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Synthesis, structural characterization, DNA binding activity studies of Cu(II), Ni(II) and Zn(II) metal complexes containing thiazole moiety

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ABSTRACT

The 2:1 Schiff base condensates of 1,2-di(thiophen-2-yl)ethane-1,2-dione with 1,4-diamino butane (L), respectively, were isolated and used to prepare Cu(II), Ni(II) and Zn(II) complexes. The resultant ligand complexes have been characterized from their elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR, mass, UV-vis spectroscopic studies. The Schiff bases and their Cu(II), Ni(II) and Zn(II) complexes have been screened for binding behaviours towards calf thymus DNA. Binding of the complexes separately with Calf Thymus DNA is monitored using UV–vis spectral titrations, viscosity measurements and circular dichroic studies. The displacement of ethidium bromide (EB) bound to DNA by the complexes; in phosphate buffer solution (pH ~ 7.2) is monitored using fluorescence spectral titrations. The DNA binding constants reveal that all these complexes interact with DNA via intercalating binding mode with binding constants (K_b) of 5.4 x 10^4 M^{-1}, 4.1 x 10^4 M^{-1} and 3.9 x 10^4 M^{-1} and K_{app} of 6.3 x 10^5 M^{-1} 6.0 x 10^5 M^{-1} and 5.8 x 10^5 M^{-1}.

Keywords: Imine, DNA binding, Viscosity, Intercalation, Thenil.

INTRODUCTION

In 1970, Crick [1] enunciated the primary basis of molecular biology, which dictated the one-way flow of genetic information from DNA to RNA to protein. They described the DNA as a double helix that adopts different three-dimensional conformations although the most common form of the double stranded DNA is the "B" form, characterized by a "right-handed" helix. The backbone of the DNA strand is composed of alternating sugar and phosphate groups with the base linked to each sugar as side chain pair, held by hydrogen bonds between specific pairs of bases. The precise base pairing in their DNA helix model suggested an obvious copying mechanism for genetic material **[**2**]**. Plenty of studies came up with that DNA is the major intracellular target of antitumor drugs because of the interaction between small molecules and these compounds can cause DNA damage in cancer cells, preventing the division of cancer cells and ending in cell death [3]. Transition metal complexes having the ability to bind and nick double stranded DNA under physiological conditions are of great importance since these could be used as diagnostic agents in medical and genomic research like foot-printing and sequence-specific binding agents, for modelling the restriction enzymes and as structural probes for therapeutic applications in cancer treatment [4-8].

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 [9]. 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 [10-12].

Thiazoles are the significant group of heterocycles containing two hetero atoms (S and N) placed in the heterocyclic ring at 1, 3-positions. Metal complexes of S and N chelating ligands have attracted considerable attention because of their interesting physico-chemical properties, pronounced biological activities [13-15] and models for metalloenzymes active sites. It is well known that N and S atoms play vital role in the coordination of metals at the active sites of numerous metallobiomolecules [16]. In addition, these classes of compounds are present in many natural and synthetic products with a broad spectrum of pharmacological activities, such as antiviral, anticancer, antibacterial, antifungal, anticonvulsant and anti-inflammatory activities that can be well illustrated by the large number of drugs in the market containing this function group [17]. These unusual structural and electronic features have led to increased interest in the synthesis of Cu(II), Ni(II) and Zn(II) complexes with mixed N,S donating chelates as structural and spectroscopic models of the active sites.

As part of our continuous interest on sulphur polydendate chelators [18-19] we synthesized a Schiff base ligand L by condensing of 1, 2-di(thiophen-2-yl)ethane-1,2-dione with 1,4-diamino butane. The characteristic resonance signals in ^{1}H NMR and ^{13}C NMR spectra indicated the presence of azomethine group as a result of condensation reaction. The stoichiometry and bonding the synthesized Cu(II), Ni(II) and Zn(II) complexes were ascertained on the basis of results by physicochemical and spectroscopic tools. Absorption and fluorescence spectroscopic studies supported that Schiff Cu(II), Ni(II) and Zn(II) complexes exhibited significant binding to calf thymus DNA. In this study, we investigated the interaction of mentioned molecules with DNA, using several spectroscopic methods including: fluorimetry, competition experiment, circular dichroism (CD) and UV absorption techniques. The Cu(II) complexes exhibited higher affinity to calf thymus DNA than the Ni(II) and Zn(II) complexes.

