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Research Article

**MIXED LIGAND COMPLEXES OF CU(II)/NI(II)/ ZN(II) IONS  
CONTAINING 1,2-DI(2-THIENYL)-1,2-ETHANEDIONE,  
HYDRAZINE HYDRATE AND METHYL  
SALICYLALDEHYDE: SYNTHESIS, CHARACTERIZATION  
AND DNA BINDING ACTIVITY.**Thiruchendil Muthu K, Iyyam Pillai S, and P. Venkatesh<sup>1\*</sup><sup>1</sup>P.G and Research Department of Chemistry, Pachaiyappa's College, Chennai-600030,  
Tamil Nadu, India.**Received:** 20 February 2016    **Accepted:** 26 February 2017    **Published:** 28 February 2017**Abstract:**

Three new metal complexes derived from the reaction of Cu(II), Ni(II) and Zn(II) acetates with the Schiff-base ligand (HL) resulted from the condensation of the 1,2-Di(2-thienyl)-1,2-ethanedione (Thenil), hydrazine hydrate and 2-Hydroxy-5-methylbenzaldehyde have been synthesized. The structure of the synthesized ligand and complexes was characterized by, elemental analyses, Mass, IR, UV-Vis., measurements. The binding properties of these complexes towards DNA have been investigated using electronic absorption, competitive fluorescence titration, viscosity measurements and circular dichroic analyses. The results obtained indicated that the complexes get bonded with DNA via an intercalation binding mode with an intrinsic binding constant,  $K_b$   $5.01 \times 10^4 M^{-1}$ ,  $4.68 \times 10^4 M^{-1}$  and  $4.21 \times 10^4 M^{-1}$  and  $K_{app}$  of  $5.82 \times 10^5 M^{-1}$ ,  $4.97 \times 10^5 M^{-1}$  and  $4.29 \times 10^5 M^{-1}$ .

**Keywords:** Thenil, DNA binding, methyl salicylaldehyde, Intercalation, hydrazine hydrate.**Corresponding author:****Dr. P.Venkatesh,**

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## INTRODUCTION:

In recent decades, hydrazone compounds have attracted lots of attention not only because of their variety of structure, but also for their various biological and chemical applications [1-2]. These properties of the hydrazones are attributed to the formation of stable chelated complexes with transition metals which catalyze physiological processes. Their metal complexes, have also found applications in various chemical processes like nonlinear optics, sensors, medicine [3]. Hydrazones and their metal complexes exhibit wide range of biological and pharmaceutical activities that includes antimicrobial, antituberculostatic, anticancer and antioxidant behaviour. In addition, hydrazones also serve as an excellent polydentate chelating agent capable of forming coordination complexes with variety of both transition and inner transition metal cations. Metal complexes derived from hydrazides of carboxylic acids have been extensively investigated due to the high physiological activities of the free ligands and the presence of a chelator group of donor atoms in the coordination sphere [4-7]. Nowadays, increasing cancer cases have been in a worrying trend and it has become one of the principal causes of death in most of the countries. It is the second most common disease after cardiovascular diseases, responsible for maximum numbers of deaths all over the world. The modernization of our society is a major factor contributing to the increasing incidence of cancer [8-10]. Though the discovery of cis-platin and other platinum containing drugs resulted in coining the terminology of elemental medicines, it exhibits high toxicity to normal cells which leads to undesirable side-effects. Moreover, majority of these medicines are inactive against many cancer cell lines and metastasis (secondary) cancers [11]. Cisplatin is ranked the second among hundreds of antitumor drugs on the market. As the first inorganic antineoplastic agent in success, cisplatin and its derivatives have attracted great attention and interest of bioinorganic chemists despite severe side effects such as nephrotoxicity and drug resistance during therapy process [12 - 13]. These shortfalls have motivated researchers to develop new and efficient alternatives to cisplatin. Developing metal complexes as drugs, however, is not an easy task. Schiff base metal complexes have received special attention because of their biological activity. Many Schiff bases are known to be medicinally important and are used to design medicinal compounds [14-15]. The chemistry of Schiff base ligands and their metal complexes have attracted a lot of interest due to their facile synthesis and wide range of applications. These compounds play an important role in the development of coordination chemistry related to catalysis, organic synthesis [16-17] antitumor, antimicrobial and cytotoxic activity [18-19].

The interaction of transition metal complexes with DNA has been intensively studied to develop newer materials for application in biotechnology and medicine in recent years. Generally, DNA is the

essential target molecule for most disease therapies such as anticancer and antiviral. Any disorder in gene expression may cause diseases and plays a secondary role in the outcome and severity of human diseases. Transition metal complexes can interact with DNA through the covalent or non-covalent modes such as groove binding, intercalation and external static electronic effects among them the most important is intercalation [20-21].

As a result, the progress of synthetic, sequence-selective binding agents for DNA targeted antitumor drugs is fundamental to promote expected applications in molecular biology, medicine and related fields. Bearing all the above information in mind, herein we have synthesized few mixed ligand complexes having as a potential drug candidate and characterized by spectral methods.

