Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(5):1159-1176

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Binding studies of some novel macroacyclic transition metal complexes towards CT-DNA via multispectroscopic techniques

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ABSTRACT

A novel macroacyclic N_2O_2 based Schiff base ligand (L), obtained by the condensation of 9,10-phenanthrenequinone and 1,8-diaminonaphthalene, has been used to synthesize the mononuclear complexes of the type [M(L)]Cl_2 [M= Co(II), Ni(II), Cu(II) and Zn(II)]. The newly synthesized ligand (L) and its complexes have been characterized with the help of elemental analyses, conductance measurements, electronic, ^{13}C-NMR, infrared and mass spectral studies. The formation of macroacyclic framework has been inferred from the appearance of imine (C=N) and (M-N) band in IR spectra and the signal observed in ^{13}C-NMR spectra. The stoichiometry and the nature of the complexes have been deduced from the results of elemental analyses and conductance data. The structural distortion in Cu(II) complex has been deduced on EPR data. The electrochemical behaviour of the Cu(II) complex has been studied by cyclic voltammetry. Absorption, Fluorescence, circular dichroism and viscosity measurement studies on the complexes proved a significant binding to calf thymus DNA.

Keywords: Macroacyclic ligand, Schiff base complex, Cyclic voltammetry, Calf thymus DNA

INTRODUCTION

Cancer is a disease that has tormented man throughout history. A manuscript named Bhrigu Samhita in almost 3000 B.C., which is perhaps one of the earliest mentions of cancer that can be found, describes the origin and treatment of cancer [1]. Platinum(II) complexes are used as anticancer drugs since long and among them cis-platin have proven to be a highly effective chemotherapeutic agent for treating various types of cancers like ovarian, testicular, head and neck cancer [2-4]. Cis-platin was initially synthesized by Peyrone in 1844, and its biological effects were accidentally discovered by B. Rosenberg and co-workers in 1965. The development of modern medicinal inorganic chemistry, stimulated by the serendipitous discovery of cis-platin has been facilitated by the inorganic chemists. And then, many successive experiments indicate that cis-platin can be used as efficient anticancer drugs through binding to DNA via an intercalation mode [5].However, its use has been limited because of many side effects such as hair follicle, neuro toxicity and the lining of gastro-intestinal tract due to drug resistance phenomenon. To overcome these side effects and limited activity of cis-platin, great efforts have been made to synthesize other alternatives such as transition metal complexes which act as better antitumor drugs [6-9]. Schiff base ligands are considered "privileged ligands" because they are easily prepared by the condensation between aldehydes/ketones and amines. The incorporation of transition metals into these compounds leads to the enhancement of their biological activities and decrease in the cytotoxicity of both metal ion and Schiff base ligand [10–12]. Further, a significant rising interest in the design of metal complexes as drugs and diagnostic agents is currently observed in the area of scientific inquiry, appropriately termed medicinal chemistry [13]. Investigations in the medicinal chemistry focus mostly on the specialization of metal species in biological media, based on the investigations of metal ions with diverse biomolecules [14-16]. Investigations on the interaction between transition metal complexes and DNA has attracted many interests due to their importance in cancer therapy and molecular biology [17-21]. Small molecules, such as transition metal complexes, can interact with DNA through the following three non-covalent modes:

intercalation, groove binding and external static electronic effects [22, 23]. DNA intercalators [24] (molecules that intercalate between DNA base pairs) have attracted particular attention due to their antitumor activity. Since the discovery of the DNA intercalation process by Lerman in 1961 thousands of organic compounds have been developed as potential anticancer agents.

In view of aforesaid importance of Schiff bases and their complexes, we report synthesis and characterization of novel macroacyclic Schiff base ligand (L) and its complexes with Co(II), Ni(II), Cu(II) and Zn(II). The comparative study of the binding affinities of Co(II), Ni(II), Cu(II) and Zn(II) complexes with calf thymus DNA is also reported.

EXPERIMENTAL SECTION

9,10-Phenanthrenequinone and 1,8-diaminonaphthalene were obtained from Sigma-Aldrich, all other reagents and solvents were purchased from Merck and used as received. CT DNA (calf thymus) was purchased from Bangalore Genei (India). Ethidium bromide (EB) was obtained from Sigma (USA).Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution were prepared by using triple distilled water. All the reactions were performed under aerobic conditions.

2.1. Characterization

The infrared spectra of the solid samples were recorded in JASCO/FT-IR 410 spectrometer in the range of 4000-400 cm^{-1}. Electronic spectra were recorded using Perkin Elmer Lambda-35 UV-Vis. spectrometer using DMSO as solvent in the range of 200-800 nm. The molar conductivity measurements of the metal complexes were carried out in ~10^{-3}M DMSO solutions using a Coronation digital conductivity meter. The ^{13}C NMR was recorded on a JEOL GSX-400 spectrometer employing $CDCl_3$ as solvent at ambient temperature. The mass spectral study was carried out using JEOL D-300 (EI) mass spectrometer. The cyclic voltammogram of 10^{-3} M solution of complexes were obtained on a CHI600A electrochemical analyzer. EPR measurements of Cu(II) complexes of all the studied ligands were recorded at liquid nitrogen temperature on a Varian E-4 X-band spectrometer using DPPH as the g-marker.

2.2. DNA binding experiments

2.2.1. Absorption spectral studies

Electronic absorption spectrum of the complex was recorded before and after addition of CT-DNA in the presence of 50 mM Tris-HCl buffer (pH 7.5). A fixed concentration of metal complexes (10 μM) was titrated with incremental amounts of CT-DNA over the range (0 – 200 μM). The equilibrium binding constant (K_b) values for the interaction of the complex with CT-DNA were obtained from absorption spectral titration data using the following equation 1 [25].

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

Where ε_a is the extinction coefficient observed for the charge transfer absorption at a given DNA concentration, ε_f the extinction coefficient at the complex free in solution, ε_b the extinction coefficient of the complex when fully bound to DNA, K_b the equilibrium binding constant, and [DNA] the concentration in nucleotides. A plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ versus [DNA] gives K_b as the ratio of the slope to the intercept. The non-linear least square analysis was performed using Origin lab, version 6.1.

2.2.2. Fluorescence spectral studies

The fluorescence spectral method using ethidium bromide (EB) as a reference was used to determine the relative DNA binding properties of the complexes to calf thymus (CT- DNA in 50 mM Tris HCl / 1 mM NaCl buffer, pH 7.5). Fluorescence intensities of EB at 610 nm with an excitation wavelength of 510 nm were measured at different complex concentrations. Reduction in the emission intensity was observed with addition of the complexes. The relative binding tendency of the complexes to CT DNA was determined from a comparison of the slopes of the lines in the fluorescence intensity versus complex concentration plot.

$$I_o/I=1 + K_{svr} \quad (2)$$

Where I_0, is the ratio of fluorescence intensities of the complex alone, I is the ratio of fluorescence intensities of the complex in the presence of CT-DNA. K_{sv} is a linear Stern–Volmer quenching constant and r is the ratio of the total concentration of quencher to that of DNA, [M] / [DNA].A plot of I_0 / I*vs.* [Complex]/ [DNA], K_{sv} is given by the ratio of the slope to the intercept. The apparent binding constant (K_{app}) was calculated using the equation K_{EB}[EB] / K_{app}[complex], where the complex concentration was the value at a 50% reduction of the fluorescence intensity of EB and K_{EB} = 1.0 x 10^7 M^{-1} ([EB] = 3.3 μM) [26].

2.2.3. CD spectral studies

Circular dichroic spectra of CT DNA in the presence and absence of metal complexes were obtained by using a JASCO J-715 spectropolarimeter equipped with a Peltier temperature control device at 25 ± 0.1 °C with a 0.1 cm path length cuvette. The spectra were recorded in the region of 220–320 nm for 200 μM DNA in the presence of 100 μM of the complexes.

2.2.4 Viscosity measurements

The binding mode of the complexes to CT-DNA by 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/η_o) 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 η_o is the viscosity of DNA alone. Viscosity values were calculated after correcting the flow time of buffer alone (t_o), $\eta = (t-t_o)/t_o$[27].

2.3. Synthesis Procedures

2.3.1. Synthesis of macroacyclic ligand (L)

2mmol (0.42 g) of 9,10-phenanthrenequinone was dissolved in warm methanol (50 mL) and a slight excess of 1,8-diaminonaphthalene 5 mmol (0.16 g) in methanol (50 mL) was added drop wise to the hot solution and few drops of Conc. HCl was added. The reaction mixture was refluxed for 3 hrs. The volume was then reduced by boiling to about 25 mL, then cooled, stirred occasionally, and allowed to stand overnight. The resulting dark brown product was collected, washed with cold methanol, and dried *in vacuo*.

(L): Yield: 74%,Dark brown compound, m.p: 228 ^{0}C, Anal. Found.(%): C, 84.63;H, 4.08;N, 5.09; Calc.(%): C, 84.74; H, 4.12; N, 5.20; EI-MS: m/z, 538.60; IR (KBr, cm^{-1}): $\nu_{(C=N)}$, 1639 cm^{-1}, $\nu_{(C=O)}$, 1684 cm^{-1}, $\nu_{(-C=C-)}$, 1581 cm^{-1}, $\nu_{(-C=C-)}$, 2953; ^{13}C NMR (δ, ppm in $CDCl_3$) 168.31 (C=N), 193.87 (C=O).

2.3.2. Synthesis of macroacyclic Schiff base complexes [**ML**]

A solution of hydrated metal chloride $CoCl_2 \cdot 6H_2O$/$NiCl_2 \cdot 6H_2O$/$CuCl_2 \cdot 2H_2O$ and $ZnCl_2$ (1 mmol) in methanol (25 ml) was added slowly to a hot solution of (L**)** (1 mmol) dissolved in 25 ml of methanol. The resulting solution was magnetically stirred under reflux for 8 h at room temperature resulting in the isolation of solid product. The product thus formed was filtered, washed with methanol and dried in vacuum over anhydrous calcium chloride.

