhibits a broad $g_{\perp}$ component [41] with the splitting of the $g_{\parallel}$ component, reflecting the coupling with the Cu(II) nucleus (I = 3/2). The $g_{\parallel}$ value at 2.19 and $g_{\perp}$ at 2.05 are quite similar to the values reported for other related square planar copper (II) complexes and are typical for axially symmetrical Cu(II) complexes with the unpaired electron in the $d_{x^2-y^2}$ orbital with $^{2}B_{1g}$ ground state [42, 43]. The order $g_{\parallel} > g_{\perp} > g_e$ (2.0023) further confirm that the ground state of the Cu(II) is predominantly $d_{x^2-y^2}$.

Mass spectra

Electrospray mass spectra in the positive ion mode were recorded for the complexes **1**–**3**. The ESI-MS spectrum of the Cu(II) complex **3** shows the molecular cation peak, $[M–Cl]^+$ at m/z = 496 corresponding to a mass loss of 35.45 due to the one chloride ion from the parent macrocycle. The spectrum shows some prominent peaks corresponding to the various fragments of the complex. The molecular cation during the fragmentation process yields the species such as; ($C_{16}H_{35}N_5OClCu + 2H^+$) 411; ($C_{16}H_{32}N_4ClCu$) 378; ($C_{12}H_{24}N_4ClCu + 2H^+$) 326 and $[C_{12}H_{24}N_4Cu + 2H^+]^{2+}$ 145 from the loss of C_4H_9NO, $C_4H_{10}N_2O_2$, $C_8H_{18}N_2O_2Cl$ and $C_8H_{18}N_2O_2Cl_2$ fragments, respectively, which confirm the structure of the complex **3**. The molecular cation peaks relating to the complexes **1** and **2** were not observed. Nevertheless, many fragments were observed in each of the spectra to characterize obvious compositions of the complexes. Peaks defining $[M–2Cl^- + 2H^+]^{2+}$ and ($C_{12}H_{24}N_4ClNi + 6H^+$) fragments were recorded at m/z 231and 326, respectively for complex **2**, while for complex **1** ($C_{12}H_{24}N_4ClCo + 3H^+$) fragment was observed at m/z 321. Other peaks at m/z 143 were ascribed to $[C_{12}H_{24}N_4Co + 2H^+]^{2+}$ and $[C_{12}H_{24}N_4Ni + 2H^+]^{2+}$ fragments for complex **1** and **2** respectively.

Solution stability studies

To confirm the stability of complexes **1** and **3** in buffered solution at various pH values, UV/VIS and cyclic voltammetric studies were performed under conditions similar to those used for DNA binding studies. UV/VIS spectra of complex **1** and **3** exhibited no change in the position of the intraligand band over a pH range 2–12 with only minor change in intensity. The complexes also display similar spectra over a period of 24 h and no precipitation was observed (Fig. 2a and b) The non-precipitation after a 24 h time period and over a broad pH range suggests the robust nature of these complexes in solution. The solution stability of complex **1** and **3** were further studied by means of cyclic voltammetery in the pH range 2–10. The voltammetric behaviour of the complexes was found to be reproducible and they exhibited quasireversibility [44] typical for one electron transfer process corresponding to M(II)/M(I) redox couple (Fig. 3a and b).

DNA binding studies

DNA is the primary intracellular target of antitumor drugs. Interaction of the complexes with DNA can induce DNA

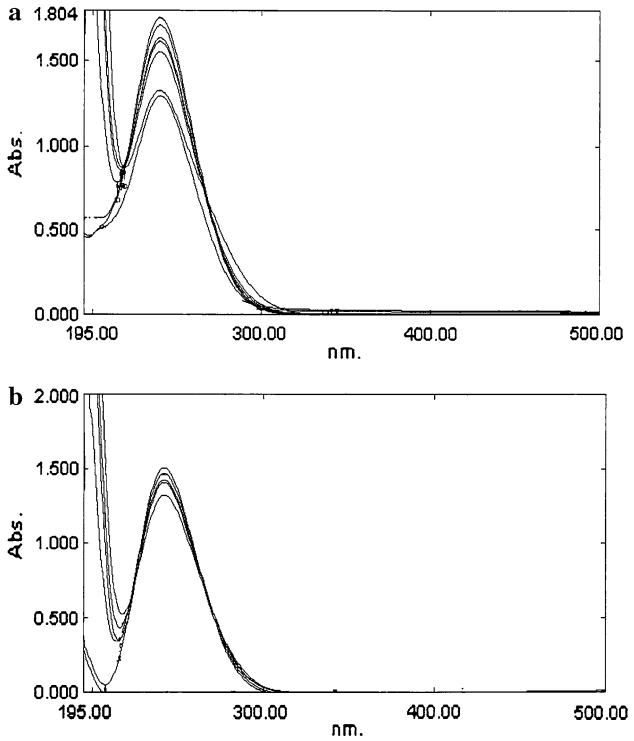


Fig. 2 (**a**) UV/VIS Absorption spectra of the Cu (II) complex **3** as a function of pH. Conditions: Tris–HCl buffer, 25°, The curves from top to bottom correspond to pH 12, 10, 8, 6, 4, 2, respectively. (**b**) UV/VIS Absorption spectra of the Cu (II) complex **3** after 24 h as a function of pH. Conditions: Tris–HCl buffer, 25°, The curves from top to bottom correspond to pH 12, 10, 8, 6, 4, 2, respectively

damage, which leads to blockage of cell division and eventually cell death. Interaction with 5′GMP provides supportive evidence in favor of the DNA binding studies.

