DNA BINDING AND CLEAVAGE STUDIES OF Cu(II), Ni(II) AND Zn(II) COMPLEXES OF N-(5-AMINOPENTYL)-3-[(5-(N-HYDROXY ACETAMIDO)-PENTYL]CARBAMOYL]PROPIONOHYDROXAMIC ACID IN AQUEOUS MEDIUM

V. Hero, S. Poornima, E. Sundaravadivel and M. Kandaswamy*

Department of Inorganic Chemistry, University of Madras, Guindy Maraimalai Campus, Chennai – 600 025, India. E-mail: mkands@yahoo.com; Fax: +91–44–22300488.

Received 08 December 2012; accepted 26 December 2012

Abstract

The synthesis and characterization of water soluble N-(5-aminopentyl)-3-[(5-(N-hydroxyacetamido) pentyl] carbamoyl] propionohydroxamic acid (L) and its [CuL], [NiL] and [ZnL] complexes are described. Cyclic voltammetric studies of [CuL] complex exhibits a quasireversible one-electron transfer process corresponding to the Cu(II)/Cu(I) redox couple with and Epc = -0.48 V and Epa = -0.40 V. [NiL] complex reveals a one electron quasireversible redox process corresponding to Ni(II)/Ni(I) redox couple with Epc = -0.61 V and Epa = -0.52 V. [NiL] complex in the cathodic region and also exhibits a quasireversible one-electron quasireversible process corresponding to Ni(II)/Ni(III) redox couple with an anodic peak potential Epa = 0.46 V and Epc = 0.37 V in the anodic region. The binding ability and mode of binding of the complexes with CT-DNA were studied using absorption, fluorescence spectral titrations and viscosity measurements. The binding constant obtained from the absorption spectral titrations for [CuL], [NiL] and [ZnL] complexes are 2.5 x 10^5, 2.8 x 10^5 and 8.7 x 10^4 M^-1 respectively. The Cu(II), Ni(II) and Zn(II) complexes cleaves the plasmid DNA. The complex [CuL] damages the plasmid DNA by oxidative cleavage and 'OH radical plays vital role in DNA damage, while [NiL] and [ZnL] complexes cleave the plasmid DNA by hydrolytic pathway. Gel electrophoresis using groove recognition agents DAPI (minor groove binder) and methyl green (major groove binder) has shown that complexes bind in the minor groove of DNA.

© 2012 Universal Research Publications. All rights reserved

Keywords: Dihydroxamic ligand, Water soluble Cu²⁺, Ni²⁺ & Zn²⁺ complexes, DNA binding studies, DNA cleavage studies.

1. Introduction

Hydroxamic acid derivatives are widely distributed in all tissues of plants, as growth factors and vitamins in microbes [1] and also a key pharmacophore in a number of important chemotherapeutic agents. Pharmacological applications of these compounds include tumor inhibitors, antimicrobial agents, antituberculous, antileukemic agents [2]. Several of the hydroxamic acid derivatives entered into human clinical trials for the treatment of cancer and arthritis [3]. They are widely known metal chelators especially iron(III), aluminium(III) and vanadium(IV). Due to their ability to chelate high density metal ions they can be used as possible imaging agents [4]. As a siderophore, the hydroxamates have an ability to form stable transition metal complexes through the formation of a five membered chelate rings [5]. They are widely used as iron chelators that solubilise iron and transport it into the cells [1]. Metal complexes can cleave the DNA under physiological conditions are of interest in the development of metal-based anticancer agents [6-8]. Numerous biological experiments have demonstrated that DNA is the primary intracellular target of anticancer drugs; interaction between small molecules and DNA can cause damage in cancer cells, blocking the division and resulting in cell death [9-11]. Metal complexes as DNA cleaving agents are more attractive compared to small organic compounds because of their unique spectroscopic and electrochemical properties. Ligands in the metal complexes play a major role in their binding to DNA. In particular, certain Ni(II) complexes, which strongly bind and cleave DNA, exhibit prominent anticancer activities and regulate apoptosis [12-13]. In our previous reports, we have demonstrated that some of the binuclear Ni(II), VO(II), Cu(II) and Zn(II) complexes display better DNA hydrolysis [14-21].
Recently, we have found that the bis-phenanthroline water bridged bicopper(II) complex [22] and a series of macrocyclic and macrobicyclic dizinc(II) complexes play a pivotal role in the DNA hydrolysis and mechanism underlying induction of cell death [23,24].

Here, we report synthesis and characterization of new water soluble N-(5-aminopentyl)-3-[(5-(N-hydroxyacetamido)pentyl)|carbamoyl] propionohydroxamic acid (L) (Scheme 1) and their Cu(II), Ni(II) and Zn(II) complexes (Scheme 2). The mode of binding interactions of synthesized complexes with DNA and nuclease activity studies has been carried out in aqueous medium.