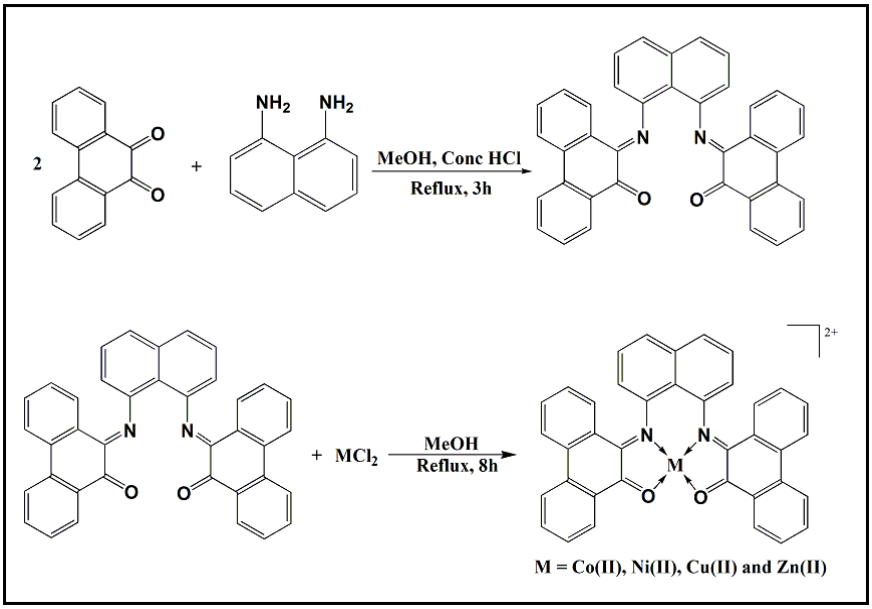

Fig. 1. Schematic representation of the synthesis of N_2O_2 based macroacyclic ligand and its metal complexes

[Co(L)]Cl_2: Yield: 76%; Dark Brown compound; Anal. Found.(%): C, 76.27; H, 3.64; N,4.61 and Co, 9.77; Calc.(%): C, 76.38; H, 3.71; N, 4.69 and Co, 9.86; IR (KBr, cm^{-1}): $\nu_{(C=N)}$, 1602 cm^{-1}, $\nu_{(C=O)}$, 1647 cm^{-1},$\nu_{(C-H)}$, 2911 cm^{-1}, $\nu_{(C=C)}$, 1563 cm^{-1}, $\nu_{(M-N)}$, 482;Λ_m($Smol^{-1}cm^2$) 127.32;UV-Vis. in DMSO, nm (transition): 230 (π-π* ligand), 350 (MLCT) and 560($^4A_{2g} \rightarrow {}^4T_{1g}$).

[Ni(L)]Cl_2: Yield: 80%; Pale Brown compound; Anal. Found.(%): C, 76.36; H, 3.70; N, 4.62 and Ni, 9.75; Calc.(%): C, 76.41; H, 3.71; N, 4.69 andNi, 9.83; IR (KBr, cm^{-1}): $\nu_{(C=N)}$, 1589 cm^{-1}, $\nu_{(C=O)}$, 1639 cm^{-1},$\nu_{(C-H)}$, 2877 cm^{-}

^{1}, $\nu_{(C=C)}$, 1568 cm^{-1}, $\nu_{(M-N)}$, 483; Λ_m($Smol^{-1}cm^2$) 121.78; UV-Vis. in DMSO, nm (transition): 247 (π-π* ligand), 380 (MLCT) and 615 ($^1A_{1g} \rightarrow {}^1B_{1g}$).

[Cu(L)]Cl_2: Yield: 82%; Black compound; Anal. Found.(%): C, 75.72; H, 3.60; N, 4.59 and Cu, 10.46; Calc.(%): C, 75.80; H, 3.68; N, 4.65 and Cu, 10.55; IR (KBr, cm^{-1}): $\nu_{(C=N)}$, 1593 cm^{-1}, $\nu_{(C=O)}$, 1636 cm^{-1},$\nu_{(C-H)}$, 2920 cm^{-1}, $\nu_{(C=C)}$, 1560 cm^{-1}, $\nu_{(M-N)}$, 490; Λ_m($Smol^{-1}cm^2$) 129.77; UV-Vis. in DMSO, nm (transition): 228 (π-π* ligand), 350 (MLCT) and 676 ($^2B_{1g} \rightarrow {}^2E_g$).

Zn(L)]Cl_2: Yield: 66%; Dark brown compound; Anal. Found.(%): C, 75.48; H, 3.56; N, 4.57 and Zn, 10.74; Calc.(%): C, 75.57; H, 3.67; N, 4.64 and Zn, 10.83; IR (KBr, cm^{-1}): $\nu_{(C=N)}$, 1604 cm^{-1}, $\nu_{(C=O)}$, 1644 cm^{-1}, $\nu_{(C-H)}$, 2908 cm^{-1}, $\nu_{(C=C)}$, 1568 cm^{-1},$\nu_{(M-N)}$, 465; Λ_m($Smol^{-1}cm^2$) 120.21.

RESULTS AND DISCUSSION

3.1. Characterization of the complexes

1,8-diaminonaphthalene reacts with 9,10-phenanthrenequinone to form the corresponding macroacyclic Schiff base (L). The metal complexes were prepared by the equimolar reaction of (L) and corresponding metal salts. The schematic representation of the synthesis of the macroacyclic ligand and its complexes is shown in Fig.1.All the four macroacyclic Schiff base complexes [M(L)]Cl_2 are found to be stable towards air, and they are soluble in DMSO and DMF but insoluble in common organic solvents. The analytical data of the complexes corresponds well with the general formula [M(L)]Cl_2, where M = Co(II), Ni(II), Cu(II) and Zn(II); L = Ligand. The molar conductance data of the Co(II), Ni(II), Cu(II) and Zn(II) chelates show that the complexes are1:2 electrolytes.

3.1.1. FT-IR Spectroscopy

The IR spectrum of the macroacyclic Schiff base ligand (L), given in Fig.2, shows the most significant C=N stretching mode at 1639 cm^{-1}, which indicates the formation of imino group [28]. The appearance of the band at 1684 cm^{-1}, assigned to the C=O stretching vibration [29], indicates the formation of the open-chain product containing terminal carbonyl groups. The C-H and C=C stretching bands of the ligand are found at2953 and 1581 cm^{-1} respectively. The IR spectra of the complexes [Co(L)]Cl_2, [Ni(L)]Cl_2, [Cu(L)]Cl_2 and [Zn(L)]Cl_2 are shown in the Figures 3, S1, S2 and S3 respectively. In the IR spectra of all the complexes, the C=N band and the terminal C=O band shifts to the lower region indicating the coordination of metal ion to imino nitrogen [30] as well as to the oxygen present in the carbonyl groups [31] of (L).Further, the IR spectra of metal complexes also show some new sharp bands in the region 482 cm^{-1}, 483 cm^{-1}, 490 cm^{-1} and 465 cm^{-1} for Co(II), Ni(II), Cu(II) and Zn(II) complexes respectively which is due to the formation of coordinate bond between the imino nitrogen and the metal [32].

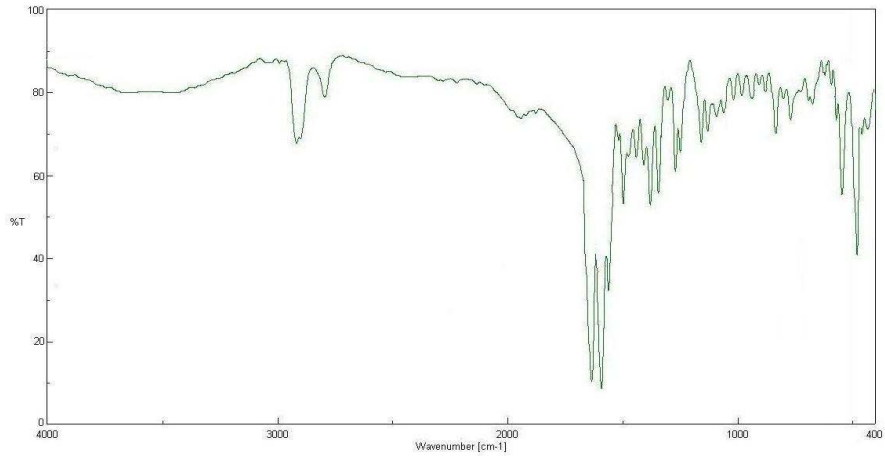

Fig. 2. FT-IR spectra of macroacyclic Schiff base ligand (L)

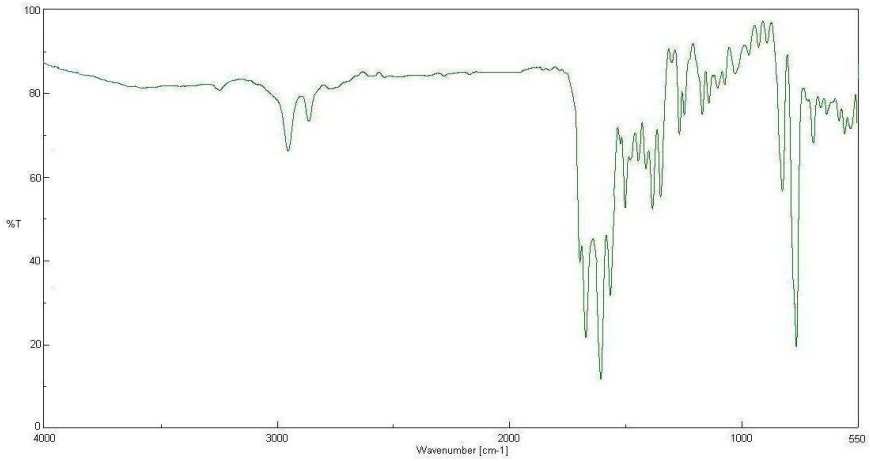

Fig. 3. FT-IR spectra of $[Co(L)]Cl_2$ complex

3.1.2. ^{13}CNMR spectroscopy

The ^{13}C NMR spectrum of the macroacyclic Schiff base ligand (L) is shown in Fig. 4. The ^{13}C NMR spectrum of (L) exhibited signals at δ 116.47, 122.57, 126.27, 127.30, 127.94, 128.17, 128.92, 131.09, 131.15, 132.47, 133.76, 145.79, 148.97, 154.20 were due to aromatic carbons (Ar-C). The imino carbon (**C**=N) atoms are observed at δ168.31. The carbonyl carbon resonates at δ 193.87.The ^{13}C NMR spectrum confirms the formation of Schiff base ligand by the condensation of 9,10-Phenanthrenequinone and 1,8-diaminonaphthalene.