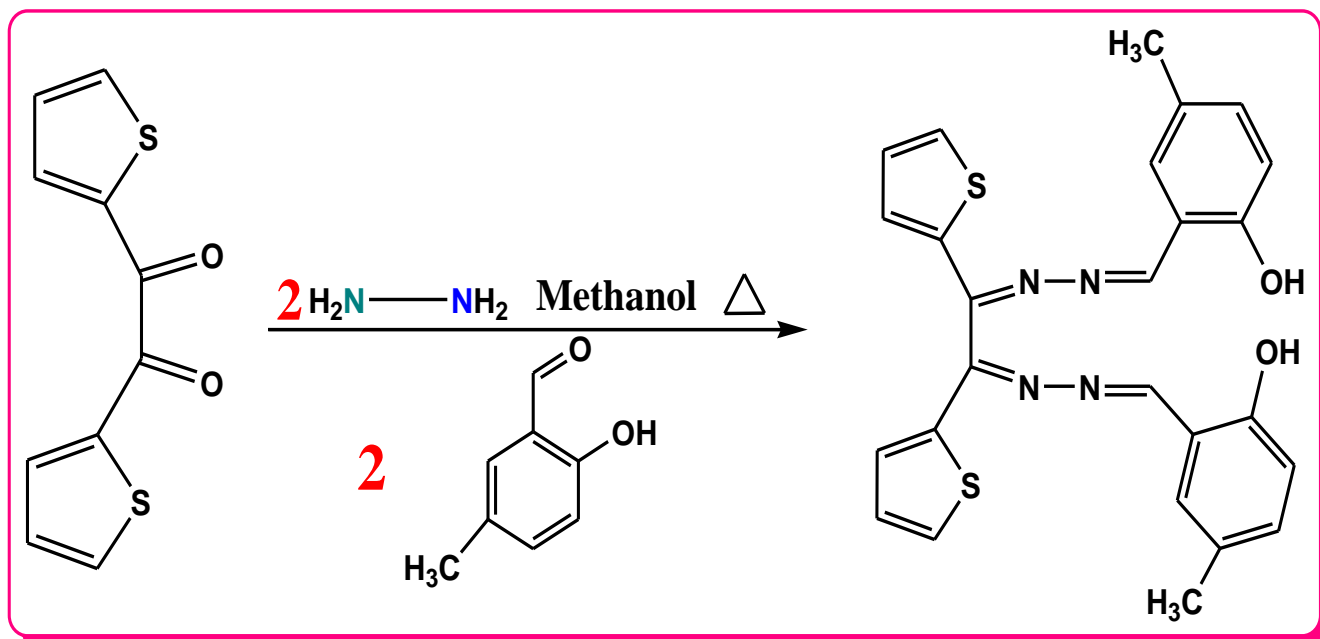
## EXPERIMENTAL PROTOCOLS:

### Reagents and instruments

All the chemicals used in the current work viz, 1,2-Di(2-thienyl)-1,2-ethanedione (Thenil), hydrazine hydrate, 2-Hydroxy-5-methylbenzaldehyde and metal(II) acetates were of analytical reagent grade (produced by Merck, Germany). 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 by means of 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  $\text{cm}^{-1}$ . UV-Vis spectra were recorded using a Perkin Elmer Lambda 35 spectrophotometer operating in the range of 200-900 nm with quartz cells and  $\epsilon$  are given in  $\text{M}^{-1}\text{cm}^{-1}$ . 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  $25 \pm 0.1^\circ\text{C}$  with 0.1 cm path length cuvette.

### Synthesis of Schiff base ligand L

A warm solution of hydrazine hydrate (0.010 mol, 0.4 ml) taken in 20ml of methanol was added slowly to a methanolic solution (~25 ml) of 1,2-Di(2-thienyl)-1,2-ethanedione (0.005 mol, 1 g) placed in round bottom flask. To this reaction mixture 2-Hydroxy-5-methylbenzaldehyde (0.010 mol, 1.36 g) in methanol is added as shown in Scheme 1. The reaction mixture was stirred for overnight followed by refluxing at  $80^\circ\text{C}$  for 6 h. Removal of solvent at reduced pressure gave the crude product. The product was washed twice with diethyl ether and recrystallized from chloroform.



