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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF Cu (II), Ni (II) AND Zn (II) COMPLEXES WITH CURCUMIN AND METHYL SALICYLALDEHYDE.

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Abstract:

Curcumin, the major active component of the spice turmeric, which is considered to be a very useful compound in health matters, is recognized as a safe component with great potential for cancer chemoprevention and cancer therapy. The paper presents the synthesis of complex combinations of Cu(II), Ni(II) and Zn(II) with Schiff base obtained by the condensation reaction of curcumin, hydrazine hydrate, methyl salicylaldehyde . The structural features have been arrived from their FT-IR, ^{1}H NMR, ^{13}C NMR, mass, UV-vis spectroscopic studies. The binding behaviours of the synthesis complexes towards calf thymus DNA have been investigated by absorption spectra, emission spectra, viscosity measurements and circular dichroic studies. The DNA binding constants reveal that all these complexes interact with DNA via intercalating binding mode with binding constants (K_b) of 4.72 x 10^4 M^{-1}, 3.89 x 10^4 M^{-1} and 3.13 x 10^4 M^{-1} and K_{app} of 5.26 x 10^5 M^{-1}, 4.37 x 10^5 M^{-1} and 3.91 x 10^5 M^{-1}.

Keywords: *Curcumin, DNA binding, Viscosity, Intercalation, methyl salicylaldehyde.*

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INTRODUCTION:

Cancer, a type of malignant growth or tumour, caused by abnormal and uncontrolled cell division is the leading cause of mortality globally. In a recent report in Nature, cancer has surpassed mortality incidence predominating all other health concerns, viz., cardiovascular diseases, cerebrovascular diseases, HIV/AIDS, lower respiratory infection including pneumonia, malaria, cirrhosis and as well as road accidents[1]. The burden of cancer has continuously increased not only in developing countries but also in Western Europe as well as in the United States. Although current strategies, including surgery, chemotherapy, radiation, and immunosuppressant, make a good progress in reducing cancer incidence and mortality rates and improving survival, cancer still accounts for more deaths than heart disease in persons younger than 85 years [2]. Medicinal researchers are continuing all over world in order to explore a safe and effective metal based biologically active compound as potential antitumor drug which is of great urgency to overcome the drug-induced cellular resistance and the efficacy of each drug against certain cancers. The treatment involves the administration of multiple drugs as it is clear that chemotherapy leads to the development of resistance. As the first generation of the platinum-based antitumor agent, cisplatin is widely used in the treatment of head and neck, testicular, small lung cell and ovarian cancers [3-6]. Although cisplatin and its derivatives are the most widely used metal-based drugs for cancer therapy, there are many drawbacks such as general toxicity, nonspecific targeting and acquired drug resistance [7-10]. The design and preparation of new transition metal complexes with low side-effects in comparison with cisplatin analogues is a very important field in medicinal chemistry [11].

Transition metal complexes are a subject of enormous research interest due to their interactions with nucleic acids. Transition metal complexes interact with DNA *via* different binding modes such as covalent, intercalation, groove or electrostatic binding. Binding studies of small molecules to DNA are very important due to their utility as DNA structural probes, DNA foot printing and sequence-specific cleavage agents and potential antitumor drugs [12 – 13]. The area of inorganic chemistry, which most widely developed in the last few decades is mainly due to coordination chemistry and applies very particularly to the coordination compounds of transition metals. The chemistry of coordination compounds has always been a challenge to the inorganic chemists. Metal ion dependent processes are found throughout the life science and vary tremendously in their function and complexity. In literature, several reports linked the significance of biological activity of metal complexes with the metal ions rather than with the ligands. [14].A major advantage of these metal-based over organic-based drugs is their ability to vary coordination number, geometry and redox states. Metals can also alter the pharmacological properties of organic based drugs by forming coordination compounds with them [15]. In topical years, the metal complexes with chelating ligands bearing O, N type donor atoms of alternative organic structures have attracted the chemists owing to their interesting medicinal applications [16]. Therefore, it is obvious that the nature of the ligands plays major role in their interaction with DNA molecule. The intend of synthetic ligands that read the information in the DNA duplex has been a central target at the interface of chemistry and biology [17]. Coordination chemistry is the most widely developed field in the last few decades. Among the complexing ligands Schiff bases are having special interest due to their preparative accessibility and structural variety. Schiff base are widely designed and prepared for its high yield and one-step procedure via condensation of amines and aldehydes / ketones [18]. The interaction of Schiff base metal complexes with DNA has been widely studied in the past decades. The binding and reaction of metal complexes with DNA has been the subject of strong research in relation to the growth of novel reagents for biotechnology and medicine [19]. In this regard, mixed ligand metal complexes play an important role in the activation of enzymes and display good nucleolytic cleavage activity. Mixed ligand complexes are used for storage as well as for transport of active material through membrane [20].

Curcumin [1,7-bis(4-hydroxy-3methoxyphenyl)-1,6-heptadiene- 3,5-dione], which is a naturally occurring yellow pigment obtainable from the rhizomes of turmeric (Curcuma longa Linn.), is a common ingredient used in spices, cosmetics and traditional Chinese medicine. The medicinal activity of curcumin has been known since ancient times and this molecule has been the object of several investigations in the field of biology, medicine and pharmacology over the last decades, such as antioxygenation, antibiosis and antitumor activities [21]. It has been found to be nontoxic to humans up to the dose of 10 g/day [22]. With this background it is strongly felt that the metal chelating ability of curcumin has to be explored with new transition metal-curcumin complexes as a bio-model for anticancer activity. The strong chelating ability of diketones has been widely investigated towards a great number of metal ions; therefore, curcumin could be of great importance in the chelate treatment of metal intoxication and overload. Of late, there have been many reports in the literature on the metal chelating properties of curcumin, employing techniques like potentiometry and absorption

spectroscopy [23]. Till date there are more than 3000 scientific publications in literature on the chemical, photophysical, biological, and anticancer effects of curcumin. Curcumin exhibits potential therapeutic application against several chronic diseases including cancer, inflammatory, neurological, cardio-vascular and skin diseases [24]. Curcumin can chelate metal ions and form metallo-complexes showing greatly effects than curcumin alone [25]. In view of aforesaid importance of Schiff bases and their complexes the present work stems from our interest to study the DNA binding copper (II), nickel(II) and zinc(II) complexes which are derived from the condensation reaction of curcumin, hydrazine hydrate and methyl salicylaldehyde and the respective metal ions.