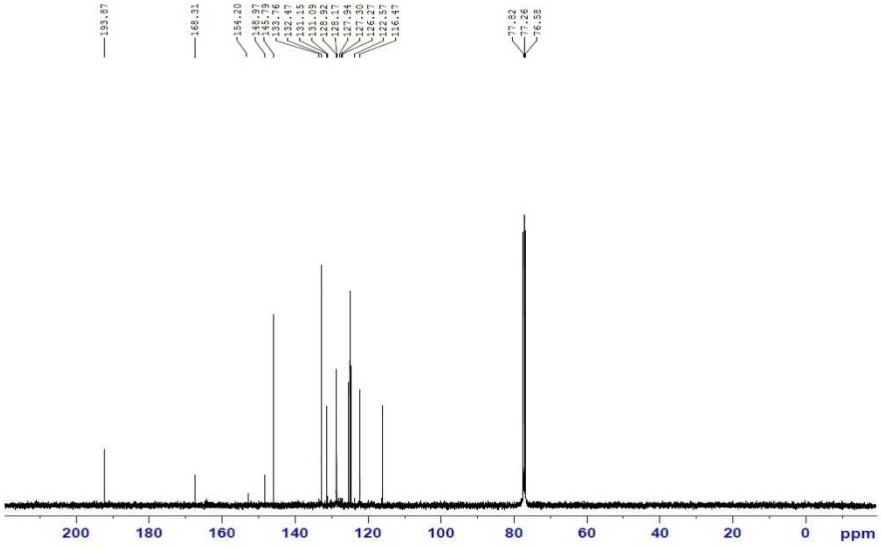

Fig. 4 ^{13}C NMR spectra of macroacyclic Schiff base ligand (L)

3.1.3. Electron Impact Mass spectral analysis

The Electron Impact mass spectrum of the macroacyclic Schiff base ligand (L) is shown in Fig. 5. The EI mass spectrum of (L) shows the molecular ion peak at m/z = 538 $[M]^+$ $(C_{38}H_{22}N_2O_2)^+$ confirms the formation of the macroacyclic Schiff base ligand (L). The peaks at m/z = 485, 440, 383, 308, 253, 236, 204, 181, 154, 136, 104 and 78 corresponds to the fragments $C_{34}H_{19}N_2O_2$, $C_{30}H_{16}N_2O_2$, $C_{26}H_{14}N_2O_2$, $C_{22}H_{13}NO$, $C_{18}H_{11}NO$, $C_{16}H_9NO$, $C_{14}H_8NO$, $C_{13}H_8O$, $C_{10}H_6N_2$, $C_{10}H_4O$, C_7H_4O and C_6H_4 respectively. This confirms the molecular structure of the macroacyclic Schiff base ligand (L).

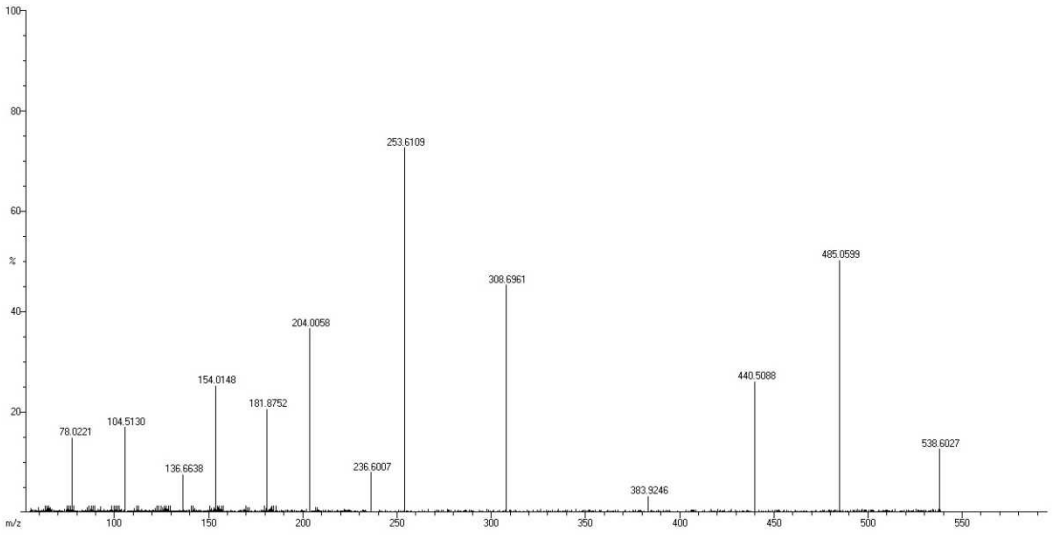

Fig. 5. Electron impact mass spectra of macroacyclic Schiff base ligand (L)

3.1.3. Electronic spectroscopy

The electronic spectra of the complexes have been measured in the range 200-800 nm in DMSO. The UV-Vis absorption bands of the complexes are obtained in the range of 200-500 nm is given in Fig. S4, and the absorption bands obtained in the range of 500 to 800 nm is shown in Fig.S5. The ligand shows absorption between 200-250 nm which is intra-ligand charge transfer π-π* transitions of the ligand [33]. The absorption bands in the region350-380 nm are attributed to the metal - ligand charge transfer bands. The $[Co(L)]Cl_2$complexhas a low intensity band at 560 nm is indicative of square planar geometry and is assigned $^4A_{2g} \rightarrow {}^4T_{1g}$ transition [34]. The absorption spectrum of the $[Ni(L)]Cl_2$ complex displays a low intensity band at 615 nm, which is due to the $^1A_{1g} \rightarrow {}^1B_{1g}$ transition, and assigned square planar geometry [35]. The absorption spectrum of the $[Cu(L)]Cl_2$complex exhibit a weak band at 676 nm demonstrating square planar geometry and is assigned to the $^2B_{1g} \rightarrow {}^2E_g$ transition [36].The complexes $[Co(L)]Cl_2$, $[Ni(L)]Cl_2$ and $[Cu(L)]Cl_2$, shows similar electronic spectra. However, they show an additional peak between 560-680 nm, which is due to the d-d transition of the metal ions, suggesting they are four coordinated environment, having square planar geometry, with dsp^2 configuration. The complex $[Zn(L)]Cl_2$which has a completely filled d^{10} electronic configuration is not expected to show any d-d electronic transition, and the complex is expected to have tetrahedral geometry [37] with sp^3 configuration.

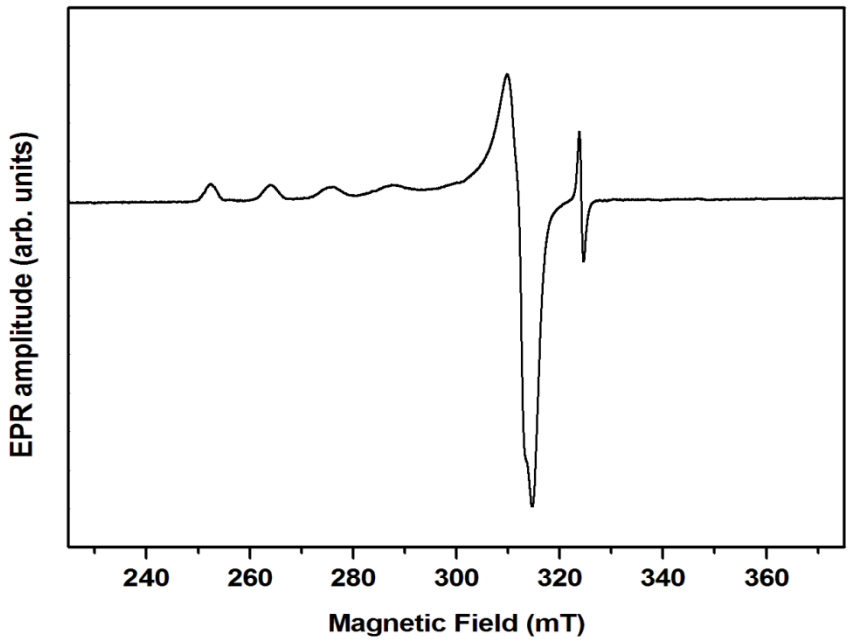

Fig. 6. EPR spectra of $[Cu(L)]Cl_2$ complex recorded in frozen DMSO at 77 K

3.2. EPR spectral analysis

The liquid nitrogen temperature (LNT) X-band EPR spectrum of the macroacyclic copper(II) complex is shown in Fig. 6. The hyperfine structure observed corresponds to N_2O_2 coordination mode in square planar complex. The spectrum for the frozen solution shows anisotropic pattern for a powder sample, show four lines with nuclear hyperfine spin 3/2 due to hyperfine splitting. i.e., four well resolved peaks [38] of low intensities in the low-field region and one intense peak in the high-field region resulting from the coupling of the unpaired electron with the nuclear spin of Cu(II). The trend $g_{\|}$ (2.237) > $g_{\perp}$(2.171) > 2 observed in these complex shows that the unpaired electron lies in the $d_{x^2-y^2}$ orbital of the Cu(II) ion having $^2B_{1g}$ as the ground state. The present EPR results show that $g_{\|}$ is equal to 2.237 which are in conformity with the presence of mixed copper-nitrogen and copper-oxygen bonds in this complex [39].