MATERIALS AND METHODS

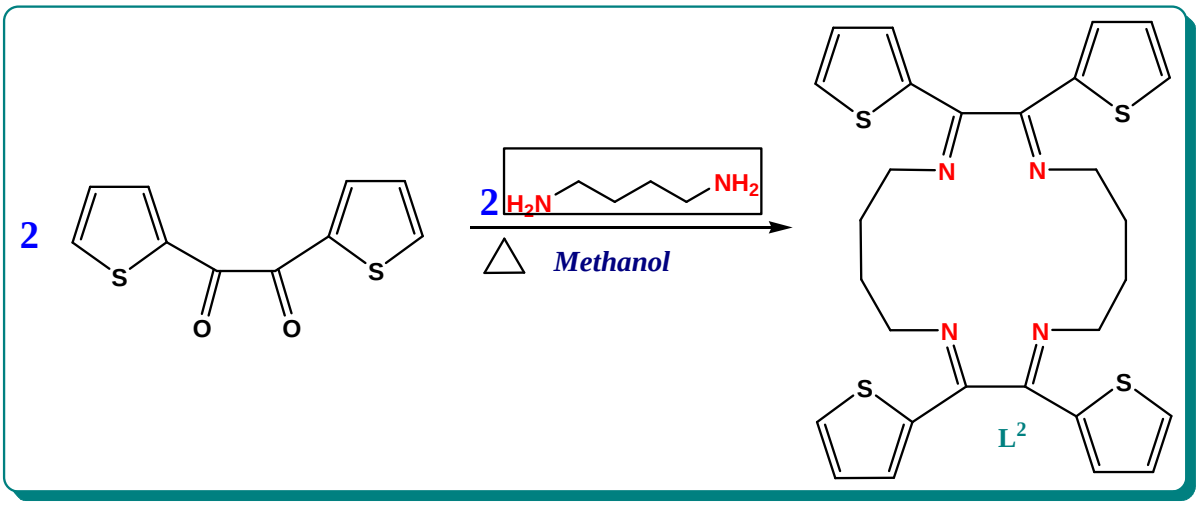
2.1 Chemicals and methods:

Thenil, metal chlorides and 1, 4-diamino butane was purchased from Aldrich. Highly polymerized calf-thymus DNA sodium salt (7% Na content) from Bangalore Genei (India). Deionized water was used in synthesis. Ethidium bromide (EtBr) was purchased from Sigma Chemical Co. Other chemicals were of reagent grade and used without further purification Elemental analysis was recorded on a Carlo Erba model 1106 elemental analyzer. FT-IR spectra (4000–400 cm^{-1}) were recorded as KBr pellets from Perkin Elmer FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 by using TMS as an internal standard using a BRUKER 500 instrument. 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}. Absorbance value of DNA in the absence and presence of complexes were made in the range of 220–300 nm. DNA concentration was fixed at 0.1 mM, while the complexes were varied from 5 µM to 20 µM. The emission spectra were recorded on a Perkin Elmer LS-45 fluorescence spectrometer. Fluorescence spectra were recorded at temperatures 310 K in the range of 510 – 670 nm upon excitation at 355 (λ_{max} was 594 nm).Mass spectral analysis was performed in Q-TOF Mass Spectrometer. Viscosity measurements were recorded using a Brookfield Programmable LV DVII+ viscometer. 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. Tris (hydroxymethyl) aminomethane–HCl (Tris–HCl) buffer solution was prepared by using deionized and sonicated triple distilled water.

2.2 General procedure for the synthesis of compounds:

2.3 Synthesis of the ligand:

A methanolic solution (20 mL) of thenil (1 gm, 0.004 mol) (was slowly added to a methanolic solution 20 mL) of 1,4-diamino butane (0.002 mol, 0.08 ml) with constant stirring. 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 as shown in Scheme 1.

