

Template synthesis of novel carboxamide dinuclear copper (II) complex: spectral characterization and reactivity towards calf-thymus DNA

Suvigya Mathur · Sartaj Tabassum

Received: 13 July 2007 / Accepted: 11 September 2007 / Published online: 26 October 2007
© Springer Science+Business Media, LLC. 2007

Abstract Dinuclear complexes Bis [aqua 1,8-(1,2-dicarboxamido benzene) 3,6-diazaoctane copper (II)/nickel (II)] tetrachloride (**1** and **2**) were synthesized by a two component one-pot metal template condensation between phthalic anhydride and 1,8-diamino 3,6-diazaoctane. Elemental analysis, molar conductance measurements, electronic absorption, infra-red, electron paramagnetic resonance, nuclear magnetic resonance, atomic absorption, and electron spray mass spectral studies have been performed to probe the nature and structure of the complexes. The interaction of copper (II) complex with calf thymus (CT-DNA) has been studied by using absorption, emission and circular dichroic spectral methods, viscometry, and cyclic voltammetry. A strong hyperchromism along with a red shift in UV bands and hypochromism in the ligand field band of the complex **1** on interaction with CT-DNA imply a covalent mode of DNA binding. This is further confirmed by studying the reactivity of complex **1** using circular dichroism and viscosity measurements. The variation in relative emission intensity of DNA-bound ethidium bromide observed upon treatment with the complex **1** parallel the trend of DNA binding studies. Cyclic voltammetry studies reveal that the complex **1** prefers to bind to DNA in Cu(II) rather than Cu(I) oxidation state.

Keywords Carboxamide derived Cu(II) complex · DNA binding studies · Absorption and emission spectroscopy · Electrochemistry · Covalent binding

Abbreviations

CD	Circular dichroism
CT DNA	Calf-thymus DNA
CV	Cyclic voltammetry
DMSO	Dimethylsulfoxide
EthBr	Ethidium bromide
IL	Intra ligand
LF	Ligand field
LMCT	Ligand to metal charge transfer
LNT	Liquid nitrogen temperature
UV	Ultra-violet
TCNE	Tetracyanoethylene

Introduction

The development of metal complexes as artificial nucleases is an area of burgeoning interest. The metal complexes as pharmaceuticals (Guo and Sadler 1999) have gained access over traditional organic dominated drugs, due to their potential use as regulators of gene expression and tools of molecular biology (Lippert 2000). The major intracellular target of anticancer metallodrugs is DNA; therefore metal complexes that can bind to specific nucleobases of DNA are of interest in the development of antitumor

S. Mathur · S. Tabassum (✉)
Department of Chemistry, Aligarh Muslim University,
Aligarh, UP 202002, India
e-mail: tsartaj62@yahoo.com

agents. Studies of metal complexes, which react at specific sites along a DNA strand as reactive models for protein–nucleic acid interactions, provide routes toward rational development of chemotherapeutic agents (Lippard 1978), sensitive chemical probes for DNA structure in solution (Barton 1986), and tools for the molecular biologist to dissect genetic systems (Dervan 1986). In this regard, transition metal complexes stand out as exceptional candidates for artificial nucleases of DNA due to their diverse ability to recognize and react selectively with individual target sites (Cowan 2001; Morrow and Iranzo 2004; Tonde et al. 2006).

Apart from the success of platinum-based drugs, some other metal compounds such as titanium and ruthenium complexes have shown potential for chemotherapy (Karidi et al. 2005). However, challenges remain to be overcome including side effects, toxicity, targeting, delivery, acquired resistance and cancer specificity. To circumvent such problems drugs based on essential metals, having reduced toxicity are required, which has led to the investigation of many copper-based drugs. Copper is a biologically relevant element and its complexes have demonstrated a wide range of pharmacological activity such as antiviral (West and Owens 1998), anticancer (Moubaraki et al. 1999) and anti-inflammatory activity (Andrade et al. 2000). It was found that copper complexes often demonstrate enhanced biological activity than the parent ligand alone (Ainscough et al. 1998). The exploration of copper complexes as chemical nucleases is well documented because they possess biologically accessible redox potential and relatively high nucleobases affinity (Pogozelski and Tullius 1998). Copper-based reactions of DNA are particularly appealing since they have the potential for application *in vivo*.

Among the new design strategies developed for anticancer drugs, the incorporation of carboxamide [$-\text{C}(\text{O})\text{NH}-$] group into the copper (II) complexes results in the synthesis of efficacious probes of DNA structure. As various anticancer agents possess an amide group like, [hexamethylene-bis-acetamide] (Fibach et al. 1977), aminopterin, D-penicillamine, phenylalanine mustard, and 6-mercaptopurine, have demonstrated an increased anticancer activity when administered as metal complexes (Dromfield et al. 1970). In addition, amide groups are also well known to offer potential binding sites for metal ion

complexation. The Lewis acid character of metal ions will also control the amide N–H deprotonation and hence can effectively generate coordinated amido species of varying reactivities (Sahu et al. 2004).

Keeping in view, the two important features such as biocompatible nature of Cu(II) complexes and biological significance of carboxamide group we have designed dinuclear carboxamido Cu(II)–Ni(II) complexes. The interaction of Cu(II) complex with CT-DNA has been investigated using a host of physical methods like absorption, emission, circular dichroic spectroscopies, viscosity measurements and electrochemical methods.

Experimental

Materials

All reagents were of the best commercial grade and were used without further purification. Phthalic anhydride, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, Tris base were procured from E. Merck and 1,8-diamino-3, 6-diazaoctane from Fluka. Disodium salt of calf-thymus DNA purchased from Sigma Chemical Company was stored at 4° and used as received.

Methods and instrumentation

Microanalysis were performed using Carlo Erba Analyzer Model 1108. Copper and nickel contents were determined on GBC 932 Plus atomic absorption spectrophotometer. 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, as KBr pellets. UV–VIS spectra were recorded on a USB 2000 Ocean Optics spectrometer and the data were reported as $\lambda_{\text{max}}/\text{nm}$. The solid state 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. ESI-MS spectra were recorded on Micromass Quattro II triple quadrupole mass spectrometer. The NMR spectra were obtained on a Bruker DRX-300 spectrometer.

All the experiments involving interaction of the complexes with CT-DNA were conducted in buffer

containing tris(hydroxymethyl)aminomethane (0.01 M) and adjusted to pH 7.2 with hydrochloric acid. The CT-DNA was dissolved in Tris–HCl buffer and was dialyzed against the same buffer overnight. Solutions of CT-DNA gave ratios of UV absorbance at 260 and 280 nm above 1.8, indicating that the DNA was sufficiently free of protein (Marmur 2004). DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient $6,600 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ at 260 nm (Reichmann et al. 1954). Concentrated stock solution of the copper complex was prepared by dissolving the complex **1** in H_2O and diluting suitably with the corresponding buffer to the required concentrations for all the experiments.