3.3 Electrochemical studies

The cyclic voltammogram of the macroacyclic copper(II) complex is shown in Fig. 7. The cyclic voltammogram of the Cu(II) complex exhibits one quasi-reversible cathodic response at E^1_{pc} = -0.6 V with corresponding anodic peak at E_{pa} = -1.03 V. This is attributed to Cu(II)/Cu(I) reduction process. The presence of more electronegative oxygen atom in the oxyimine moiety decreases the electron density around Cu(II) ion and favours only the reduction process [40].

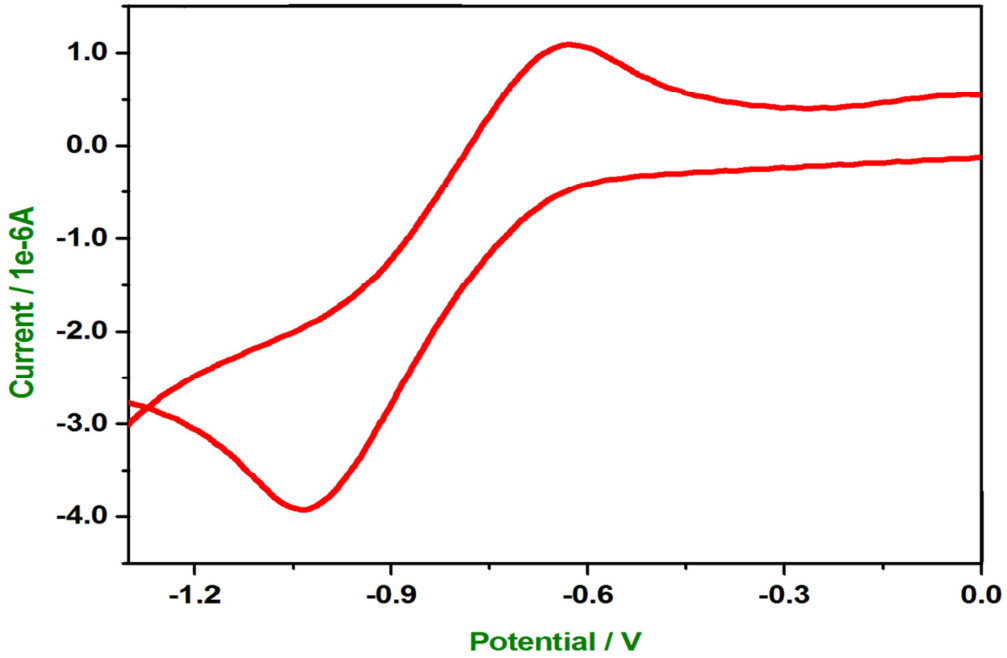

Fig. 7. Cyclic voltammogram of [Cu(L)]Cl_2complex

3.4. DNA binding studies

3.4.1. Absorption spectral studies

Electronic absorption spectroscopy was used to study the interaction of metal complexes with DNA [41]. The absorption spectral traces of the complexes with increasing concentration of CT DNA are shown in Figures S6, S7, S8 and S9 respectively. Generally, a complex binding to DNA *via* intercalation usually results in hypochromism and bathochromism involving strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The DNA-binding constant of metal(II) complexes are usually determined by the MLCT band because no light absorption for DNA is found in the range of wavelength over 350 nm and thus no interference exists. The extent of the hypochromism in the visible MLCT band is consistent with the strength of intercalative interaction [42]. On increasing the concentration of CT-DNA, the absorption bands of the complexes were affected, resulting in the tendency of hypochromism and a remarkable red shift of 8-11 nm were observed in all the complexes, Co(L)]Cl_2, [Ni(L)]Cl_2, [Cu(L)]Cl_2 and [Zn(L)]Cl_2. A red shift can be directly linked with π* orbital of intercalated compound couples with the π–orbital of the base pairs, thus decreasing π-π* transition energy. On the other hand, the coupling π-orbital is partially filled by electrons, thus decreasing transition probabilities and concomitantly resulting in hypochromism [43]. Further, a plot of DNA]/(ϵ_a-ϵ_f) versus [DNA] was drawn to elucidate the DNA binding affinities of the complexes, and is provided in Fig. 8. In order to quantitatively compare the binding affinity of complexes with CT-DNA the intrinsic binding constants K_b of the complexes were determined. The binding Constants (K_b) of the metal complexes were given in Table 1. The Kb values obtained here are lower than those reported for classical intercalators (EB and [Ru(phen)(dppz)] whose binding constants are on the order of 10^6–10^7

(mol L^{-1})$^{-1}$) [44, 45] and also compared to other reported later 3d metal complexes (Kb = 0.88–3.83 x 10^4 (mol L^{-1})$^{-1}$). It is clear that the hypochromism and K_b values are not enough evidence, but these results can suggest an intimate association of the compounds with CT-DNA and indicate that the binding strength of complex is in the order Cu(II) complex>Ni(II) complex> Co(II) complex> Zn(II) complex. From these experiment, the Cu(II) complex has high binding constant value indicating the effective intercalation among the Co(II), Ni(II) and Zn(II) complexes.

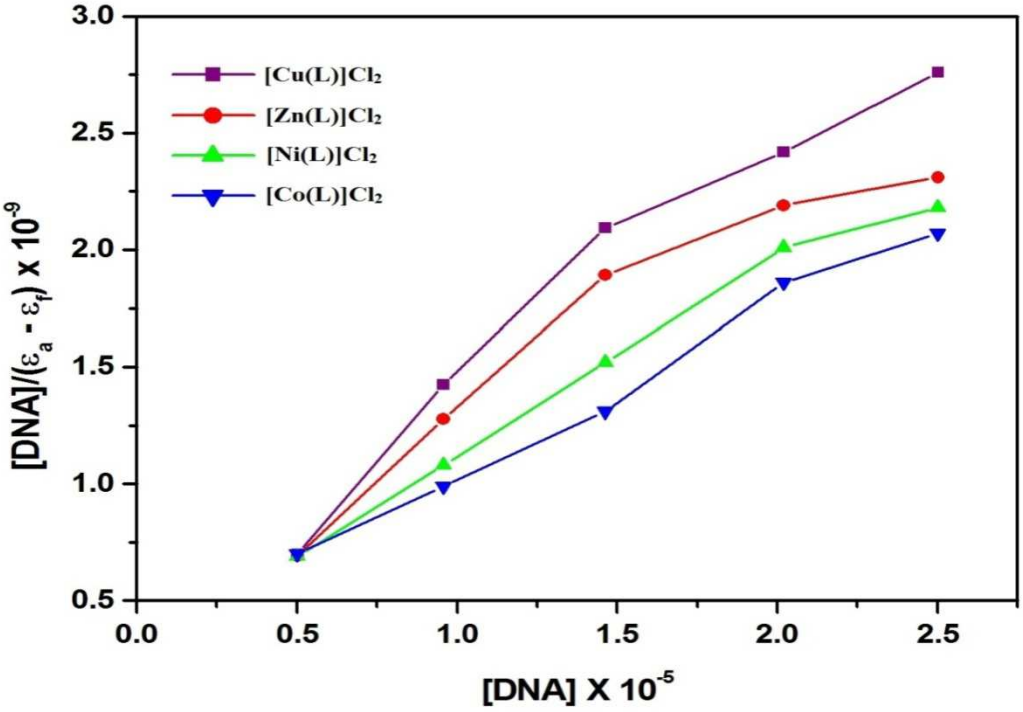

Fig. 8. The plots of [DNA] / (ϵ_a-ϵ_f) versus [DNA] for the titration of DNA with[Co(L)]Cl_2, [Ni(L)]Cl_2,[Cu(L)]Cl_2 and [Zn(L)]Cl_2complexes

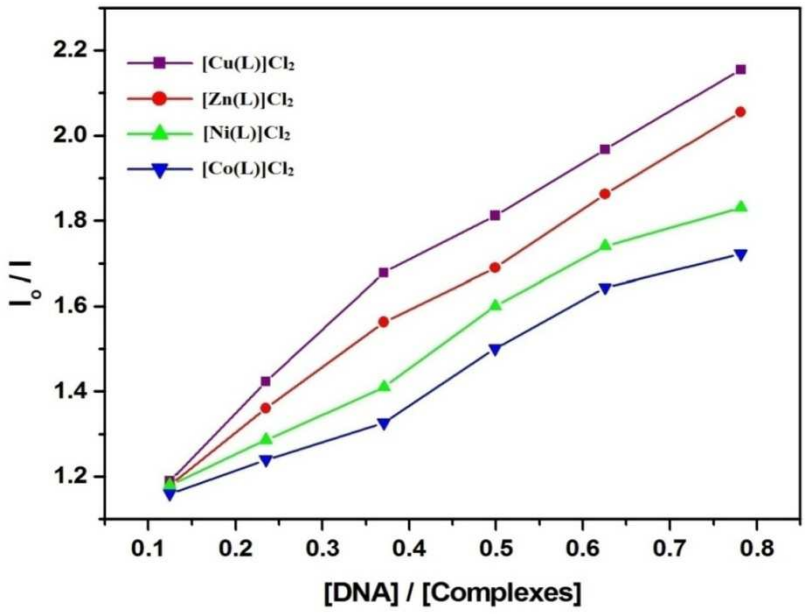

Fig. 9. The plots of emission intensity I_o / I *vs* [DNA] / [complexes]

3.4.2. Emission spectral studies

The emission spectral method is used to study the relative binding of the complexes to CT-DNA. The emission intensity of ethidium bromide (EB) is used as a spectral probe as EB shows no apparent emission intensity in buffer solution because of solvent quenching and an enhancement of the emission intensity when intercalatively bound to DNA [46]. The binding of the complexes to DNA decreases the emission intensity of EB. The fluorescence quenching of EB bound to DNA by the complexes are shown in Figures S10, S11, S12 and S13 respectively. The

fluorescence intensity at 613 nm (λex= 518 nm) of EB in the bound form was plotted against the compound concentration. To compare the DNA binding affinities of the complexes, the plot of I_o/I *vs* [DNA]/[complex], was drawn and is given in Fig. 9. From the graph, the apparent binding constants (K_{app}) of the complexes were calculated and given in Table 2. The maximum value obtained for copper complex is in consistent with the absorption spectral measurements.