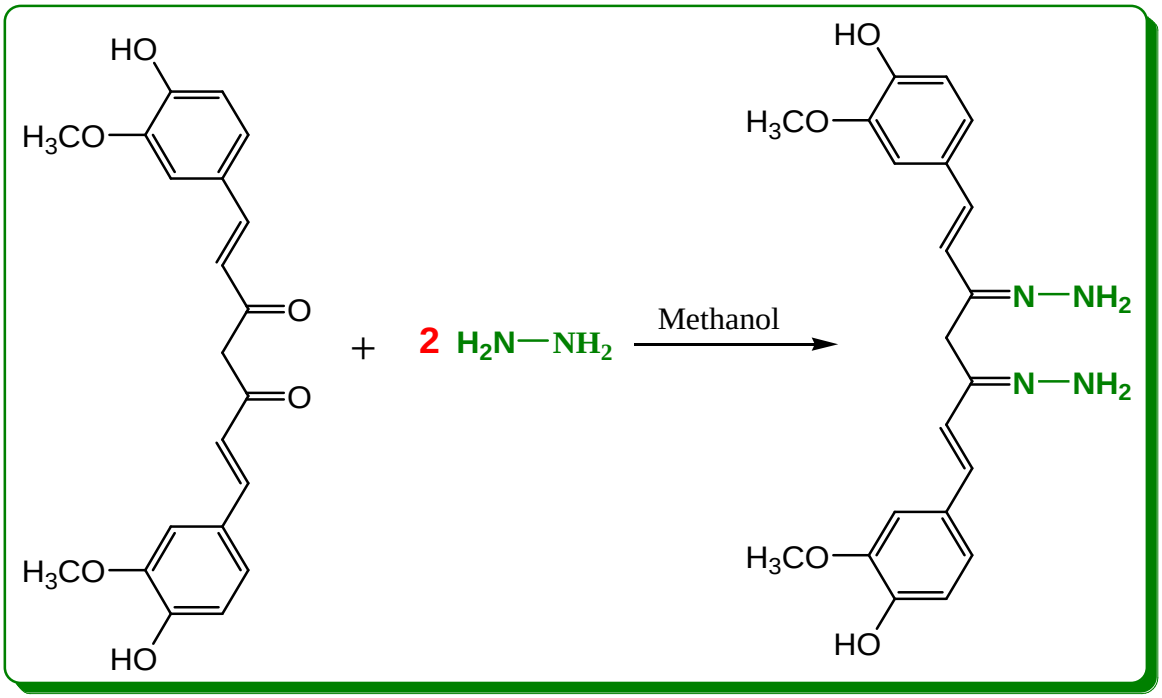
MATERIALS AND MEASUREMENT:

All reagents, curcumin, methylsalicylaldehyde, hydrazine hydrate and metal (II) chlorides were of Merck products and they were used as supplied. Commercial solvents were distilled and then used for the preparation of ligands and their complexes. DNA was purchased from Bangalore Genei (India). Tris (hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution was prepared using deionized and sonicated triple distilled water. The IR spectral studies were carried out in the solid state as pressed KBr pellets using a Perkin-Elmer FT-IR spectrophotometer in the range of 400-4000 cm-1. The ^{1}H NMR and ^{13}C NMR were obtained using Bruker AM-500 instrument at 500 and 125 MHz respectively. UV-Vis spectra were recorded using a Perkin Elmer Lambda 35 spectrophotometer operating in the range of 200-900 nm with quartz cells and ε are given in $M^{-1}cm^{-1}$. The emission spectra were recorded on Perkin Elmer LS-45 fluorescence spectrometer. Viscosity measurements were recorded using a Brookfield Programmable LV DVII+ viscometer. The electro spray mass spectra were recorded on a Q-TOF micro mass spectrometer. Circular dichoric spectra of CT-DNA were obtained using a JASCO J-715 spectropolarimeter equipped with a Peltier temperature control device at 25 ± 0.1°C with 0.1 cm path length cuvette.

General Procedure for the Synthesis of Compounds:

Synthesis of the Ligand:

A methanolic solution (20 mL) of hydrazine hydrate (0.002 mol, 0.270 gm) was slowly added to a methanolic solution (20 mL) of curcumin (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.



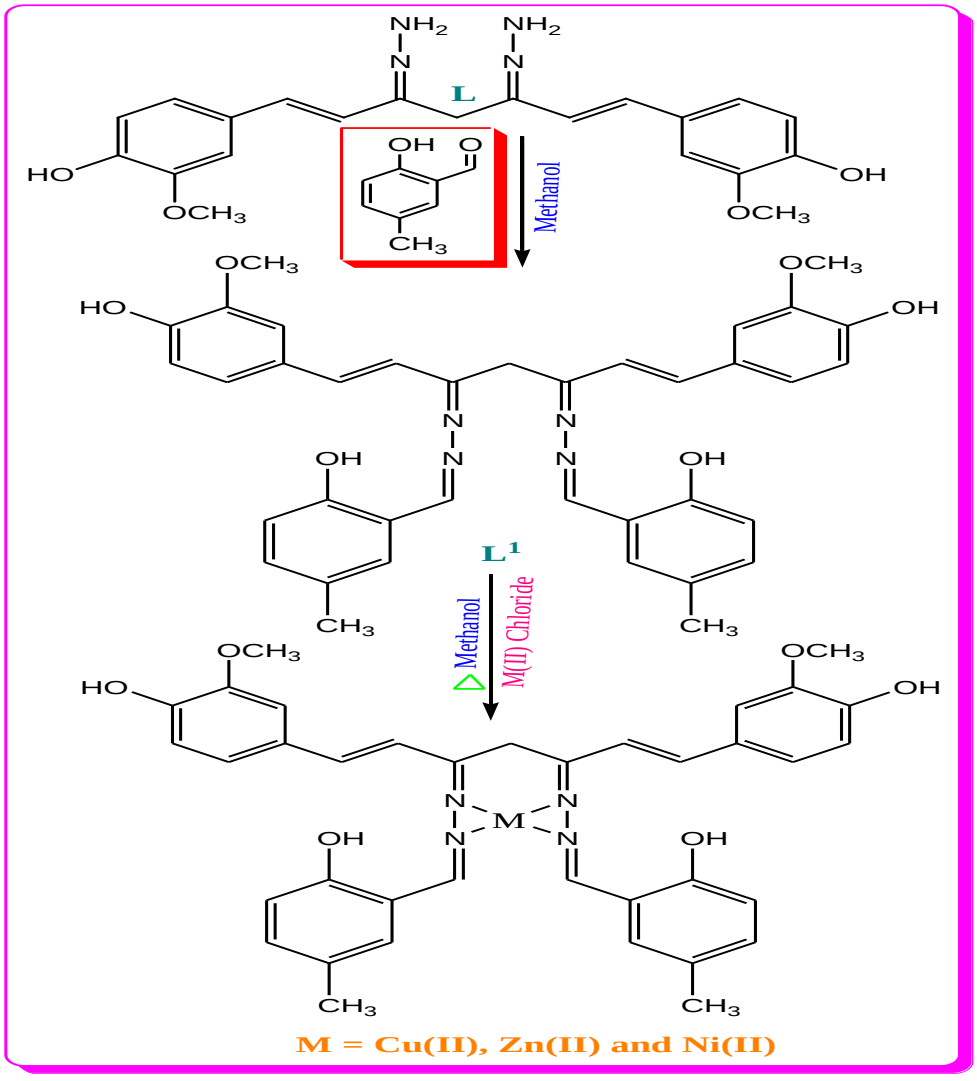
Scheme 1: Synthesis of Schiff base Ligand.

Synthesis of Schiff Base Ligand L1 and its Metal Complexes

A methanolic solution (20 mL) of ligand L (0.0025 mol 1 gm) was slowly added to a methanolic solution (20 mL) of methyl salicylaldehyde (0.58 gm, 0.0050 mol) with constant stirring as shown in Scheme 2. 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.

All complexes were synthesized using the same procedure as given below:

A methanolic solution (20 mL) of ligand (L) (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 (L1) and their Respective Metal Complexes.