Absorption titration

The absorption spectral titration of the complexes with CT-DNA was followed by monitoring the absorbance of intraligand bands (Fig. 4a and b). Any interaction between the complex and the DNA is expected to perturb the ligand centered spectral transitions of the complexes. Intensity of the spectral band of the complexes at 224 and 240 nm were found to increase with the increasing concentration of the DNA. The complexes **1** and **3** exhibited hyperchromism of 27.7 and 23.3% when saturated around [DNA]/[complex] = 0.75 and 0.58, respectively. The hyperchromic and hypochromic effect are the spectral features of DNA concerning its double helix structure. The "hyperchromic effect" which results from the structural damage of DNA observed in these complexes is indicative of strong binding of the complexes to CT-DNA. However, no red shift was observed in the absorption traces, which ruled out coordinate covalent binding with N7 base moieties of DNA. Complex-interaction occurs with exterior phosphates of DNA primarily via electrostatic attraction [45]. Nevertheless, DNA double helix possesses many hydrogen bonding sites positioned on the edges of the DNA bases, it is quite probable that the coordinated –NH– groups and the dangling –OH groups could form hydrogen bonds with the DNA base pairs [46], contributing to the overall hyperchromism. No significant change was observed in the absorbance of LF transition of the complexes on the addition of CT-DNA revealing that the binding of metal complexes via N7 of guanine is quite unlikely. This was evidenced by the UV/VIS absorption spectral studies of the complexes with 5′GMP. The present ligand framework lacks extended π-systems; the intercalative binding mode is therefore, overruled.

In order to further compare quantitatively the affinity of complexes bound to CT-DNA the intrinsic binding constants K_b of the complexes were also determined (Fig. 5a and b). The binding constants obtained for **1** and **3** using Eq. (1) are $2.94 \times 10^4\ M^{-1}$ and $2.71 \times 10^4\ M^{-1}$, respectively, revealing that both the complexes bind strongly to CT-DNA with almost same affinity.

Interaction studies with 5′GMP

Interaction of metal complexes with nucleotides is notably important to illustrate the binding sequence with DNA at the molecular level. Previously, it has been observed that interaction of 5′GMP with Cu^{2+} ion occurs mainly via N7 site [47] while O6 position is favoured in other early transition metal ions [48]. The absorption spectra of complexes **1** and **3** on interaction with 5′GMP show hyperchromism in the intraligand bands, with a red shift (bathochromic) of 5 nm for the complex **3** (Fig. 6a and b). However, no distinct spectral changes were observed in the LF bands, obscuring the possibility of coordination of the metal ions either to N7 or O6 of GMP. The hyperchromicity implies that the interaction between the complexes and the GMP is a combination of electrostatic and hydrogen bonding with the oxygen of the negatively charged phosphate group to the –NH– of the complex [49] or possibly via O6 atom of nucleobase with pendant –OH of the complex. The functionalized –OH appendage facilitates in carrying the metal ion to the site in the major groove.

^{1}H and ^{31}P NMR spectroscopic studies with 5′GMP

The conclusive evidence for the interaction of complexes **1** and **3** with 5′GMP was further obtained by ^{1}H NMR and

^{31}P NMR spectroscopy, being most sensitive and a reliable technique (Fig. 7a and b). The ^{1}H NMR of 5′GMP in D_2O solvent records the proton resonance of guanine H_8 at 8.07 ppm and ribose H_1'–H_5' at 3.8–5.8 ppm, respectively. The resonance of 2-NH_2 was obscured due to the exchange of protons with deuterium solvent. For paramagnetic complexes, the chemical shift of protons adjacent to the metal centre will be significantly perturbed and there is an apparent line broadening. On interaction of Co(II) complex **1** with 5′GMP, there is no line broadening however, H_2' resonance of ribose records an increase in intensity while the other peaks of ribose show downfield shift. The G-H_8 signal has diminished but does not display significant downfield shift (8.07 ppm in free 5′GMP to 8.1 ppm in **1** + 5′GMP bound), which shows non-involvement of N7 position of guanine in coordination [50]. These results are also consistent with UV/VIS interaction studies of complex **1** with DNA and 5′GMP. ^{31}P NMR of 5′GMP records a signal at 3.65 ppm, which is completely quenched on addition of complex **1** sugestive of involvement of phosphate moiety via electrostatic binding mode.

