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Design, synthesis, characterization and DNA interaction of new Schiff base metal complexes

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ABSTRACT

A new set of Schiff base complexes derived from thenil and 1,3-diaminepropane have been synthesized and characterized by infrared (IR), mass and UV–Visible spectral analysis. The spectroscopic data of metal complexes indicated that the metal ions are complexed with azomethine nitrogen atoms. The interaction of the synthesized complexes with CT (Calf - Thymus) DNA was investigated by utilizing absorption, fluorescence spectroscopy (ethidium bromide displacement assay) Viscosity and Circular dichroic analysis. The obtained results suggest that the complexes synthesized bind to DNA via an intercalative mode and can quench the fluorescence intensity of EB bound to DNA. Considerable hypochromicity and red shifts were observed in the UV absorption band of the synthesized complexes. Binding constants (K_b) of DNA with the complexes were calculated to be 5.01 x 10^4 M^{-1}, 3.93 x 10^4 M^{-1} and 3.45 x 10^4 M^{-1}. and K_{app} of 5.82 x 10^5 M^{-1}, 4.86 x 10^5 M^{-1} and 4.04 x 10^5 M^{-1}.

Keywords: Thenil, 1,3-diaminepropane, DNA binding, Intercalative mode, Schiff base.

INTRODUCTION

Cancer is one of the fatal diseases, which claims over 6 million people each year worldwide and still increasing. The majority of drugs used for the treatment of cancer today are not 'cancer cell specific' and potently cytotoxic against normal cells. So, the most rapidly developing area of pharmaceutical research is the discovery of new drugs for cancer today. To date, cisplatin is considered to be one of the most effective and widely used anticancer drugs. Second generation platinum drugs including cisplatin and oxaliplatin have been developed for clinical application [1]. The efficacy of these drugs, including cisplatin, however, is reduced by increasing tumor resistance and in the case of cisplatin, high toxicity. This in turn affects the administration of the drugs [2]. Hence, more-efficacious, target-specific, less-toxic and non-covalently DNA-binding cytotoxic drugs are required to be developed. It is well-known that the modes of DNA non-covalent interaction with transition-metal complexes include electrostatic effect, groove binding and intercalation. Both the planarity of ligand and the coordination geometry of the metal ion play important roles in deciding the intercalating ability of complexes to DNA [3-5]. With this aim, several classes of metal complexes have been synthesized using various ligands and metal ions, and their anticancer activity has been successfully evaluated both in vitro and in vivo. Among the non-platinum complexes for metal based chemotherapy, copper, nickel and zinc complexes have been much explored due to the fact that all the metals are bio essential elements responsible for numerous bioactivities in living organism. Transition metals of Cu(II) ion plays a vital role for the development of connective tissue, nerve coverings and bone in humans. It acts as a reductant in the enzymes superoxide dismutase, cytochrome oxidase, lysil oxidase, dopamine hydroxylase and several other oxidases which reduce molecular oxygen. In biological systems, it is also a substantial catalytic co–factor for a variety of metabolic reactions, electron transfer and oxygen transport proteins such as azurin, plastocyanin, laccase and hemocyanin [6]. Nickel is present in the active sites of several important classes of metalloproteins, as either a homodinuclear or a

heterodinuclear species [7]. Zinc is also one of the essential metal ions for the growth and repair of our body tissues and it serves an important structural role in DNA binding proteins and stabilizing the acceptable binding sites [8].

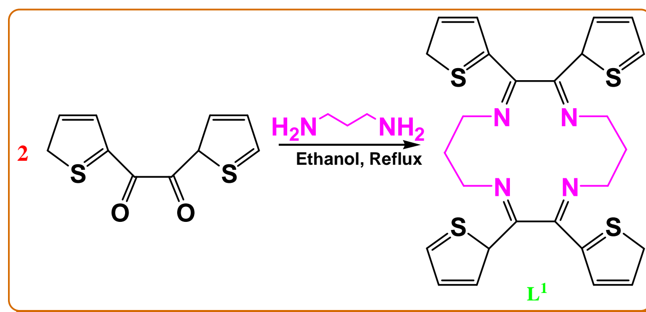
DNA is an important drug target and it regulates many biochemical processes that occur in the cellular system. The different loci present in the DNA are involved in various regulatory processes such as gene expression, gene transcription, mutageneis, carcinogenesis, etc [9].Transition metal complexes bind to DNA by both covalent and non-covalent interactions. Covalent binding involves the coordination of the nitrogenous base or the phosphate moiety of the DNA to the central metal ion and is possible in complexes where the metal is coordinatively unsaturated or is coordinated to substitutionally labile ligands. The three different non-covalent binding modes are intercalation, which involves the stacking of the molecule between the base pairs of DNA, groove binding, which comprises the insertion of the molecule into the major or minor grooves of DNA and electrostatic or external surface binding. Upon binding to DNA, the small molecules are stabilized through a series of weak interactions such as π-stacking interactions of aromatic heterocyclic groups between the base pairs (intercalation), hydrogen bonding and van der Waals interactions of functional groups bound along the groove of the DNA helix [10]. The binding mode and strength are sensitively dependent on the shape, planar area, size and electron density of the interacting aromatic rings. So, a systematic study of the influence of varying parameters on the interaction of metal complexes with DNA would be valuable in the rational design of new drugs and therapeutic reagents targeted to DNA, and it is possible to systematically vary parameters of interest by changing the properties of the intercalating groups [11-13].

Recently, our group has continuously been interested in introducing the new schiff base complexes of N_4 systems. For examples, we have reported the synthesis, DNA binding of N_4 containing Schiff base Cu(II) ,Ni(II), Zn(II) complexes [14,15]. Here in, we report the synthesis, spectral characterization and DNA binding activities of new schiff base ligand and their respective copper, nickel and Zinc(II) complexes.