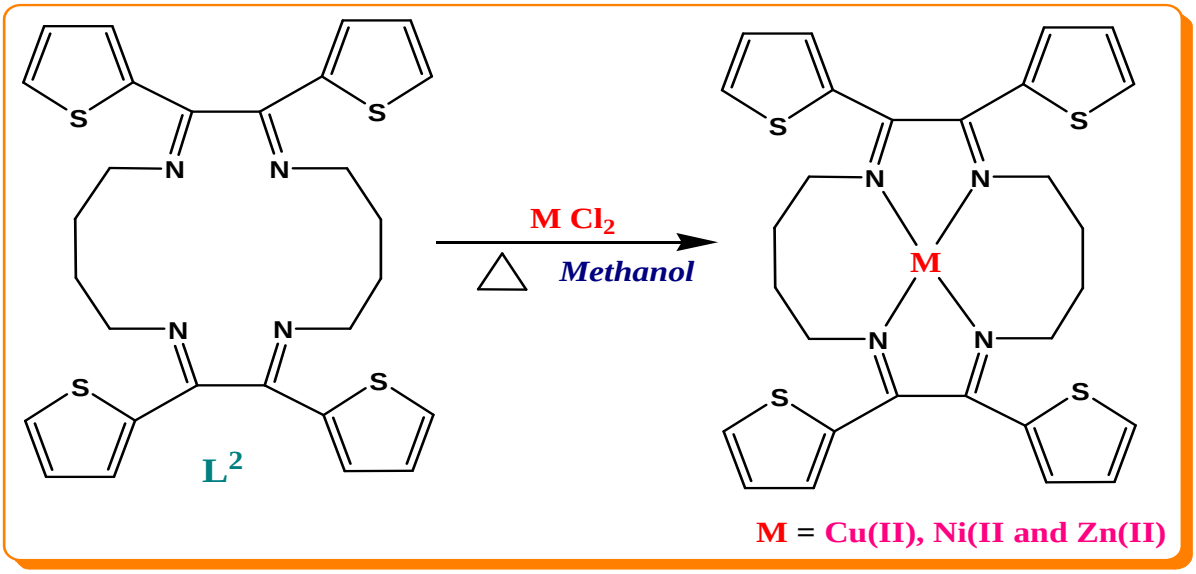


Scheme 1. Synthesis of Schiff base Ligand (L)

2.3 Synthesis of the complexes L:

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 metal complexes

2.4 DNA binding experiments.

2.4.1 Absorption spectral studies

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, εf 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 [20].

2.4.2 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)

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

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) [21].

2.4.3 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) [22]. 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.

2.4.4 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 AND DISCUSSION

3.1 Structural characterization of the Schiff base ligand (L) and their complexes.

3.1.1 FT-IR spectral analysis.

In the IR spectra of Schiff base ligand **L**, a strong band around 1630-1640 cm^{-1} was due to the azomethine linkage and absence of free amine groups around 3240 cm^{-1} conforms the condensation between thenil and 1,4-diamino butane as represented in Figure 1. The bands attributed to $\nu_{(S-H)}$ were found around 2650 cm^{-1} and the other band exhibited at 1100 cm^{-1} which is due to $\nu_{(C-S)}$. In the IR spectra of Schiff base complexes showed a strong band around 1612-1614 cm^{-1} was due to the azomethine linkage to metal complexes. The important strong band observed around at 420 - 430 cm^{-1} for all the complexes are assignable to $\upsilon_{(M-N)}$ vibrations pyridine ring coordination peak [23]. 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 as represented in Figure 2 and Figure S1and S2.

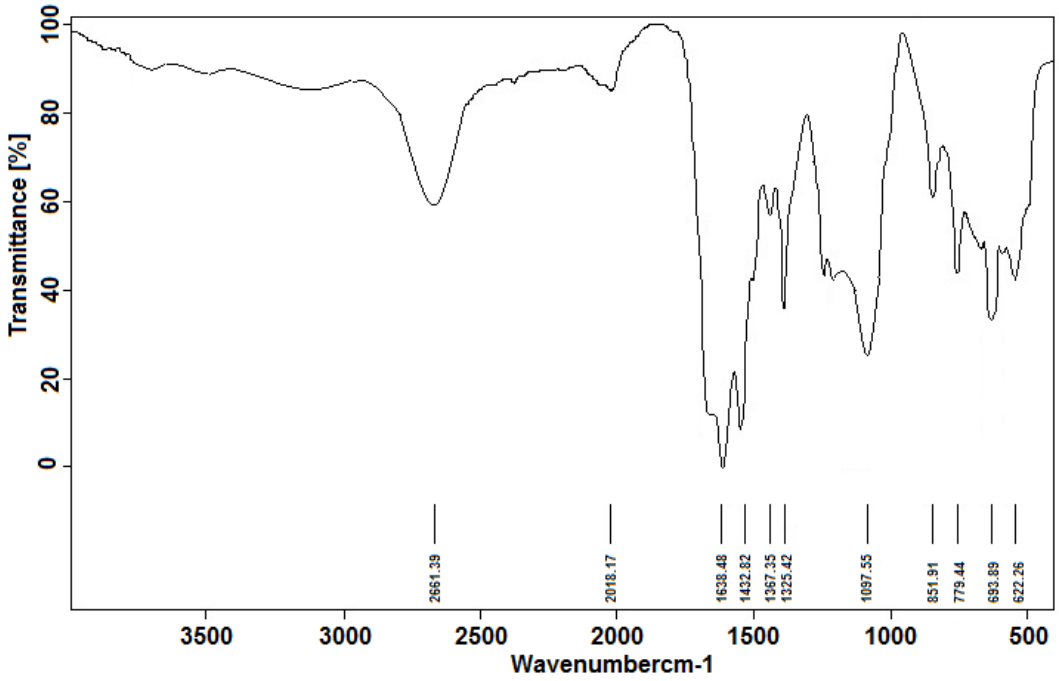


Figure 1. FT-IR spectrum of the Schiff base ligand (L)