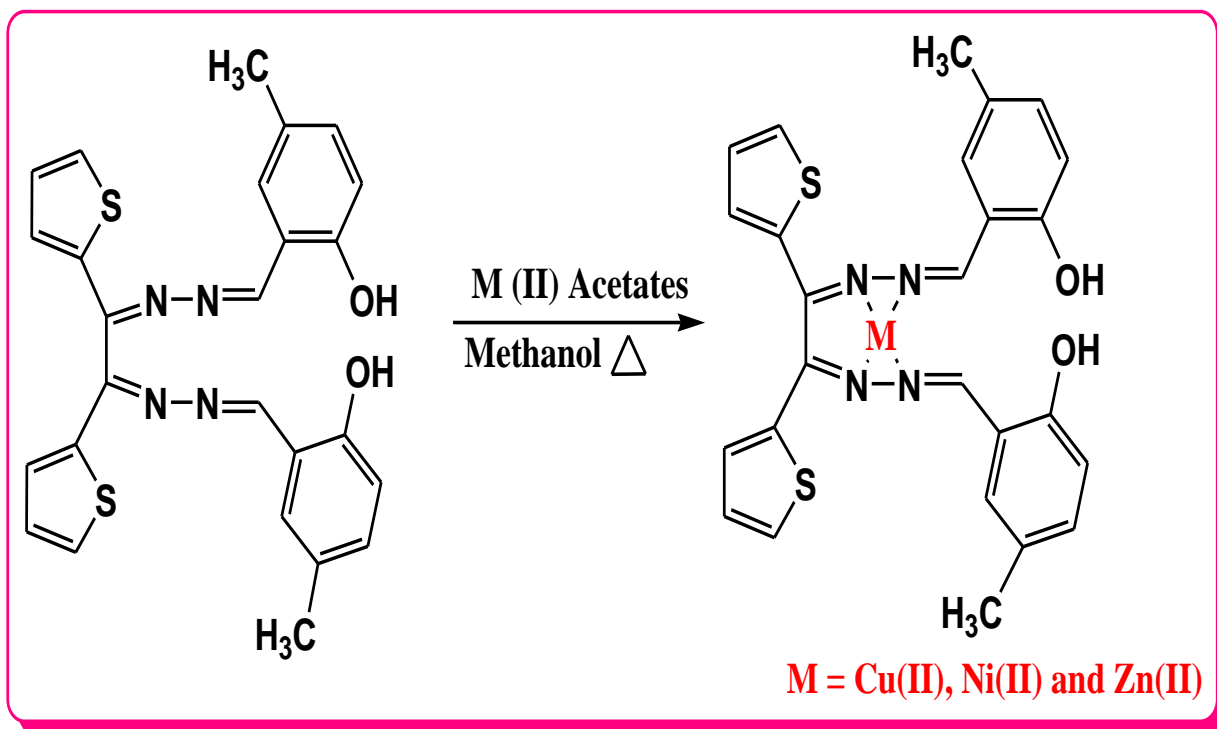
Scheme 1: Synthesis of Schiff base ligand L.

**Synthesis of Schiff base metal complexes.**

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

To a warm methanolic solution (~25 ml) of the metal acetates (0.001 mol) taken in round bottom flask was added slowly a methanolic solution (~20 ml) of ligand L (0.001 mol, ~ 0.5 gm). The

reaction mixture was stirred for 6 h followed by refluxing for 4 h. The coloured solid product was isolated on slow evaporation over a period of four days as shown in Scheme 2. The product was washed several times with methanol and dried in vacuo.



Scheme 2: Synthesis of Schiff base metal complexes.

### DNA binding experiments

#### Absorption spectral studies

CT-DNA (20 mg) was dissolved in Tris-HCl buffer (100 mL, 50 mM Tris-HCl, 50 mM NaCl, pH = 7.2) and kept at 4 °C for less than 4 days. The absorption ratio A<sub>260</sub>/A<sub>280</sub> was within the range of 1.8–2.0. The DNA concentration was determined via absorption spectroscopy using the molar absorption coefficient of 6,600 M<sup>-1</sup> cm<sup>-1</sup> (260 nm) for CT-DNA [22]. Absorption titration experiments were performed by varying the concentration of CT-DNA (0, 40, 80, 120, 160, 200, 300 and 400 μM) while keeping the drug and its metal complexes concentration constant (40 μM). The reference solution was the corresponding buffer solution. Upon measuring the absorption spectra, an equal amount of CT-DNA solution was added to both the compound solution and the reference solution to eliminate the absorbance of DNA itself. The solutions were allowed to incubate for 10 min at room temperature before the absorption spectra were recorded. Each sample solution was scanned in the range of 200–400 nm. The titration processes were repeated until no change in the spectra, indicating binding saturation had been achieved. The equilibrium binding constant (K<sub>b</sub>) values for the interaction of the complex with CT-DNA were obtained from absorption spectral titration data using the following equation (1) [23].

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

(1)

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

#### Fluorescence emission spectral studies

The enhanced fluorescence of EB in the presence of DNA can be quenched by the addition of a second molecule [24]. The extent of fluorescence quenching of EB bound to CT-DNA can be used to determine the extent of binding between the second molecule and CT-DNA. Competitive binding experiments were carried out in the buffer by keeping [DNA]/[EB] = 1 and varying the concentrations of the compounds. The fluorescence spectra of EB were measured using an excitation wavelength of 520 nm, and the emission range was set between 550 and 750 nm. Quenching of the fluorescence of Eth-Br bound to DNA were measured with increasing amount of metal

complexes as a second molecule and Stern–Volmer quenching constant K<sub>sv</sub> was obtained from the following equation [25].

$$I_0/I = 1 + K_{sv}r \quad (2)$$

Where I<sub>0</sub> is the ratio of fluorescence intensities of the complex alone, I is the ratio of fluorescence intensities of the complex in the presence of CT-DNA. K<sub>sv</sub> 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<sub>0</sub> / I vs. [complex] / [DNA], K<sub>sv</sub> is given by the ratio of the slope to the intercept. The apparent binding constant (K<sub>app</sub>) was calculated using the equation K<sub>EB</sub>[EB] / K<sub>app</sub>[complex], where the complex concentration was the value at a 50% reduction of the fluorescence intensity of EB and K<sub>EB</sub> = 1.0 × 10<sup>7</sup> M<sup>-1</sup> ([EB] = 3.3 μM).