Table 1 Binding Constants of complexes

Complexes	Binding Constant (K_b) M^{-1}
$[Co(L)]Cl_2$	2.54×10^4
$[Ni(L)]Cl_2$	1.24×10^5
$[Cu(L)]Cl_2$	5.27×10^5
$[Zn(L)]Cl_2$	1.64×10^4

Table 2 Apparent Binding Constants of complexes

Complexes	Apparent Binding Constant (K_b) M^{-1}
$[Co(L)]Cl_2$	9.8×10^4
$[Ni(L)]Cl_2$	2.6×10^5
$[Cu(L)]Cl_2$	5.7×10^5
$[Zn(L)]Cl_2$	3.5×10^4

3.4.3. CD spectral studies

Circular dichroism (CD) is the technique commonly used to study DNA transitions as well as to identify different DNA structures. The UV circular dichroic spectrum of CT DNA exhibits band at 290 nm due to base stacking and negative band at 240 nm due to helicity of B-DNA [47] are very sensitive towards the interaction with such small molecules. Incubation of the DNA with the complexes $[Ni(L)]Cl_2$, $[Cu(L)]Cl_2$ and $[Zn(L)]Cl_2$ induced considerable changes in CD spectrum. The effect of the complexes on the CD spectrum of CT DNA has been illustrated in Fig. 10. The examination indicates that the positive band slightly increased in intensity and the negative band decreased in intensity after the binding of the complexes with DNA however no considerable shift in λ_{max} could be observed. This suggests that the DNA binding of the complexes induces certain conformational changes, such as the conversion from a B-like structure to a more Z-like structure within the DNA molecule [48]. From the results of absorption, emission and CD spectroscopic studies, we conclude that all the complexes can effectively bind to CT-DNA *via*. Intercalative mode.

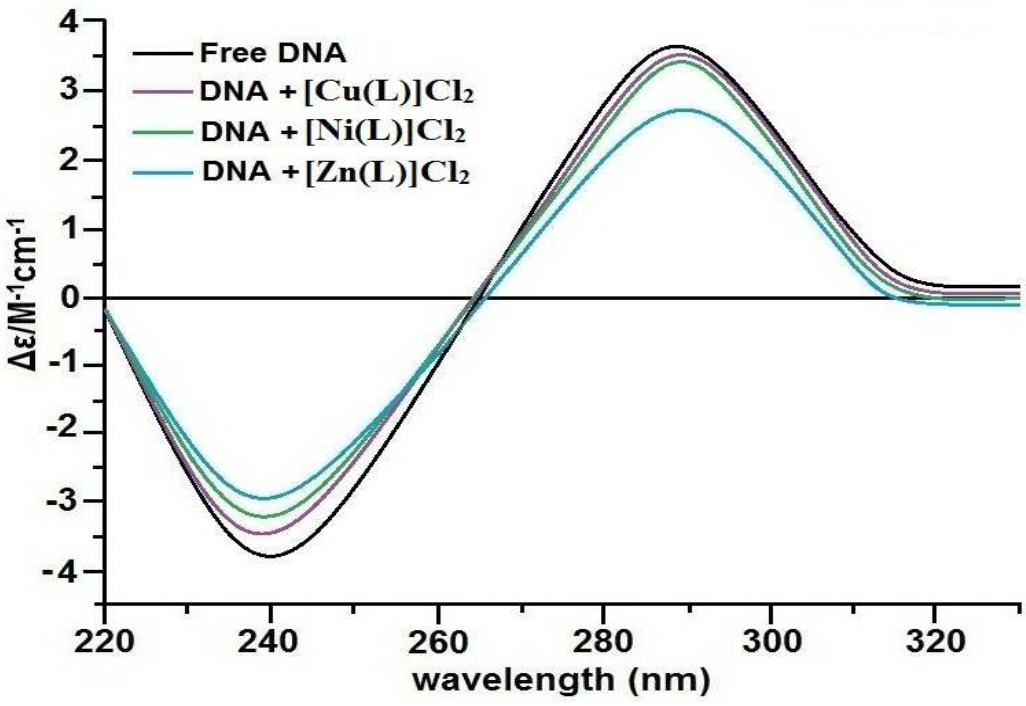

Fig. 10. CD spectra recorded over the wavelength range 220-320 nm for solutions containing 2:1 ratio of CT-DNA (200 µM) and mononuclear $[Ni(L)]Cl_2$, $[Cu(L)]Cl_2$ and $[Zn(L)]Cl_2$ complexes (100 µM)

3.4.4. Viscosity measurements

In order to elucidate the binding mode of macroacyclic complexes $[Ni(L)]Cl_2$, $[Cu(L)]Cl_2$ and $[Zn(L)]Cl_2$ with DNA, viscosity of DNA solutions containing varying amount of added complexes were measured. Classical intercalative mode causes a significant increase in viscosity of DNA solution due to separation of base pairs at intercalation sites and increase in overall DNA length [49].The effects of metal complexes on the viscosities of CT-DNA are shown in Fig.11. With the ratios of the investigated compounds to CT-DNA increasing, the relative viscosities of DNA increased steadily, indicating that there exist intercalation between the compounds and DNA helix. The increased degree of viscosity, which may depend on the binding affinity of compounds to DNA, follows the order $[Cu(L)]Cl_2$>$[Ni(L)]Cl_2$>$[Zn(L)]Cl_2$. On the basis of the electronic absorption titration, emission and CD spectral studies together with the viscosity measurements, we find that all four metal complexes bind to CT-DNA via an intercalative binding mode and the Cu(II) complex binds to CT-DNA more strongly.

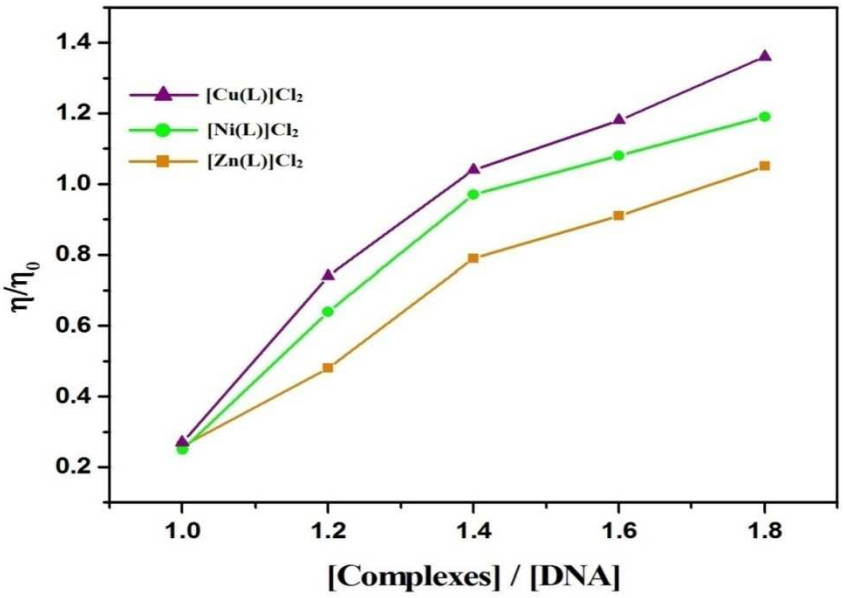

Fig. 11. Viscosity measurements of complexes $[Ni(L)]Cl_2$, $[Cu(L)]Cl_2$ and $[Zn(L)]Cl_2$

CONCLUSION

A macroacyclic ligand and its complexes have been synthesized and characterized by electronic absorption spectra, IR, ^{13}C NMR, and mass spectral analysis. All the metal ions are four coordinate and the geometry can be described as square planar. The molar conductance values reveal that all the complexes are 1:2 electrolytes in nature. DNA-binding properties of the metal(II) complexes with DNA have been investigated by UV-Vis spectra, emission spectra, CD spectra and viscosity measurements. Results indicate that all the four complexes bind to CT-DNA via an intercalative mode.