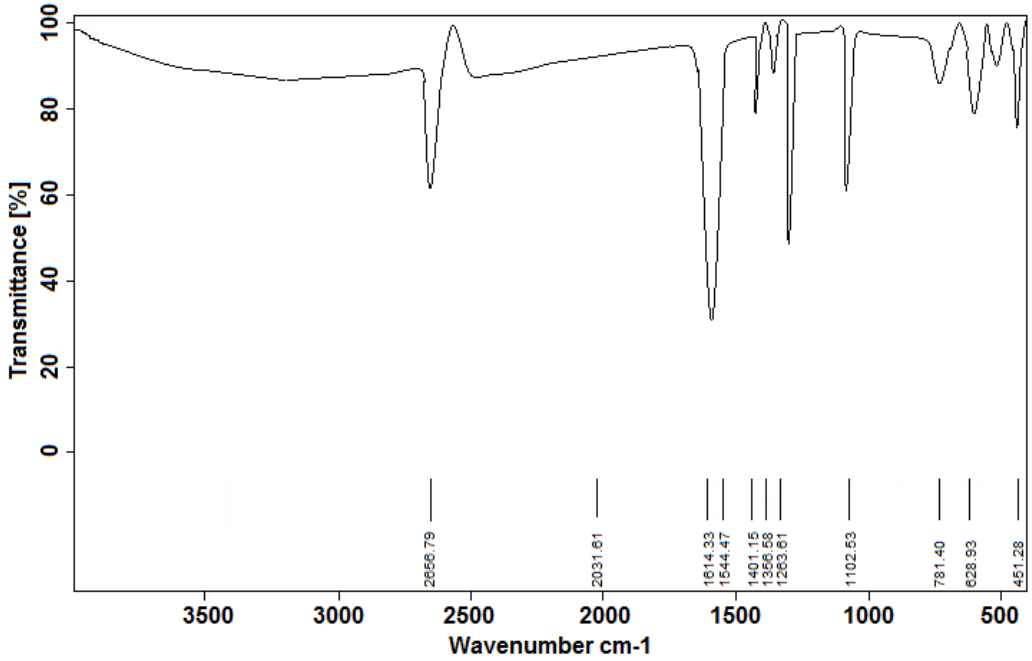


Figure 2. FT-IR spectrum of Cu(II) complex

3.1.2 ESI-MS spectrum

The molecular ion peak [M^+] at m/z = 548 confirms the molecular weight of the macrocyclic Schiff base ligand $C_{28}H_{28}N_4S_4$. The peaks at m/z = 471, 384, 302, 248, 220, 202 and 54 corresponds to the various fragments $C_{20}H_{24}N_4S_2$, $C_{16}H_{22}N_4S$, $C_{12}H_{16}N_4S$, $C_{12}H_{20}N_4$ and $C_{10}H_{26}N_4$ respectively as shown in Figure 3. This confirms the molecular structure of the ligand L. The molecular ion peak [M^+] at m/z = 612 confirms the molecular weight of the macrocyclic Schiff base Cu(II) complex $C_{28}H_{28}N_4S_4Cu$. The peaks at m/z = 530, 448, 365, 283, 227 and 171 corresponds to the various fragments $C_{24}H_{26}N_4S_3Cu$, $C_{20}H_{24}N_4S_2Cu$, $C_{16}H_{22}N_4SCu$, $C_{12}H_{20}N_4Cu$, $C_8H_{12}N_4Cu$ and $C_4H_4N_4Cu$ respectively as shown in Figure 4 and Figure S3and S4. The molecular ion peak [M^+] at m/z = 607 and 614 confirms the molecular weight of the macrocyclic Schiff base Ni(II) and Zn(II) complex $C_{28}H_{28}N_4S_4M$ [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.