2. Experimental

2.1 Materials and instrumentation

The supercoiled pBR322 DNA was purchased from Bangalore Genei (India). Superoxide dismutase (SOD), ethidium bromide (EB) and L-histidine, DAPI were obtained from Sigma. Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution was prepared by using deionized and sonicated triple distilled water. NMR spectra were recorded using Varian Unity Plus 400 MHz Spectrometer. Mass spectra were recorded using LC/MS API 2000 AB SCIEX with positive ionisation mode using ESI probe. IR Spectra were recorded using Perkin Elmer FTIR model SPECTRUM I, using sample dispersed in KBr pellet. UV–Visible spectra were recorded using Perkin Elmer Lambda 35 spectrophotometer operating in the range 200–1000 nm with quartz cells and ε values are given in M$^{-1}$ cm$^{-1}$. Electrochemical measurements were carried out by using CHI 620 Electrochemical analyzer containing a glassy carbon electrode cell setup comprised of glassy carbon electrode as working, platinum wire auxiliary and saturated Ag/AgCl electrodes. Tetra(n-butyl)ammonium perchlorate (TBAP) used as the supporting electrolyte in electrochemical measurements. perchlorate salts are potentially explosive; hence, care should be taken in handling TBAP.

2.2 Synthesis

2.2.1 Preparation of N-benzoylcarboxamidino-5-hydroxylaminopentane-1-ol (1)

Benzylchloroformate (180 g, 1.055 moles) in 200 ml of toluene was slowly added with stirring, to an aqueous solution containing 5-aminopentan-1-ol (100 g, 0.969 moles), sodium carbonate (123.2 g, 1.162 moles) in water (1000 ml) at 25 ºC. The reaction mixture was stirred for 30 min, the organic layer was separated. The product was isolated as white solid in 95 % yield (218.5 g) on evaporation of the solvent from organic layer under reduced pressure, and then precipitated with 200 ml of hexane. The product was isolated, washed with hexane and dried in vacuo for overnight. Melting point: 45 ºC. Elemental analysis data: calculated (%) for C$_{13}$H$_{19}$NO$_3$ (237): C 65.80, H 8.07, N 5.90. Found (%): C 65.78; H 8.02, N 5.89. ESI MS: 237 [M]$^+$. IR Spectra: 1535 cm$^{-1}$ for ν N-H bending, 1685 cm$^{-1}$ for ν C=O str, 2863-2943 cm$^{-1}$ C-H str, 3335 cm$^{-1}$ for ν N-H str and 3400 cm$^{-1}$ for ν O-H str.

H NMR (CDCl$_3$): δ 7.3-7.4 (m, 5H, phenyl protons), δ 5.1 (s, 2H, methylene protons attached to phenyl ring), δ 4.8 (s, 1H, OH proton), δ 3.2 (t, 2H, J = 5.1 Hz, methylene protons), δ 2.9 (t, 2H, J = 6 Hz, methylene protons), δ 1.6 (m, 4H, methylene protons), δ 1.4 (m, 2H, methylene protons).

2.2.2 Preparation of N-benzoylcarboxamidino-5-hydroxylaminopentane (3)

5-benzoylcarboxamidino-5-methanesulfonate (2) was prepared by reacting methanesulfonyl chloride (64 g, 0.558 moles) in 50 ml of methyl chloride with I (100 g, 0.42 moles), triethylamine (64 g, 0.63 moles) in 300 ml of methyl chloride at -5 ºC. The reaction mixture was allowed to stir for 30 min and quenched in 500 ml of water. The organic layer was evaporated under reduced pressure to attain compound (2)

Compound (2) was dissolved in 1000 ml of methanol and then reacted with an aqueous solution containing hydroxylamine sulphate (415.5 g, 2.53 moles), sodium hydroxide (200 g, 5 moles) in 600 ml of water for 3 h at 60 ºC. The reaction mixture was cooled to 25 ºC, treated with 1000 ml of methyl chloride, 3000 ml of water and then layers were separated. The product was isolated as white solid in 71 % yield (75.5 g) on evaporation of the solvent from organic layer under reduced pressure, and then precipitated with 500 ml of isopropyl ether. The product was isolated, washed with isopropyl ether, and dried in vacuo for overnight. Melting point: 107 ºC. Elemental analysis data: calculated (%) for C$_{13}$H$_{19}$NO$_3$ (252): C 61.88, H 7.99, N 11.10. Found (%): C 61.86, H 7.90, N 11.00. ESI MS: 253 [M+H]$^+$. IR Spectra: 1690 cm$^{-1}$ for ν C=O str, 2933 cm$^{-1}$ -CH str, 3257 cm$^{-1}$ for ν N-H str and

(a) Cyclic voltammograms of 1 = [CuL] and 2 = [NiL] complexes in the cathodic region, (b) Cyclic voltammogram of [NiL] complex in the anodic region.
Absorption spectra of (a) [CuL], (b) [NiL] and (c) [ZnL] complex (10 μM) in the absence and presence of increasing amounts of CT-DNA (0 - 250 μM) at room temperature in 50 mM Tris-HCl buffer pH=7.2. Plot of [DNA]/(ε - εf) vs [DNA] for absorption titration of CT-DNA and (d) [CuL], (e) [NiL] and (f) [ZnL] complexes. The arrow shows the intensity changes on increasing the DNA concentration.