DNA binding experimental

Electronic absorption titrations

Absorption spectral titration experiments were performed on USB 2000 Ocean Optics spectrophotometer. Maintaining a constant concentration of the Cu(II) complex ($1.6 \times 10^{-4} \text{ M}$) while varying the nucleic acid concentration ($0.8\text{--}4.1 \times 10^{-4} \text{ M}$). This was achieved by dissolving an appropriate amount of the Cu(II) complex and DNA stock solutions while maintaining the total volume constant. This results in a series of solutions with varying concentrations of DNA but with a constant concentration of the complex. The absorbance (A) of the most shifted band of investigated complex 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 Cu(II) complex with CT-DNA was determined using the equation,

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

where ε_a , ε_f , and ε_b correspond to $A_{\text{obsd}}/[\text{Cu}]$, the extinction coefficient for free copper complex, and the extinction coefficient for the copper complex in the fully bound form, respectively. A plot of $[\text{DNA}]/(\varepsilon_a - \varepsilon_f)$ versus $[\text{DNA}]$, where $[\text{DNA}]$ is the concentration of DNA in the base pairs, gives K_b as the ratio of slope to the intercept (Wolfe et al. 1987).

Luminescence titration

Emission intensity measurements were carried out using Hitachi F-2500 spectrofluorometer at 25° . The tris buffer was used as a blank to make preliminary adjustments. Luminescence titration quenching experiments were conducted by adding aliquots of $0\text{--}1.2 \times 10^{-4} \text{ M}$ solutions of the copper (II) complex to samples containing $2 \times 10^{-5} \text{ M}$ ethidium bromide and $0.3 \times 10^{-4} \text{ M}$ CT-DNA in Tris–HCl buffer. The Stern–Volmer equation (Lakowicz and Webber 1973)

$$I_0/I = 1 + K r_{\text{Cu}} \quad (2)$$

where I_0 and I are the emission intensities in the absence and the presence of the complex, respectively. K is a linear Stern–Volmer quenching constant and r_{Cu} is the ratio of total concentration of complex to that of DNA.

Circular dichroism

Circular dichroic spectra of CT-DNA were obtained by using a JASCO J-715 CD spectropolarimeter at 25° in the region of 220–320 nm. All experiments were done using a quartz cell of 1 cm path length. Each CD spectrum was collected after averaging over at least 4 accumulations using a scan speed of 100 nm min^{-1} and a 1 s response time.

Viscosity measurements

Viscosity measurements were carried out using Ostwald's viscometer at $29.00 \pm 0.01^\circ$. 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 $([\text{Cu}]/[\text{DNA}])$, (Cohen and Eisenberg 1969) 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 observed flow time of DNA containing solution ($t > 100 \text{ s}$) corrected for the flow time of buffer alone (t_0), $\eta = t - t_0$.

Cyclic voltammetry

Cyclic voltammetric studies were performed on a CH Instrument Electrochemical analyzer in a single

compartmental cell with 0.4 M KNO₃ 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. All electrochemical data were collected at 25° and are uncorrected for junction potentials. 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

Bis [aqua 1,8-(1,2-dicarboxamido benzene) 3,6-diazaoctane copper (II)] tetrachloride (1)

1,8-diamino-3,6-diazaoctane (2.9 ml, 20 mmol) was added drop wise to the ethanolic solution of CuCl₂ · 2H₂O (3.4 g, 20 mmol) in 1:1 molar ratio. The resulting deep blue solution, λ_{max} = 536 nm corresponding to formation of copper(II) 1,8-diamino-3,6-diazaoctane dichloride complex was stirred at 25° for 30 min and subsequently phthalic anhydride (2.9 g, 20 mmol) dissolved in 25 ml ethanol was added to it. The reaction mixture was refluxed for ca. 1 h and a light blue solid product was obtained. The product was filtered, washed with ethanol and dried in vacuo: **1** (85%), Mp 195°. Anal. Calc. for C₂₈H₄₄N₈O₆ · Cu₂Cl₄ C 39.3, H 5.1, N 13.1, Cu(II) 14.7; found: C 39.2, H 5.1, N 13.0, Λ_M (H₂O): 240 Ω^{-1} cm² mol⁻¹ (1:4 electrolyte). UV–VIS, 25°, 1 × 10⁻³ M, H₂O, λ_{max} in nm, (ϵ M⁻¹ cm⁻¹): 245(22,000), 268 (2,350), 619 (350). IR (as KBr pellet): 3,152 ν (N–H of carboxamide moiety), 1,713 ν (C=O), 1,398 ν (C–N), 2,877 ν (CH₂), 2,933 ν (CH), 416 ν (Cu–N). ESI-MS: m/z [C₂₈H₄₀N₈O₄Cu₂]Cl₄ 821.: Cu(II) 14.6%.

Bis [aqua 1,8-(1,2-dicarboxamido benzene) 3,6-diazaoctane nickel (II)] tetrachloride (2)

This green color compound was obtained with a procedure analogous to that for **1**, using NiCl₂ · 6 H₂O (4.7 g, 20 mmol): **2** (77%). Mp 210°. Anal. Calcd. for C₂₈H₄₄N₈O₆Ni₂Cl₄: C 39.7, H 5.2, N 13.2, Ni(II)13.7; found: C 39.6, H 5.2, N 13.1, Λ_M

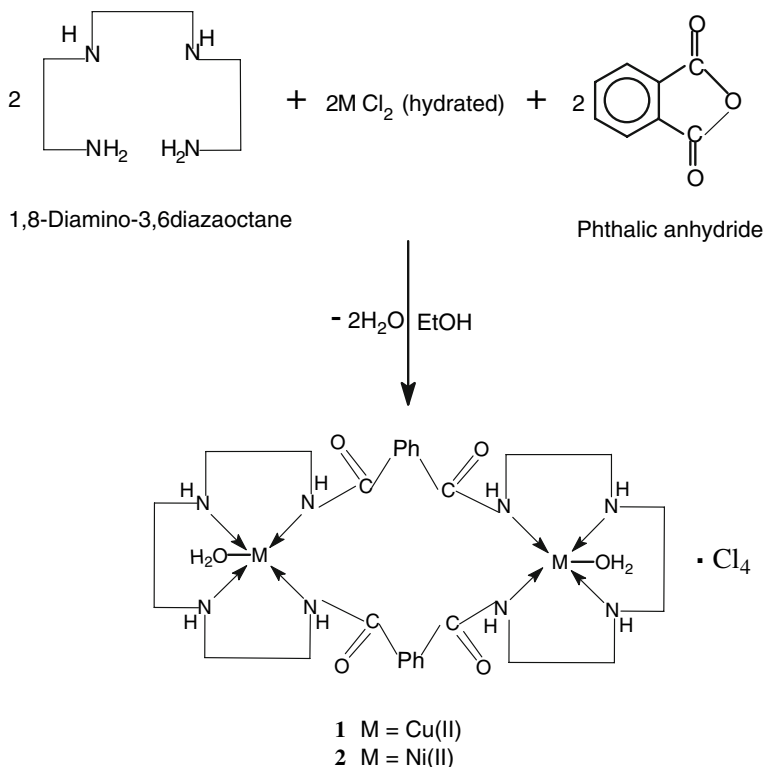
(DMSO): 325 Ω^{-1} cm² mol⁻¹ (1:4 electrolyte). UV–VIS 25°, 1 × 10⁻³ M, H₂O, λ_{max} in nm (ϵ M⁻¹ cm⁻¹), 244 (1,800), 265 (1,950), 576 (200). IR (as KBr pellet): 3,263 ν (N–H of carboxamide moiety), 1,704 ν (C=O), 1,394 ν (C–N), 2,880 ν (CH₂), 2,952 ν (CH), 433 ν (Ni–N). ¹H-NMR (300 MHz, DMSO-d₆, 25°): 8.1 (–NH of amide); 7.8–7.4 (arom. H); 4.2–3.1 (CH₂ adjacent to amide –NH); 3.0 (NCH₂); 2.9–2.6 (NCH₂CH₂). ¹³C-NMR (75.44 MHz): 167.8 (>C=O); 122–134 (arom. C); 35.3–40.3 (N–C–N). ESI-MS: m/z [C₂₈H₄₀N₈O₄Ni₂]Cl₄ 811. Ni(II) 13.6%.