EXPERIMENTAL SECTION

Chemicals and methods:
Thenil, metal chlorides and 1,3-diaminepropane was purchased from Aldrich. The CT - DNA was purchased from Bangalore Genei (India). Elemental analysis was recorded on a Carlo Erba model 1106 elemental analyzer. FT-IR spectra were obtained from Perkin Elmer FTIR spectrometer with the samples prepared as KBr pellets. UV–Visible spectra were recorded using Perkin Elmer Lambda 35 spectrophotometer operating in the range of 200–1000 nm with quartz cells and ε values are expressed in M^{-1} cm^{-1}. The emission spectra were recorded on a Perkin Elmer LS-45 fluorescence spectrometer. Mass spectral analysis was performed in Q-TOF Mass Spectrometer. Viscosity measurements were recorded using a Brookfield Programmable LV DVII+ viscometer. Tris (hydroxymethyl) aminomethane–HCl (Tris–HCl) buffer solution was prepared by using deionized and sonicated triple distilled water.

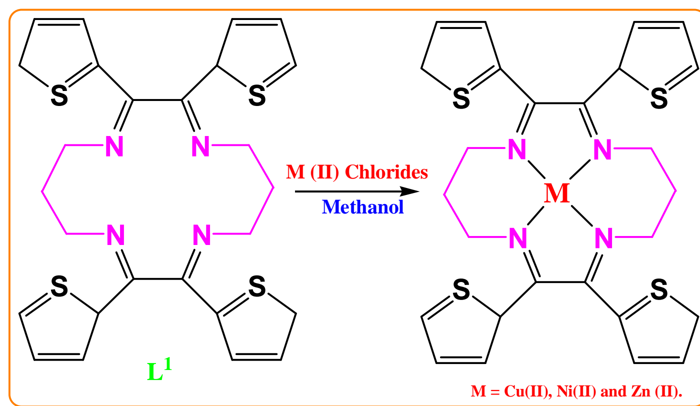
General procedure for the synthesis of compounds:
Synthesis of the ligand:
A methanolic solution (20 mL) of thenil (0.002 mol, 0.270 gm) was slowly added to a methanolic solution (20 mL) of 1,3-diaminepropane (1 gm, 0.0054 mol) with constant stirring as shown in Scheme 1. This reaction mixture was stirred for 6 h, and then refluxed for 8 h on water bath. Removal of solvent at reduced pressure gave the crude product. The product was washed twice with diethyl ether and recrystallized from chloroform.



Scheme 1: Synthesis of Schiff base Ligand

All complexes were synthesized using the same procedure as given below:
A methanolic solution (20 mL) of ligand (L^1) (1.0 gm, 0.0020 mol) was added slowly to an equimolar amount of appropriate metal chloride salts in methanol (20 mL) with constant stirring. The mixture was stirred for 4 h, and the reaction was carried out for 6 h under reflux as represented in Scheme 2. After cooling the reaction mixture to room

temperature, the resulting product was washed with diethyl ether and dried in vacuo. Finally the complexes were washed with petroleum ether and dried in vacuum desiccators over anhydrous $CaCl_2$.



Scheme 2: Synthesis of Schiff base Ligand (L^1) and their respective metal complexes

DNA binding experiments.
Absorption spectra:
Absorption spectra were recorded on Perkin Elmer Lambda 35 spectrophotometer operating in the range of 200–1000 nm with quartz cells. Absorption titrations were performed by keeping the concentration of the complexes constant (40 μM), and by varying [CT DNA] from DNA (0, 40, 80, 120, 160, 200, 300 and 400) mM.. For the complexes the binding constants (K_b), have been determined from the spectroscopic titration data using the following equation:

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

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]/ (ε_a - ε_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 [16].

Fluorescence spectroscopy
The relative bindings of complexes to CT-DNA were studied with an EB-bound CT-DNA solution in 5 mM Tris–HCl/50 mM NaCl buffer (pH=7.2). The fluorescence spectra were recorded at room temperature with excitation at 530 nm and emission at about 612 nm. The experiments were carried out by titrating complexes into EB–DNA solution containing 5×10^{-5}M EB and 5×10^{-5}M CT-DNA. Quenching of the fluorescence of EthBr bound to DNA were measured with increasing amount of metal complexes as a second molecule and Stern–Volmer quenching constant K_{sv} was obtained from the following equation: (2)

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

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. Ksv 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 $K_{EB} = 1.0 \times 10^7 M^{-1}$ ([EB] = 3.3 μM) [17].

Viscosity measurements
Viscosity experiments were carried out at 30.0ºC ± 0.1ºC. CT-DNA samples of approximately 0.5mM were prepared by sonicating in order to minimize complexities arising from CT-DNA flexibility and by varying the concentration of the complexes (0.01 mM, 0.02 mM, 0.03 mM, 0.04 mM, 0.05 mM) [18]. Flow time was measured with a digital stopwatch three times for each sample and an average flow time was calculated. 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.

CD spectrophotometric studies

The CD spectra of CT-DNA in the presence or absence of complex were collected in Tris–HCl buffer (pH=7.2) containing 50 mM NaCl at room temperature. The spectra were recorded in the region of 220–320 nm for 200 µM DNA in the presence of 100 µM of the complexes. Each CD spectrum was collected after averaging over at least three accumulations using a scan speed of 100 nm min^{-1} and a 1 s response time. Machine plus cuvette base lines, and CD contribution by the CT-DNA and Tris buffer were subtracted and the resultant spectrum zeroed 50 nm outside the absorption bands. 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.

RESULTS

FT-IR spectral analysis

The IR spectra of metal complexes are like to each other, except for slight shifts and intensity changes of few vibration peaks caused by different metal(II) ions, which indicate that the complexes have similar structure. Fig. 1 and 2 shows FT-IR spectra of L^1 and the complexes. In order to study the bonding mode of Schiff base ligand L^1 to the metal complexes, the IR spectrum of the free ligand is compared with the spectra of the complexes. A sharp band at 1644 cm-1 in the IR spectrum of the Schiff base ligand (CH=N) shifts downward by about 1625 - 1615 cm^{-1} in all the complexes indicating coordination through azomethine nitrogen. The unaltered position of bands around 2650 cm-1 due to SH moiety of the thenil in all the metal complexes indicates that these groups are not involved in coordination. Additional support for the formation of the complexes were provided by the existence of medium intensity bands in the region 460 - 480 cm^{-1} assigned to the M-N [19].