Compared with the ^{1}H NMR spectra of 5′GMP and **1** + 5′GMP, the ^{1}H NMR spectra of complex **3** + 5′GMP displayed remarkable changes. The H_8 signal due to 5′GMP at 8.07 ppm disappears, while peaks due to H_1'–H_5' showed significant line broadening with downfield shifts. These observations depict that the complex **3** shows tendency for coordination to the N7 position of the guanine compared to complex **1**. However, phosphate binding of complex **3** to 5′GMP cannot be overruled owing to the absence of any signal in the ^{31}P NMR of **3** + 5′GMP. These NMR results strongly support the

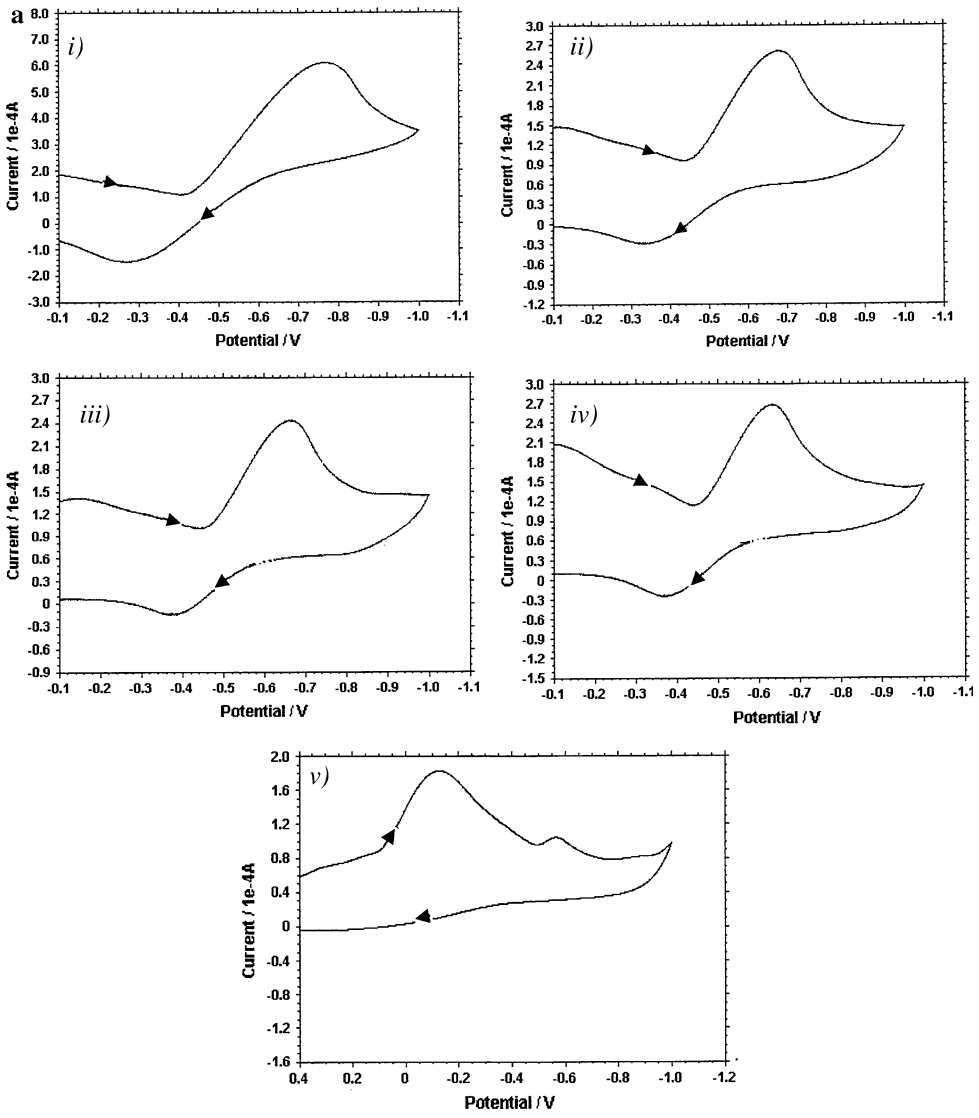


Fig. 3 Cyclic voltammetric data for (**a**) complex **1** (**b**) complex **3** at pH (i) 2, (ii) 4, (iii) 6, (iv) 8, (v) 10. Conditions: 5 mM Tris–HCl/50 mM KCl buffer, 25°, scan rate 0.1 VS^{-1}