Results and discussion

The synthetic procedure for the complexes is illustrated in Scheme 1. CHN analysis and spectral data (UV–VIS, IR, EPR, ¹H, ¹³C NMR, and atomic absorption) were consistent with the proposed formulation of the dinuclear complexes. The new complexes **1** and **2** are air and moisture stable solids with solubility in water and DMSO, respectively but they are insoluble in methanol and diethyl ether. The molar conductance value of complexes **1**, **2** in water and DMSO, respectively indicates that these complexes are 1:4 electrolytes. Analytical data revealed that complexes contain two metal centers and a water molecule in the coordination sphere; these indications were further supported by atomic absorption data and IR spectral bands. DNA binding studies were performed with the Cu(II) complex and analogous Ni(II) complex was synthesized only for NMR studies.

IR spectral studies

The IR spectra of the complexes **1** and **2** showed characteristic bands in the region 3,150–3,268 cm⁻¹ ascribed to ν (N–H) of carboxamide moiety (Garg et al. 2004). A medium intensity sharp band was observed in the region 1,704–1,713 cm⁻¹ assigned to ν (C=O) of carboxamide linkage (Jain et al. 2004). Previous reports on carboxamide ligand also support these assignments (Alcock et al. 2005) and confirm the non-involvement of >C=O group in coordination with the metal ion. Additionally, signals at ~1,394, ~2,877, and ~2,933 cm⁻¹ were attributed to ν (C–N), ν (CH₂), and aromatic ν (CH) vibrations,

Scheme 1


respectively. A broad band in the range 3,300–3,500 cm^{-1} , assigned to $\nu(\text{O-H})$ of the water molecule present in the lattice or coordinated to metal ion in complexes (Chauhan and Arjmand 2005). The far IR spectra of the complexes **1** and **2** exhibited absorptions at 416 and 433 cm^{-1} , ascribed to $\nu(\text{Cu-N})$ and $\nu(\text{Ni-N})$, respectively.

Absorption spectral studies

The absorption spectra of the complexes **1** and **2** (1×10^{-3} M) were recorded at 25° in H_2O and DMSO, respectively in 200–800 nm as depicted in Fig. 1. The solution spectra of the complexes were observed as three distinct bands. Complex **1** displayed a weak broad band in the range 580–660 nm due to d–d transition of the cupric ion. This band was assigned to $d_{xz}, d_{yz} \rightarrow d_{x^2-y^2}$ transition characteristic of square pyramidal geometry Cu(II) complexes (Du et al. 2002). The diamagnetic Ni(II) complex **2** showed a d–d band at 576 nm, assigned to ${}^3\text{B}_1(\text{F}) \rightarrow {}^3\text{E}(\text{F})$ transition. The λ_{max} value is consistent with a pentacoordinate environment around the Ni(II) ion (Herrera et al. 2003). In addition, complex **1** and **2**

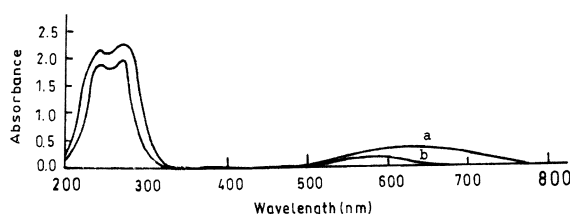


Fig. 1 Absorption spectra of (a) complex **1** (1×10^{-3} M) in H_2O and (b) complex **2** (1×10^{-3} M) in DMSO in the spectral range 220–800 nm

displayed two strong bands in the UV region one at 245 nm attributed to intraligand charge transfer transitions and another at 268 nm assigned to ligand to metal charge transfer transition.

NMR spectral studies

The complex **2** was characterized by ${}^1\text{H}$ and ${}^{13}\text{C}$ NMR spectroscopy in DMSO-d_6 . The ${}^1\text{H}$ NMR spectrum of the complex **2** showed signals for aliphatic and aromatic protons with chemical shift values in accordance with the proposed structure. The

^1H NMR spectrum revealed characteristic signal at 8.1 ppm of the amide $-\text{NH}$ (Rowland et al. 2002). Multiplets in the range of 7.8–7.4 ppm arise due to aromatic protons. Signals due to CH_2 protons (adjacent to amide $-\text{NH}$ proton) were observed from 4.2 to 3.1 ppm (Chavez et al. 1996). Furthermore, resonances at 3.0 and 2.9–2.6 ppm were assigned to (NCH_2) and NCH_2CH_2 protons of presence of 1,8-diamino-3,6-diazaoctane (Dong et al. 2004).

The ^{13}C NMR spectrum of complex **2** exhibited a characteristic signal at 167.8 ppm due to $>\text{C}=\text{O}$ functional group of amide linkage (Shirin et al. 2002). Additionally multiplets in the region 122–134 ppm were ascribed to aromatic carbons. Signals in the range 35.3–40.3 ppm were assigned to $\text{N}-\text{C}-\text{C}-\text{N}$ carbon atoms (Spingler and Pieve 2005). Thus ^1H and ^{13}C NMR data are in well agreement with the proposed structure.

EPR spectral studies

The X-band electron-paramagnetic-resonance (EPR) spectrum of complex **1** was recorded at frequency of 9.1 GHz under the magnetic field strength $3,000 \pm 1,000$ gauss using TCNE as field marker at LNT. Generally, the spectral parameters reflect the usual increase of the equatorial ligand field (LF) in four coordinate complexes versus the five coordinate counterparts. The complex **1** showed an anisotropic spectrum with different g_{\parallel} and g_{\perp} values. The EPR spectrum of complex **1** exhibited $g_{\parallel} = 2.201$, $g_{\perp} = 2.044$ values, and $g_{\text{av}} = 2.093$ computed from the formula $g_{\text{av}}^2 = g_{\parallel}^2 + 2g_{\perp}^2/3$. The values g_{\parallel} and g_{\perp} were in good agreement with an essentially $d_{x^2-y^2}$ copper (II) ground and were anticipated for square pyramidal geometry (Chauhan and Arjmand 2006). The order $g_{\parallel} > g_{\perp} > g_e$ (2.0023) further confirm that the ground state of copper (II) is predominantly $d_{x^2-y^2}$. The g values are also related to the axial symmetry parameter, G , by the expression $G = (g_{\parallel} - 2)/(g_{\perp} - 2)$. The G value measures the extent of the exchange interaction between two copper centers in the polycrystalline solids. If $G < 4$, considerable exchange interaction occurs, whereas if $G > 4$ exchange interaction is negligible. In the present case $G = 4.568$ which confirm that the two Cu(II) centers are too far apart to interact with each other (Rowland et al. 2001).