3320 cm⁻¹ for ν O-H str. ¹H NMR (CDCl₃): δ 7.3-7.4 (m, 5H, phenyl protons), δ 5.1 (s, 2H, methylene protons attached at phenyl ring), δ 4.85 (s, 1H, OH proton), δ 3.6 (t, 2H, J = 4.2 Hz, methylene protons), δ 3.2 (t, 2H, J = 4.8 Hz, methylene protons), δ 1.5-1.6 (m, 4H, methylene protons), δ 1.3-1.5 (m, 2H, methylene protons).

2.2.5 Preparation of N-(5-benzylxocarbonylaminopentyl)-N-(hydroxy)acetamide (6)

Acetyl chloride (40.2 g, 0.512 moles) was slowly added with stirring, to a reaction mixture of 3 (100 g, 0.396 moles), sodium carbonate (42 g, 0.396 moles) in 200 ml of N,N-dimethyl acetamide at -10 ºC and then stirred for 30 min. The reaction mixture was then treated with 100 ml of aqueous ammonium hydroxide solution for 30 min at 25 ºC. 1000 ml of water and 1000 ml of ethyl acetate were added to the reaction mass and then reaction mass pH was adjusted to 1.5 with diluted hydrochloric acid solution. The organic layer was separated and the product was isolated as solid in 90 % yield (105 g) on evaporation of the solvent from organic layer under reduced pressure and then precipitated with 500 ml of isopropyl ether. The product was isolated, washed with isopropyl ether and dried in vacuo. Melting point: 94ºC. Elemental analysis data: calculated (%) for C₁₂H₂₂N₂O₆ (294): C 61.21, H 7.53, N 9.52. Found (%): C 61.12, H 7.51, N 9.49. ESI MS: 295 [M+H]+. IR Spectra: 1 593 cm⁻¹ for ν CO str (carboxylic acid), 1686 cm⁻¹ for ν CO str (amide), 2879-2947 cm⁻¹ C-H str, 3147 cm⁻¹ for ν N-H str and 3345 cm⁻¹ for ν O-H str. ¹H NMR (CDCl₃): δ 8.5 (1H, s, NH proton), δ 7.3-7.4 (m, 5H, phenyl protons), δ 5.0 (s, 2H, methylene protons attached to phenyl ring), δ 4.9 (s, 1H, NOH proton), δ 3.6 (t, 2H, J = 6 Hz, methylene protons), δ 3.2 (t, 2H, J = 4.5 Hz, methylene protons), δ 2.8 (t, 2H, J = 4.7 Hz, methylene protons), δ 2.6 (t, 2H, J = 9 Hz, methylene protons), δ 1.7 (t, 2H, J = 4.5 Hz, methylene protons), δ 1.5 (t, 2H, J = 4.65 Hz, methylene protons) and δ 1.3 (m, 2H, methylene protons).

2.2.4 Preparation of N-(5-benzylxocarbonylaminopentyl)-tetrahydro-3, 6-dioxo-1,2-oxazine (5)

Compound 4 (100 g, 0.283 moles) was reacted with N,N'-Dicyclohexylcarbodiimide (64.2 g, 0.311 moles) in 1000 ml of methylene chloride at 5 ºC. The temperature of the reaction mixture was raised to 25 ºC, stirred for 3 h and then cooled to -10 ºC. The resultant reaction mass was filtered to remove dicyclohexyl urea. The product was isolated as white solid in 95 % yield (90 g) on evaporation of the solvent from the filtrate under reduced pressure and then precipitated with 500 ml of isopropyl ether. The product was isolated, washed with isopropyl ether and dried in vacuo. Melting point: 85ºC. Elemental analysis data: calculated (%) for C₁₇H₂₃N₂O₆ (334): C 61.07, H 6.63, N 8.38. Found (%): C 61.0, H 6.59, N 8.3. ESI MS: 334 [M]+. IR Spectra: 1770 cm⁻¹ for ν C=O str (carboxylic acid), 1657 cm⁻¹ for ν CO str (amide), 2863-2935 cm⁻¹ C-H str, 3300 cm⁻¹ for ν N-H str. ¹H NMR (CDCl₃): δ 7.3-7.4 (m, 5H, phenyl protons), δ 5.1 (s, 2H, methylene protons attached to phenyl ring), δ 3.75 (t, 2H, J = 5.4 Hz, methylene protons ), δ 3.2 (t, 2H, J = 4.9 Hz, methylene protons), δ 2.8 (t, 2H, J = 3.15 Hz, methylene protons), δ 2.7 (t, 2H, J = 2.4 Hz, methylene protons), δ 1.7 (t, 2H, J = 4.2 Hz, methylene protons), δ 1.5 (t, 2H, J = 5.3 Hz, methylene protons) and δ 1.3 (m, 2H, methylene protons).
2.2.6 Preparation of N-(5-benzyloxy carbonylamino pentyl) -3 -[ (5-(N-hydroxy acetamido) pentoxy carbamoyl) propionyloxy hydrazine acid (8)