DNA Binding Experiments

Absorption Spectral Studies:

Absorption titration experiments were performed by maintaining the metal complex concentration as constant at 40 mM while varying the concentration of the CT-DNA (0, 40, 80, 120, 160, 200, 300 and 400) mM. While measuring the absorption spectra, equal quantity of CT-DNA was added to both the complex solution and the reference solution to eliminate the absorbance of CT-DNA itself. From the absorption data, the intrinsic binding constant (K_b) was determined from a plot of [DNA]/(ε_a-ε_f) versus [DNA] using equation (1)

[DNA]/ (ε_a - ε_f) = [DNA]/ (ε_b - ε_f) + 1/K_b (ε_b - ε_f)--(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 [26].

Fluorescence Spectral Analysis:

The emission spectrum is obtained by setting the excitation monochromator at the maximum excitation wavelength and scanning with emission monochromator. Often an excitation spectrum is first made in order to confirm the identity of the substance and to select the optimum excitation wavelength. Further experiments were carried out to gain support for the mode of binding of complexes with CT-DNA. Non-fluorescent or weakly fluorescent compounds can often be reacted with strong fluorophores enabling them to be determined quantitatively. On this basis molecular fluorophore EthBr was used which emits fluorescence in presence of CT-DNA due to its strong intercalation. 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)

$I_o/I=1 + K_{sv}$. ----- (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 x 10^7 M^{-1} ([EB] = 3.3 μM) [27].

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. 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.

Viscosity Measurements

Viscosity experiments were carried out at 30.0°C ± 0.1°C. 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) [28]. 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.

RESULTS AND DISCUSSIONS

FT-IR Spectral Analysis

In order to find binding modes of Schiff base ligand and methyl-salicylaldehyde with transition metal ions, IR spectra of compounds were recorded. The IR spectra of the ligand L showed a broad band in the region3473 cm^{-1}, assignable to intra-molecular hydrogen bonded –OH groups. The phenyl group shows C–H stretching at 3026 cm^{-1}. In the spectra of Schiff base ligand and their complexes, disappearance of carbonyl band and a new strong sharp band that appears at 1642 cm^{-1} region is attributed to the ν(C=N) band, confirming the formation of the Schiff base ligand. The spectrum of the ligand L and L1 shows–C=N bands in the region 1640 cm^{-1}, which is shifted to lower frequencies in the spectra of the complexes 1621 cm^{-1} indicating the involvement of –C=N nitrogen in coordination to the metal ion. In order to study the bonding mode of Schiff base to the metal complexes, the IR spectrum of the free ligand is compared with the spectra of the complexes. Assignment of the proposed coordination sites is further supported by the appearance of medium bands around 512 cm^{-1} which could be attributed to ν M–N. 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. The representative spectrum of the ligand is shown in Fig 1.

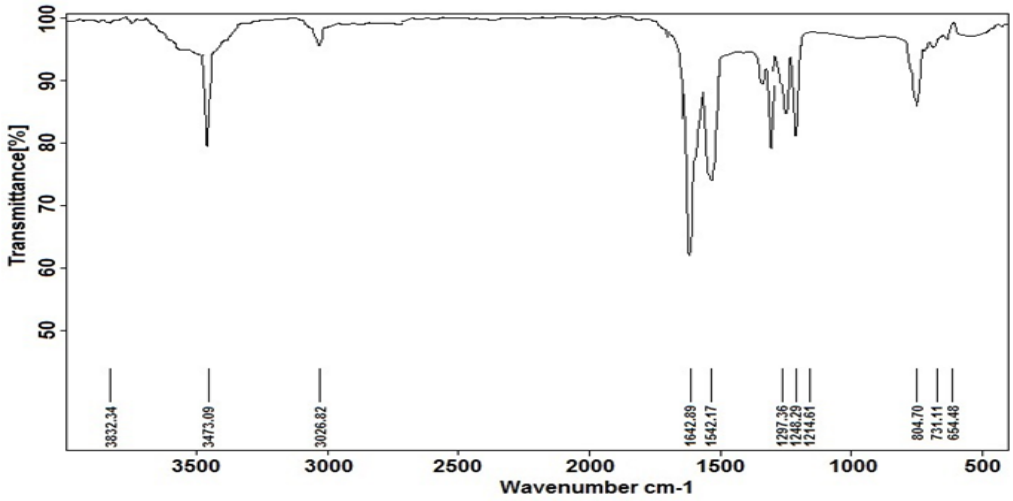


Fig 1: FT-IR Spectrum of the Schiff Base Ligand (L).

Mass Spectral Analysis:

The molecular ion peak [M^+] at m/z = 396 confirms the molecular weight of the Schiff base ligand L $C_{21}H_{24}N_4O_4$. The peaks at m/z = 366, 288, 232, 192, 154 and 124 corresponds to the various fragments $C_{20}H_{22}N_4O_3$, $C_{15}H_{20}N_4O_2$, $C_{12}H_{16}N_4O$, $C_{10}H_{16}N_4$, $C_7H_{14}N_4$ and $C_7H_{12}N_2$ respectively as shown in Fig. 2. This confirms the molecular structure of the ligand L. The molecular ion peak [M^+] at m/z = 632 confirms the molecular weight of the Schiff base ligand L1 $C_{37}H_{36}N_4O_6$. The peaks at m/z = 544, 480, 288, 232, 180 and 124 corresponds to the various fragments $C_{33}H_{28}N_4O_6$, $C_{33}H_{28}N_4$, $C_{17}H_{28}N$, $C_{13}H_{20}N_4$, $C_9H_{16}N_4$ and $C_5H_8N_4$ respectively as shown in Fig. S1. This confirms the molecular structure of the ligand L1.

The molecular ion peak [M^+] at m/z = 696 confirms the molecular weight of the Schiff base Cu(II) complex $C_{37}H_{36}N_4O_6Cu$. The peaks at m/z = 608, 544, 448, 351, 295, 243, 187 and 122 corresponds to the various fragments $C_{33}H_{28}N_4O_4Cu$, $C_{33}H_{28}N_4Cu$, $C_{25}H_{28}N_4Cu$, $C_{17}H_{18}N_4Cu$, $C_{13}H_{20}N_4Cu$, $C_9H_{16}N_4Cu$, $C_5H_8N_4Cu$ and H_4N_4Cu respectively as shown in Fig. 3, Fig. S2 & S3. The molecular ion peak [M^+] at m/z = 698 and 691, confirms the molecular weight of the Schiff base Zn(II) and Ni(II) complex $C_{37}H_{36}N_4O_6M$ [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.

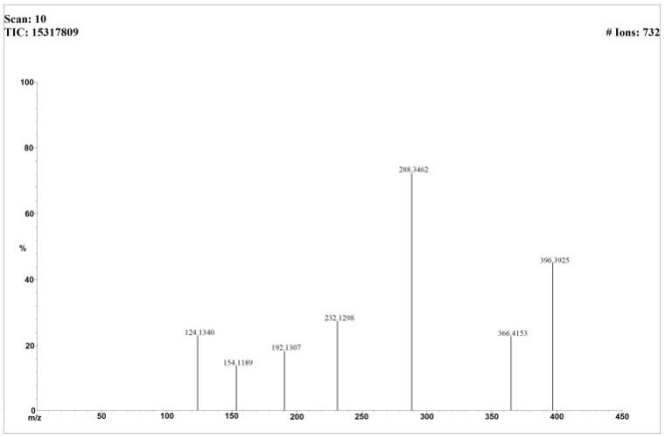


Fig 2: ESI-Mass Spectrum of Schiff Base Ligand (L).