DNA binding studies

DNA binding is the critical step for many cytotoxic metal complexes as DNA is the primary intracellular target of antitumor drugs. Coordination compounds offer many binding modes to polynucleotides, including outer-sphere non-covalent binding, metal coordination to nucleobases and phosphate backbone interactions. When the present dinuclear Cu(II) complex interacts with DNA, the two labile water molecules in the complex are replaced by a nucleophile on DNA, usually a nitrogenous base such as guanine N_7 , leading to a strong covalent bonding of the complex with DNA (Grover et al. 1994). In addition, incorporation of more than one Cu(II) center in a single complex produces enhanced electrostatic interactions to the anionic DNA phosphate backbone and facilitates its binding to DNA (Humphreys et al. 2002). The mode and propensity of binding of the coordination compounds to calf-thymus DNA can be detected by a number of techniques, and we report herein absorption titration, fluorescence spectroscopy, circular dichroism studies, cyclic voltammetry and viscosity measurement on complex **1**.

Absorption studies

Electronic absorption spectroscopy is universally employed to determine the binding of complexes with the DNA helix. Any interaction between the complex and DNA is expected to perturb the ligand centered spectral transitions of the complex (Uma et al. 2005). The absorption spectrum of complex **1**, exhibited bands around 245, 268, and 619 nm. On the incremental addition of CT-DNA ($0.8\text{--}4.1 \times 10^{-4}$ M) to the complex **1** (1.6×10^{-4} M), a considerable increase in molar absorptivity (Fig. 2) accompanied by a red shift of 7 and 12 nm, respectively of the $\pi \rightarrow \pi^*$ absorption bands was observed. The hyperchromic and hypochromic effect are the spectral features of DNA binding concerning its double helix structure (Yang et al. 1994). Therefore, the observed hyperchromic changes in the UV spectrum of the complex **1** suggest strong binding of CT-DNA, probably due to covalent bonding (Fig. 3).

Structurally, complex **1** provides two water molecules directly attached to the metal ion, which can be replaced by a nucleophile in DNA, usually a

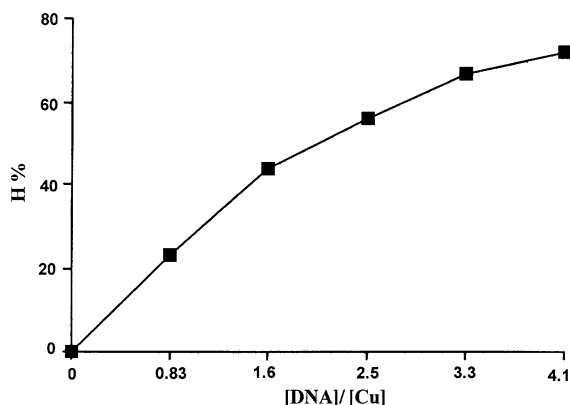


Fig. 2 % Hyperchromism of complex **1** upon addition of CT-DNA at 245 nm

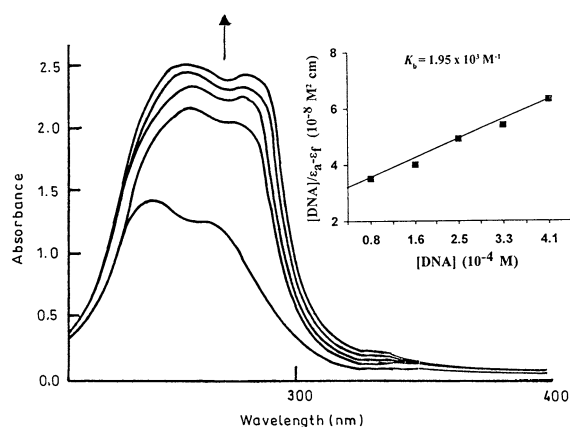


Fig. 3 UV spectral traces of complex **1**, in Tris-HCl buffer (0.01 M, pH 7.2) upon addition of CT-DNA. Inset: Plots of $[DNA]/(\epsilon_a - \epsilon_f)$ versus $[DNA]$ for the titration of CT-DNA with complex **1**, and linear fitting of the data. [Complex **1**] 1.6×10^{-4} M, $[DNA]$ $0.8\text{--}4.1 \times 10^{-4}$ M

nitrogenous base such as guanine (N_7) leading to a strong binding of the complex **1** (Ito et al. 2005). The present ligand system lacks extended π -systems thus ruling out the possibility of any intercalative

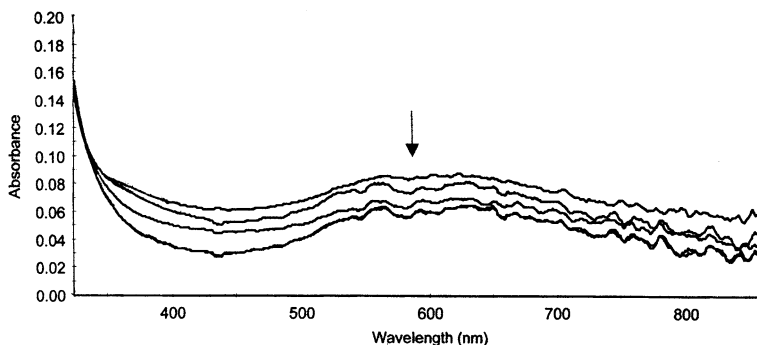
interactions but have the potential to bind to DNA covalently. Furthermore, appreciable changes are observed also in the LF band of the complex **1**. Intensity of the LF band at 619 nm was found to decrease (hypochromism) with the addition of CT-DNA as shown in Fig. 4. These results further support that the complex **1** is coordinated presumably to a DNA base such as guanine (N_7) (Raja et al. 2005). Additionally, a strong hydrophobic interaction between the macrocyclic moiety and the hydrophobic interior access in DNA is plausible (Liu et al. 2002). It is interesting that the complex **1** may sterically clash with the DNA surface, and the coordinated $-NH-$ and carbonyl oxygens of complex **1** may involve in hydrogen bonding with the N_7/O_6 sites of the intrastrand guanine bases. Our results are consistent with a number of earlier reports on DNA interaction (Liu et al. 2003).

To enable quantitative estimation of DNA binding affinity of complex **1**, the intrinsic binding constant K_b was determined using Eq. 1 by concomitant recording of the UV traces with increasing concentration of DNA. The intrinsic binding constant K_b for complex **1** was determined as $K_b = 1.95 \times 10^3 \text{ M}^{-1}$ which is of lower magnitude than those of classical intercalator (EthBr-DNA $1.4 \times 10^6 \text{ M}^{-1}$ in 25 mM Tris-HCl/40 mM NaCl buffer pH = 7.9, $3.0 \times 10^6 \text{ M}^{-1}$ in 5 mM Tris-HCl/50 mM NaCl buffer, pH = 7.2). Therefore, it is reasonable to assume that complex **1** binds covalently to CT-DNA. Nevertheless, hydrogen-bonding interactions cannot be ruled out.