#### Viscosity measurements

Viscosity measurements were carried out using a capillary viscometer at a constant temperature (25.0 ± 0.1 °C). Flow times were measured with a digital stopwatch, and each sample was measured three times, and then an average flow time was calculated. To find the binding mode of the complexes towards CT-DNA, viscosity measurements were carried out on CT-DNA (0.5 mM) by varying the concentration of the complexes (0.01 mM, 0.02 mM, 0.03 mM, 0.04 mM, 0.05 mM). Data were presented as (η/η<sub>0</sub>) versus binding ratio of concentration of complex to that of concentration of CT-DNA, where η is the viscosity of DNA in the presence of complex and η<sub>0</sub> is the viscosity of DNA alone.

#### 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.

## RESULTS:

### Structural characterization of the Schiff base ligand and their respective metal complexes.

#### FT-IR spectral analysis

The ligand and their respective metal complexes were synthesized in a very facile and essentially identical method. The IR spectra of the Schiff base complexes were compared with the free ligand in order to determine the coordination sites that may involved in chelation. The position and or the intensities of these peaks are expected to be changed upon chelation. The IR spectra of the ligand and metal complexes were recorded in the range of 400–4000 cm<sup>-1</sup> as shown in Figure 1, 2 and S1, S2. The FT-IR spectrum of all compounds

shows strong bands around  $[3400 - 3300 \text{ cm}^{-1}]$  and  $850 \text{ cm}^{-1}$  region which were assignable to phenolic -OH and  $\nu(\text{C-S-C})$  vibration group. Practically no effect on these frequencies after complexation indicates non involvement of these groups in coordination. In the free ligand, the spectrum showed a strong band at  $1645 \text{ cm}^{-1}$  in the spectrum assignable to  $\nu(\text{C=N})$  of azomethine. On the other hand, in the coordination complexes this band was shifted to lower frequencies ( $15-25 \text{ cm}^{-1}$ )

indicating the participation of azomethine nitrogen in coordination to metal ions [26]. The bands appearing in the region  $1480-1440 \text{ cm}^{-1}$  and  $1100-1064 \text{ cm}^{-1}$  were usual modes of phenyl ring vibrations [27]. The new bands in the region of  $478-498 \text{ cm}^{-1}$  in all the complexes are assigned to stretching frequencies of  $(\text{M-N})$  bonds respectively [28]. Thus, the IR spectral data results provide strong evidences for the complexation of Schiff-bases with metal(II) ions.

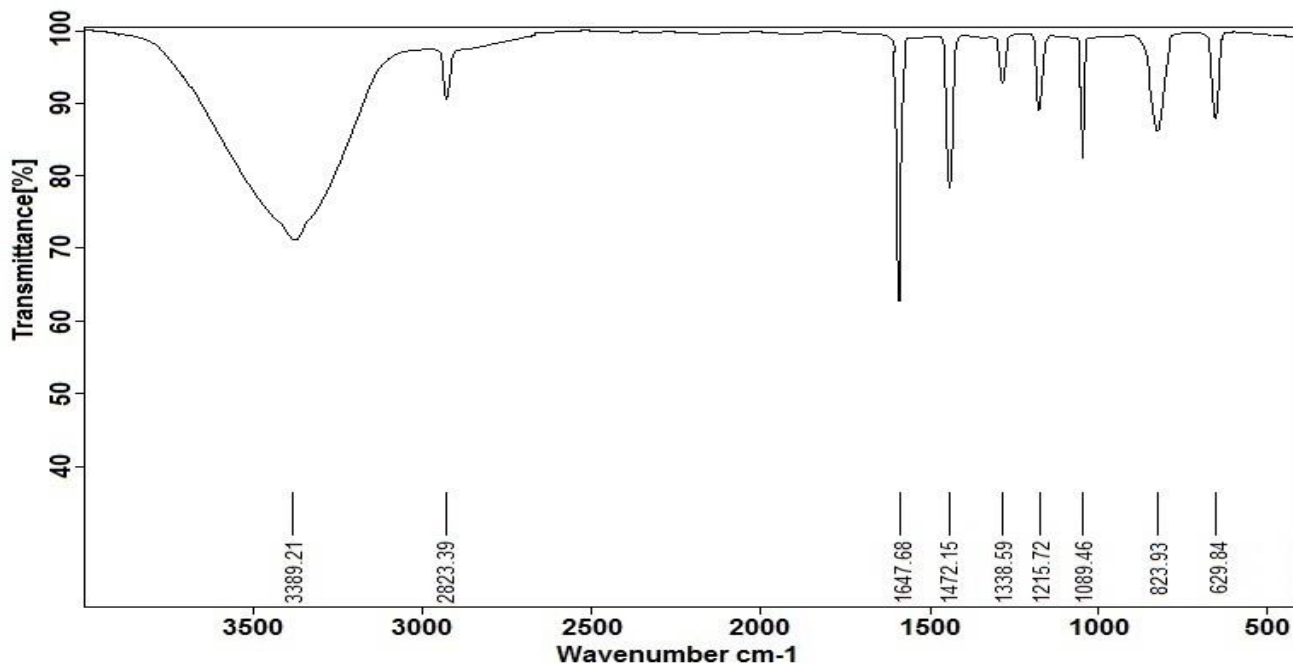


Fig 1: FT-IR spectrum of the Schiff base ligand (L).