N-(5-aminopentyl)-N-(hydroxy)acetamide (7) was prepared by hydrogenating a mixture of 6 (12 g, 0.04 moles), sodium hydroxide (1.8 g, 0.045 moles), 0.6 g of 10 % palladium on carbon in 200 ml of methanol at 25 °C for 45 min under 50 psi hydrogen pressure. The reaction mass was filtered and the filtrate pH was adjusted to 7.5 with sulfuric acid and then evaporated under reduced pressure. Elemental analysis data: calculated (%) for C16H16O2N (160): C 52.48, H 10.07, N 17.48. Found (%): C 52.42, H 10.01, N 17.41. ESI MS: 161 [M+H]+. 1H NMR (D2O): δ 3.4 (t, 2H, J = 4.26 Hz, methylene protons), δ 7.2 (s, 2H, J = 4.65 Hz, methylene protons), δ 3.8 (s, 3H, methyl protons), δ 1.4 (m, 4H, methylene protons), δ 1.1 (m, 2H, methyl protons). N-(5-aminopentyl)-N-(hydroxy)acetamide (7) was treated with 10 ml of water and 60 ml of acetone at 20 °C. The resultant slurry was filtered, filtrate was reacted with 5 (17.4 g, 0.052 moles) in 60 ml of acetone at 25 °C for 15 h. The precipitated white compound was filtered, washed with acetone, water and recrystallised from methanol. The product was isolated as off white solid in 85 % (8.0 g) yield. The precipitated white compound was filtered, washed with acetone, water and recrystallised from methanol. The product was isolated as off white solid in 72 % (14.5 g) yield. Melting point: 148 °C. Elemental analysis data: calculated (%) for C16H16O2N (160): C 52.48, H 10.07, N 17.48. Found (%): C 52.42, H 10.01, N 17.41. ESI MS: 161 [M+H]+. 1H NMR (D2O): δ 3.4 (t, 2H, J = 4.26 Hz, methylene protons), δ 7.2 (s, 2H, J = 4.65 Hz, methylene protons), δ 3.8 (s, 3H, methyl protons), δ 1.4 (m, 4H, methylene protons), δ 1.1 (m, 2H, methyl protons). ESI MS: 361 [M+H]+ (360): C 53.31, H 8.95, N 15.54. Found (%): C 53.29, H 8.9, N 15.44. ESI MS: 361 [M+H]+. IR Spectra: 1530 cm−1 for ν C=O str, 1622 cm−1. H NMR (D2O): δ 8.1 (s, 1H, NH proton), δ 3.3 (t, 4H, J = 4.6 Hz, methylene protons), δ 2.87 (t, 2H, J = 2.8 Hz, methylene protons), δ 2.6 (t, 2H, J = 5.7 Hz, methylene protons), δ 2.1 (t, 2H, J = 5.1 Hz, methylene protons), δ 1.8 (s, 3H, methyl protons), δ 1.34–1.54 (m, 6H, methylene protons), δ 1.1–1.25 (m, 2H, methylene protons), δ 0.9–1.08 (m, 4H, methylene protons). 13C NMR (D2O): δ 173.7, 172.9, 170 (carbonyl carbons), δ 50.5, 48.2, 47.1, 29.786, 26.152, 24.336, 23.723, 23.556 (methylene carbons), δ 19.0 (methyl carbon).

2.2.7 Synthesis of metal complexes using dihydroxamate ligand, N-(5-aminopentyl)-3-[ [(5-(N-hydroxy acetamido) pentoxy carbamoyl) propionyloxy hydrazine acid (L)