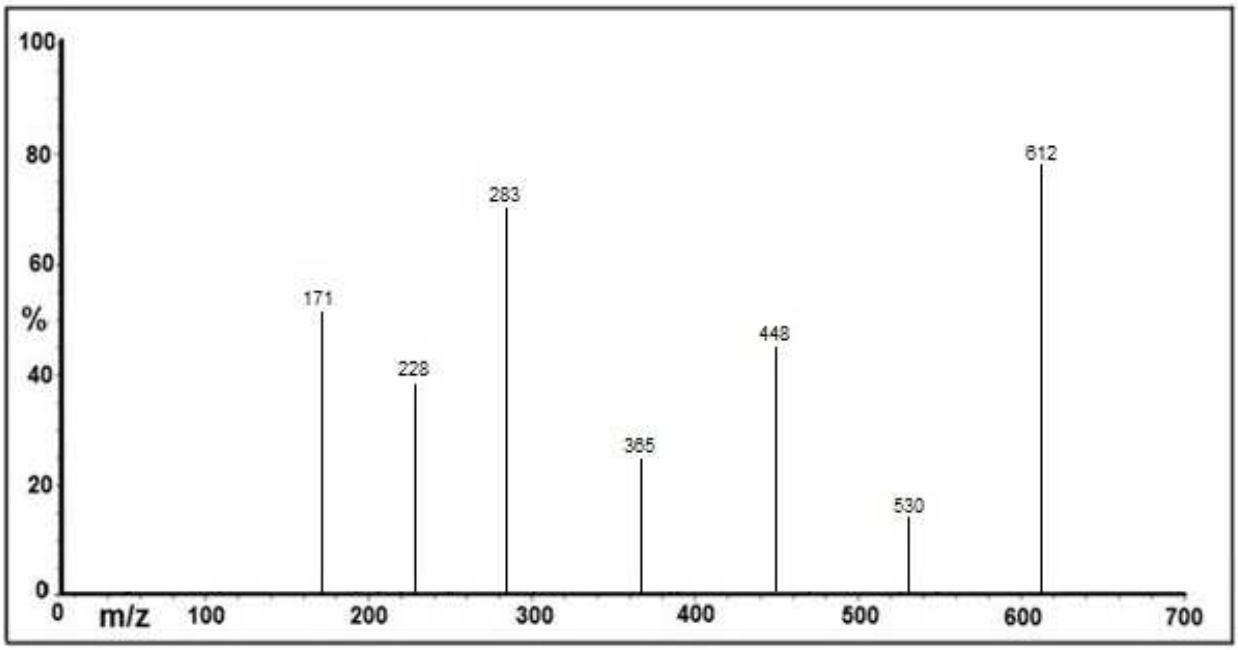


Figure 3. ESI-Mass spectrum of Schiff base ligand (L)

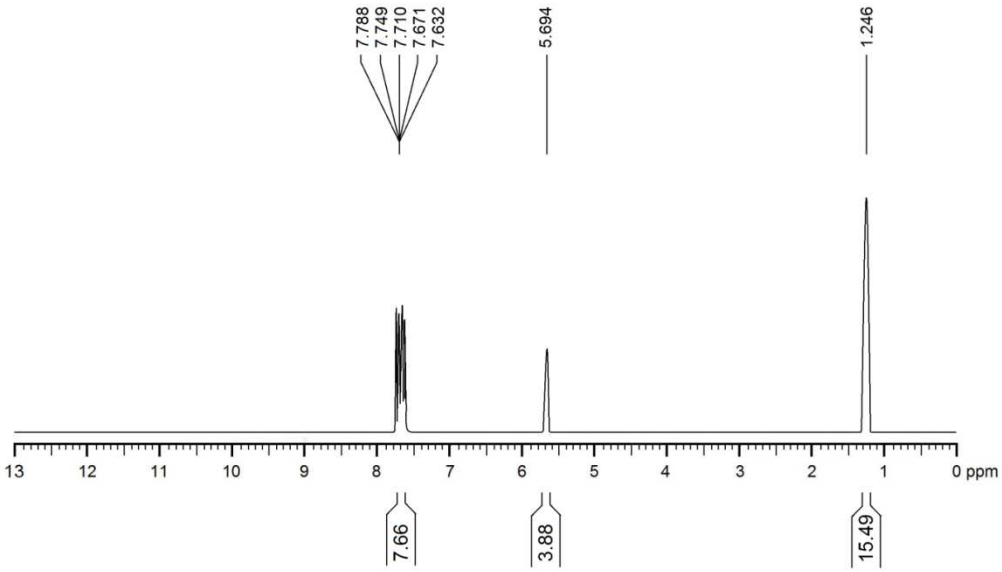


Figure 4. ESI-Mass spectrum of Cu(II) complex

3.1.3 The ^{1}H-NMR spectra

The ^{1}H NMR spectrum showed the peaks of the 16 protons as singlet around at δ 1.24 ppm corresponding aliphatic diamino butane. The singlet (4 H) in the region around δ 5.69 ppm corresponds to the SH protons [24]. The multiplet around the region of δ 7.63 – 7.78 ppm is due to the protons in the thiophene ring as depicted in Figure 5.