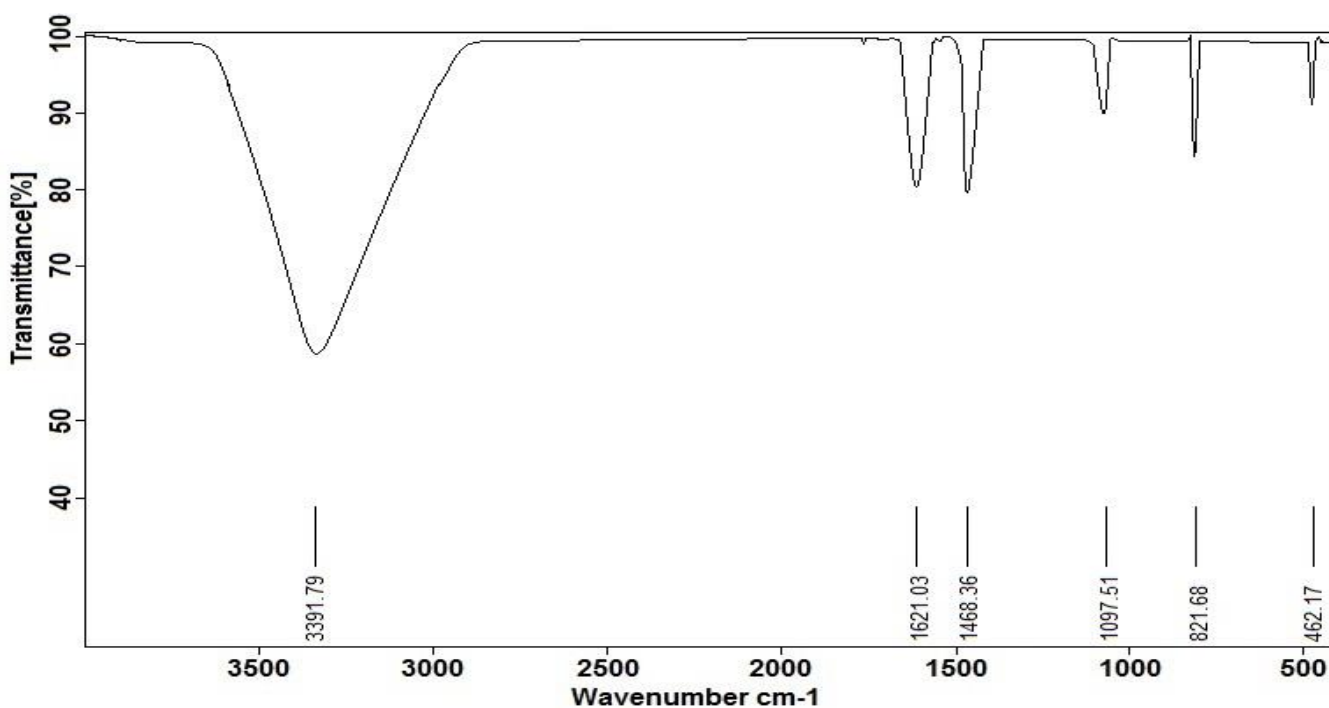


Fig 2: FT-IR spectrum of Cu(II) complex.

**UV-Vis spectral analysis:**

The electronic spectra of the ligand and their respective complexes were recorded in DMSO at room temperature as shown in Figure 3a and 3b. In the spectrum of the Schiff base ligand, the bands appeared in the region of 350-390 and 250 - 300 nm were assignable to the  $n-\pi^*$  transition of the azomethine group and for the  $\pi-\pi^*$  transition of the aromatic ring. In the formation of the complexes, notably these bands are shifted to lower wavelength, suggesting that the nitrogen atom of

the azomethine group is coordinated to the metal ion. 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  ${}^2B_{1g} \rightarrow {}^2E_g$  and  ${}^2B_{1g} \rightarrow {}^2B_{2g}$  transitions [29] in a distorted square planar Cu(II) environment. The Ni(II) complex is diamagnetic and the band around 440 nm could be assigned to  ${}^1A_{1g} \rightarrow {}^1B_{1g}$  transition, consistent with other square-planar nickel(II) complexes [30].