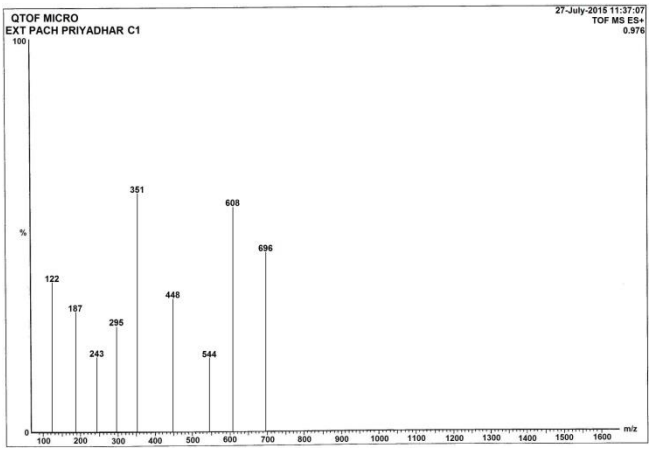


Fig 3: ESI-Mass Spectrum of Cu (II) Complex.

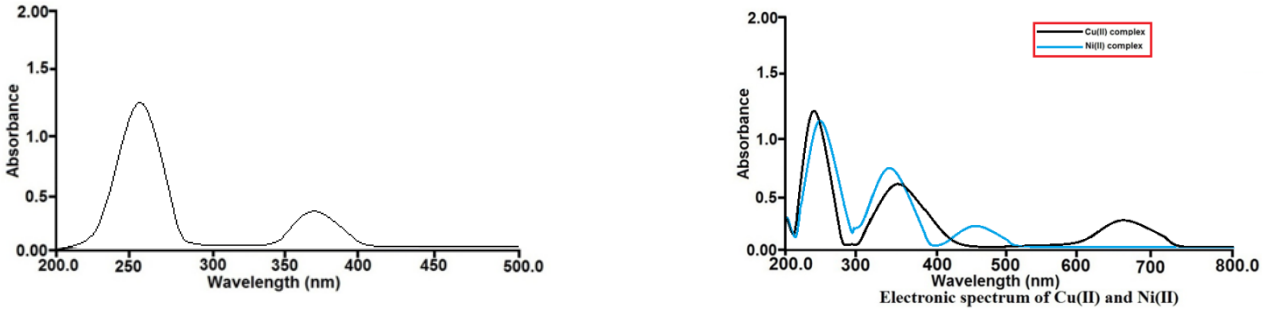


Fig 4: Electronic Spectrum of the (a) ligand (L), (b) Cu (II) and Ni(II) Complexes.

UV-Vis Spectral Analysis:

The electronic spectra of the ligand and their respective complexes were recorded in DMSO at room temperature as shown in Fig. 4 (a) and (b). In the spectrum of the ligand, the bands in the 350-390 nm range are assigned to the n-π* transitions of the azomethine group. During the formation of the complexes, these bands are shifted to lower wavelength, suggesting that the nitrogen atom of the azomethine group is coordinated to the metal ion. The values in the 250 - 300 nm range are attributed to the π-π* transition of the aromatic ring. In the spectra of the complexes, these bands are shifted slightly to lower wave length. The electronic spectrum of the Cu (II) complex in DMSO exhibits a d–d band at 690 nm which can be assigned to the combination of $^2B_{1g}$ ^2E_g and $^2B_{1g}$ $^2B_{2g}$ transitions [29] in a distorted square planar copper (II) environment. The Ni (II) complex is diamagnetic and the band around 440 nm could be assigned to $^1A_{1g}$ $^1B_{1g}$ transition, consistent with other square-planar nickel (II) complexes [30].

NMR Spectral Analysis:

^{1}H NMR spectra of Schiff base ligand L showed peak at δ 1.292 ppm as singlet is due to the presence of CH_2. The six methyl protons in curcumin were observed as singlet around δ 3.37 ppm. The doublets around δ 5.45 ppm are owing to the presence of ethylene protons. The NH_2 protons were observed around δ 7.43 ppm as singlet. Other peaks around δ 6.52 – 6.40 ppm as multiplet is due to the presence of protons in benzene ring. The Fig. 5 shows the ^{1}H NMR spectra Schiff base ligand L. The ^{13}C NMR spectra of schiff base ligand L is depicted in Fig. 6. In the spectra the peak at δ 18.7 ppm is due to the presence of CH_2 group. The peak at δ 56.2 ppm is due to the presence of methyl carbon. The peak at δ 135.9 and 139.0 ppm is due to the presence of ethylene carbon atoms. The other peaks are owing to the presence of aromatic carbons.

^{1}H NMR spectra of Schiff base ligand L1 showed peak at δ 1.26 ppm as singlet is due to the presence of CH_2. The six methyl protons in salicylaldehyde were observed as singlet around δ 2.934 ppm. The six methyl protons in curcumin moiety were observed as singlet around δ 3.46 ppm. The doublets around δ 5.56 ppm are owing to the presence of ethylene protons. Other peaks around δ 6.52 – 6.40 ppm as multiplet is due to the presence of protons in benzene ring. In the spectra the NH_2 proton was disappeared and showed a new peak at δ 8.36 ppm corresponding to imine group. The Fig.S4 shows the ^{1}H NMR spectra Schiff base ligand L1. The ^{13}C NMR spectra of schiff base ligand L1 is depicted in Fig. S5. In the spectra the peak at δ 18.02 ppm is due to the presence of CH_2 group. The peak at δ 54.39 ppm is due to the presence of methyl carbons in curcumin moiety. The peak at δ 129.5 and 138.2 ppm is due to the presence of ethylene carbon atoms. The other peaks are owing to the presence of aromatic carbons. Furthermore the peak at δ 168.57 is owing to the presence of imine group. In the spectra of ligand L1, the peak at 23.71 ppm is due to the presence of methyl carbons in methyl salicylaldehyde.

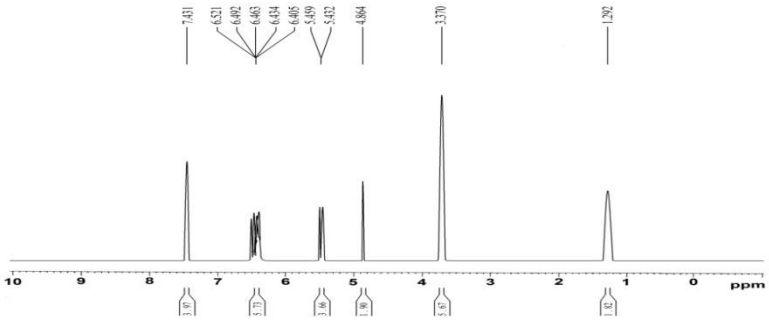


Fig 5:^{1}H-NMR Spectrum of Schiff Base Ligand (L).