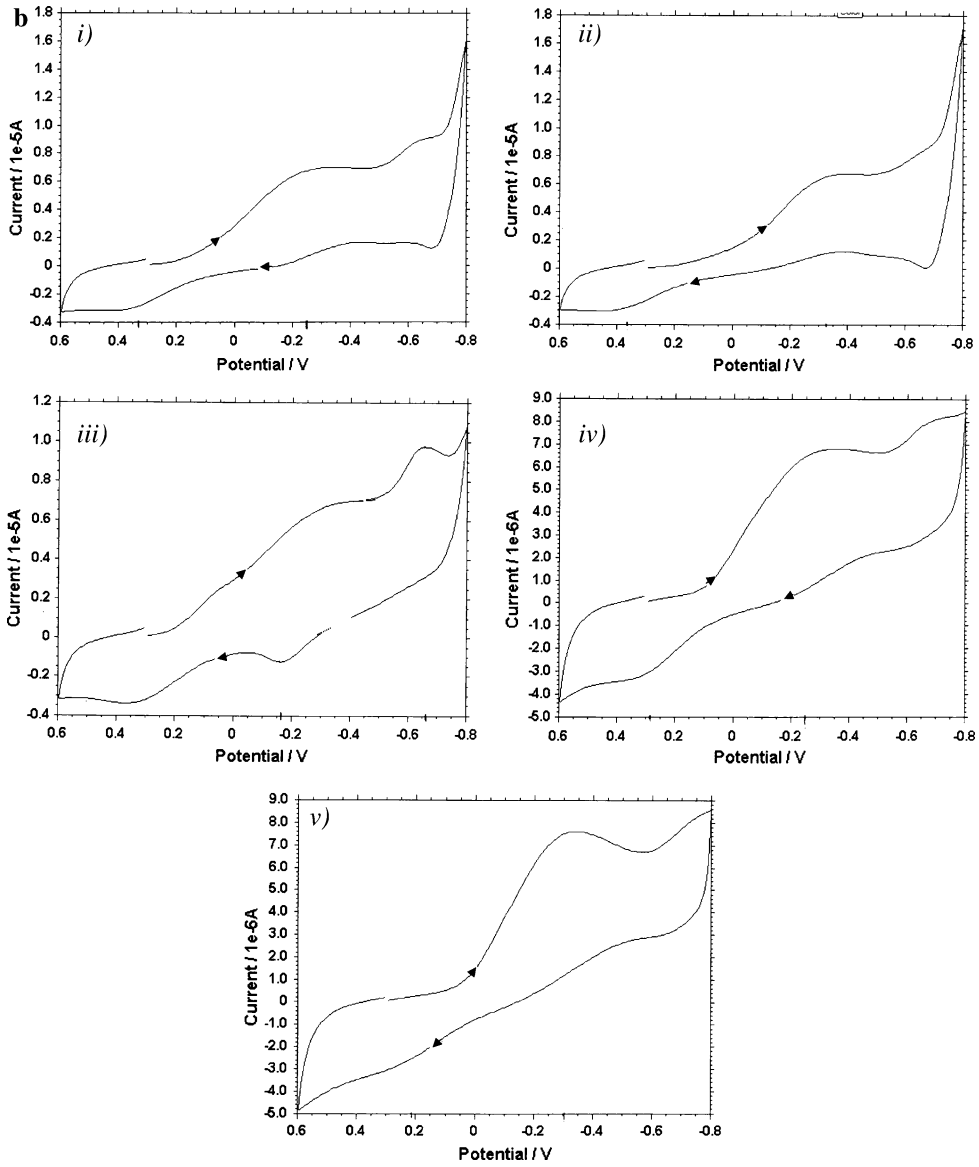


Fig. 3 continued

phosphate binding of complexes **1** and **3** with 5′GMP, and simultaneous coordination of complex **3** to the N7 atom of nucleobase.

Fluorescence spectroscopic studies

To exclude the possibility of the intercalative binding mode an ethidium bromide assay was carried out. Ethidium bromide is a conjugate planar intercalating molecule emitting intense fluorescence when bound to DNA [51]. Decrease in emission intensity results when a second DNA binding molecule either replaces EthBr or accepts the excited state electron from EthBr [52]. Complexes **1** and **3** do not display luminescence either alone or in tris buffer. The addition of complex **1** and **3** to the EthBr-DNA system resulted in the reduction in the emission intensity (Fig. 8a and b). As complexes **1** and **3** bind to DNA primarily via surface binding, they cannot displace the strongly DNA bound EthBr. So the observed quenching occurs through the photoelectron transfer mechanism [53]. The larger quenching extent of complex **3** than the complex **1** is expected, as the reduction of the DNA-bound complex **1** ($E_{1/2} = -0.285$ V) is more difficult than the complex **3** ($E_{1/2} = -0.180$ V). The relative fluorescence intensity, plotted as a function of CT-DNA concentration (in terms of [M]/[DNA]) (Fig. 9a and b) is in good agreement with the linear Stern-Volmer equation (2), which also proves that the complexes bind to DNA. The value of the fluorescence quenching constant K_{sv} obtained as the slope of I_0/I vs r