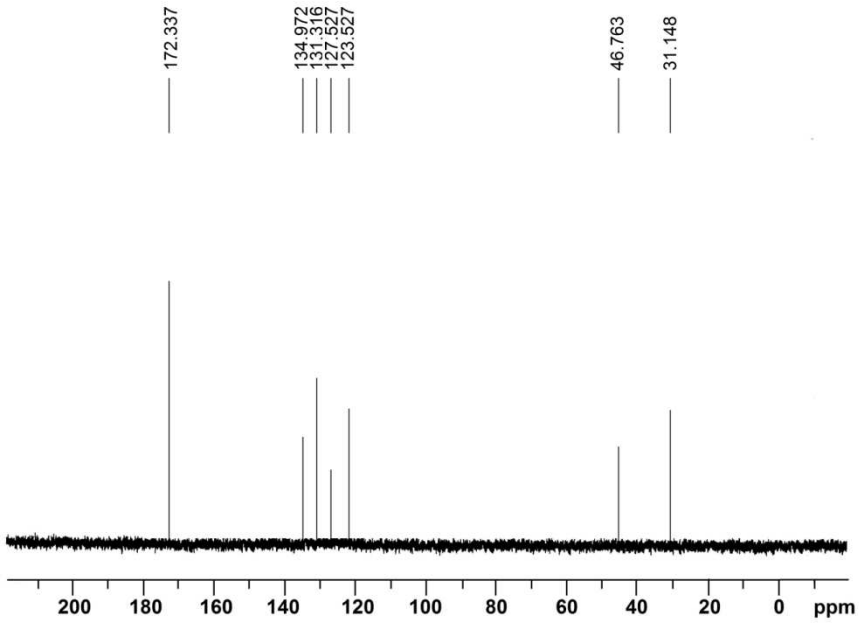


Figure 5. ^{1}H-NMR spectrum of Schiff base ligand (L)

3.1.4 The ^{13}C-NMR spectra

In the ^{13}C-NMR, the signal of carbon atoms owing to azomethine group was observed around 172 ppm. The signals related to the carbon atoms at the thiophene ring are seen around 123, 127, 131 and 134 ppm. The signal around δ 46 ppm corresponds to the methylene (CH_2) carbon of the diamino butane moiety as represented in Figure 6.

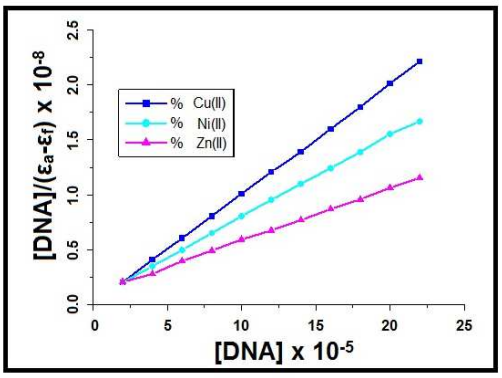


Figure 6. ^{13}C-NMR spectrum of Schiff base ligand (L)

3.2 DNA binding experiments

3.2.1 Absorption spectral studies

UV–vis absorption studies were performed to further ascertain the predicted binding trend of Cu(II), Ni(II) and Zn(II) complexes with DNA. The interactions of complexes in the absence and presence of increasing amount CT-DNA (at a constant concentration of complexes) are given in Figures 7 and S5 and S6 respectively. The electronic absorption spectroscopy is one of the most common method to study the DNA-binding properties of M(II) complexes, since there are strong MLCT (metal-to-ligand), LMCT (ligand-to-metal) and charge transfer (intraligand) features were observed in the absorption spectra of these complexes. 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 suggested an intimate association of the compounds with CT-DNA and it is also likely that these compounds bind to the helix *via* intercalation [25**]**. After the compounds intercalate to the base pairs of DNA, the π* orbital of the intercalated compounds could couple with π orbitals of the base pairs, thus decreasing the π → π* transition energies. Therefore, these interactions resulted in the observed hypochromism

[26]. The Cu(II) complex showed more hypochromicity than the other complexes, indicating that the binding strength of the copper(II) complexes are much stronger than that of the other synthesized complexes.

The intrinsic binding constant (K_b) for the association of complexes with CT-DNA (Figure 7 Inset) were found to be 5.4 x 10^4 M^{-1} for Cu(II), 4.1 x 10^4 $M^{-1}$for Ni(II) 3.9 x 10^4 M^{-1} for Zn(II) respectively which are comparable to that observed for typical classical intercalators, it suggests a mode of intercalative binding that involves a stacking interaction between the complexes and the base pairs of DNA. From the electronic absorption studies, though it has been found that the three compounds can bind to DNA by intercalation, the binding mode need to be proved through some more experiments.

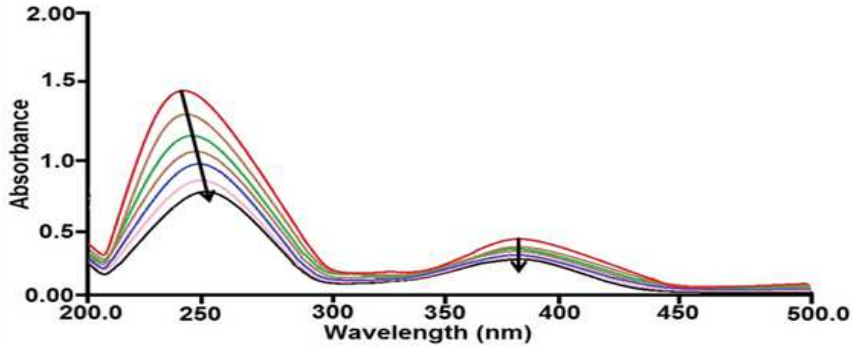


Figure 7. Absorption spectra of complex Cu(II) (1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10^{-3} M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5). The Inset shows the plots of [DNA] / (ϵ_a-ϵ_f) versus [DNA] for the titration of DNA with Cu(II),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