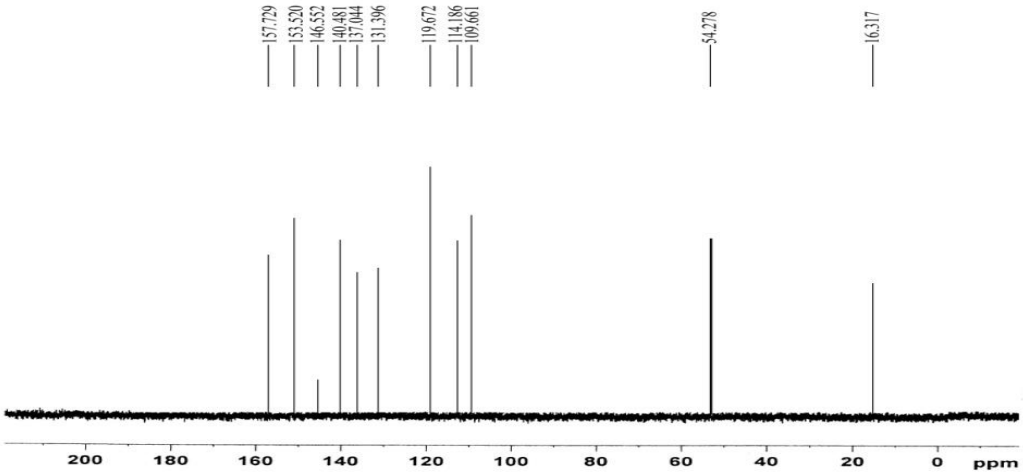


Fig 6: ^{13}C-NMR spectrum of Schiff base ligand (L).

DNA Binding Experiments

Absorption Spectral Studies

Absorption titration can be used to observe the interaction of molecules with DNA. Generally, the binding of an intercalative molecule to DNA is always accompanied by hypochromism and/or significant bathochromism in the absorption spectra due to strong stacking interactions between the aromatic chromophore of the compounds and DNA base pairs [31]. Therefore, in order to obtain evidence for the binding ability of each compound to CT DNA, spectroscopic titration of compound solutions with CT DNA should be performed [32]. The electronic absorption spectra of complexes in the absence and presence of CT DNA are given in Fig. 7, Fig. S6 and S7. With increasing concentration of CT-DNA, for the Cu(II), Ni(II) and Zn(II) complexes, the absorption bands at 383 nm respectively also show hypochromism. The prominent shift in the spectra also suggests the tight complexation of synthesized molecule with DNA, which resulted in the change in the absorption maxima of the DNA. These results suggest an intimate association of the compounds with CT-DNA and it is also likely that compounds bind to the helix via intercalative mode.

In order to obtain information on the binding affinity of the three compounds towards CT-DNA, the intrinsic binding constants (K_b,insets in Fig. 7) were estimated using Eq. (1). The spectroscopic changes suggest that the intrinsic binding constants (K_b) of Cu(II), Ni(II) and Zn(II) complexes are 4.72 x 10^4 M^{-1}, 3.89 x 10^4 M^{-1} and 3.13 x 10^4 M^{-1}.

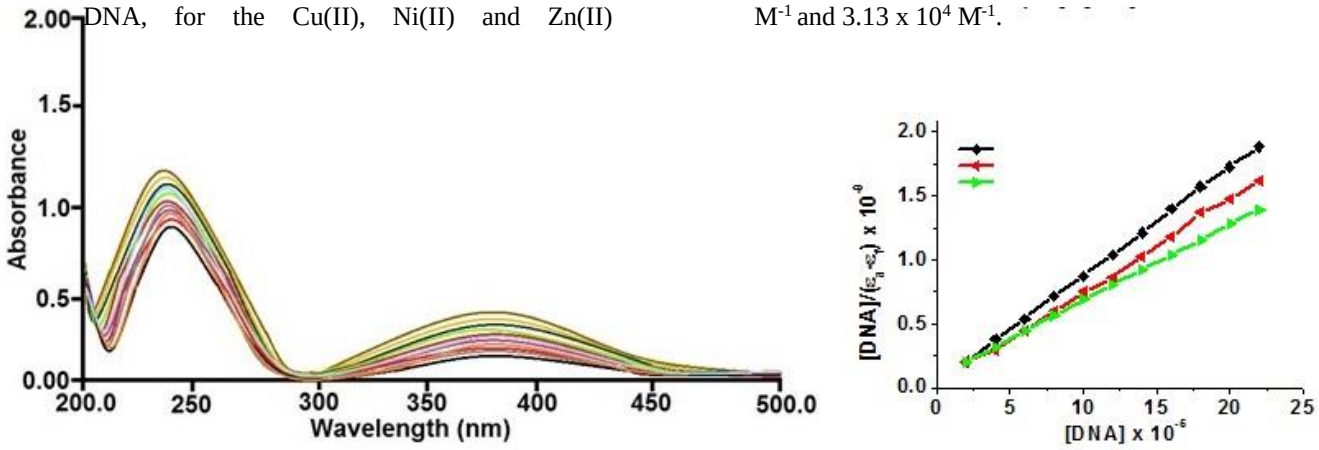


Fig 7: Absorption spectra of complex Cu(II) (1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10^{-3} M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5). The Inset shows the plots of [DNA] / (ϵ_a-ϵ_f) versus [DNA] for the titration of DNA with Cu(II), Ni(II) and Zn(II) complexes.

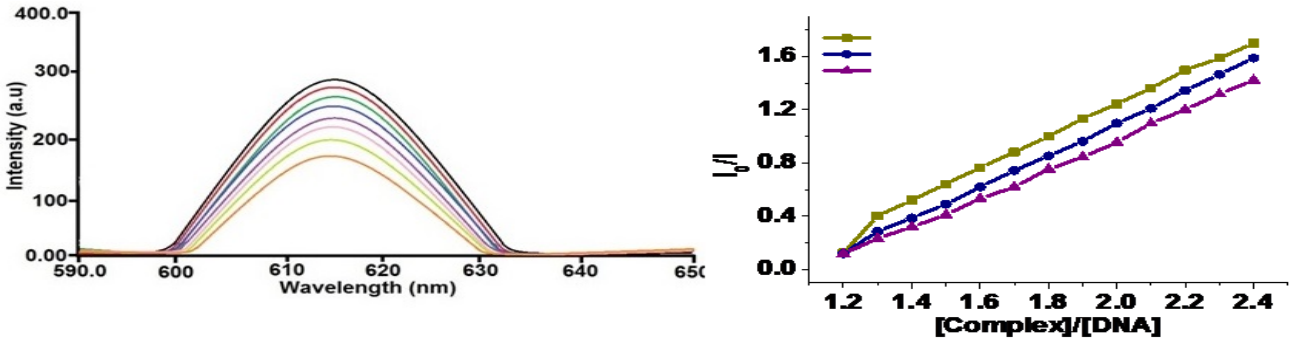


Fig 8: Emission spectrum of EB bound to DNA in the presence of Cu(II): ([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-25 µM, λex= 440 nm). Inset shows the plots of emission intensity I_o / I *vs* [DNA] / [complex] for the titration of DNA with Cu(II), Ni(II) and Zn(II) complexes.

Fluorescence Measurements:

In order to further investigate the interaction modes of the Schiff base complexes with DNA, a competitive binding experiment using EB as a probe was carried out. EB is an intercalator that gives a significant increase in fluorescence emission when bound to DNA and it can be quenched by the addition of second DNA binding molecule by either replacing the EB and/or by accepting the excited-state electron of the EB through an electron transfer mechanism. The enhanced fluorescence can be quenched on addition of the second molecule which could replace the bound EB or break the secondary structure of the DNA, which can be used to monitor the extent and mode of binding thereby indicating the ability of a molecule to prevent intercalation of EthBr to DNA [33]. The fluorescence quenching of EB bound to CT-DNA by the complexes was shown in Fig. 8, Fig. S8 and S9. The fluorescence quenching spectra illustrated that upon increasing the concentration of the compounds the emission band at 616 nm exhibited hypochromism. The results show that the fluorescence intensity of DNA-EB decreases remarkably with the addition of the complex which indicate that the complex binds to DNA by intercalation or partial intercalation replacing EB from the DNA structure. The constant value of K_{sv} is the slope of the regression line and the quenching constant of the complexes were found to be K_{app} of 5.26 x 10^5 M^{-1}, 4.37 x 10^5 M^{-1} and 3.91 x 10^5 M^{-1}, as shown in inset of Fig. 8 indicating a strong binding of copper(II) complex with CT-DNA. The observations of the above experiments suggest intercalative binding mode of the complexes with CT-DNA and corroborate well with the results of absorbance studies.