Emission spectral studies

As the complex **1** is non-emissive both in the presence and absence of CT-DNA, competitive

Fig. 4 Absorption spectral traces of complex **1**, in Tris-HCl buffer (0.01 M, pH 7.2) upon addition of CT-DNA in the LF band. [Complex **1**] 1.6×10^{-4} M, $[DNA]$ $0.8\text{--}4.1 \times 10^{-4}$ M



ethidium bromide (EthBr) binding studies were undertaken to gain support for the mode of binding of the complex **1** with DNA. The molecular fluorophore EthBr emit intense fluorescence in the presence of CT-DNA due to its strong intercalation between the adjacent DNA base pairs and stabilization of its excited state (Lepecq and Paoletti 1967). The study involves addition of the complexes to DNA pretreated with EthBr and then measurement of emission intensities of DNA-bound EthBr. The extent of quenching of fluorescence of EthBr bound to DNA would reflect the extent of binding of complexes to DNA (Baguley and LeBert 1984).

Two mechanisms have been proposed to account for the quenching of EthBr emission, the replacement of the molecular fluorophores (if complexes binds to DNA more strongly than EthBr) and/or by electron transfer. The non replacement-based quenching has been correlated with DNA-mediated electron transfer from the excited EthBr to an acceptor (e.g., cupric ion, Cu^{2+}) (Raja et al. 2005). The emission intensity of DNA-bound EthBr is quenched considerably on addition of complex **1** (Fig. 5). As complex **1** binds to DNA via covalent bonding, it cannot displace the strongly DNA-bound EthBr. So the observed quenching is obviously due to the facile intramolecular photo induced electron transfer from the excited EthBr to complex **1** bound to DNA ($E_{1/2}$ 140 mV) (Selvi et al. 2005). Perhaps, it is possible that the low energy ligand based π^* orbitals in complex **1** facilitate the photo induced electron transfer. Besides, the more rigid and shorter A-like conformation of DNA, is likely stabilized by the complex **1** through hydrophobic interaction in DNA grooves is also not suitable for intercalative interaction by EthBr leading to its destabilization of the excited state of bound EthBr (Selvakumar et al. 2006).

The fluorescence-quenching curve of EthBr bound to DNA by the complex **1** is shown in Fig. 6. The quenching plot illustrate that the quenching of EthBr bound to DNA by the complex **1** is in good agreement with the linear Stern–Volmer equation. The value of quenching constant K for the complex **1** estimated using Eq. 2 is 0.16, suggesting the strong interaction of the complex **1** with DNA, which is consistent with the above absorption spectral results.

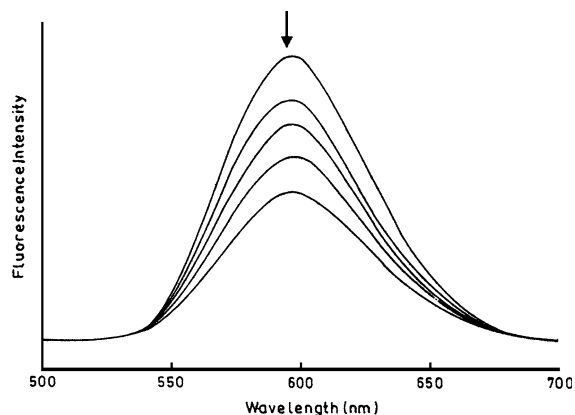


Fig. 5 Emission spectra of EthBr bound to DNA in the presence of complex **1** in Tris–HCl buffer. $[\text{EthBr}] = 2 \times 10^{-5} \text{ M}$, $[\text{DNA}] = 0.3 \times 10^{-4} \text{ M}$, $[\text{complex}] = 0\text{--}1.2 \times 10^{-4} \text{ M}$. $\lambda_{\text{ex}} = 310 \text{ nm}$. $\lambda_{\text{em}} = 599 \text{ nm}$. Arrow shows the intensity change upon increasing concentration of the complex **1**

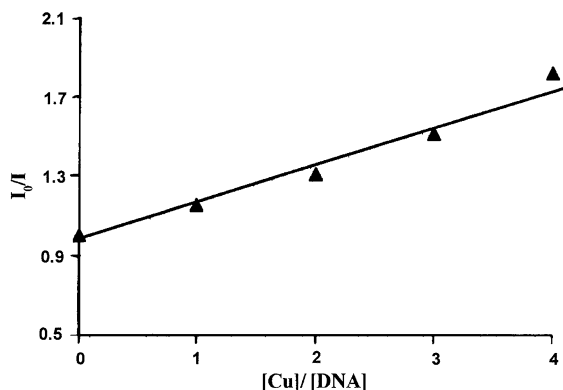


Fig. 6 Fluorescence quenching curve of DNA bound EthBr by complex **1**. $[\text{EthBr}] = 2 \times 10^{-5} \text{ M}$, $[\text{DNA}] = 0.3 \times 10^{-4} \text{ M}$, $[\text{complex}] = 0\text{--}1.2 \times 10^{-4} \text{ M}$. $\lambda_{\text{ex}} = 310 \text{ nm}$. $\lambda_{\text{em}} = 599 \text{ nm}$

Circular dichroic spectral studies

Circular dichroic spectral technique is useful in diagnosing changes in DNA morphology during complex–DNA interactions, as the band due to base stacking (278 nm) and that due to right handed helicity (248 nm) are quite sensitive to the mode of DNA interaction with complexes (Ivanov et al. 1973). On interaction with metal complexes, the changes in the CD spectrum of DNA may correspond to the changes in DNA structure (Lincoln et al. 1997). Simple groove binders and electrostatic interaction of complexes show less or no perturbation on the base-stacking and helicity bands, while

intercalators enhance the intensities of both the bands stabilizing the right-handed B conformation of CT-DNA as observed for classical intercalator (Collins et al. 1994).

Upon addition of complex **1**, the CD spectrum of DNA undergoes changes in both the positive and negative bands (Fig. 7, curve b). There is however, no shift in the band positions. In the presence of complex **1**, the positive band showed remarkable decrease in molar ellipticity revealing significant Cu(II) complex–CT-DNA interaction (Arjmand and Chauhan 2005). The extended π systems reduce the helical twist angle of the DNA base pairs and increase the intensity of the base-stacking band (Norden and Tjernelund 1982). Thus the decrease in the stacking band on addition of complex **1** rules out the intercalation mode of binding. Furthermore, decrease in the intensity of DNA helicity band indicates the hydrophobic interaction of $-\text{NH}-$ groups with DNA. Such spectral changes are characteristic of B to A conformational change (Ramakrishnan and Palaniandavar 2005). These CD spectral results support that the complex **1** interact with CT-DNA, and that the binding event induces certain conformational changes in DNA.