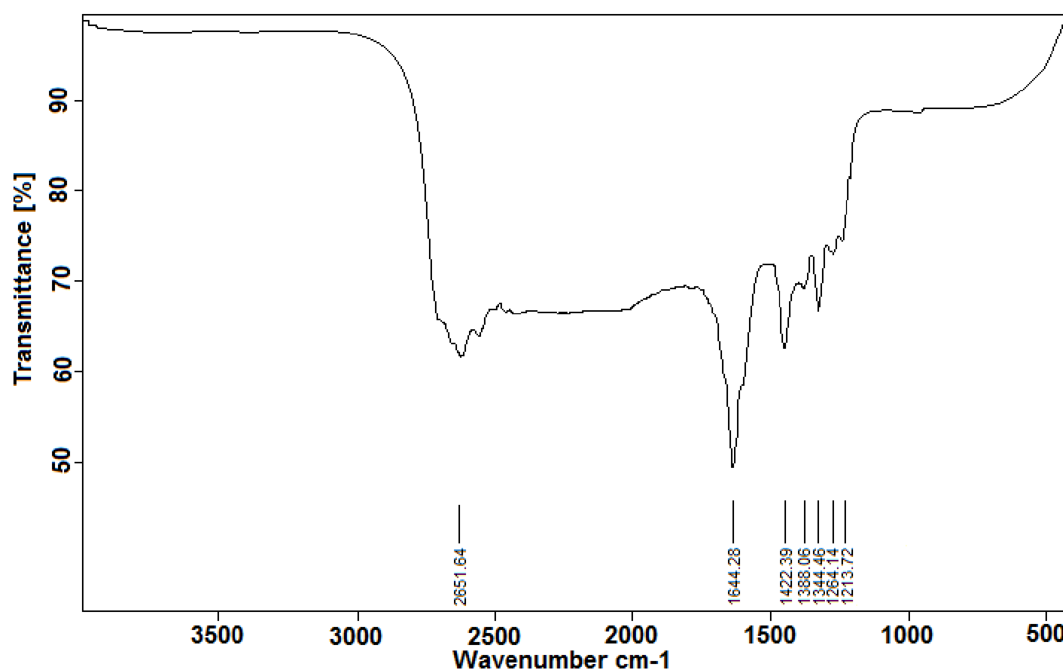


Fig. 1 FT-IR spectrum of the Schiff base ligand (L^1)

Mass spectra.

The molecular ion peak [M^+] at m/z = 524 confirms the molecular weight of the macrocyclic Schiff base ligand $C_{26}H_{28}N_4S_4$. The peaks at m/z = 386, 248, 192, 108 and 54 corresponds to the various fragments $C_{20}H_{26}N_4S_2$, $C_{14}H_{24}N_4$, $C_{10}H_{16}N_4$ and $C_2H_2N_2$ respectively as shown in Fig.3. This confirms the molecular structure of the ligand.

The molecular ion peak [M^+] at m/z = 588 confirms the molecular weight of the macroacyclic Schiff base Cu(II) complex $C_{26}H_{28}N_4S_4Cu$. The peaks at m/z = 501, 450, 386, 312, 281, 255 and 129 corresponds to the various fragments $C_{20}H_{26}N_4S_2Cu$, $C_{14}H_{24}N_4Cu$ and $C_{10}H_{16}N_4Cu$ respectively as shown in Fig.4 and Fig. S1 and S2. The molecular ion peak [M^+] at m/z = 590 and 583, confirms the molecular weight of the macroacyclic Schiff base Zn(II) and Ni(II) complex $C_{24}H_{20}N_4S_4M$ [M = Zn and Ni]. The type of fragmentation observed in Zn(II) and Ni(II) complex was similar with that of the Cu(II) complex.