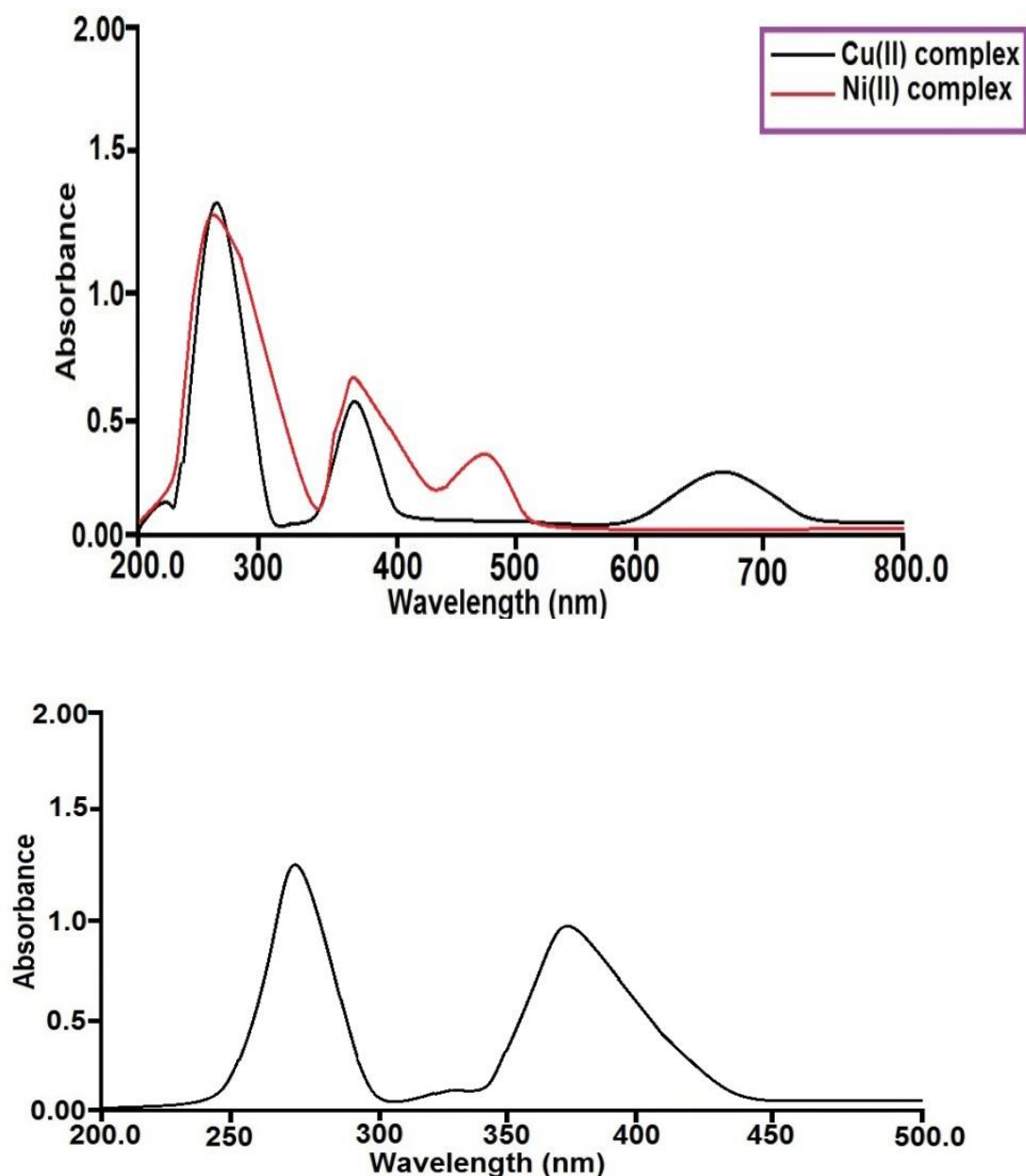


Fig 3: Electronic spectrum of the (a) ligand (L), (b) Cu(II) and Ni(II) complexes.



**Mass spectral analysis:**

The molecular ion peak  $[M^+]$  at  $m/z = 486$  confirms the molecular weight of the Schiff base ligand L  $C_{26}H_{22}N_4O_2S_2$ . The peaks at  $m/z = 422, 360, 278, 196, 110$  and  $88$  corresponds to the various fragments  $C_{21}H_{18}N_4O_2S_2$ ,  $C_{16}H_{16}N_4O_2S_2$ ,  $C_{12}H_{14}N_4O_2S$ ,  $C_8H_{12}N_4O_2$ ,  $C_6H_6N_4$  and  $C_2H_8N_4$  respectively as shown in Figure 4.

The molecular ion peak  $[M^+]$  at  $m/z = 550$  confirms the molecular weight of the Schiff base Cu(II) complex  $C_{26}H_{22}N_4O_2S_2Cu$ . The peaks at  $m/z = 458,$

$365, 297, 229, 173$  and  $123$  corresponds to the various fragments  $C_{20}H_{18}N_4OS_2Cu$ ,  $C_{14}H_{14}N_4S_2Cu$ ,  $C_{11}H_{14}N_4SCu$ ,  $C_8H_{14}N_4Cu$ ,  $C_4H_6N_4Cu$  and  $H_4N_4Cu$  respectively as shown in Figure 5. The molecular ion peak  $[M^+]$  at  $m/z = 545$  and  $551$ , confirms the molecular weight of the Schiff base Ni(II) and Zn(II) complex  $C_{26}H_{22}N_4O_2S_2M$  [ $M = Ni(II)$  and  $Zn(II)$ ] as represented in Figure S3 and S4. The type of fragmentation observed in Ni(II) and Zn(II) complex was similar with that of the Cu(II) complex.