Metal complexes were prepared by a general procedure in which L (2.0 g, 11 mmol) in methanol was treated with metal (Cu(II), Ni(II), Zn(II)) acetylacetone (11 mmol) in ethyl acetate under stirring for 20 h at 25 °C. Layers were separated and the aqueous layer was washed with ethyl acetate. The product was isolated as solid by lyophilization of aqueous layer. Thus obtained copper(II), nickel(II) and zinc(II) complexes were characterized by Elemental analysis, ESI-MS, FTIR, UV-Visible spectroscopic techniques. For [CuL] obtained yield 60% (2.5 g), Elemental analysis: calculated (%) for C8H10CuN3O4 (421): C 45.54, H 7.17, N 13.28. Found (%): C 45.51, H 7.12, N 13.0. IR Spectra: 1640 cm−1 for ν C=O str (amide), 2932 cm−1 C-H str, 3092 cm−1 for ν N-H str and 3433 cm−1 for ν O-H str. UV-Visible: [λ/nm (ε/M cm)] in H2O: 211 (1,03,000), 614 (230). ESI MS: 422 [CuL+1]⁺. For [NiL] obtained yield 74% (3.1 g), Elemental analysis: calculated (%) for C8H10NiN3O4 (417): C 46.18, H 7.02, N 13.46, Found (%): C 46.12, H 7.0, N 13.39. IR Spectra: 1651 cm−1 for ν C=O str (amide), 2932 cm−1 C-H str, 3283 cm−1 for ν N-H str. UV-Visible: [λ/nm (ε/M cm)] in H2O: 225 (99,000), 308 (93,000), 655 (110), 749 (80) and 1073 (100). ESI MS: 417 [NiL+1]⁺. For [ZnL] obtained yield 71% (3.0 g), Elemental analysis: calculated (%) for C8H10Zn3O4N3 (423): C 45.34, H 7.13, N 13.22. Found (%): C 45.31, H 7.12, N 13.19. IR Spectra: 1625 cm−1 for ν C=O str (amide), 2935 cm−1 C-H str, 3435 cm−1 for ν O-H str. UV-Visible: [λ/nm (ε/M cm)] in H2O: 220 (2,91,000), 312 (12,000). ESI MS: 423 [ZnL]⁺.

2.3 DNA binding experiments

2.3.1 Absorption spectral studies

Absorption spectral titrations were carried out in (50 mM Tris-HCl buffer, pH 7.2) buffer at room temperature to investigate the binding affinity between CT - DNA and complex. The concentration of CT - DNA was determined from the absorption intensity at 260 nm with a ε value [25] of 6600 M−1 cm−1. Absorption titration experiments were performed by varying the concentration of the CT - DNA (0 - 200 μM) keeping the complex concentration (10 μM) as constant. The absorbance (A) was recorded after each addition of CT - DNA. In order to eliminate the absorbance of the CT-DNA an equal amount of the same was added to both the compound solution and the reference solution. The intrinsic binding constant, Kd was determined for the complexes [CuL], [NiL] and [ZnL] was determined from the spectral titration data using the following equation [26].

$$[\text{DNA}] / ([\text{DNA}] - \epsilon_1) = [\text{DNA}] / ([\text{DNA}] - \epsilon_2) + 1 / K_d ([\text{DNA}] - \epsilon_2)$$ (a)

Here, $\epsilon_1$, $\epsilon_2$, and $\epsilon_0$ corresponds to $A_{obsd}$ / [complex],


61
extinction coefficient for the free complex, and extinction coefficient for the complex in the fully bound form, respectively.

2.3.2 Fluorescence spectral studies

The competitive binding experiments were carried out in Tris – HCl buffer (pH 7.2) by keeping EB - DNA solution containing [EB] = 2 µM and [DNA] = 280 µM as constant and varying the concentration of complex (0 - 280 µM). Fluorescence intensities at 610 nm (excited at 510 nm) were measured after each addition of complex. Stern – Volmer quenching constant Ksv of the complexes [CuL], [NiL] and [ZnL] to CT-DNA were determined from the equation \( I/I_0 = 1 + K_{sv}r \) where I0 and I are fluorescence intensities of EB-DNA in absence and presence complex, respectively. Ksv is a linear Stern – Volmer quenching constant and r is the ratio of the total concentration of complex to that of DNA, [M]/[DNA]. In the linear fit plot of I/I0 vs [complex]/[DNA], Ksv is given by the ratio of slope to intercept. The apparent binding constant (Kapp) was calculated from the equation Kapp[EB]/Kapp[complex] where Kapp = 1 x 10^6, [EB] = 2 µM, and [complex] is the concentrations of the complex at 50% reduction of the emission intensity [27].

2.3.3 Viscosity measurements

To further clarify the binding mode of the present nickel complexes to CT-DNA, viscosity measurements were carried out on CT - DNA (0.5 mM) by varying the concentration of the complex (0.01 mM, 0.02 mM, 0.03 mM, 0.04 mM, 0.05 mM). Data were presented as (η/ηo) versus binding ratio of concentration of complex to that of concentration of CT-DNA, where η is the viscosity of DNA in the presence of complex and ηo is the viscosity of DNA alone.

2.4 DNA cleavage experiments

The cleavage of plasmid DNA was monitored by agarose gel electrophoresis. The cleavage of supercoiled pBR322 DNA by copper (II), nickel (II) and zinc (II) complexes were studied in a medium of 50 mM Tris-HCl buffer (pH = 7.2) in the presence of H2O2. The sample was incubated for 1 h at 37 °C and the reaction quenched by 1 µl of loading buffer. All the samples were finally loaded on 0.8 % agarose gel containing EB (1 µg ml\(^{-1}\)). Electrophoresis was carried out at 50 mV in TAE buffer. Resulting bands were visualized by UV light and photographed. Cleavage mechanistic investigation of pBR322 DNA was done using different reagents such as DMSO, ethanol, NaN3, L - histidine, SOD, KI and EDTA added to pBR322 DNA prior to the addition of complexes. The selectivity of groove binding of the complexes was obtained by gel electrophoresis of plasmid DNA in presence of groove recognition agents like DAPI and methyl green.