3.2.2 Fluorescence spectral studies

The fluorescence quenching experiments were performed to get an estimate on the relative binding affinity of the complexes to CT-DNA with respect to EB. It is well known [27] that free EB displays a decrease in emission intensity in Tris. HCl buffer medium because of quenching by solvent molecules. However EB strongly fluoresces in the presence of DNA, due to complete intercalation between the adjacent DNA base pairs, a process that can be reversed by addition of a competing molecule (fluorescence quenching) [28]. Fixed amount of DNA was titrated with increasing amount of Cu(II), Ni(II) and Zn(II) complexes as depicted in Figure 8 and Figure S7 and S8. Titration of CT-DNA lead an appreciable reduction in emission intensity upon addition of Cu(II), Ni(II) and Zn(II) complexes to CT-DNA pretreated with EB fluorophore resulting in decrease in binding of EB to DNA. The fluorescence of EB tends to increase after interacting with DNA. If metal complex interact with DNA, it leads to decrease the fluorescence intensity of EB-DNA system [29].

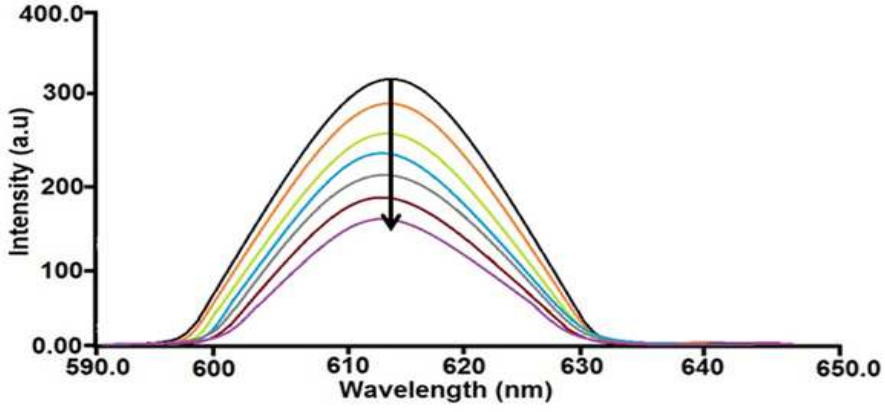


Figure 8. Emission spectrum of EB bound to DNA in the presence of Cu(II) complex ([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-25 µM, λex= 430 nm). Arrow shows the absorbance changing upon increasing complex concentrations. Inset shows the plots of emission intensity I_o / I *vs* [DNA] / [complex] for the titration of DNA with Cu(II), Ni(II) and Zn(II) complexes.

The quenching efficiency is calculated bythe quenching constants K_{app} of all the complexes were calculated according to the classical Stern–Volmer equation. The quenching plots illustrate that the binding of DNA by the complexes is in good agreement with the linear Stern–Volmer equation. In the linear fit plot of I_0/I vs [complex], the

K values calculated for complexes are 6.3 x 10^5 M^{-1} for Cu(II), are 6.0 x 10^5 M^{-1} for Ni(II) and are 5.8 x 10^5 M^{-1} for Zn(II) respectively as shown in the Figure 8 inset. The above results showed that all the complexes could replace EB from the DNA–EB system, and a complex-DNA system was formed. The reduced emission of the DNA–EB system was caused by EB being expelled from the hydrophobic environment into the water solution [30]. Hence, the fluorescence studies indicated that Cu(II), Ni(II) and Zn(II) complexes binds to DNA by intercalation.

3.2.3 Viscosity study

To further reinforce our findings obtained by the studies given in preceding sections, we have carried out viscosity measurements for Schiff base complexes with CT-DNA. Hydrodynamic measurement such as viscosity study is least ambiguous and the most critical test of binding mode in solution in the absence of crystallographic structural data [31]. A classical intercalation model demands that the DNA helix lengthens as base pairs are separated to accommodate the bound ligand, leading to the increase of DNA viscosity. In contrast, a partial, non-classical intercalation of ligand could bend (or kink) the DNA helix, reducing its length and, concomitantly, its viscosity [32]. A series of solutions was made which contained a fixed concentration of DNA and various concentrations of the synthesized complex. Then, the viscosity measurements were conducted at room temperature. Figure 9 displays the changes in relative viscosity of DNA with increasing concentrations of Cu(II), Ni(II) and Zn(II) complexes. It was observed that the relative viscosity of DNA increased steadily with increasing the amounts of Cu(II), Ni(II) and Zn(II) complexes. These results suggested an intercalative binding mode of the complexes with DNA and also hold up the results obtained from absorption and emission studies.