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Fig. S1. FT-IR spectra of $[Ni(L)]Cl_2$ complex

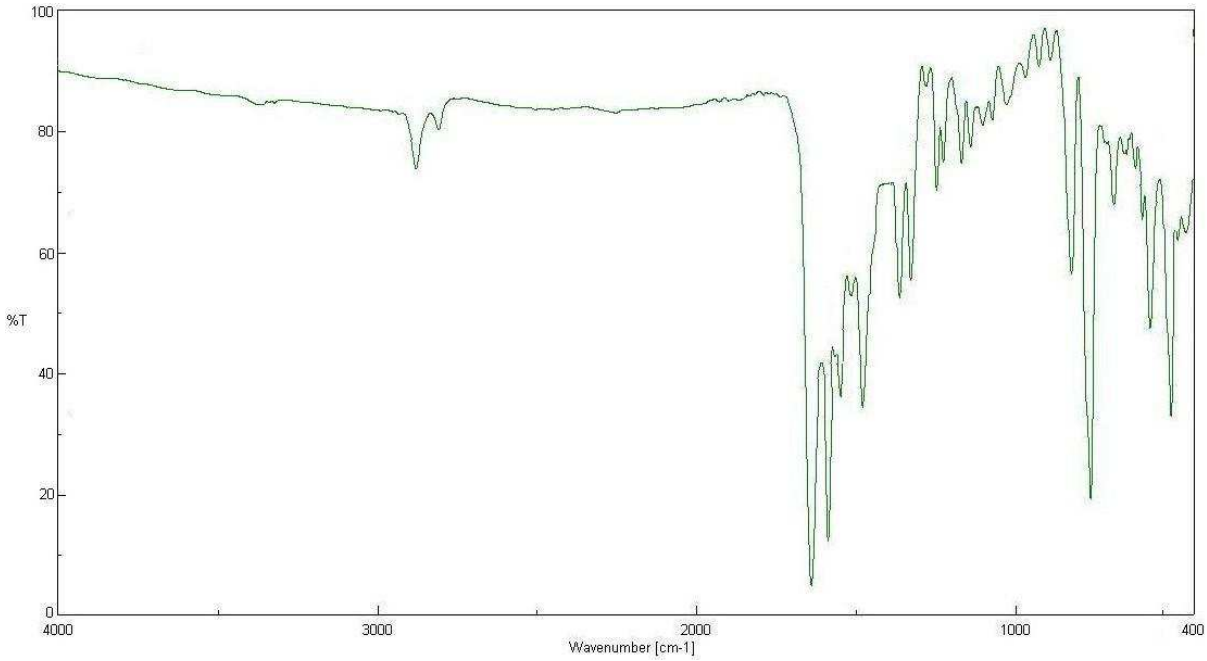

Fig. S2. FT-IR spectra of $[Cu(L)]Cl_2$ complex

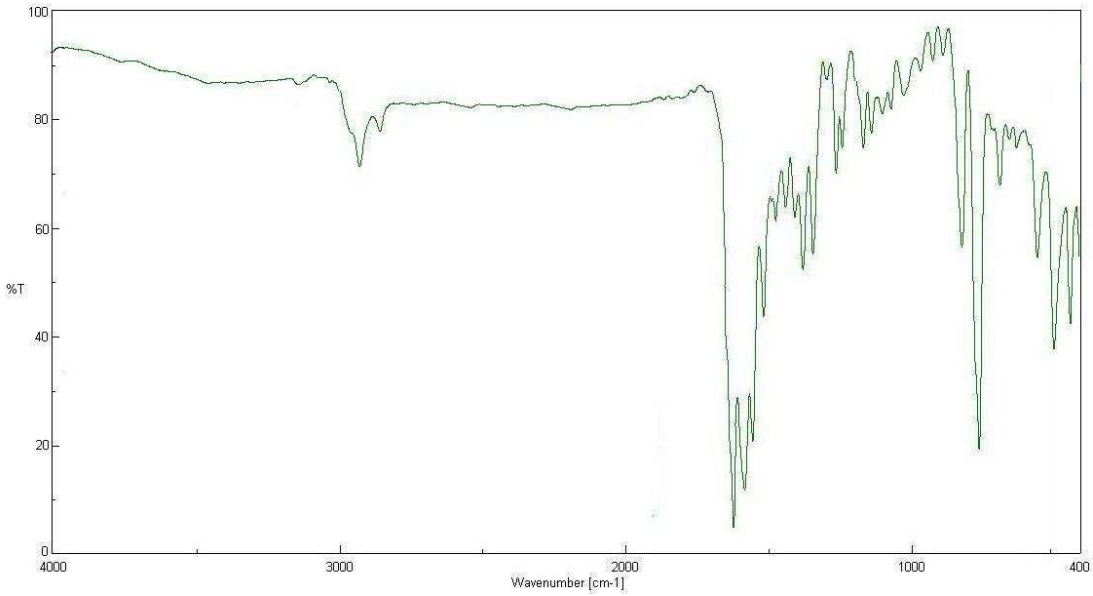

Fig. S3. FT-IR spectra of $[Zn(L)]Cl_2$ complex

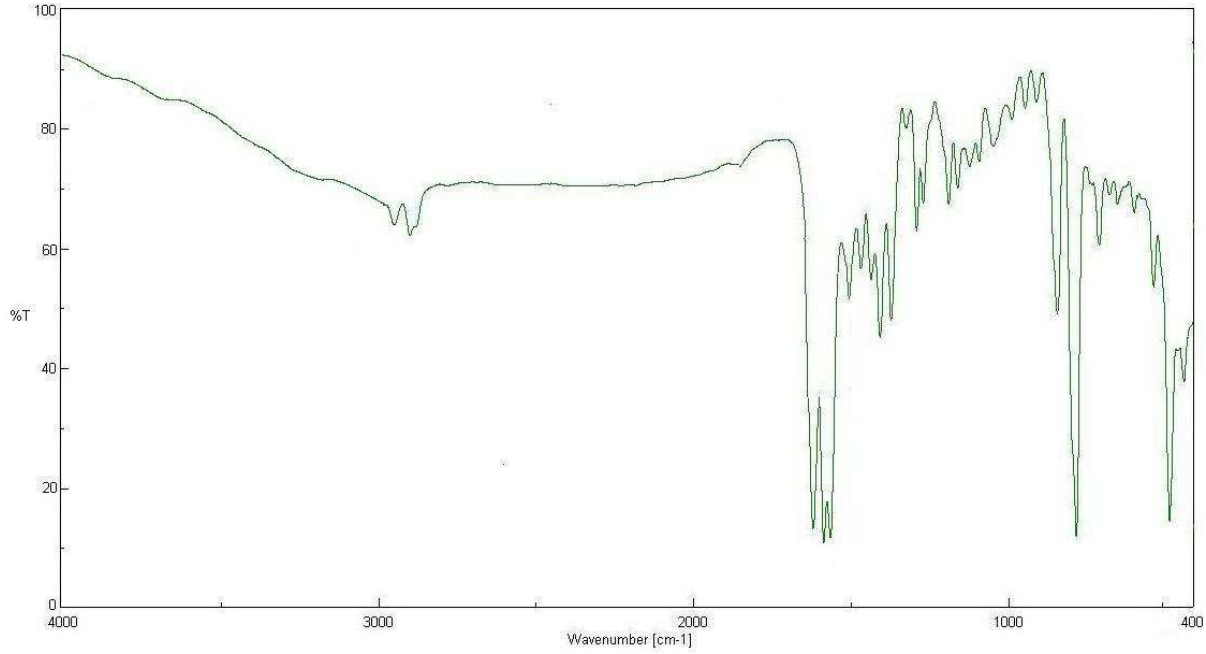

Fig.S4. Electronic spectrum of $[Co(L)]Cl_2$, $[Ni(L)]Cl_2$&$[Cu(L)]Cl_2$(200 to 500 nm)

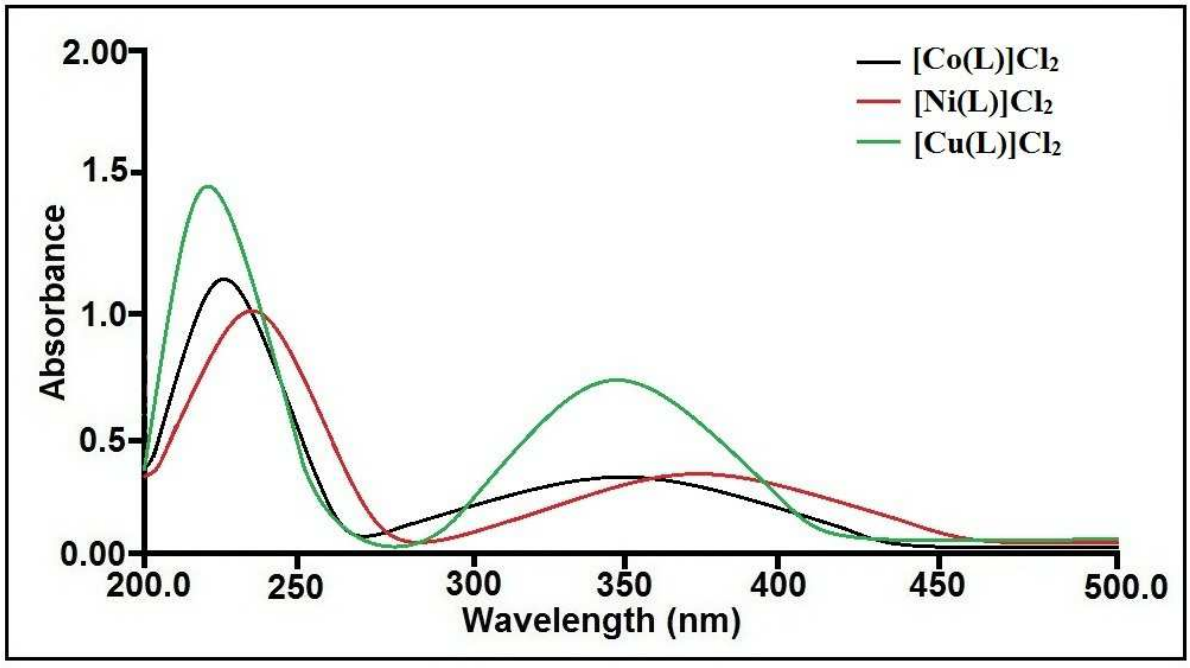

Fig. S5. Electronic spectrum of $[Co(L)]Cl_2$, $[Ni(L)]Cl_2$&$[Cu(L)]Cl_2$(500 to 800 nm)