3. Results and discussion

The ligand N- (5-aminopentyl) -3- [(5-(N-hydroxyacetamido) -pentyl) carbamoyl] propiono hydroxamic acid (L) and their [CuL], [NiL] and [ZnL] complexes was synthesized and characterized by elemental analysis and spectroscopic techniques. The selected IR and NMR peaks of synthesized compounds were given in experimental section. The electronic spectra of aqueous solution of [CuL] complex exhibit absorption maxima at 614 nm d-d transition of copper ion in visible region. This suggests that the coordination geometry around the metal ion may be distorted square pyramidal. [NiL] complex exhibit absorption maxima at 655, 749 and 1073 nm due to d-d transition of nickel ion. This suggests that the coordination geometry around the metal ion may be distorted octahedral. The ESI-MS spectra of 1, 5 and 8 compounds showed a molecular ion peak [M\(^+\)] at m/z 237, 334 and 494 respectively that are equivalent to their molecular weights. Compounds 3, 4, 6, 7 and L showed a peak at m/z 253, 353, 295, 161 and 361 respectively corresponds to their [M+1]\(^+\) ion. The [CuL], [NiL] and [ZnL] complexes showed a peak at m/z 422, 417 and 423 corresponds to their [CuL+1]\(^+\), [NiL+1]\(^+\), and [ZnL]\(^+\) ions.

3.1 Electrochemical studies

The electrochemical behavior of [CuL] and [NiL] complexes has been studied by cyclic voltammetry in water containing 0.1 M TBAP. The electrochemical data are summarized in table 1. The mononuclear [CuL] complex exhibits a quasireversible redox wave for a one-electron transfer process corresponding to the Cu(II)/Cu(I) redox couple with an anodic peak potential Epa of -0.40 V and cathodic peak potential of -0.48 V Vs Ag/AgCl. The cyclic voltammogram of [NiL] complex also reveals a one-electron quasireversible reduction wave corresponding Ni(II)/Ni(I) redox couple with Epa = -0.52 V and Epc = -0.61 V Vs Ag/AgCl. [NiL] complex exhibits a quasireversible redox wave for a one-electron transfer process corresponding to the Ni(II)/Ni(III) redox couple with an anodic peak potential Epa = 0.46 V and Epc = 0.37 V Vs Ag/AgCl.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Epc (V)</th>
<th>Epa (V)</th>
<th>ΔE (mV)</th>
<th>E(1/2) (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[CuL]</td>
<td>-0.48</td>
<td>-0.40</td>
<td>80</td>
<td>-0.44</td>
</tr>
<tr>
<td>[NiL]</td>
<td>-0.61</td>
<td>-0.52</td>
<td>90</td>
<td>-0.56</td>
</tr>
</tbody>
</table>

Measured by Cyclic Voltammograms at 100 mVs, E Vs Ag/AgCl. Conditions: GC working and Ag/AgCl reference electrodes; supporting electrolyte, TBAP; [complex] = 1 x 10\(^{-3}\) M; [TBAP] = 1 x 10\(^{-1}\) M.

3.2 DNA Binding studies

3.2.1 Absorption spectral studies

Interaction of metal complexes with CT-DNA can be monitored by absorption spectral titrations. The absorption spectra of the new synthesised complexes in absence and presence of DNA is shown in Fig. 2. An aqueous solution of [CuL], [NiL] and [ZnL] complexes showed a band at 220 nm due to the intraligand charge transitions and exhibited 49%, 37% and 18% hypochromism respectively with an increase in the concentration of CT-DNA upto 250 µM and are shown in Fig. 2a, 2b and Fig. 2c. The intrinsic binding constant Ks has been obtained from the changes in intraligand charge transfer transition around 220 nm and the values are given in table 2. These values are ~100 times lower than reported for typical classical intercalators (eg. ethidium bromide, 10\(^6\) M\(^{-1}\)) [28].
Emission spectra of EB bound to DNA in Tris–HCl buffer (pH 7.2) in the absence and presence of the complexes (a) [CuL], (b) [NiL] and (c) [ZnL]. [EB] = 2 µM, [DNA] = 280 µM, [Complex] = 0 to 280 µM. (λex = 520 nm). The arrow shows the intensity changes on increasing the complex concentration. The plot of I/I₀ vs. [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by complex (d) [CuL], (e) [NiL] and (f) [ZnL].