Viscometry studies

To throw further light on the DNA binding mode, viscosity measurements on the metal complexes

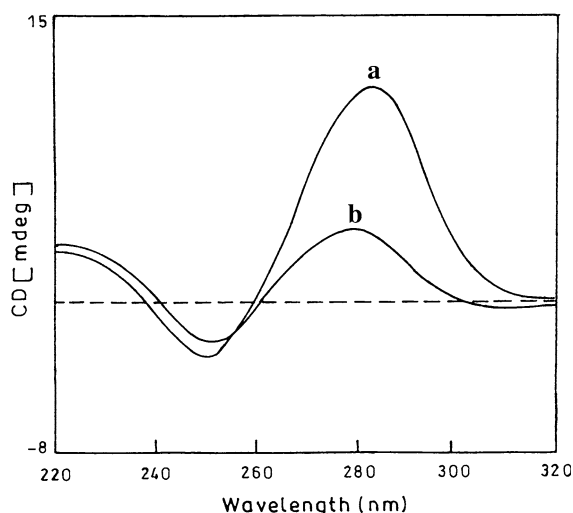


Fig. 7 CD spectra of (a) CT-DNA alone (1×10^{-4} M, Tris–HCl buffer, 25°, pH 7.2) and (b) CT-DNA in presence of complex **1** (1×10^{-4} M)

bound to DNA has been undertaken, as they are sensitive to DNA length change and are the most critical tests of binding in solution in the absence of crystallographic structural data (Satyanaryana et al. 1993). The DNA viscosity is enhanced significantly due to complete or partial intercalation of small molecules in the DNA base stacking but it is slightly disturbed by electrostatic or covalent binding of molecules (Maheswari and Palaniandavar 2004). The changes in the specific relative viscosity of DNA on addition of increasing concentrations of complex **1** are shown in Fig. 8. The decrease in relative viscosity of DNA observed for the complex **1** suggest covalent binding of the complex **1** with CT-DNA, which produced bends or kinks in the DNA and thus reduced its effective length and concomitantly its viscosity (Raja et al. 2005). The result suggests that complex **1** may bind to DNA covalently.

Redox studies

Electroanalytical techniques are the most effective and versatile methods available for the mechanistic study of redox systems. Cyclic voltammetry has been employed to study the interaction of the complex **1** with CT-DNA with a view to further explore the DNA binding modes assessed from the absorption, emission and viscometric studies. The cyclic voltammogram of the complex **1** in the absence of CT-DNA (Fig. 9, curve a) reveal a non-Nernstian but a fairly reversible/quasi-reversible one electron redox process involving the Cu(II)/Cu(I) couple, as judged from the peak potential separation (ΔE_p) and formal potential

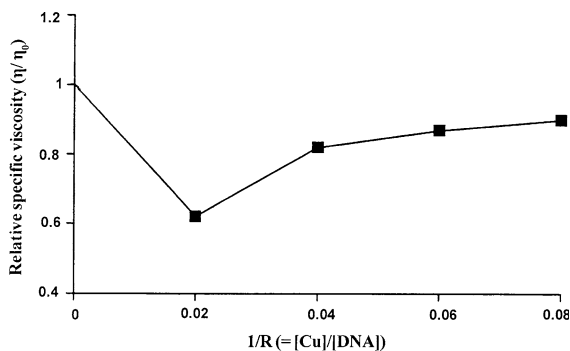
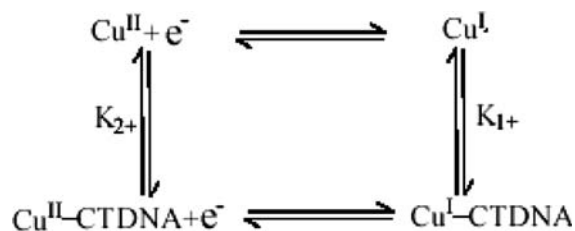


Fig. 8 Effects of increasing amount of complex **1** on the relative viscosity of CT-DNA at $29.00 \pm 0.01^\circ$. [DNA] = 4×10^{-4} M, pH 7.2

($E_{1/2}$) of 110 mV (59 mV for one electron transfer process) and 230 mV, respectively. At different scan rates the cyclic voltammogram did not show any major changes.

Keeping all the parameters constant ($T = 25^\circ$, scan rate 0.1 Vs^{-1} , potential range 0.60 to -0.10 V), complex **1** experienced a significant reduction in cathodic peak current on addition of DNA (Fig. 9, curve b) is due to the slow diffusion of an equilibrium mixture of the free and DNA-bound complex **1** to the electrode surface (Hirohama et al. 2005). Further, the observed shift (90 mV) in $E_{1/2}$ value to more negative potential suggest that both Cu(II) and Cu(I) forms of the complex **1** bind to DNA but with Cu(II) displaying higher DNA binding affinity than Cu(I) form (Grover et al. 1994). This is illustrated by the ratio of the equilibrium constants (K_+/K_{2+}) for the binding of Cu(I) and Cu(II) species to DNA. The value has been estimated from the net shift in $E_{1/2}$ on the addition of DNA assuming reversible electron transfer using the following equation: $E_b^0 - E_f^0 = 0.0591 \log (K_+/K_{2+})$ where E_b^0 and E_f^0 are the formal potentials of the Cu(II)/Cu(I) couple in the bound and free forms, respectively, and K_+ and K_{2+} the corresponding binding constants for the binding of the 1+ and 2+ species to DNA, respectively (Scheme 2). The K_+/K_{2+} value for complex **1** is 0.03, which is less than unity suggesting preferential



Scheme 2

stabilization of Cu(II) form over Cu(I) form on binding to DNA (Mahadevan and Palaniandavar 1998).

Conclusions

The spectroscopic and analytical data presented above clearly indicate that the dinuclear Cu(II)/Ni(II) complexes resulting from the reaction between 1,8-diamino-3,6-diazaoctane, $\text{CuCl}_2 \cdot 4\text{H}_2\text{O}/\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and phthalic anhydride contain two benzene-1,2 dicarboxamide units closed by $(\text{CH}_2)_2\text{-NH-}(\text{CH}_2)_2\text{-NH-}(\text{CH}_2)_2$ links. The dinuclear Cu(II) complex is highly water soluble and exhibit square pyramidal geometry with two water molecules coordinated to metal ion. The biological target of this complex is unknown, however, we have shown here that the complex **1** is able to bind to CT-DNA under physiological pH 7.2. Supported by the various DNA binding experiments, it is likely that complex **1** interacts covalently with DNA, by replacement of the coordinated H_2O molecules with DNA bases. In addition, the presence of coordinated -NH- groups facilitates hydrogen-bonding interactions with DNA. Therefore, the DNA binding studies of complex **1** could provide information good enough for the rational design of new hydro soluble DNA binding agent. The final scope of this work is to better understand the interaction of water-soluble copper complex with DNA in view of its potential application in chemotherapy.

Acknowledgements We are grateful to the Third World Academy of Science, Italy (Grant No. 01-268RG/CHE/AS) for generous financial support to purchase electrochemical analyzer and Ocean Optics USB-2000 spectrometer. Thanks to Regional Sophisticated Instrumentation Center, Central Drug Research Institute, Lucknow, for providing CHN-analysis data, ES-IMS and NMR spectra and Regional Sophisticated Instrumentation Center, Indian Institute of

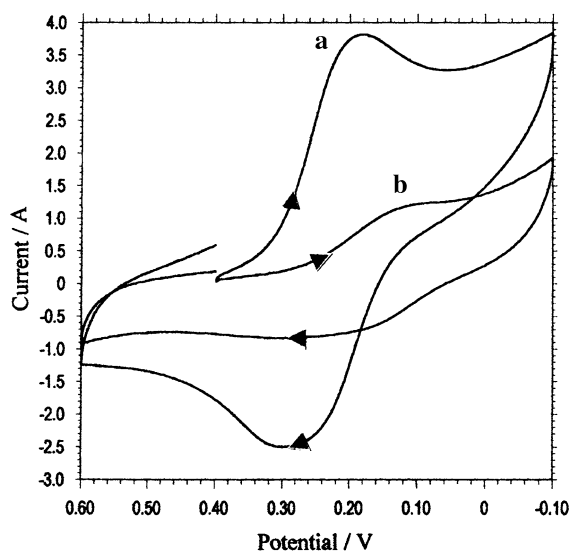


Fig. 9 CV (scan rate 0.1 Vs^{-1} , H_2O , 25° , pH 7.2) of (a) complex **1** alone and (b) complex **1** in presence of CT-DNA. [Complex **1**] $1 \times 10^{-3} \text{ M}$, [DNA] $6 \times 10^{-3} \text{ M}$

Technology, Bombay, for EPR measurements. The authors gratefully acknowledge Dr. R. P. Roy, Cell Biology Lab, National Institute of Immunology, New Delhi, for providing access to the CD facility and Dr. Javed Mussarat, Department of Microbiology, Aligarh Muslim University, Aligarh, for providing atomic absorption facility.