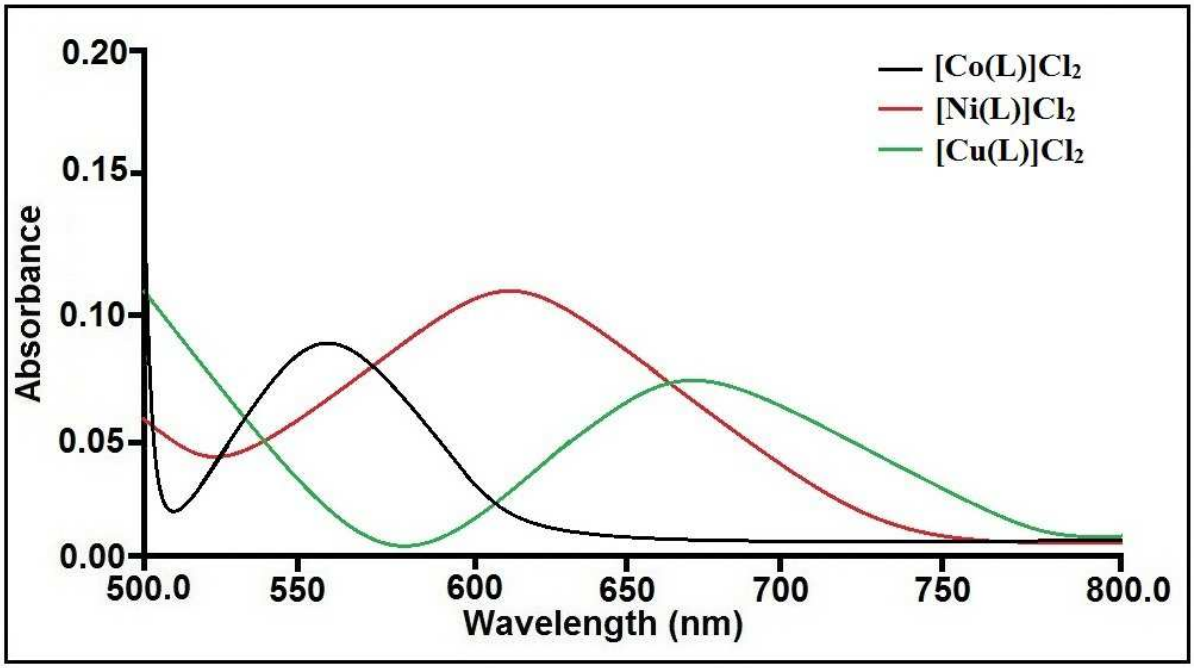

Fig.S6. Absorption spectra of complexes $[Co(L)]Cl_2$(1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-25 x 10^{-5} M) at room temperature in 50 mMTris-HCl/NaCl buffer (pH = 7.5). Arrow shows the absorbance changing upon increasing DNA concentrations

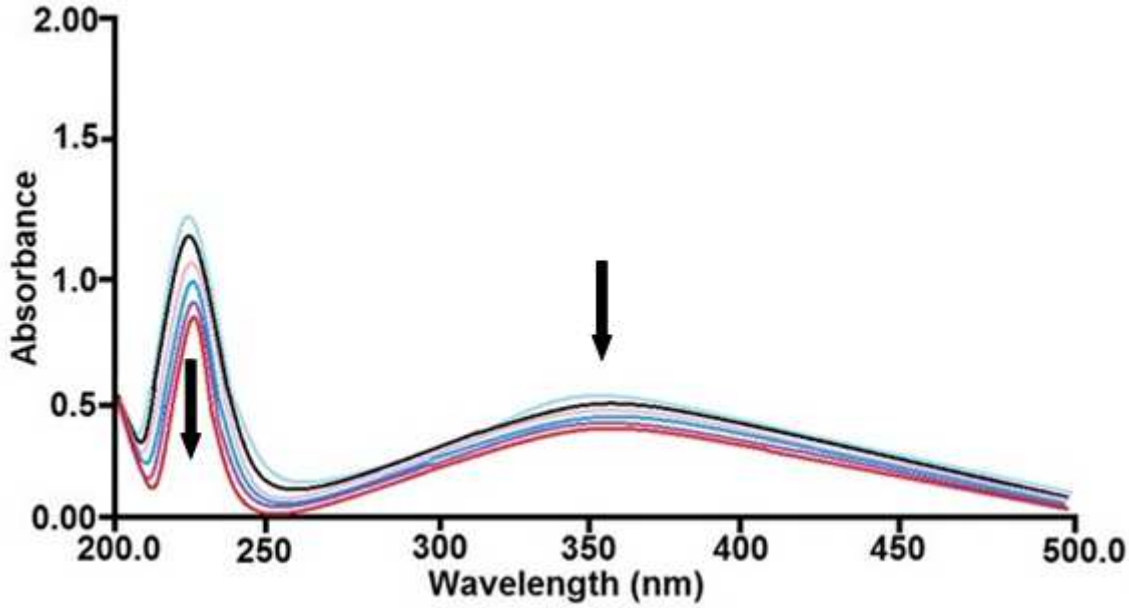

Fig. S7. Absorption spectra of complexes $[Ni(L)]Cl_2$(1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-25 x 10^{-5} M) at room temperature in 50 mMTris-HCl/NaCl buffer (pH = 7.5). Arrow shows the absorbance changing upon increasing DNA concentrations

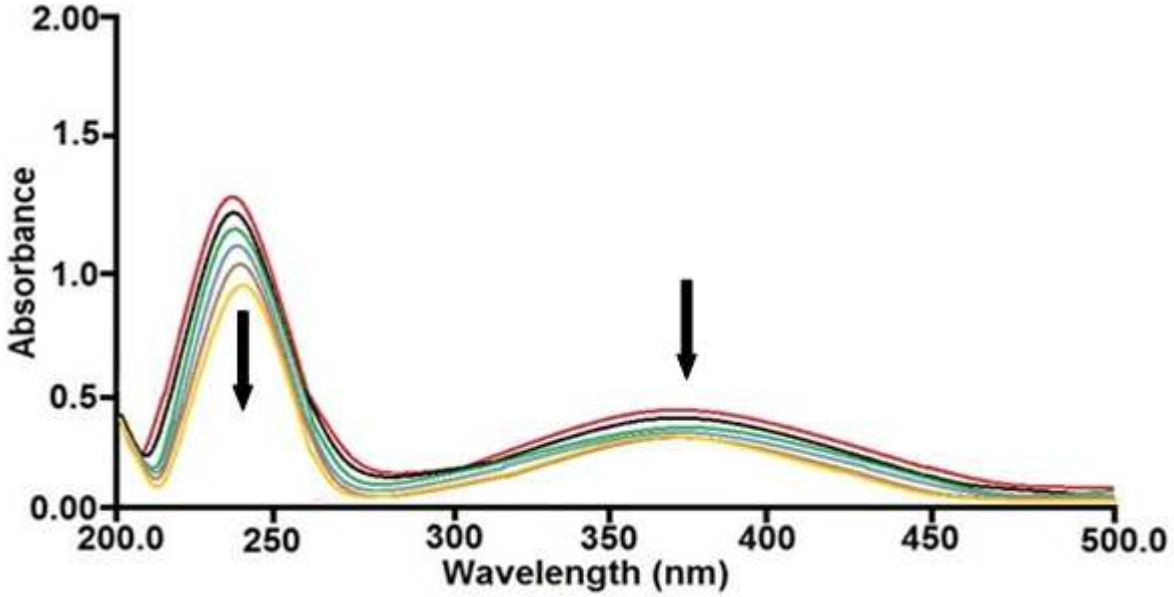

Fig. S8. Absorption spectra of complexes $[Cu(L)]Cl_2$(1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-25 x 10^{-5} M) at room temperature in 50 mMTris-HCl/NaCl buffer (pH = 7.5). Arrow shows the absorbance changing upon increasing DNA concentrations

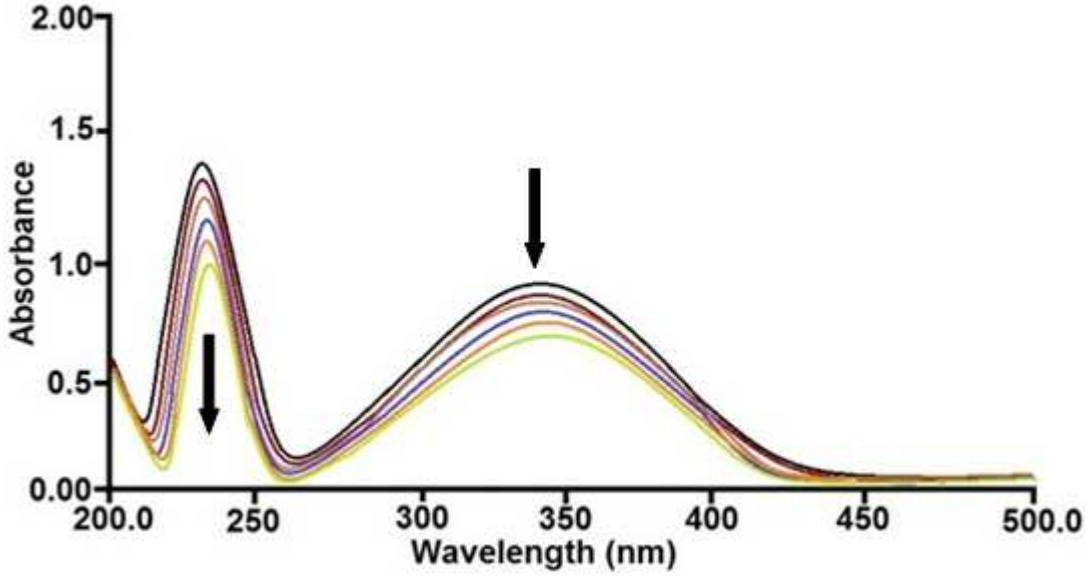

Fig. S9. Absorption spectra of complexes [Zn(L)]Cl_2(1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-25 x 10^{-5} M) at room temperature in 50 mMTris-HCl/NaCl buffer (pH = 7.5). Arrow shows the absorbance changing upon increasing DNA concentrations