3.2.2 Fluorescence spectral studies
In competitive ethidium bromide (EB) binding studies, the complexes [CuL], [NiL] and [ZnL] were added to DNA pretreated with EB ([EB] = 2 µM, [DNA] = 280 µM) and then emission intensities of DNA-induced EB were measured (Fig. 3). Addition of a complex would quench the EB emission by either replacing DNA-bound EB (if it binds to DNA more strongly than EB) and/or by accepting the excited state electron from EB [29]. The non-replacement-based quenching has been suggested with DNA-mediated electron transfer from the excited ethidium bromide to acceptor metal complexes. As there is no complete quenching of the EB-induced emission intensity, an intercalative mode of DNA-binding of [CuL], [NiL] and [ZnL] observed values is ruled out. Further, the quenching extents “Ksv” for [CuL], [NiL] and [ZnL] have been estimated by using Stern–Volmer equation and K_app values are also calculated from fluorescence spectral titrations and are given in table 2. These values are lower than the classical intercalators which suggested that the complexes [CuL], [NiL] and [ZnL] may be bind to DNA grooves.

3.2.3 Viscosity measurements
Mode of binding of complexes to CT-DNA was investigated by using the viscosity measurements also. Complex that interact in the DNA grooves by partial or non classical intercalation leads no change in relative viscosity of CT-DNA solution when an increasing the concentration of complex. A classical intercalative mode causes a significant increase in viscosity of DNA solution due to increase in separation of base pairs at intercalation sites and hence an increase in overall DNA length [30-31]. To explore the interaction between the complexes to CT-DNA, viscosity measurements were carried out by keeping the DNA concentration as constant and varying the concentration of complex. An increasing amount of complex into CT-DNA no change in the relative viscosity of DNA solution and are shown in Fig. 4. The data suggest that the new synthesized complexes interact to grooves of DNA base pairs.

3.3 DNA cleavage studies
The nuclease activity of complexes has been studied, supercoiled pBR322 DNA (150 µg/µL) was incubated with complex in aqueous buffer solution (5 mM Tris–HCl/40 mM NaCl, pH 7.2). The DNA-cleaving ability of complexes was demonstrated initially by a plasmid relaxation assay, in which the conversion of supercoiled form (Form I) to nicked circular (Form II) and linear open circular (Form III) DNA were monitored.

Mechanistic investigations were done using various additives to understand the nature of the reactive species.
A + e was observed in the DNA + \[ZnL\] complex (75 μM) + ex (75 μM) + increasing DNA incubated with re•, can efficiently inhibit DNA cleavage, indicating Cu(II) complexes play the key role in forming a stable complex Cu(II) species that was responsible for the DNA cleavage, we investigated in the presence of hydroxyl radical scavengers (DMSO, KI), singlet oxygen quenchers (NaN\(_2\), L-histidine, and ethanol), superoxide scavenger (SOD) and chelating agent (EDTA) under our experimental conditions. From the Fig. 5 there was no apparent inhibition in the DNA cleavage activity of the \[CuL\] complex in the presence of singlet oxygen quenchers such as NaN\(_2\) and L-histidine and superoxide scavenger (SOD). Complete inhibition in DNA cleavage was observed in the presence of hydroxyl radical (\(\cdotOH\)) scavengers such as KI, DMSO and in presence of chelating agent EDTA (0.5 mM) (Fig. 8, Lane 2). The mechanistic results indicate the formation of \(\cdotOH\) as the DNA cleaving agent. The EDTA, a Cu(II) specific chelating agent that strongly binds to Cu(II) forms a stable complex, can efficiently inhibit DNA cleavage, indicating Cu(II) complexes play the key role in the cleavage.

Changes in relative viscosity of CT-DNA on increasing amounts of complex

<table>
<thead>
<tr>
<th>Complexes</th>
<th>(K_w)</th>
<th>(K_{sv})</th>
<th>(K_{app})</th>
</tr>
</thead>
<tbody>
<tr>
<td>[CuL]</td>
<td>2.5 x 10(^2)</td>
<td>0.14</td>
<td>1.8 x 10(^4)</td>
</tr>
<tr>
<td>[NiL]</td>
<td>2.8 x 10(^2)</td>
<td>0.14</td>
<td>1.8 x 10(^4)</td>
</tr>
<tr>
<td>[ZnL]</td>
<td>8.7 x 10(^2)</td>
<td>0.11</td>
<td>2.5 x 10(^4)</td>
</tr>
</tbody>
</table>

The cleavage of supercoiled pBR322 DNA by complexes \[NiL\] and \[ZnL\] and their mechanistic study of DNA cleavage has shown in Fig. 6 and Fig. 7 respectively. \[NiL\] and \[ZnL\] complexes cleave the pBR322 DNA and no apparent inhibition was observed in presence of hydroxyl radical scavenger (DMSO, KI), singlet oxygen quencher (NaN\(_2\), L-histidine, ethanol) and superoxide scavenger (SOD). These results rules out the possibility of cleavage by hydroxyl radical, singlet oxygen and superoxide anion, respectively. This fact implies that the DNA cleavage reaction by complex \[NiL\] and \[ZnL\] should be due to hydrolytic mechanism. The EDTA efficiently inhibits the DNA cleavage activity of the \[NiL\] and \[ZnL\] complexes (Fig. 8, Lane 3 and Lane 4).