References

- Ainscough EW, Brodie AM, Denny WA, Finlay GJ, Ranford JD (1998) Nitrogen, sulfur and oxygen donor adducts with copper(II) complexes of antitumor 2 formylpyridinethiosemicarbazone analogs: physicochemical and cytotoxic studies. *J Inorg Biochem* 70:175–185
- Alcock NW, Clarkson G, Glover PB, Lawrance GA, Moore P, Napitupulu M (2005) Complexes of 2,6-bis[N-(2'-pyridylmethyl)carbamyl]pyridine: formation of mononuclear complexes, and self-assembly of double helical dinuclear and tetranuclear copper(II) and trinuclear nickel(II) complexes. *Dalton Trans* 518–527
- Andrade A, Namora SF, Woisky RG (2000) Synthesis and characterization of a diruthenium-ibuprofenato complex: comparing its anti-inflammatory activity with that of a copper(II)-ibuprofenato complex. *J Inorg Biochem* 81:23–27
- Arjmand F, Chauhan M (2005) Binding studies of asymmetric pentacoordinate Cu (II) complexes containing phenanthroline and ethylenediamine moiety with calf thymus DNA. *Helv Chim Acta* 88:2413
- Baguley BC, LeBert M (1984) Quenching of DNA-ethidium fluorescence by amsacrine and other antitumor agents: a possible electron-transfer effect. *Biochemistry* 23: 937–943
- Barton JK (1986) Metals and DNA: molecular left-handed complements. *Science* 223:727–734
- Bromfield RJ, Dainty RH, Gillard RD, Heaton BT Growth of Microorganisms in the Presence of Transition Metal Complexes: the Antibacterial Activity of *trans*-Dihalo-genotetrapyridinerhodium (III) Salts (1970) *Nature* 223:375
- Chauhan M, Arjmand F (2005) Synthesis, characterization and interaction of a new chiral trinuclear complex [bis(aquodiaminotryptophanato) Cu^{II}-Sn^{IV}]chloride with calf thymus DNA. *Transition Met Chem* 30:481–487
- Chauhan M, Arjmand F (2006) Chiral and achiral macrocyclic copper (II) complexes: synthesis, characterization and comparative binding studies with calf thymus DNA. *Chem Biodivers* 3:660
- Chavez FA, Olmstead MM, Mascharak PK (1996) Synthesis, structure, and properties of {N,N'-Bis[2-(2-pyridyl)ethyl]pyridine-2,6-dicarboxamido}copper(II). *Inorg Chem* 35: 1410–1412
- Cohen G, Eisenberg H (1969) Viscosity and sedimentation study of sonicated DNA-proflavine complexes. *Biopolymers* 8:45–55
- Collins JG, Shields TP, Barton JK (1994) ¹H-NMR of Rh(NH₃)₄phi³⁺ Bound to d(TGGCCA)₂: classical intercalation by a nonclassical octahedral metalointercalator. *J Am Chem Soc* 116:9840–9846
- Cowan JA (2001) Chemical nucleases. *Curr Opin Chem Biol* 5:634–42
- Dervan PB (1986) Design of sequence-specific DNA-binding molecules. *Science* 223:464–471
- Dong Y, Lindoy LF, Turner P, Wei G (2004) Three-ring, branched cyclam derivatives and their interaction with nickel(II), copper(II), zinc(II) and cadmium(II). *Dalton Trans* 1264–1270
- Du M, Guo YM, Bu XH, Ribas J, Monfort M (2002) Structural and first magnetic characterization of unique mono-μ-chloro bridged dinuclear CuII complexes with heterocycle-functionalized diazamesocyclic ligands. *New J Chem* 26:939–945
- Fibach E, Reuben RC, Rifkind RA, Marks PA (1977) Effect of hexamethylene bisacetamide on the commitment to differentiation of murine erythroleukemia cells. *Cancer Res* 37:440–444
- Garg BS, Sarbhai M, Kumar DN (2004) Spectral and thermal characterization of new biomimetic polydentate amide ligands. *Indian J Chem* 43A:1106
- Grover N, Welch TW, Fairley TA, Cory M, Thorp HH (1994) Covalent binding of aquaruthenium complexes to DNA. *Inorg Chem* 33:3544–3548
- Guo Z, Sadler PJ (1999) Metals in medicine. *Angew Chem Int Ed Engl* 38:1512–1531
- Herrera AM, Staples RJ, Kryatov SV, Nazarenko AY, Akimova EVR (2003) Nickel(II) and copper(II) complexes with pyridine-containing macrocycles bearing an aminopropyl pendant arm: synthesis, characterization, and modifications of the pendant amino group. *Dalton Trans* 846–856
- Hirohama T, Kuranuki Y, Ebina E, Sugizaki T, Arii H, Chikira M, Selvi PT, Palaniandavar M (2005) Copper(II) complexes of 1,10-phenanthroline-derived ligands: studies on DNA binding properties and nuclease activity. *J Inorg Biochem* 99:1205–1219
- Humphreys KJ, Johnson AE, Karlin KD, Rokita SE (2002) Oxidative strand scission of nucleic acids by a multinuclear copper(II) complex. *J Biol Inorg Chem* 7:835–842
- Ito T, Thyagarajan S, Kenneth KD, Rokita SE (2005) Recognition of guanines at a double helix-coil junction in DNA by a trinuclear copper complex. *Chem Commun* 4812–4814
- Ivanov VI, Minchenkova LE, Schyolkina AK, Poletayer AI (1973) Different conformations of double-stranded nucleic acid in solution as revealed by circular dichroism. *Biopolymers* 12:89–110
- Jain SL, Bhattacharyya P, Milton HL, Slawin AMZ, Crayston JA, Woollins JD (2004) New pyridine carboxamide ligands and their complexation to copper(II). X-Ray crystal structures of mono-, di-, tri- and tetranuclear copper complexes. *Dalton Trans* 862–871
- Karidi K, Garoufis A, Tsipis A, Hadjiliadis N, Dulk, Hde, Reedjik J (2005) Synthesis, characterization, in vitro antitumor activity, DNA-binding properties and electronic structure (DFT) of the new complex *cis*-(Cl₂Cl)[Ru(II)Cl₂(NO⁺)(terpy)]Cl. *Dalton Trans* 1176–1187
- Lakowicz JR, Webber G (1973) Quenching of fluorescence by oxygen. Probe for structural fluctuations in macromolecules. *Biochemistry* 12:4161–4170
- Lepecq JB, Paoletti C (1967) A fluorescent complex between ethidium bromide and nucleic acids. Physical-chemical characterization. *J Mol Biol* 27:87