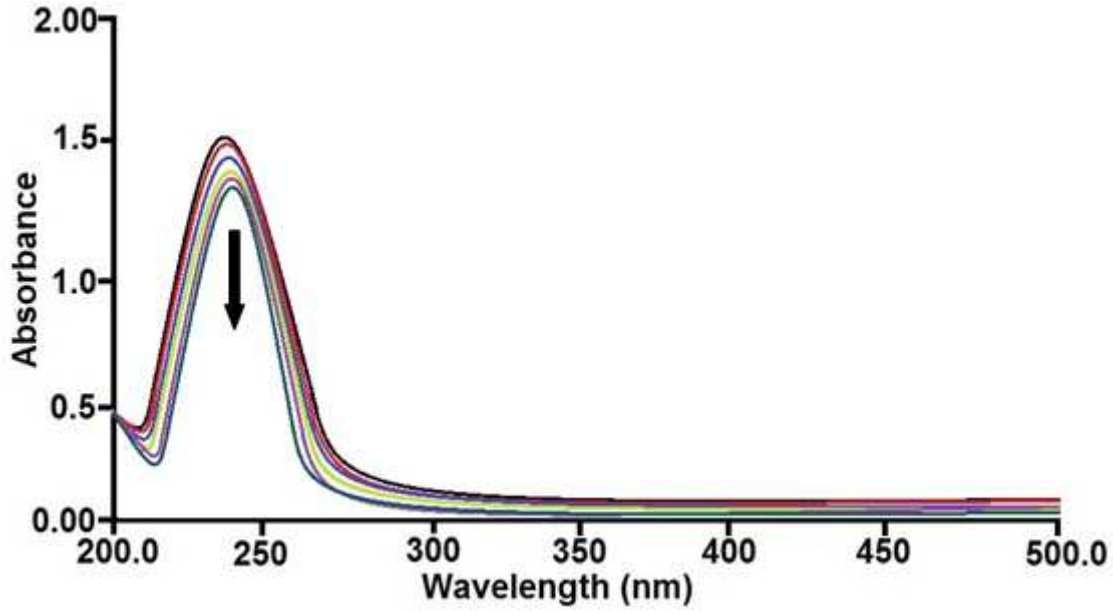

Fig.S10. Emission spectrum of EB bound to DNA in the presence of [Co(L)]Cl_2([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-30 µM, λ_{ex}= 430 nm). Arrow shows the absorbance changing upon increasing complex concentrations

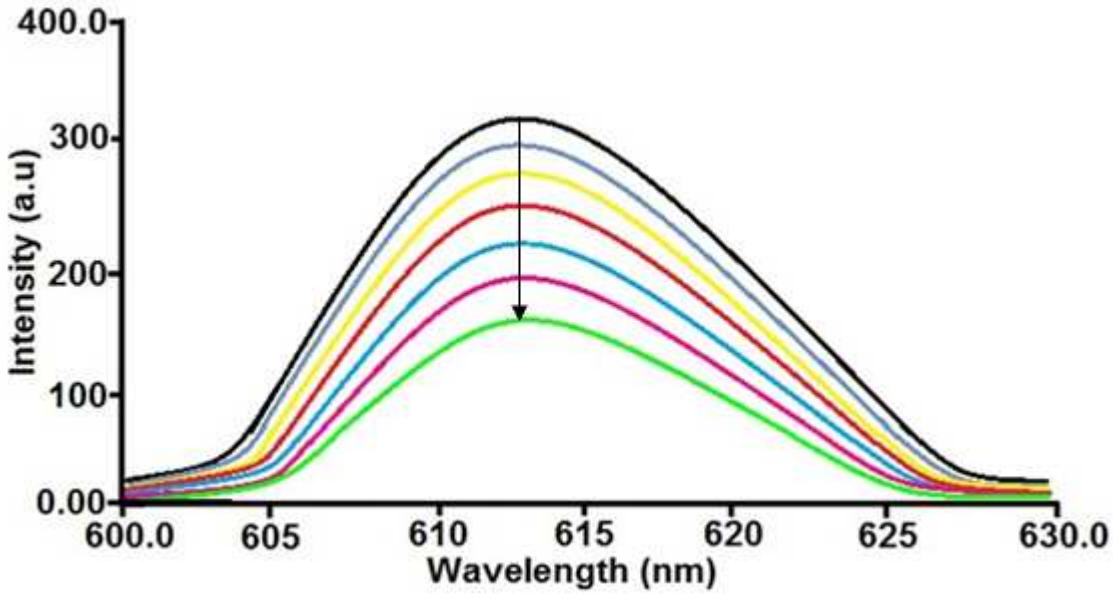

Fig. S11. Emission spectrum of EB bound to DNA in the presence of $[Ni(L)]Cl_2$([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-30 µM, λ_{ex}= 430 nm). Arrow shows the absorbance changing upon increasing complex concentrations

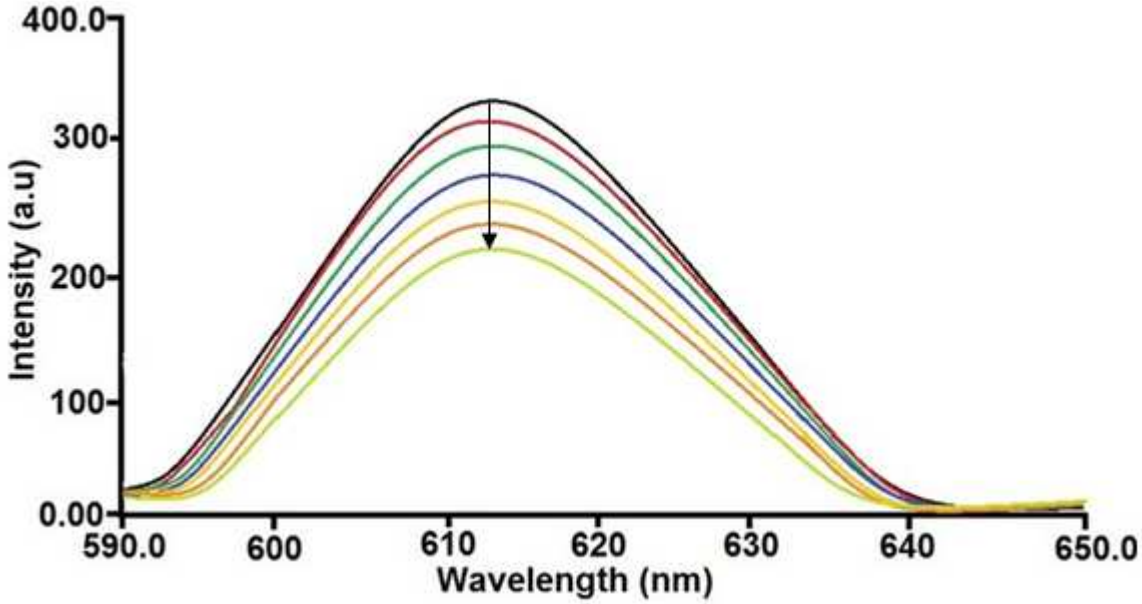

Fig. S12. Emission spectrum of EB bound to DNA in the presence of $[Cu(L)]Cl_2$([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-30 µM, λ_{ex}= 430 nm). Arrow shows the absorbance changing upon increasing complex concentrations

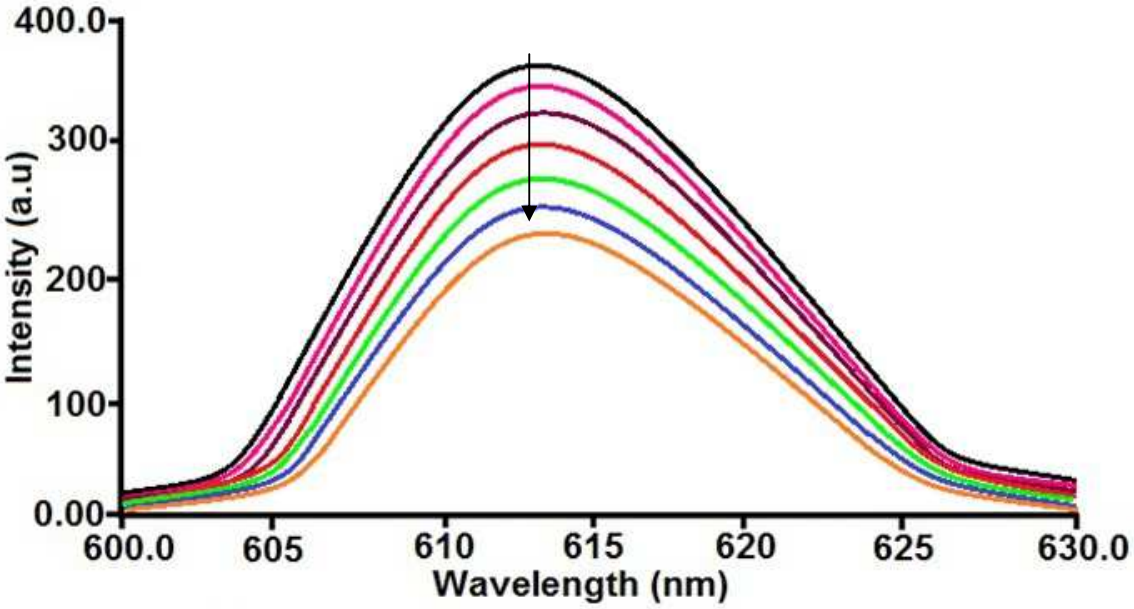

Fig. S13. Emission spectrum of EB bound to DNA in the presence of $[Zn(L)]Cl_2$([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-30 µM, λ_{ex}= 430 nm). Arrow shows the absorbance changing upon increasing complex concentrations