Cleavage of supercoiled pBR322 DNA incubated with \[ZnL\] complex (75 μM) in Tris – HCl (pH = 7.2) at 37 ºC for 3 h. Lane 1, DNA control, Lane 2, DNA + [ZnL] complex (75 μM) + NaN\(_2\) (0.5 mM), Lane 4, DNA + [ZnL] complex (75 μM) + DMSO (4 μl), Lane 6, DNA + [ZnL] complex (75 μM) + SOD (4 μl), Lane 7, DNA + [ZnL] complex (75 μM) + KI (0.5 mM).

Cleavage of supercoiled pBR322 DNA incubated with \[CuL\] (50 μM) in the presence of hydrogen peroxide in Tris – HCl buffer (pH = 7.2) at 37 ºC for 1 h. Lane 1, DNA control, Lane 2, DNA + \[CuL\] complex (50 μM) + H\(_2\)O\(_2\) (0.08 %), Lane 3, DNA + \[CuL\] complex (50 μM) + H\(_2\)O\(_2\) (0.08 %) + KI (50 μM), Lane 4, DNA + \[CuL\] complex (50 μM) + H\(_2\)O\(_2\) (0.08 %) + NaN\(_2\) (0.5 mM), Lane 5, DNA + \[CuL\] complex (50 μM) + H\(_2\)O\(_2\) (0.08 %) + DMSO (4 μl), Lane 6, DNA + \[CuL\] complex (50 μM) + H\(_2\)O\(_2\) (0.08 %) + SOD (4 units), Lane 8, DNA + \[CuL\] complex (50 μM) + H\(_2\)O\(_2\) (0.08 %) + ethanol (1 μL, 10 %).

Cleavage of supercoiled pBR322 DNA incubated with \[NiL\] (75 μM) in Tris – HCl (pH = 7.2) at 37 ºC for 3 h. Lane 1, DNA control, Lane 2, DNA + \[NiL\] complex (75 μM), Lane 3, DNA + \[NiL\] complex (75 μM) + NaN\(_2\) (0.5 mM), Lane 4, DNA + \[NiL\] complex (75 μM) + DMSO (4 μl), Lane 5, DNA + \[NiL\] complex (75 μM) + L-histidine (50 μM), Lane 6, DNA + \[NiL\] complex (75 μM) + SOD (4 units), Lane 7, DNA + \[NiL\] complex (75 μM) + KI (0.5 mM).

Minor groove binding agent DAPI and major groove binding agent methyl green were used to explore the potential interacting site of the complexes with plasmid pBR322 DNA. Fig. 9, there was no apparent inhibition in the DNA cleavage activity of complexes, when methyl
green (major groove binding agent) treated with pBR322 DNA prior to addition of complexes and external additives (Lane 2, 4, 7). Addition of DAPI (minor groove binding agent) to pBR322 DNA prior to addition of complexes inhibit the DNA cleavage activity of complexes [CuL], [NiL] and [ZnL] are shown in Fig. 9, Lane 3, 5, 7. This result clearly suggests that the complexes prefer to bind to pBR322 DNA minor groove.

4. Conclusions

The ligand (L) and their [CuL], [NiL] and [ZnL] complexes were synthesized and characterized by elemental analysis and spectroscopic techniques, the binding properties of the complexes [CuL], [NiL] and [ZnL] with CT - DNA were investigated by spectroscopic titrations and viscosity measurements. The intrinsic binding constant $K_b$ is $2.5 \times 10^4, 2.8 \times 10^4$ and $8.7 \times 10^4$ M$^{-1}$ for [CuL], [NiL] and [ZnL] complexes respectively obtained from absorption spectroscopic titrations. All the complexes have been found to promote DNA cleavage. [CuL] complex cleaves the plasmid DNA by oxidative path involving •OH as the reactive species responsible for the nuclease activity. Mechanistic studies suggest that [NiL] and [ZnL] complexes cleave the plasmid DNA by hydrolytic pathway. Additionally, the notable evidence for the groove binding of the complexes was provided by gel electrophoresis of plasmid DNA in presence of groove recognition agents.
DAPI and methyl green suggesting a minor groove selectivity of the complexes.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>EB</td>
<td>Ethidium bromide</td>
</tr>
<tr>
<td>CT-DNA</td>
<td>Calf thymus Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DAPI</td>
<td>4',6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>KI</td>
<td>Potassium iodide</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutases</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>Epa</td>
<td>Anodic peak potential</td>
</tr>
<tr>
<td>Epc</td>
<td>Cathodic peak potential</td>
</tr>
</tbody>
</table>

**Acknowledgement**

S.P. is thankful to URF, University of Madras, Maraimalai campus, Chennai – 600 025 for a fellowship. We thank the Department of Science and Technology (DST–FIST), New Delhi, Government of India, for financial support.

**References**


---


Source of support: Nil; Conflict of interest: None declared