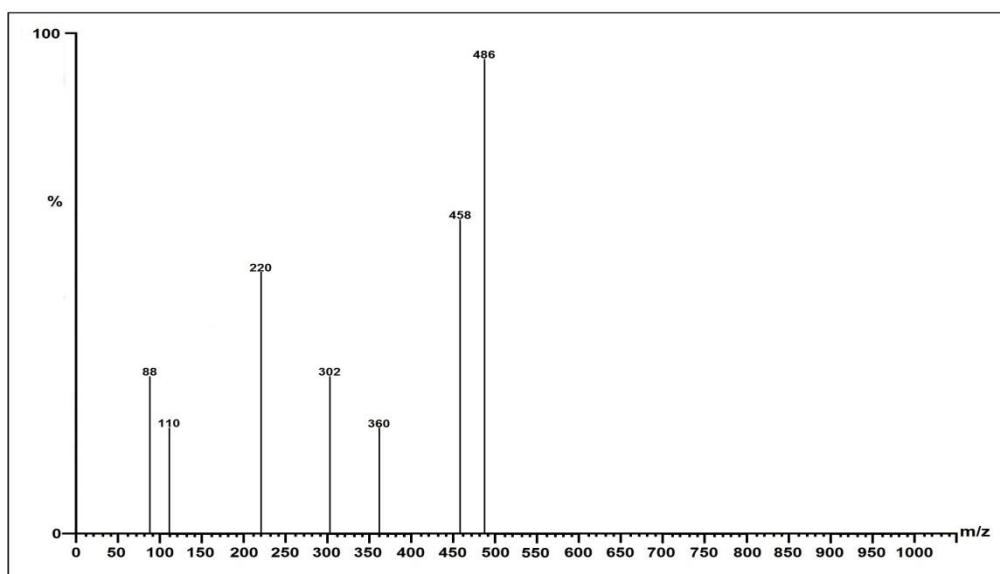


Fig 4: ESI-Mass spectrum of Schiff base ligand (L).

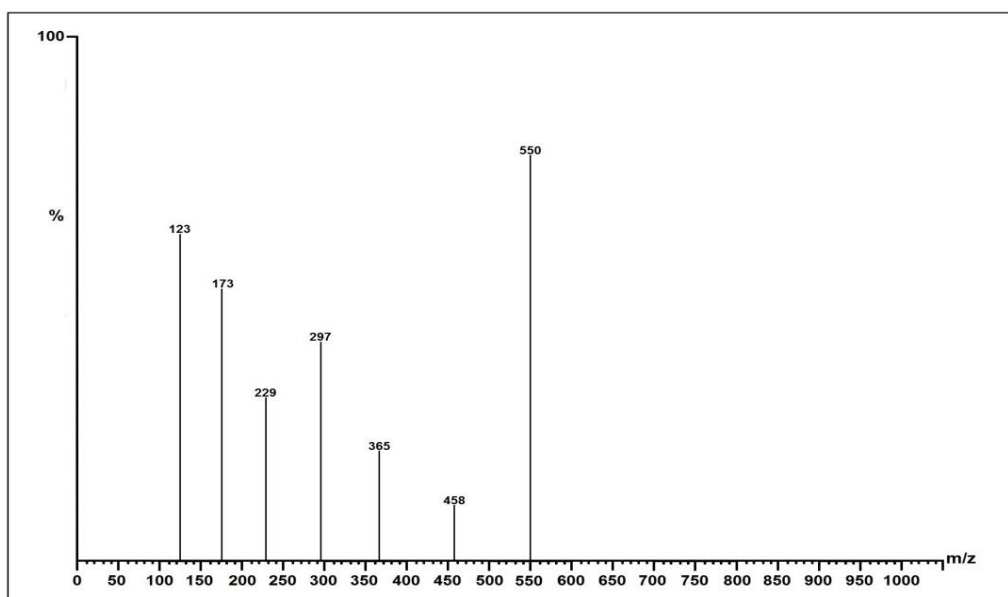


Fig 5: ESI-Mass spectrum of Cu(II) complex.

### DNA binding experiments

#### Absorption spectral studies

Electronic absorption spectroscopy has been widely employed to determine the binding ability of metal complexes with DNA [31]. DNA binding experiments were carried out in 0.5mL Tris-HCl/NaCl buffer [50mM Tris-HCl and 5mM NaCl (pH 7.2)] using DMF solution (15 $\mu$ L) of the complexes. Absorption titration measurements were done by varying the concentration of CT DNA but keeping the metal complex concentration as constant. Drugs binding with DNA via intercalation usually result in hypochromism and bathochromism of the absorption bands due to strong stacking interactions between aromatic chromophore of molecule and the base pairs of DNA. On the other hand, the absorption intensities of drugs are increased (hyperchromism) upon increasing the concentration of CT DNA due to a damage of the CT-DNA double-helix structure. The extent of the hyperchromism is indicative of the partial or non-intercalative binding modes, such as electrostatic forces, vander Waals interaction, hydrogen bonds and hydrophobic interaction [32].

In the present study, the interaction of the complexes in DMF solutions with calf thymus DNA was investigated by the changes of absorbance at 275 and 380 nm with increasing concentration of CT-DNA. The representative absorption spectra of Cu(II) complex in presence and absence of CT-DNA are shown in Figure 6.

The absorption spectra of other complexes are given in supplementary file (Figure S5 and S6). With increasing concentration of CT-DNA the absorption bands of the complexes were affected resulting in the tendency of hypochromism and a minor red shift was observed in all the complexes. The observed spectral effect was ascribed to a strong interaction between the electronic state of the intercalating chromophore and that of the DNA bases. The strength of this electronic interaction is attended to decrease as the cube of the distance between the chromophore and the DNA bases [33]. In order to compare quantitatively the binding strength of the complexes, the intrinsic binding constants  $K_b$  of the complexes with DNA were obtained by monitoring the changes in absorbance with increasing concentration of DNA. Intrinsic binding constants  $K_b$  of Cu, Ni and Zn complexes are obtained as  $5.01 \times 10^4 \text{ M}^{-1}$ ,  $4.68 \times 10^4 \text{ M}^{-1}$  and  $4.21 \times 10^4 \text{ M}^{-1}$  respectively as represented in the inset of Figure 6. The significant difference in DNA-binding affinity of the three metal(II) complexes could be understood as a result of the fact that the complex with higher numbers of metal(II) chelates showed stronger binding affinity with DNA. Our results are consistent with earlier reports on preferential binding to CT-DNA in the metal complexes [34]. These results suggest a close association of the compounds with CT-DNA and it is also probable that compounds bind to the helix via intercalative mode.