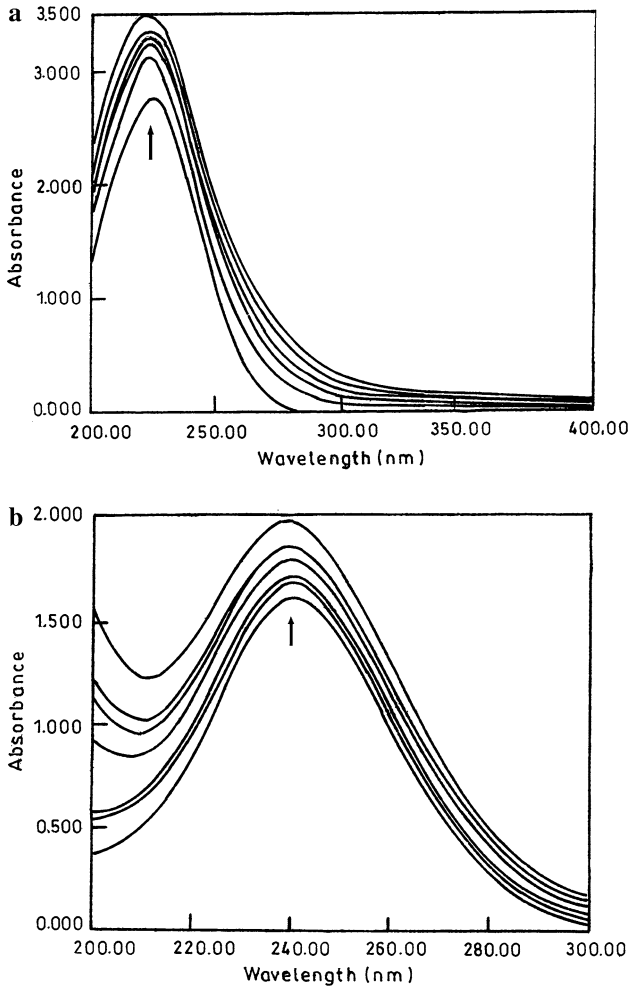


Fig. 4 Absorption spectral traces of (**a**) Complex **1** (**b**) Complex **3** in Tris–HCl buffer upon addition of CT-DNA. Arrows show the absorbance changes upon increasing concentration of the CT-DNA

(=[complex]/[DNA]) for complexes **1** and **3** are found to be 0.3 and 0.5, respectively.

Viscosity measurements

To obtain further support for the binding modes of the complexes with DNA, viscosity measurements were carried out as hydrodynamic measurements sensitive to length changes are regarded as the most critical tests of a binding model in solution in the absence of crystallographic structural data [54]. For DNA binding of a complex, a partial or a non-classical mode of binding could bend or kink the DNA helix, reduce its effective length and concomitantly its viscosity [55]. The effect of the complexes **1** and **3** on the viscosity of the CT-DNA is shown in Fig. 10. The relative specific viscosity decreases steadily, which imply complexes bind to CT-DNA via electrostatic mode

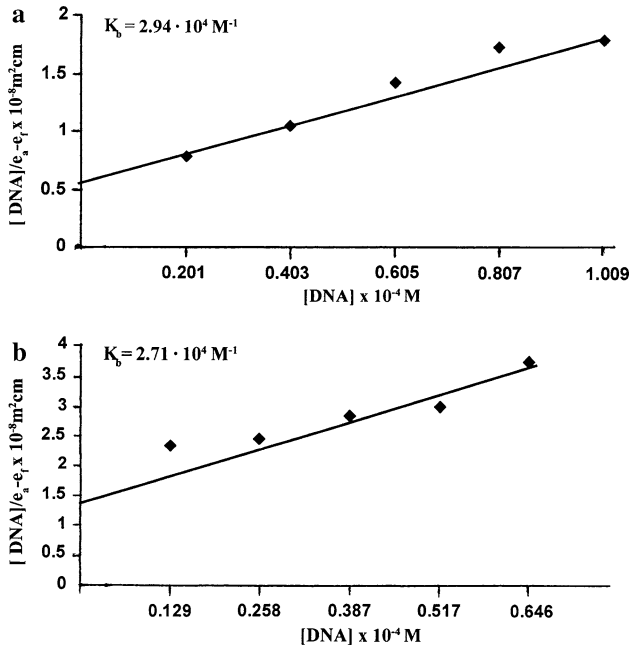


Fig. 5 Plots of $[DNA]/\varepsilon_a - \varepsilon_f$ vs [DNA] for the titration of CT-DNA with (**a**) complexes **1** (**b**) complex **3** experimental data points; full lines, linear fitting of the data. [complex] = 1.33×10^{-4} M

[56]. Moreover, the decreasing pattern of DNA viscosity occurs probably due to the insertion of the flexible –OH pendant arm into the DNA helix permitting the hydrogen bond interaction with the DNA base pairs [57]. These results are consistent with observed hyperchromic effect of **1** and **3** bound to CT-DNA.

Cyclic voltammetry

The cyclic voltammograms of complexes **1** and **3** were studied in aqueous solution containing 5 mM Tris–HCl/50 mM KCl buffer obtained at 0.1 VS^{-1} scan rate in the absence and presence of CT-DNA. Both the complexes display quasi-reversible electrochemical waves for M(II)/M(I) couple. The CV response of the complex **1** in the absence of DNA (Fig. 11a) shows reduction of Co(II) to Co(I) at a cathodic peak potential of −0.400 V and the corresponding oxidation wave at −0.110 V. The separation of anodic and cathodic peak potentials (ΔE_p) is −0.01 V and the ratio of anodic to cathodic peak currents I_{pa}/I_{pc} is 0.133, indicating a quasi-reversible redox process. The formal potential $E_{1/2}$ estimated, as the average of E_{pa} and E_{pc} is −0.405 V. Complex **3** exhibited one quasi-reversible redox couple corresponding to the Cu(II)/Cu(I) redox state with $I_{pa}/I_{pc} = 0.322$ and the E_{pc} and E_{pa} values of −0.440 and 0.020 V respectively (Fig. 11b) For this couple, ΔE_p and the formal potential $E_{1/2}$ are 0.460 and −0.210 V, respectively.