Viscosity Measurements

Viscosity measurements are used to investigate the binding modes of complex with DNA. Optical photophysical probes provide necessary, but not sufficient, clues to support a binding model. To further clarify the interactions between the complexes and DNA, viscosity measurements were carried out. Viscosity measurements that are sensitive to length change are regarded as the least ambiguous and the most critical tests of a binding model in a solution in the absence of crystallographic structural data [34]. To know the nature of DNA binding of the mixed ligand complexes, viscosity of CT DNA has been measured in the presence of varying amount of complexes, the relative viscosity of DNA increased steadily with increasing amount of complexes. The results indicate that the presence of the metal complex increases the viscosity of the DNA solution, as illustrated in Fig. 9. As general rule metal complexes can increase the viscosity of DNA when they intercalate into the double-stranded DNA (or) bind to the phosphate group of DNA backbone [35]. The plots show that the relative viscosity increases with increase in concentration of complexes. The observed order of DNA-binding in viscosity measurements suggests that metal complexes bind DNA with a moderate intercalative mode.

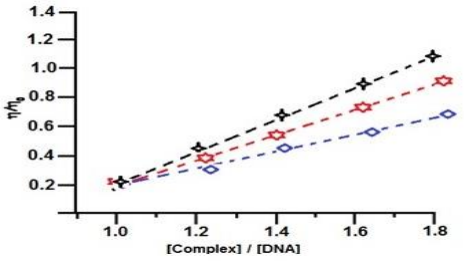


Fig 9: Viscosity measurements of the Cu(II), Ni(II) and Zn(II) complexes.

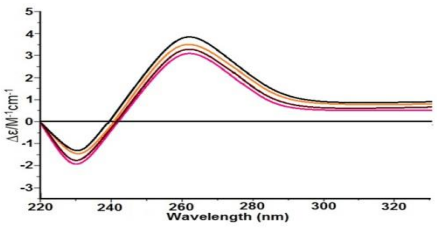


Fig10: CD spectra recorded over the wavelength range 220-320 nm for solutions containing 2:1 ratio of CT-DNA (200 µM) and Cu(II), Ni(II) and Zn(II) complexes (100 µM).

Circular Dichoric Spectral Studies:

Circular dichroism is a useful technique to assess whether nucleic acids undergo conformational changes as a result of complex formation or changes in the environment. A solution of CT-DNA exhibits a positive band (275 nm) from base stacking interactions and a negative band (245 nm) from the right-handed helicity of DNA [36]. The CD spectra of CT-DNA titrated with the complexes are shown in Fig. 10. The positive band showed significant increase of ellipticity accompanied by a slight redshift with increasing [complex]/[DNA] ratio. This indicated the intercalative effect of the complexes in base stacking of the DNA, where the complex stacked into the base pairs of duplex DNA, and prevented the neighbouring base pairs from close stacking, thus leading to an enhancement in positive band [37].

CONCLUSION

The synthesized ligand and its metal complexes have been characterized by physico-chemical methods like IR, ^{1}H NMR, ^{13}C NMR and mass spectra. The spectroscopic data of the metal complex indicate that the metal ions were complexed with nitrogen of the imine. In vitro DNA binding studies employing various optical techniques were carried out to examine the propensity of complexes towards CT DNA. The DNA binding experiment results suggest that the complex binding with DNA is by an intercalative mode. The present work would be helpful to understand the interaction mechanism of the complexes with DNA and useful in the development of new chemotherapy drugs. Among the investigated complexes, the one containing copper as the central metal ion showed better binding affinity than the other two complexes containing Zinc and nickel ions as metal counterparts respectively.

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Supplementary Figure captions.

Figure S1. Mass spectrum of Schiff base ligand (L1).

.**Figure S2.** Mass spectrum of Ni(II) complex.

Figure S3. ESI-Mass spectrum of Zn(II) complex

Figure S4. ^{1}H-NMR spectrum of Schiff base ligand (L1).

Figure S5. ^{13}C-NMR spectrum of Schiff base ligand (L1).

Figure S6. 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).

Figure S7. 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).

Figure S8. 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).

Figure S9. 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).

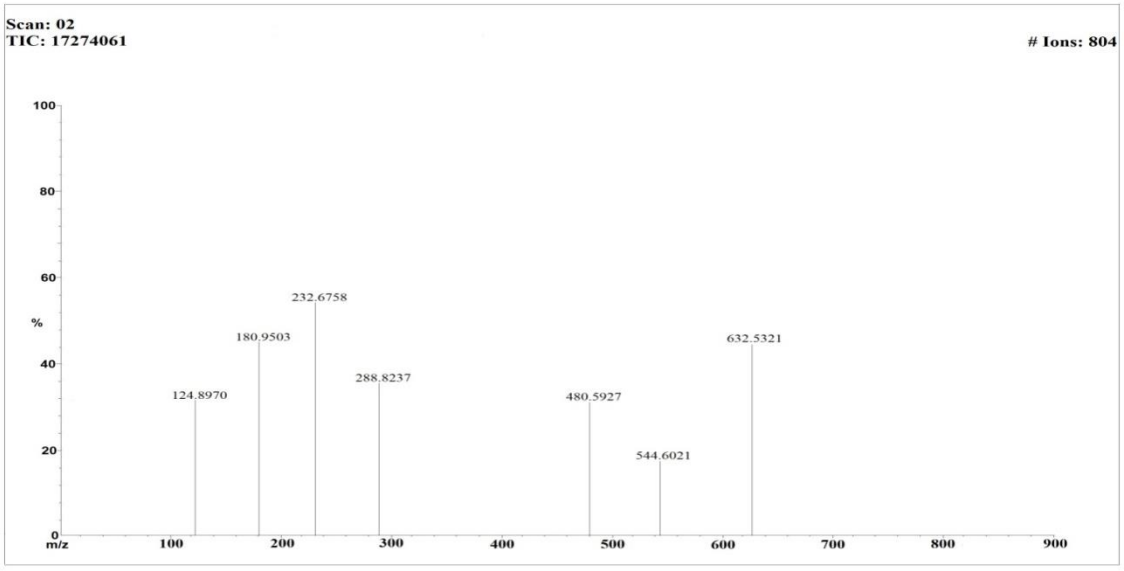


Figure S1: Mass spectrum of Schiff base ligand (L1).