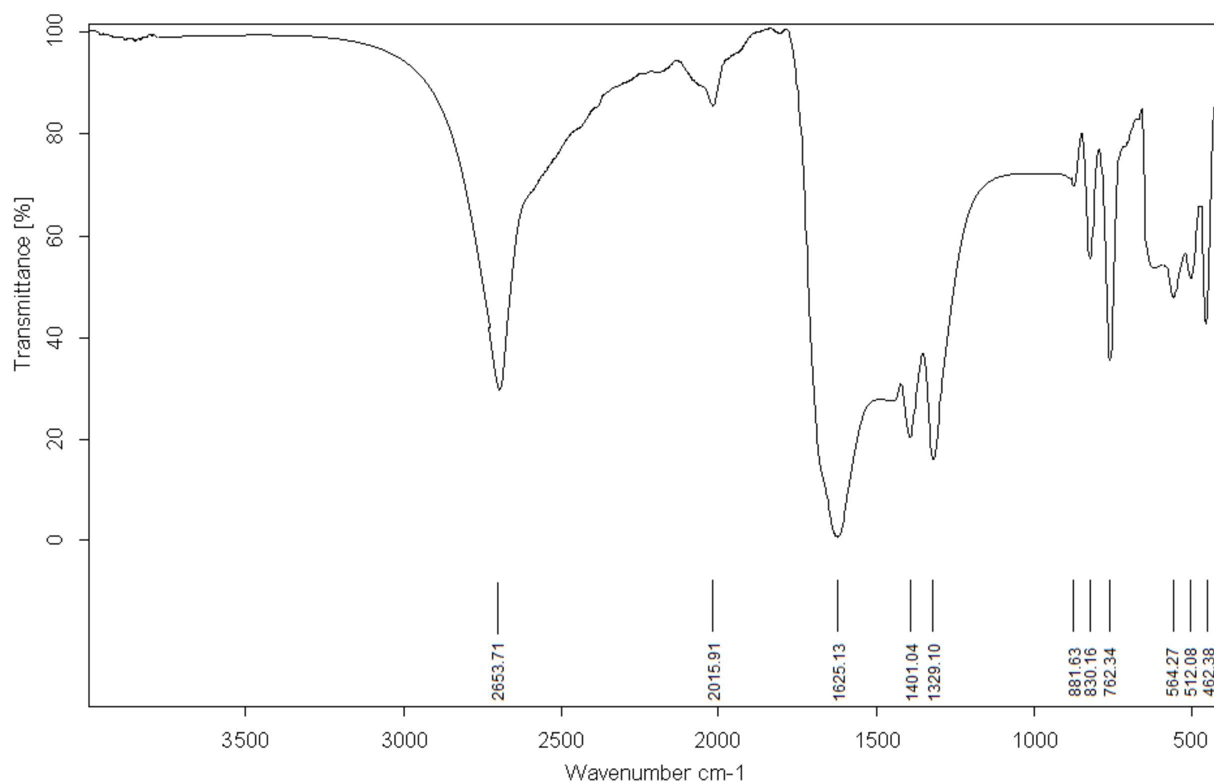


Fig. 2 FT-IR spectrum of the Schiff base Copper complex

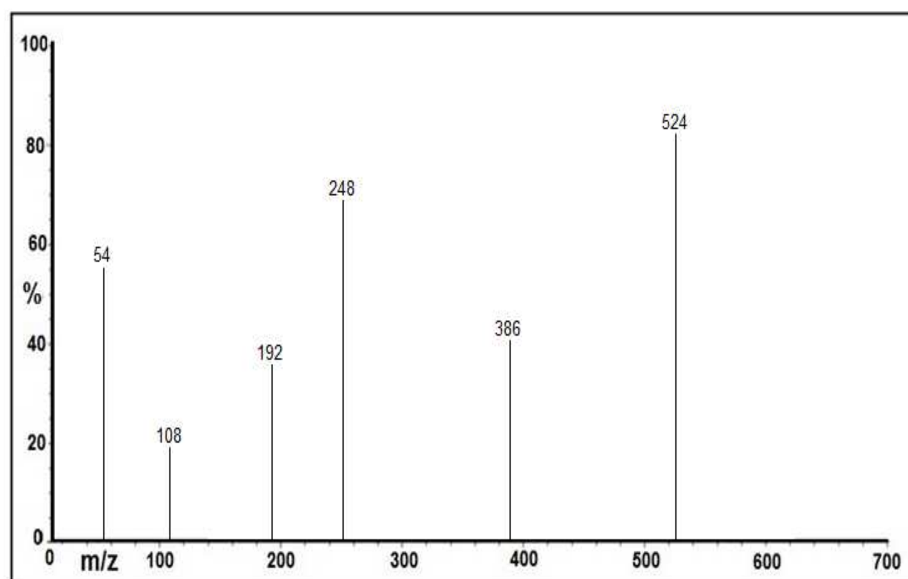


Fig. 3 Mass spectrum of Schiff base ligand (L^1)

Electronic spectral analysis of the metal complexes

The electronic spectra provide quick and reliable information in the evaluation of results furnished by other methods of structural investigations like FT-IR and Mass spectroscopy about the ligand arrangements in transition metal complexes. The electronic absorption spectra of the synthesized schiff base ligand and their respective complexes were recorded in acetonitrile solution in the range of 200 - 800 nm regions. The electronic spectra of the free Schiff base ligand (L^1) exhibited two characteristic bands around 272 nm and 348 nm as shown in Figure 5 (a). The band present in the region 270 nm are assigned to intraligand π-π^* transition while the band at 350 nm is assigned to the n- π^* transition which is characteristic of azomethine (C=N) function of the schiff base [20]. The spectra of the mononuclear Cu(II) and Ni(II) complex showed absorption bands at 425 and 630 nm which could be attributed to the $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions characterized Cu(II) ion in a square-planar geometry. The electronic

spectrum of Ni(II) complex exhibited two d-d bands around 450 nm and 680 nm (Figure 5 b) corresponding to $^1A_{1g} \rightarrow {}^1A_{2g}$ and $^1A_{1g} \rightarrow {}^1B_{1g}$ revealing the square planar geometry [21].

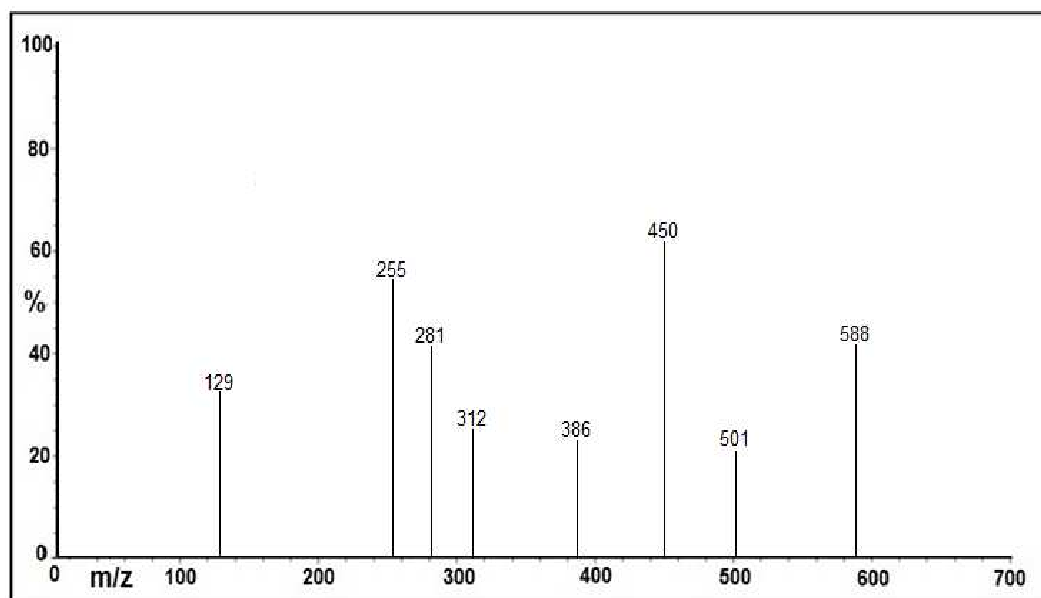


Fig. 4 ESI-Mass spectrum of Cu(II) complex

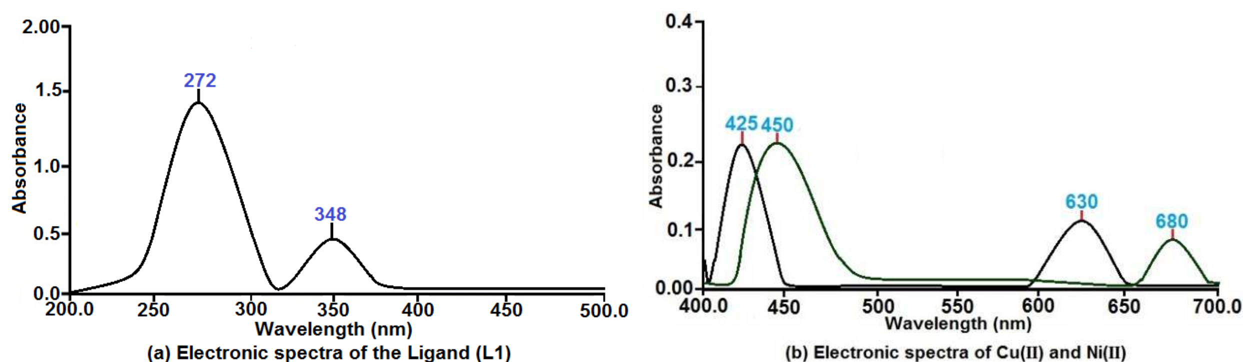


Fig. 5 Electronic spectrum of the (a) ligand (L), (b) Cu(II) and Ni(II) complexes