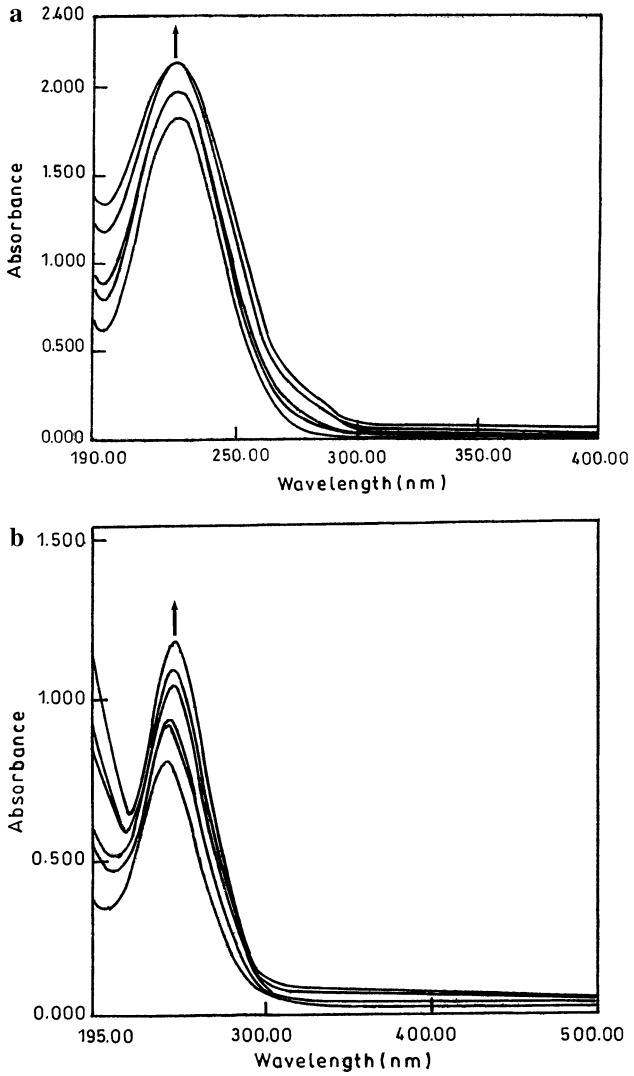


Fig. 6 Absorption spectral traces of (**a**) Complex **1** and (**b**) complex **3** in H_2O upon addition of 5′GMP