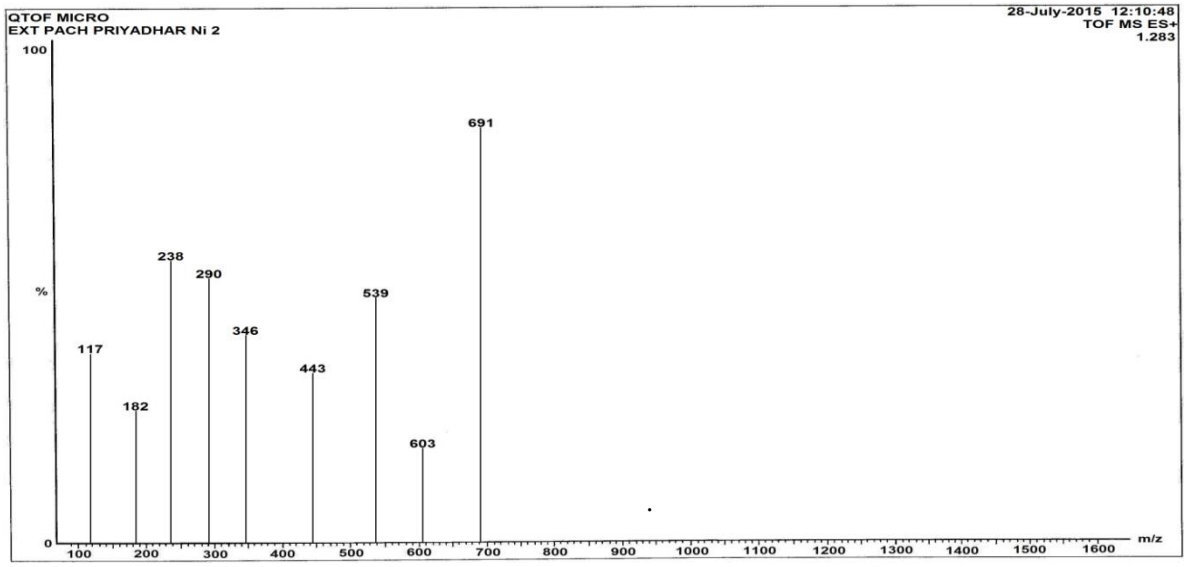


Figure S2: Mass spectrum of Ni(II) complex.

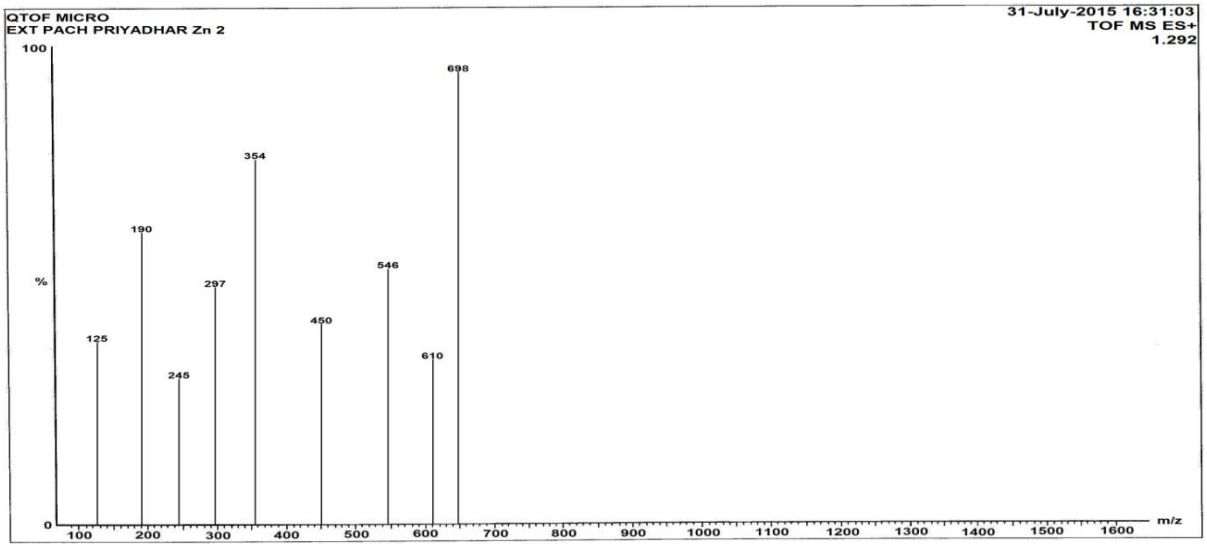


Figure S3: ESI-Mass spectrum of Zn(II) complex

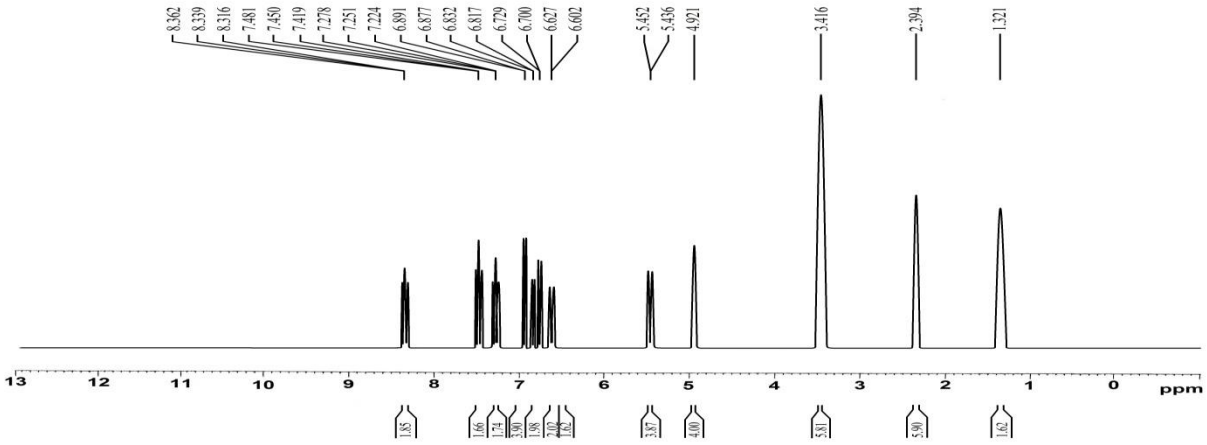


Figure S4: ^{1}H-NMR spectrum of Schiff base ligand (L1).

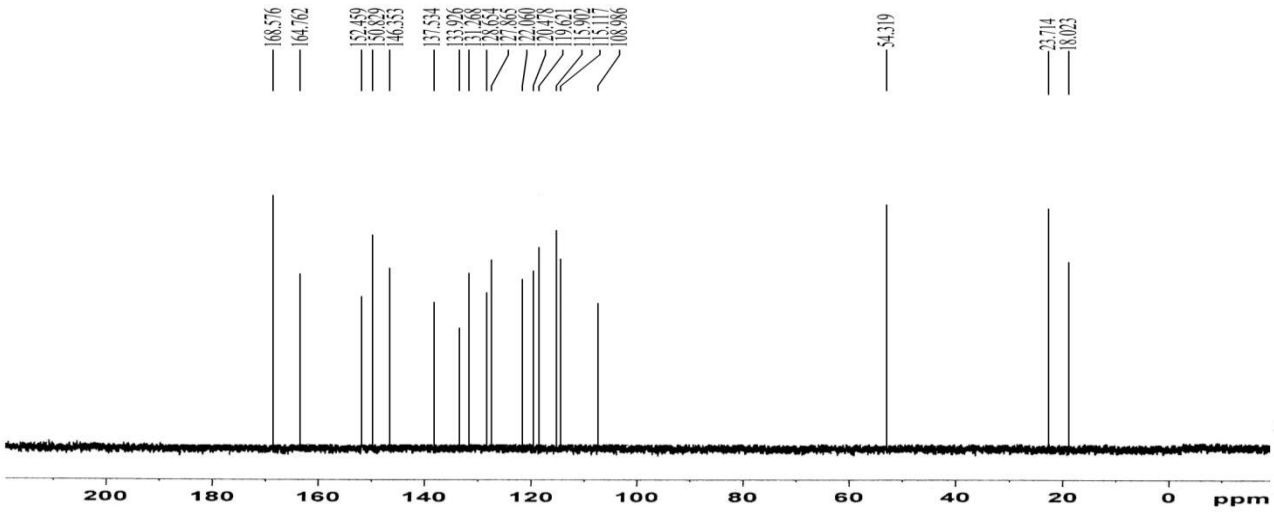


Figure S5: ^{13}C-NMR spectrum of Schiff base ligand (L1).