DNA binding experiments

Absorption spectral studies

Electronic absorption spectroscopy is universally employed to determine the binding characteristics of metal complex with DNA. The absorption spectrum of the complexes in absence and presence of CT-DNA are shown in Fig. 6 and Fig S3 and S4. When an incremental concentration of CT-DNA solution was added to a fixed concentration of the metal complexes solution (20mM), a decrease in absorption intensities (hypochromism) of LMCT band at 340 nm and hyperchromism at 270 nm with a slight red shift was observed. The coupling π orbital was partially filled by electrons, thus decreasing the transition probabilities and concurrently resulting in hypochromism [22]. Hypochromism and bathochroism is usually observed when a complex binds to DNA through intercalation as a consequence of strong staking interaction between an aromatic chromophore and a base pair of DNA. The extent of the hypochromism commonly reflects the intercalative binding strength [23].On the other hand, according to the literature report, hypochromism indicates a strong interaction between the electronic states of the chromophore and that of DNA bases. Since the decrease in the strength of the electronic interaction is expected as cube of the distance between the chromophore and the DNA bases [24]. The observed large hypochromism in our experiment strongly suggests a close adjacency of complex to the DNA bases.

In order to affirm quantitatively the affinity of the complex bound to DNA, the intrinsic binding constants K_b of the complex with DNA was obtained by monitoring the changes in absorbance at 277 nm for the title complex with increasing concentration of DNA as shown in inset of fig.6. The K_b values obtained for the synthesized complexes are as follows: for Cu(II) complex, (ii) for Ni(II) complex 5.01 x 10^4 M^{-1}, 3.93 x 10^4 M^{-1} and for Zn (II) complex is 3.45 x 10^4 M^{-1}.

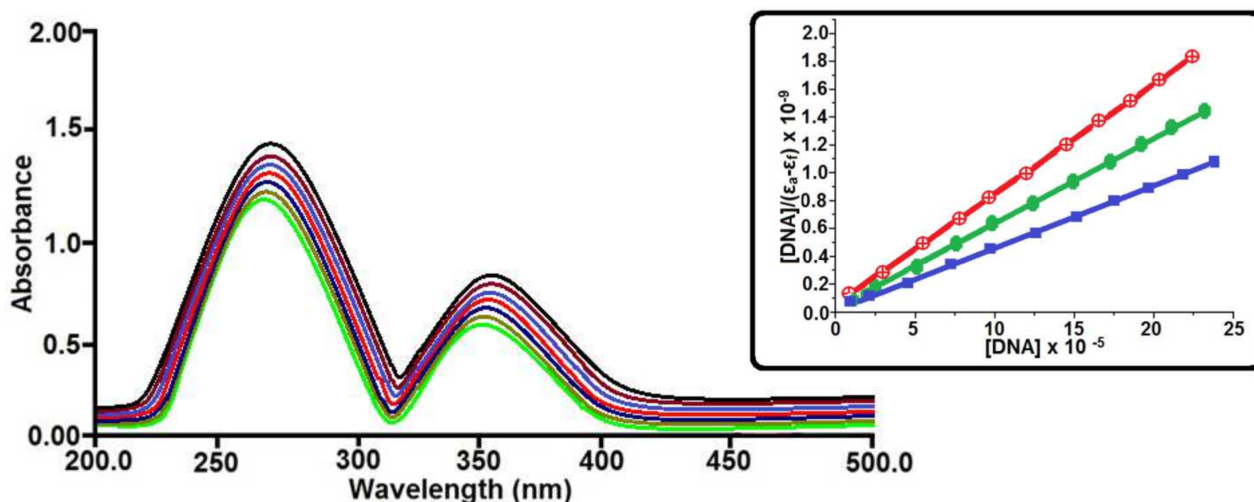


Fig. 6 Absorption spectra of complex Cu(II) (1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10^{-3} M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5). The Inset shows the plots of [DNA] / (ϵ_a-ϵ_f) versus [DNA] for the titration of DNA with Cu(II), Ni(II) and Zn(II) complexes

Fluorescence spectral studies

The fluorescence spectroscopy technique is an effective method to study metal interaction with DNA. Ethidium bromide (EB) is one of the most sensitive fluorescence probes that can bind with DNA [25]. EB does not fluoresce, but the emission intensity of EB in the presence of DNA is greatly enhanced, due to strong intercalation between the adjacent DNA base pairs. It was previously reported that this enhanced fluorescence can be quenched, at least partly, by the addition of a second molecule [26]. The extent of fluorescence quenching of EB bound to DNA is utilized to determine the extent of binding between the second molecule and DNA. In our experiments, as depicted in Fig. 7 for complexes the fluorescence intensity of EB show a remarkable decreasing trend with the increasing concentration of the complexes, indicating that some EB molecules are released from EB-DNA complex after an exchange with the complexes synthesized which result in the fluorescence quenching of EB. This may be due either to the metal complex competing with EB for the DNA-binding sites thus displacing the EB (whose fluorescence is enhanced upon DNA binding) or it should be a more direct quenching interaction on the DNA itself. The extent of quenching of the fluorescence of ethidium bromide bound to DNA would reflect the extent of DNA binding of the complexes. The quenching plots (insets of respective Fig. 7) illustrate that the fluorescence quenching of EB bound to DNA by the synthesized complexes is in linear agreement with the Stern–Volmer relationship, which corroborates that the two complexes bind to DNA. The apparent binding constants (K_{app}) have been estimated as for Cu(II) complex is 5.82 x 10^5 M^{-1}, for Ni(II) complex is 4.86 x 10^5 M^{-1} and for Zn(II) complex is4.04 x 10^5 M^{-1}.