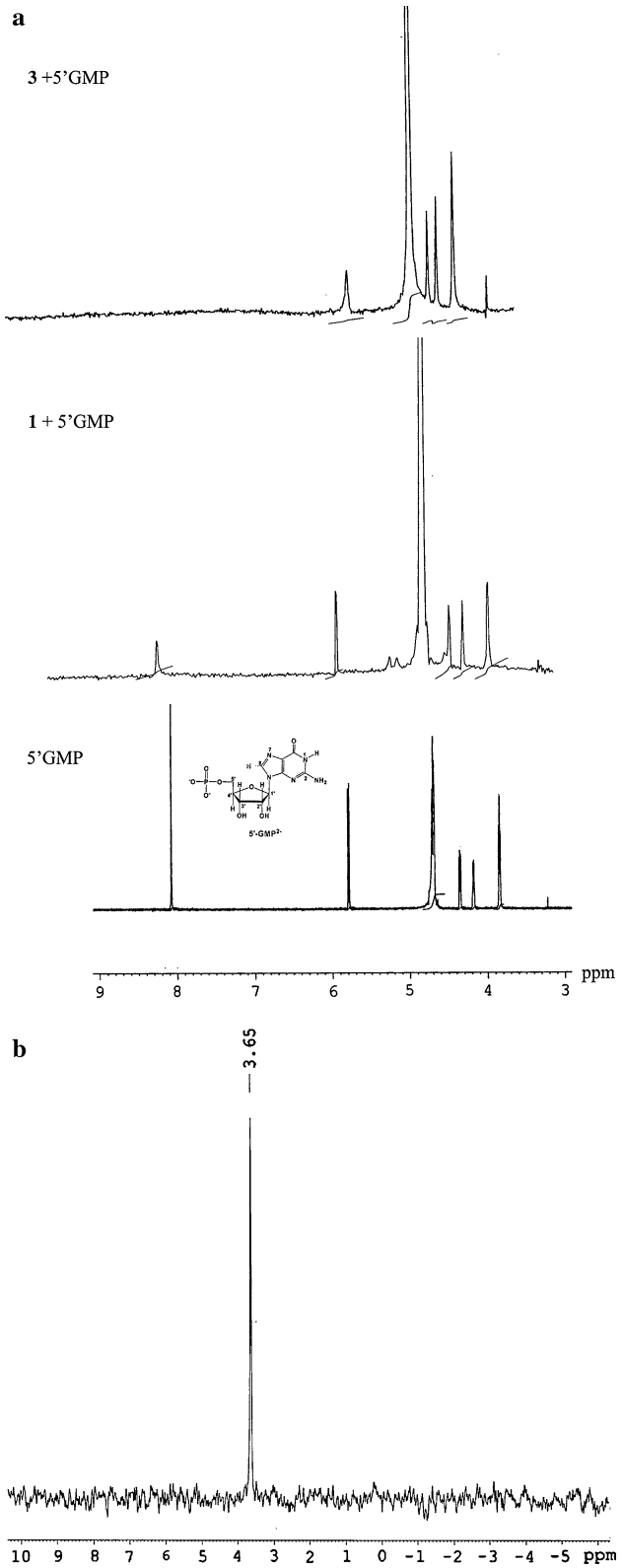


Fig. 7 (**a**) ^{1}H NMR spectra of 5′GMP and the reaction of complex **1** and **3** (2.5 mM) with 5′GMP (5 mM) in D_2O at 25° (**b**) ^{31}P NMR spectrum of 5′GMP in D_2O at 25°

Keeping all the parameters constant, quasi-reversibility of the electron transfer was maintained in the presence of DNA. Addition of DNA to complex **1** and **3** resulted in reduction in anodic and cathodic peak currents coupled with shifts in E_{pc}, E_{pa} and $E_{1/2}$ values. However, for complex **1** the anodic peak appeared weakly. The apparent reduction in the peak currents of **1** and **3** was attributed to the diffusion of the metal complexes bound to the large slowly diffusing DNA molecule [58]. The shift in the $E_{1/2}$ values indicates the binding of the complexes **1** (−0.285 V) and **3** (−0.180 V) to the DNA surface. These electrochemical results are in agreement with the above spectral results supporting that the complexes bind to DNA surface involving electrostatic interaction.

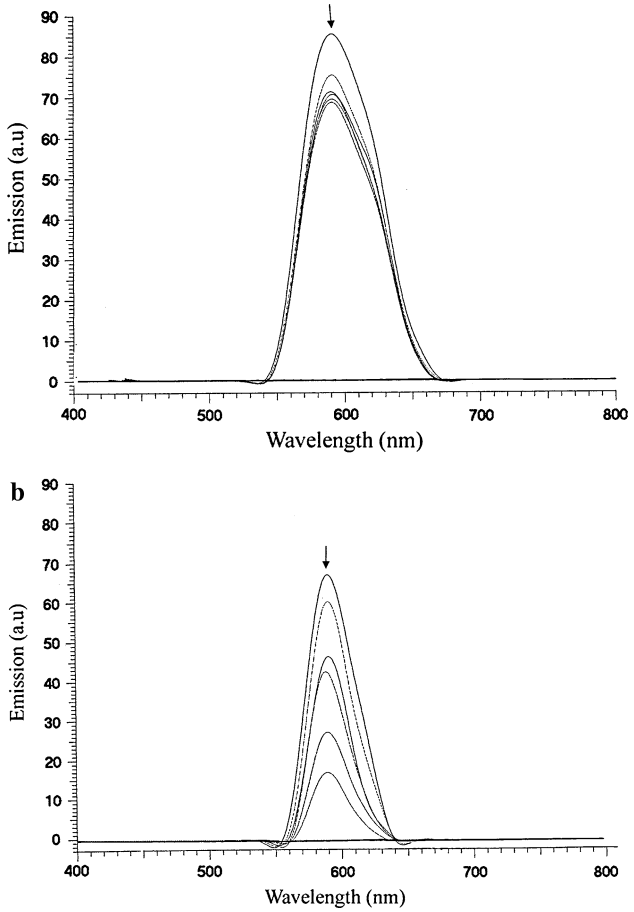


Fig. 8 Emission spectra of EB bound to DNA in the presence of (**a**) complex **1** (**b**) complex **3** in Tris–HCl buffer. Arrows show the intensity changes upon increasing concentration of the complexes