- Lincoln P, Tuite E, Norden B (1997) Short-circuiting the molecular wire: cooperative binding of Δ -[Ru(phen) $_{2}$ dppz] $^{2+}$ and Δ -[Rh(phi) $_{2}$ bipy] $^{3+}$ to DNA. *J Am Chem Soc* 119:1454–1455
- Lippard SJ (1978) Platinum complexes: probes of polynucleotide structure and antitumor drugs. *Acc Chem Res* 11:211–217
- Lippert B (2000) Multiplicity of metal ion binding patterns to nucleobases. *Coord Chem Rev* 200–202:487–516
- Liu J, Zhang H, Chen C, Deng H, Lu T, Ji L (2003) Interaction of macrocyclic copper(II) complexes with calf thymus DNA: effects of the side chains of the ligands on the DNA-binding behaviors. *Dalton Trans* 114–119
- Liu J, Zhang T, Lu T, Qu L, Zhou H, Zhang Q, Ji L (2002) DNA-binding and cleavage studies of macrocyclic copper(II) complexes. *J Inorg Biochem* 91:269–276
- Mahadevan S, Palaniandavar M (1998) Spectroscopic and voltammetric studies on copper complexes of 2,9-Dimethyl-1,10-phenanthrolines bound to calf thymus DNA. *Inorg Chem* 37:693–700
- Maheswari PU, Palaniandavar M (2004) DNA binding and cleavage properties of certain tetramine ruthenium(II) complexes of modified 1,10-phenanthrolines—effect of hydrogen-bonding on DNA-binding affinity. *J Inorg Biochem* 98:219–230
- Marmur J (2004) Procedure for isolation of deoxyribonucleic acid from microorganism. *J Mol Biol* 3:208–214
- Morrow JR, Iranzo O (2004) Synthetic metallonucleases for RNA cleavage. *Curr Opin Chem Biol* 8:192–200
- Moubaraki B, Murray KS, Ranford JD, Vittal JJ, Wang X, Xu Y (1999) Preparation, characterisation and structures of copper(II) complexes of an asymmetric anti-cancer drug analogue. *J Chem Soc Dalton Trans* 3573–3578
- Norden B, Tjernerfeld F (1982) Structure of methylene blue-DNA complexes studied by linear and circular dichroism spectroscopy. *Biopolymers* 21:1713–1734
- Pogozelski WK, Tullius TD (1998) Oxidative strand scission of nucleic acids: routes initiated by hydrogen abstraction from the sugar moiety. *Chem Rev* 98:1089–1108
- Raja A, Rajendiran V, Maheswari PU, Balamurugan R, Kilner CA, Halcrow MA, Palaniandavar M (2005) Copper(II) complexes of tridentate pyridylmethylethylenediamines: role of ligand steric hindrance on DNA binding and cleavage. *J Inorg Biochem* 9:1717–1732
- Ramakrishnan S, Palaniandavar M (2005) Mixed-ligand copper(II) complexes of dipicolylamine and 1,10-phenanthrolines: the role of diimines in the interaction of the complexes with DNA. *J Chem Sci* 117:179–186
- Reichmann ME, Rice SA, Thomas CA, Doty P (1954) A further examination of the molecular weight and size of Desoxypentose nucleic acid. *J Am Chem Soc* 76: 3047–3053
- Rowland JM, Olmstead MM, Mascharak PK (2002) Chiral monomeric and homochiral dimeric copper(II) complexes of a new chiral ligand, N-(1,2-Bis(2-pyridyl)ethyl)pyridine-2-carboxamide: an example of molecular self-recognition. *Inorg Chem* 41:1545–1549
- Rowland JM, Thornton ML, Olmstead MM, Mascharak PK (2001) Structure variation due to ligand flexibility: syntheses and structures of the copper(II) complexes [Cu(APPy)] and [Cu $_{2}$ (AEPy) $_{2}$] where APPyH $_{2}$ = Bis[3-(2-pyridinecarboxamido)propyl]-methyl amine and AEPyH $_{2}$ = Bis[3-(2-pyridine-carboxamido)ethyl]-methylamine. *Inorg Chem* 40:1069–1073
- Sahu M, Pradhan GC, Mohanty P, Dash AC (2004) Phenolamide chelates of cobalt(II), nickel(II), copper(II) and zinc(II) as catalysts for hydrolysis of active esters. Part II. Kinetics and mechanism of hydrolysis of 4-nitrophenylacetate by the metal(II) chelates of 1,2-bis(2-hydroxybenzamido) ethane and 1,3-bis(2-hydroxy-benzamido) propane. *Indian J Chem* 43A:1228–1232
- Satyanaryana S, Dabrowiak JC, Chaires JB (1993) Tris(phenanthroline)ruthenium(II) enantiomer interactions with DNA: mode and specificity of binding. *Biochemistry* 32:2573–2584
- Selvakumar B, Rajendiran V, Maheswari PU, Evans HS, Palaniandavar M (2006) Structures, spectra, and DNA-binding properties of mixed ligand copper(II) complexes of iminodiacetic acid: the novel role of diimine co-ligands on DNA conformation and hydrolytic and oxidative double strand DNA cleavage. *J Inorg Biochem* 100:316–330
- Selvi PT, Evans HS, Palaniandavar M (2005) Synthesis, structure and DNA interaction of cobalt(III) bis-complexes of 1,3-bis(2-pyridylimino)isoindoline and 1,4,7-triazacyclononane. *J Inorg Biochem* 99:2110–2118
- Shirin Z, Thompson J, Sands LL, Yap GPA, Rheingold AL (2002) C $_{2}$ -Symmetric ligands containing hydrogen bond donors: synthesis and properties of Cu(II) complexes of 2,6-bis[N,N'-(2-carboxamidophenyl)carbamoyl]pyridine. *J Chem Soc Dalton Trans* 1714–1720
- Spingler B, Pieve CD (2005) Induction of B- to Z-DNA transition by copper and zinc complexes with C(15) substituted macrocyclic pentaaza ligands. *Dalton Trans* 1637–1643
- Tonde SS, Kumbhar AS, Padhye SB, Butcher RJ (2006) Self-activating nuclease activity of copper (II) complexes of hydroxyl-rich ligands. *J Inorg Biochem* 100:51–57
- Uma V, Kanthimathi M, Weyhermuller T, Nair BU (2005) Oxidative DNA cleavage mediated by a new copper (II) terpyridine complex: crystal structure and DNA binding studies. *J Inorg Biochem* 99:2299–2307
- West DX, Owens MD (1998) Copper(II) complexes of formyl- and acetylpyrazine N(4)-(2-methylpyridinyl)-, N(4)-(2-ethylpyridinyl)- and N(4)-methyl-(2-ethylpyridinyl)thiosemicarbazones. *Transition Met Chem* 23:87–91
- Wolfe A, Shimer GH, Meehan T (1987) Polycyclic aromatic hydrocarbons physically intercalate into duplex regions of denatured DNA. *Biochemistry* 26:6392–6396
- Yang P, Guo ML, Yang BS (1994) Interaction of titanocen-edichloride on DNA. *Chin Sci Bull* 12:987–1002