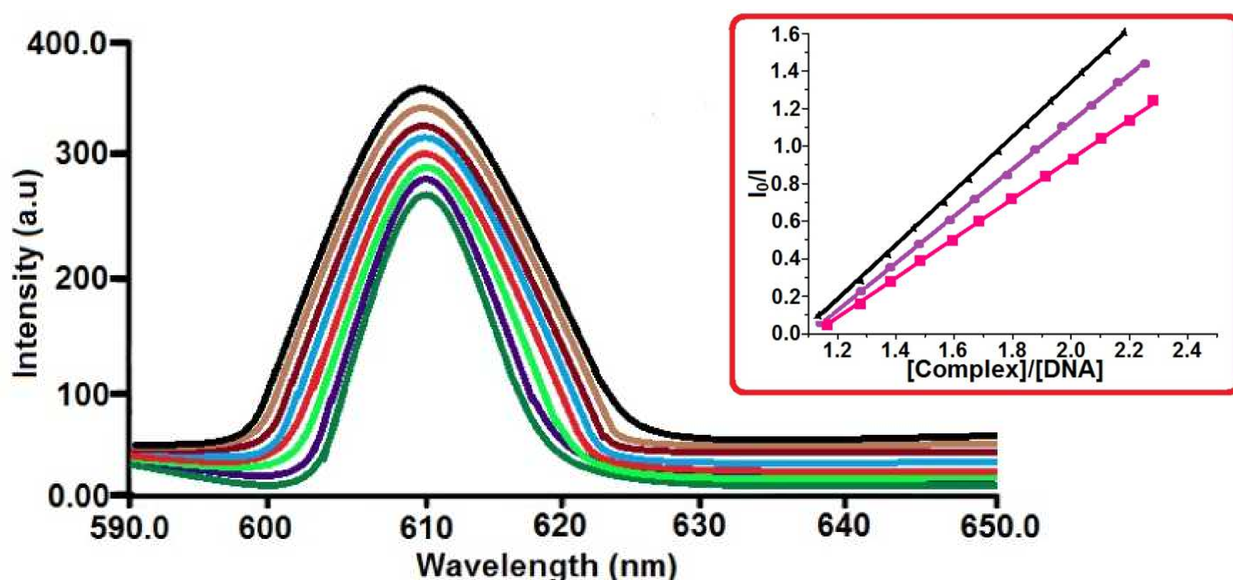


Fig. 7 Emission spectrum of EB bound to DNA in the presence of Cu(II): ([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-25 µM, λex= 440 nm). Inset shows the plots of emission intensity I_o / I *vs* [DNA] / [complex] for the titration of DNA with Cu(II), Ni(II) and Zn(II) complexes

Viscosity measurements

Furthermore, the interactions between the complexes and DNA were investigated by viscosity measurements. Optical photophysical probes provided necessary, but not sufficient clues to support a binding model. Hydrodynamic measurements that were sensitive to length change (i.e., viscosity and sedimentation) were regarded as the least ambiguous and the most critical tests of binding mode in solution in the absence of crystallographic structural data [27]. A classical intercalation mode resulted in the lengthening of the DNA helix as the base pairs were separated to accommodate the binding complex leading to an increase in DNA viscosity. In contrast, a partial and/or non-classical intercalation could bind (or kink) the DNA helix and reduce its effective length and, concomitantly, its viscosity. [28]. The effect of increasing concentrations of com plexes 1 and 2 on the specific relative viscosity of DNA is shown in Fig. 8. The relative viscosity of DNA increases steadily with an increase in the concentration of the complexes and this result parallels the pronounced absorbance hypochromism that was observed for the complexes and further confirms their intercalative mode of binding. The results suggest that complex partial intercalate between the base pairs of DNA, which is consistent with our foregoing hypothesis.