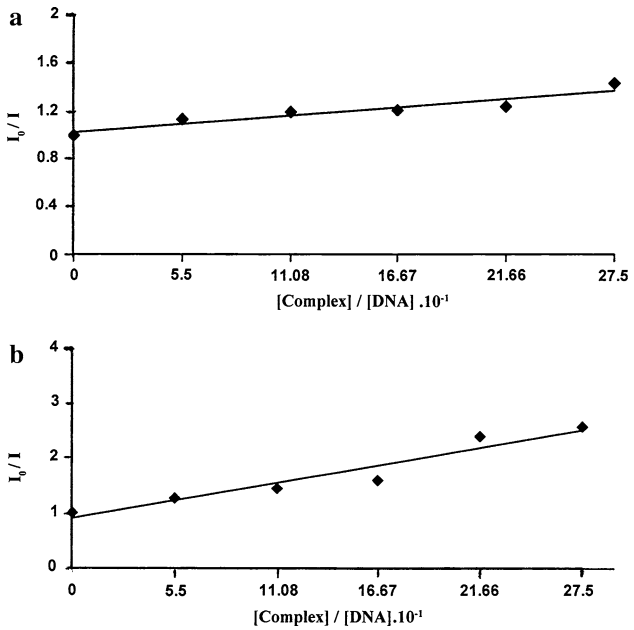


Fig. 9 Fluorescence quenching curves of DNA bound EB by (**a**) complex **1** and (**b**) complex **3**, [complex] 6.6 × 10^{-6} M–33.0 × 10^{-6} M, [CT-DNA] 1.2 × 10^{-5} M

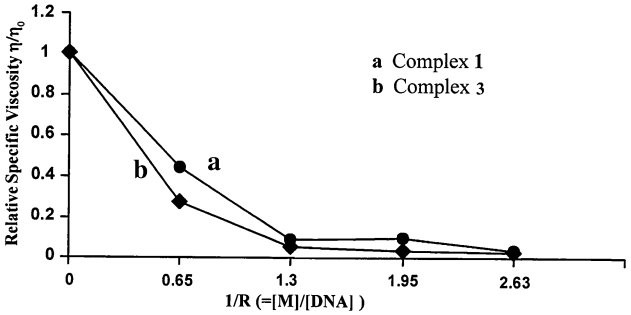


Fig. 10 Effects of increasing amount of complex **1** (●), complex **3** (■) on the relative viscosity of CT-DNA at 29 ± 0.1 °C

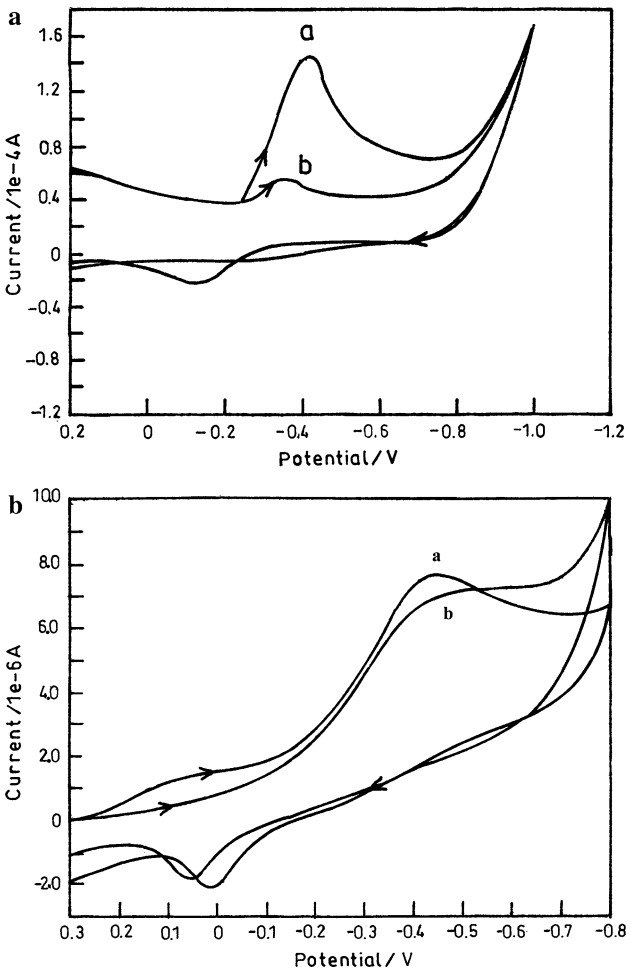


Fig. 11 Cyclic voltammograms (5 mM Tris–HCl/50 mM KCl buffer, 25°) of (**a**) complex **1** and (**b**) complex **3** in absence (curve a) and presence (curve b) of CT-DNA

Conclusions

The template synthesis and characterization of new water-soluble macrocyclic complexes of 4,15-bis(2-hydroxyethyl)-2,4,6,13,15,17-hexaazatricyclodocosane have been

described .The high water solubility of the complexes makes them suitable for biological investigations under different solution conditions. Interaction studies of complexes **1** and **3** with CT-DNA and 5′GMP were carried out using many important biophysical techniques. The results reveal that the complexes interact directly with exterior phosphates of DNA via electrostatic attraction in combination with hydrogen bond formation, which may be facilitated in complexes bearing molecular topology with –NH– and –OH groups, in addition complex **3** displays inclination towards the N7 position of the guanine nucleobase. This dual binding affinity could enhance the local concentration of the metal complex around DNA and consequently the cleavage activity. Experiments with 5′GMP are in concord with the proposed binding mode and rule out the presence of any direct coordinate covalent bond formation. This work features novel design of potential metal-based anticancer drugs and in future warrants evaluation of these complexes in cell based/ in vivo experimental protocols.

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