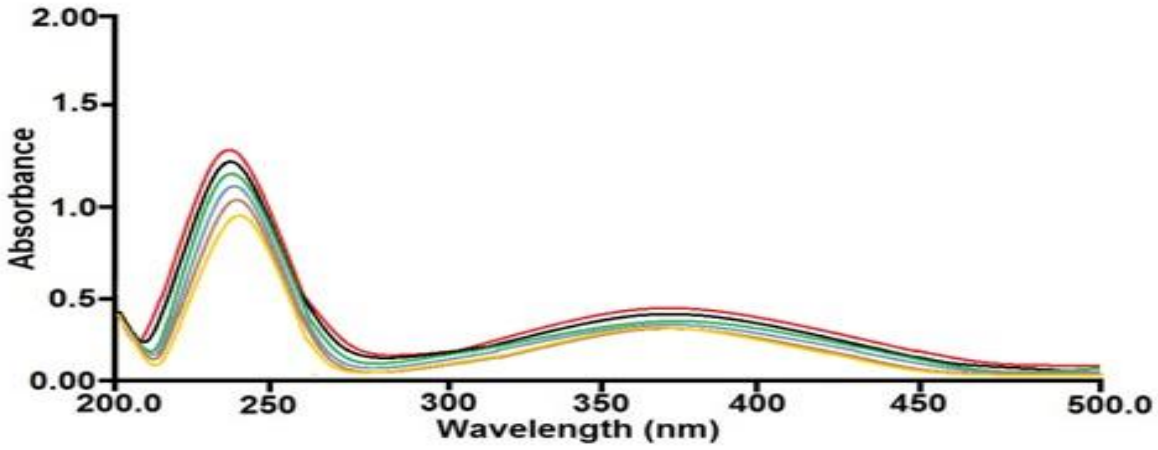


Figure S6: Absorption spectra of complexes Ni(II), (1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10^{-3} M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5).

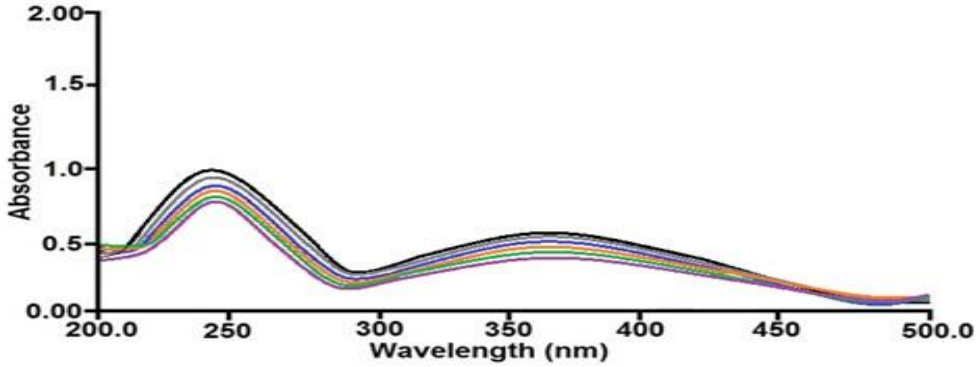
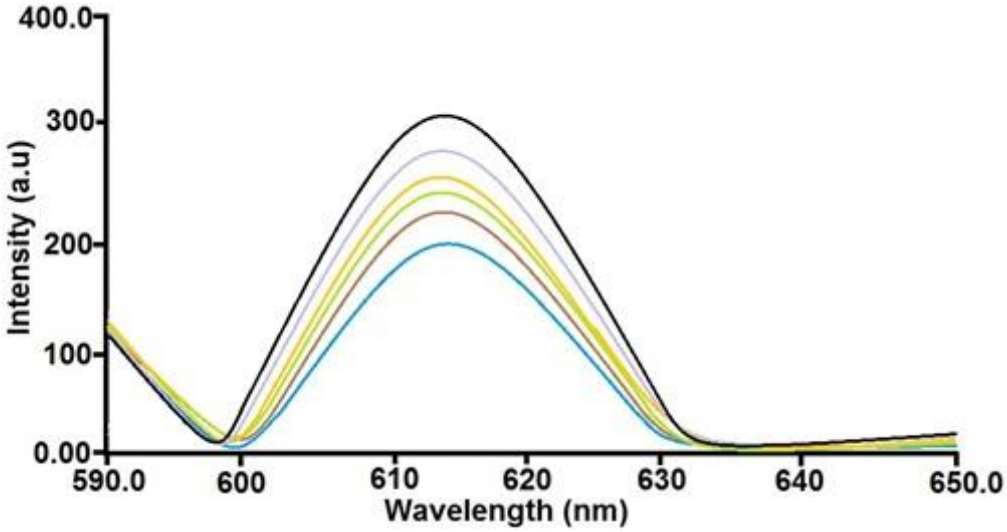


Figure S7: Absorption spectra of complexes Zn(II), (1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10^{-3} M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5).



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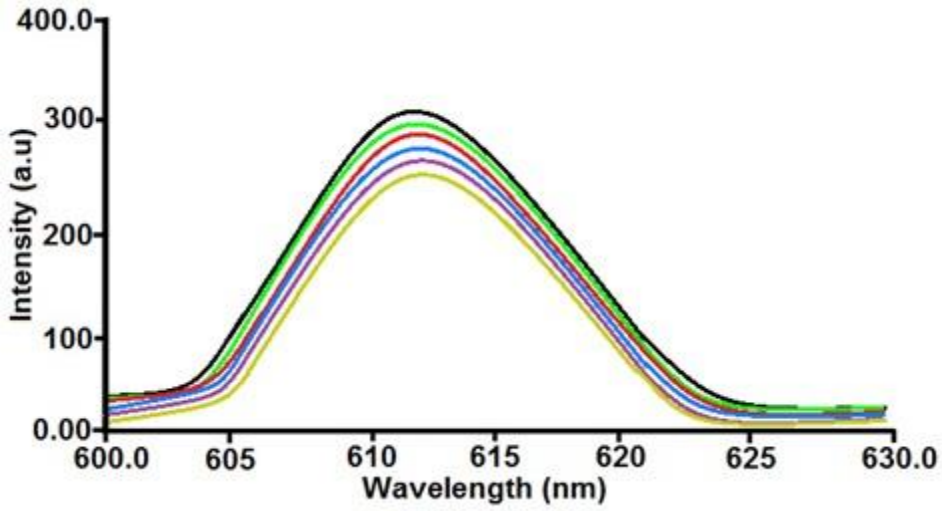

Figure S9: Emission spectrum of EB bound to DNA in the presence of Zn(II); ([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-25 µM, λ_{ex}= 440 nm).