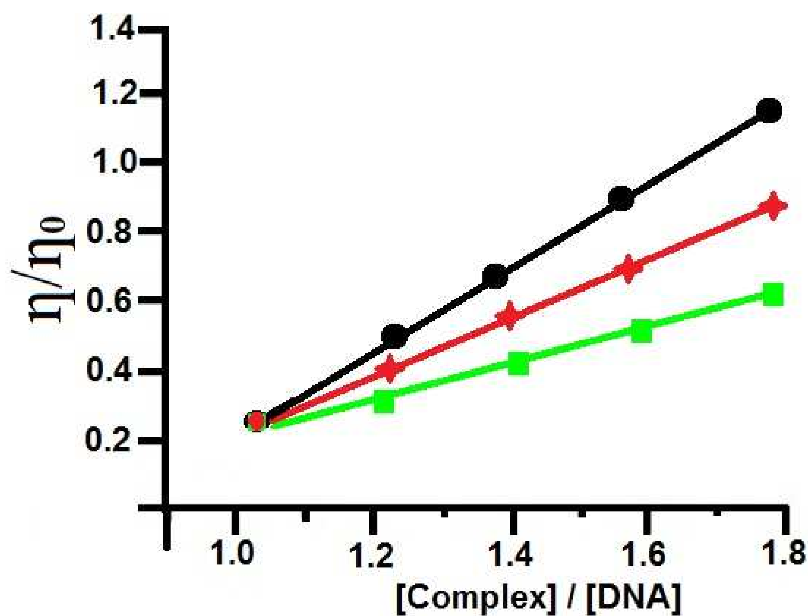


Fig. 8 Viscosity measurements of the Cu(II), Ni(II) and Zn(II) complexes

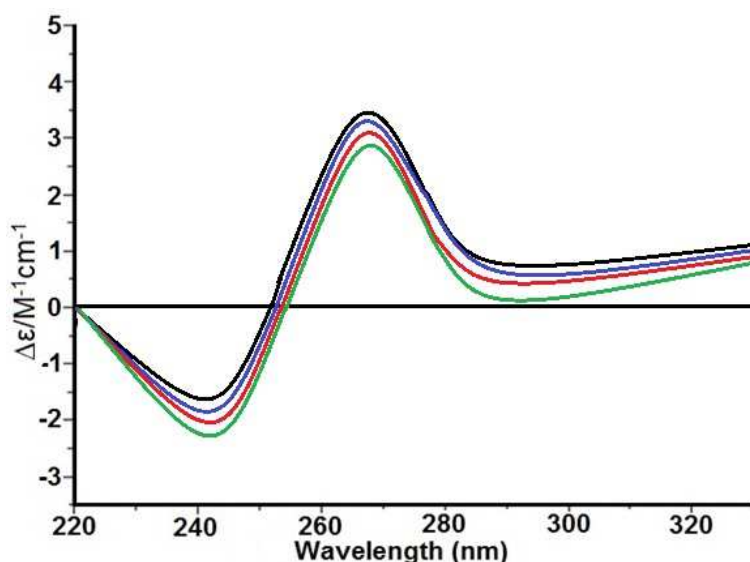


Fig. 9 CD spectra recorded over the wavelength range 220-320 nm for solutions containing 2:1 ratio of CT-DNA (200 µM) and Cu(II), Ni(II) and Zn(II) complexes (100 µM)

Circular Dichroism

Circular dichroic spectral techniques may give us useful information on how the conformation of the DNA chain is influenced by the bound complex. The CD spectrum of CT DNA consists of a positive band at 275 nm that can be due to base stacking and a negative band at 245 nm that can be due to helicity and it is also characteristic of DNA in a right-handed B form [29]. CD spectra of CT-DNA in the UV region show a distinct change in the spectral band corresponding to the B-DNA conformation (Fig. 9). The observed decrease in the positive DNA dichroic signal is likely due to a transition from the extended nucleic acid double helix to the more denatured structure [30]. It should be noted that hydrophobic base stacking in oligomers and polymers results in close contacts and Coulombic interactions that give rise to intense CD bands corresponding to each base transition [31]. Therefore the intercalated complexes which disrupt interactions between DNA bases and weaken base stacking should induce a decrease in the intensities of CD bands. It should be noted that, significant reductions in molar ellipticity in the negative band (245 nm) when complexes are present are related to destabilization and helix unwinding [32]. The results suggest that complex partial intercalate between the base pairs of DNA, which is consistent with our foregoing hypothesis.

CONCLUSION

Taken together, three new schiff base complexes have been synthesized and characterized using various spectroscopic techniques like IR, mass and absorption spectroscopy. In this work, we explored the binding interaction of the synthesized Cu(II), Ni(II) and Zn(II) complexes with CT-DNA in physiological buffer using UV–Vis and fluorescence spectroscopic techniques. The intercalative binding of mentioned complex with DNA was deduced by taking account of relevant UV–Vis absorption spectra, circular dichroism, fluorescence spectra and viscosity measurements.

Abbreviations

DNA	- Deoxyribose nucleic acid
CT	- Calf Thymus DNA
Cu	- Copper
Ni	- Nickel
Zn	- Zinc
Tris-HCl	-Tris(hydroxymethyl)aminomethane
EB	- Ethidium Bromide
CD	- Circular Dichroism