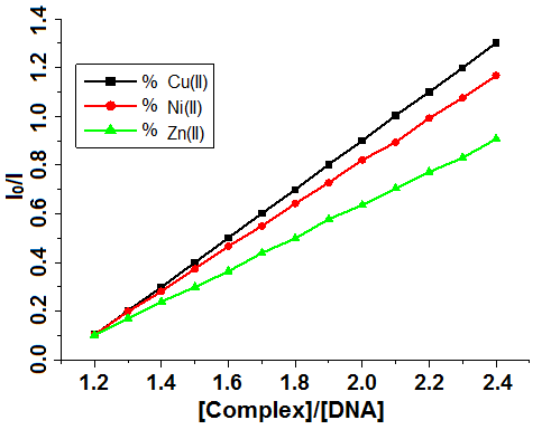


Figure 9. Viscosity measurements of the Cu(II), Ni(II) and Zn(II) complexes

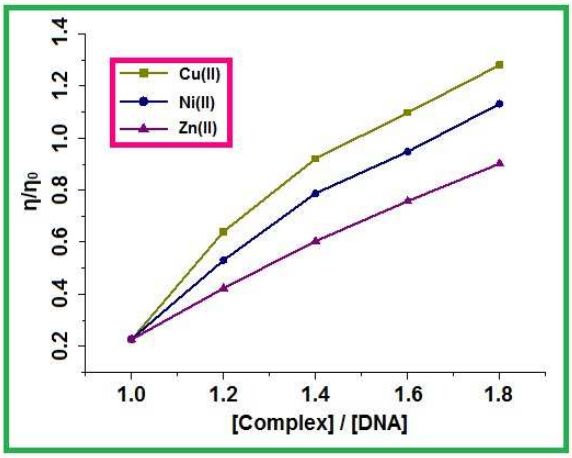
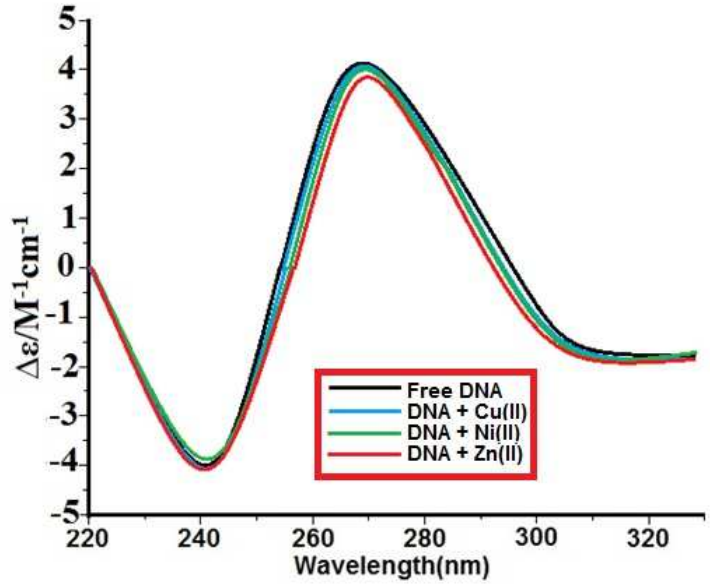


Figure 10. CD spectra recorded over the wavelength range 220-320 nm for solutions containing 2:1 ratio of CT-DNA (200 µM) and mononuclear Cu(II), Ni(II) and Zn(II) complexes (100 µM)

3.2.4 Circular dichoric spectral studies

CD spectroscopy is a useful technique to analyze interactions between complex and CT-DNA. It is also useful because CD signals are quite sensitive to the mode of DNA interaction with small molecules [33]. 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 [34]. Simple groove binding and electrostatic interactions with small molecules show less of a perturbation or no perturbation whatsoever on the base stacking and helicity bands [35]. The interaction between the complexes and CT-DNA was further studied by CD spectroscopy as shown in Figure 10. In this case the CD spectra of CT-DNA in the presence of both 1 and 2 showed a considerable increase in the intensity of the band at 275 nm, whereas the band at 248 nm did not show any significant change. The intensity increase of the band at 275 nm was less pronounced for Cu(II) complex than for Ni(II) and Zn(II) complexes. This indicates that the synthesized schiff base complexes might interact with the DNA double strands by the intercalative mode between the base pairs of DNA strands without any significant change in the right handed helicity of the DNA.



CONCLUSION

In the present study, novel Schiff base ligand L and their Cu(II), Ni(II) and Zn(II) complexes were prepared and characterized by physico-chemical methods. Furthermore, we explored the binding interaction of the complexes CT-DNA in physiological buffer using UV–Vis, fluorescence, viscosity and circular dichoric spectral studies. The DNA binding experiments using electronic spectral technique show the hypochromism at d–d transition region and hypochromism and red shift at the charge transfer region. UV–Vis absorption spectra, circular dichroism, fluorescence spectra and viscosity measurements confirm the intercalative binding mode of complexes. 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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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