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SUPPLEMENTARY FIGURES

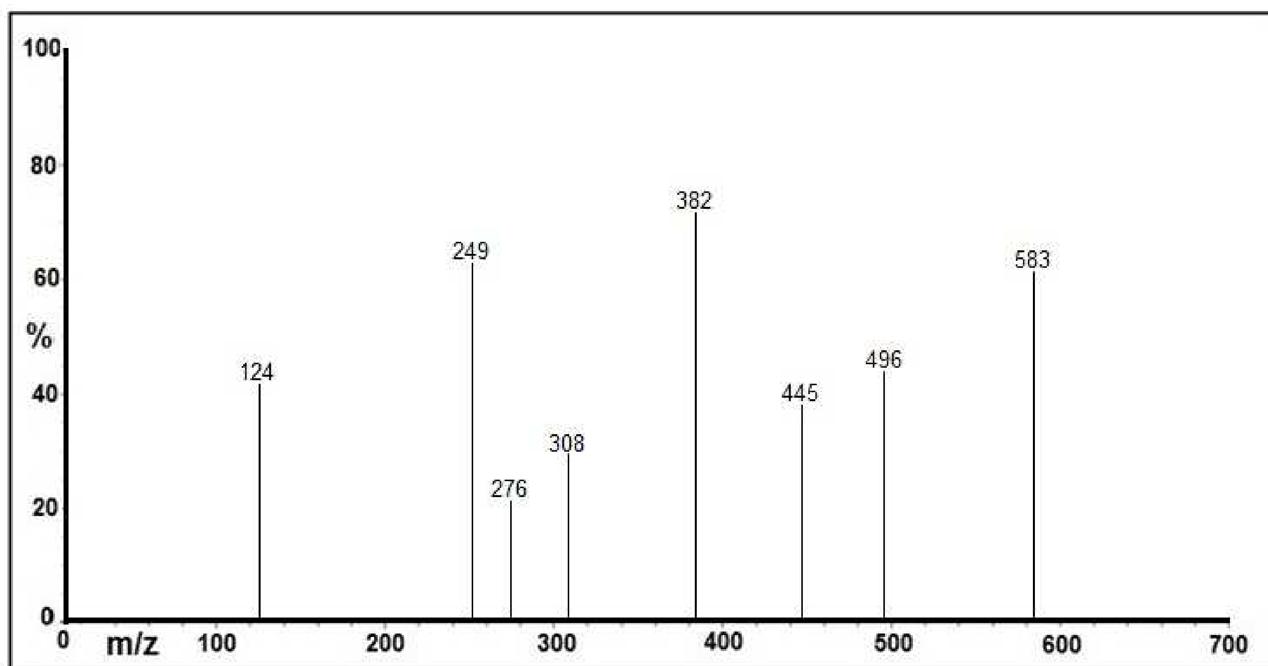

Fig. S1 Mass spectrum of Ni(II) complex

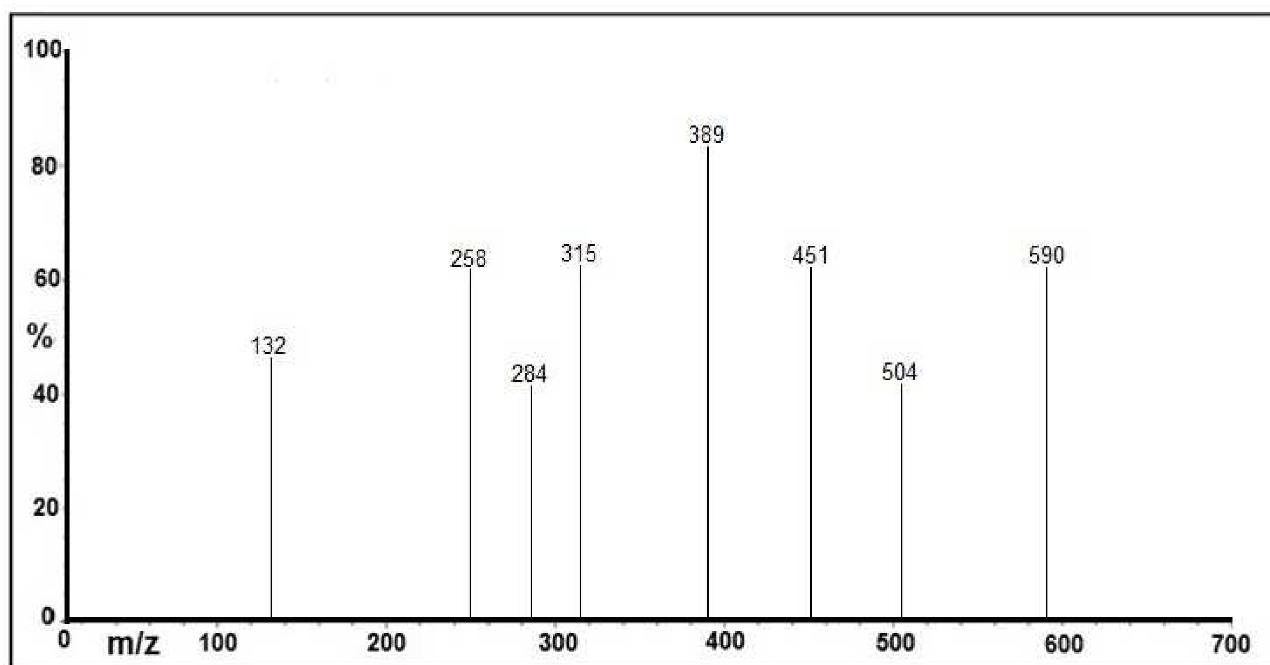

Fig. S2 Mass spectrum of Zn(II) complex

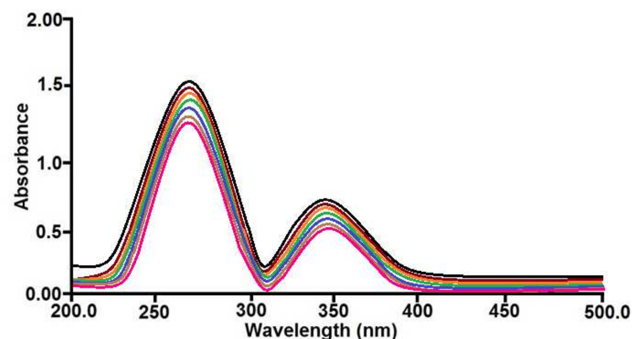

Fig. S3 Absorption spectra of complexes Ni(II), (1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10^{-3} M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5)

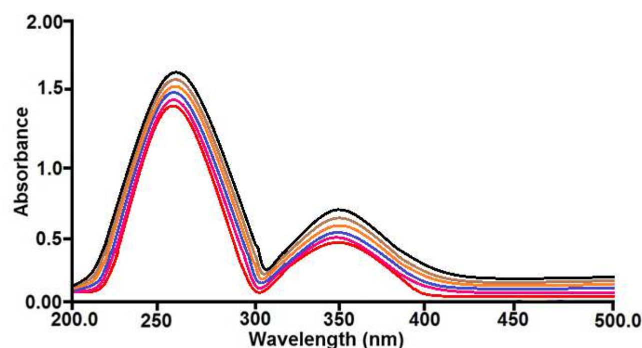

Fig. S4 Absorption spectra of complexes Zn(II), (1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10^{-3} M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5)

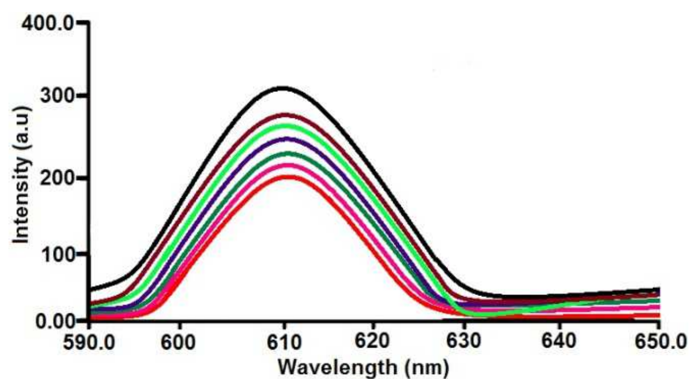

Fig. S5 Emission spectrum of EB bound to DNA in the presence of Ni(II); ([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-25 µM, λ_{ex}= 440 nm)

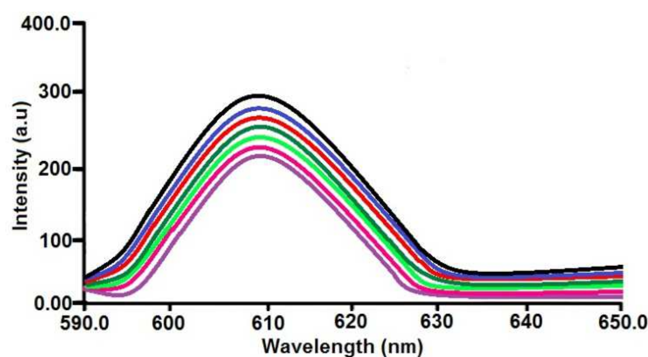

Fig. S6 Emission spectrum of EB bound to DNA in the presence of Zn(II); ([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-25 µM, λ_{ex}= 440 nm)