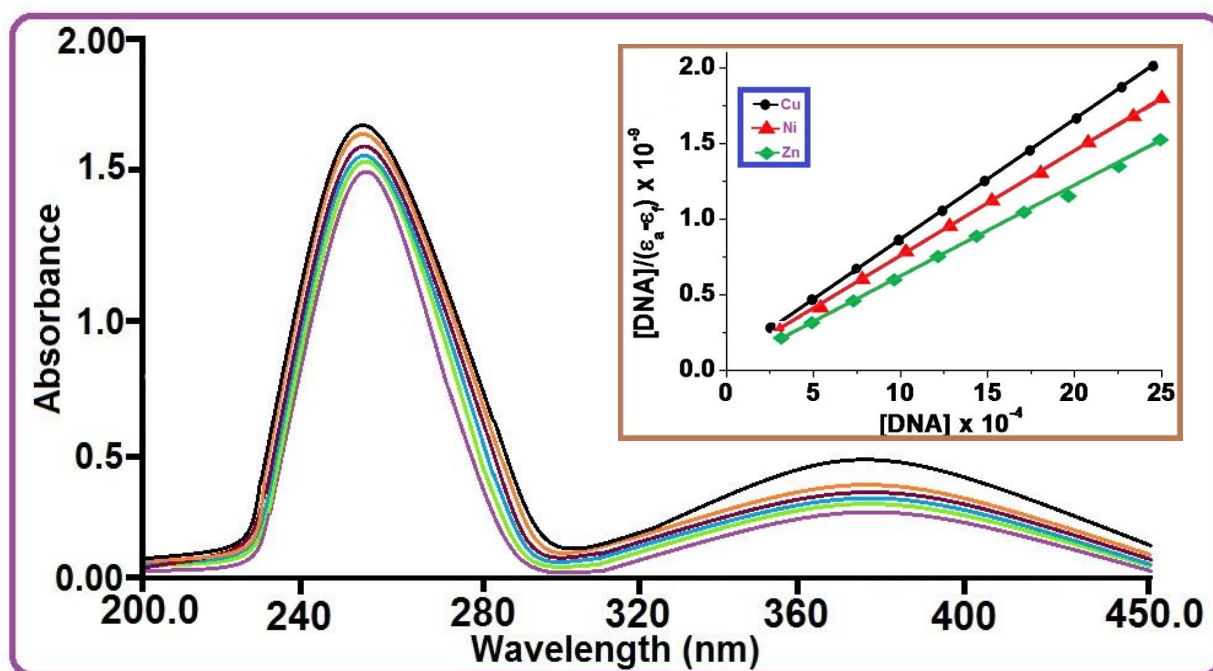


Fig 6: Absorption spectra of complex Cu(II) ( $1 \times 10^{-5} \text{ M}$ ) in the absence and presence of increasing amounts of CT-DNA ( $0-2.5 \times 10^{-3} \text{ M}$ ) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5). The Inset shows the plots of  $[\text{DNA}] / (\epsilon_a - \epsilon_f)$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



### Emission spectral studies

The DNA binding of the complexes has also been studied by monitoring the emission intensity of ethidium bromide (Eth-Br) bound to CT DNA as a function of the concentration of the added complex. Though the emission intensity of Eth-Br in buffer medium is quenched by solvent molecules, it is enhanced when it is strongly stacked in between adjacent DNA base pairs [35]. Addition of a second molecule, which may bind to DNA more strongly than EB, results in a decrease in the DNA-induced EB emission due to the replacement of EB and/or electron transfer [36].

Complexes 1–3 show no fluorescence at room temperature in solution or in the presence of CT DNA, and their binding to DNA cannot be directly predicted through the emission spectra. Therefore competitive EB binding studies may be carried out in order to examine the binding of each compound with DNA. The emission spectra of EB bound to DNA in the absence and presence of the complex are given in Figure 7 and Figure S7, S8. It can be

noted that the fluorescence intensity of the EB-DNA solutions decreases with the addition of the complex obviously. The results suggest that the complex can replace the EB and bind to the DNA molecule. The results suggest that the complex can replace the EB and bind to the DNA molecule. The quenching plots (insets in Figure 7) illustrate that the fluorescence quenching of EB bound to DNA by Cu(II), Ni(II) and Zn(II) complexes in linear agreement with the Stern–Volmer equation, which confirms that the complexes bound to DNA. The  $K_{app}$  values for Cu(II), Ni(II) and Zn(II) complexes are found to be  $5.82 \times 10^5 \text{ M}^{-1}$ ,  $4.97 \times 10^5 \text{ M}^{-1}$  and  $4.29 \times 10^5 \text{ M}^{-1}$  respectively. The attained results are in consistent with that of absorption spectroscopic studies. Anyway, it may be concluded that the entire complexes bound to DNA via the similar mode and the quenching constants of the synthesized complexes reveals that the interaction of the compound with DNA should be intercalation.

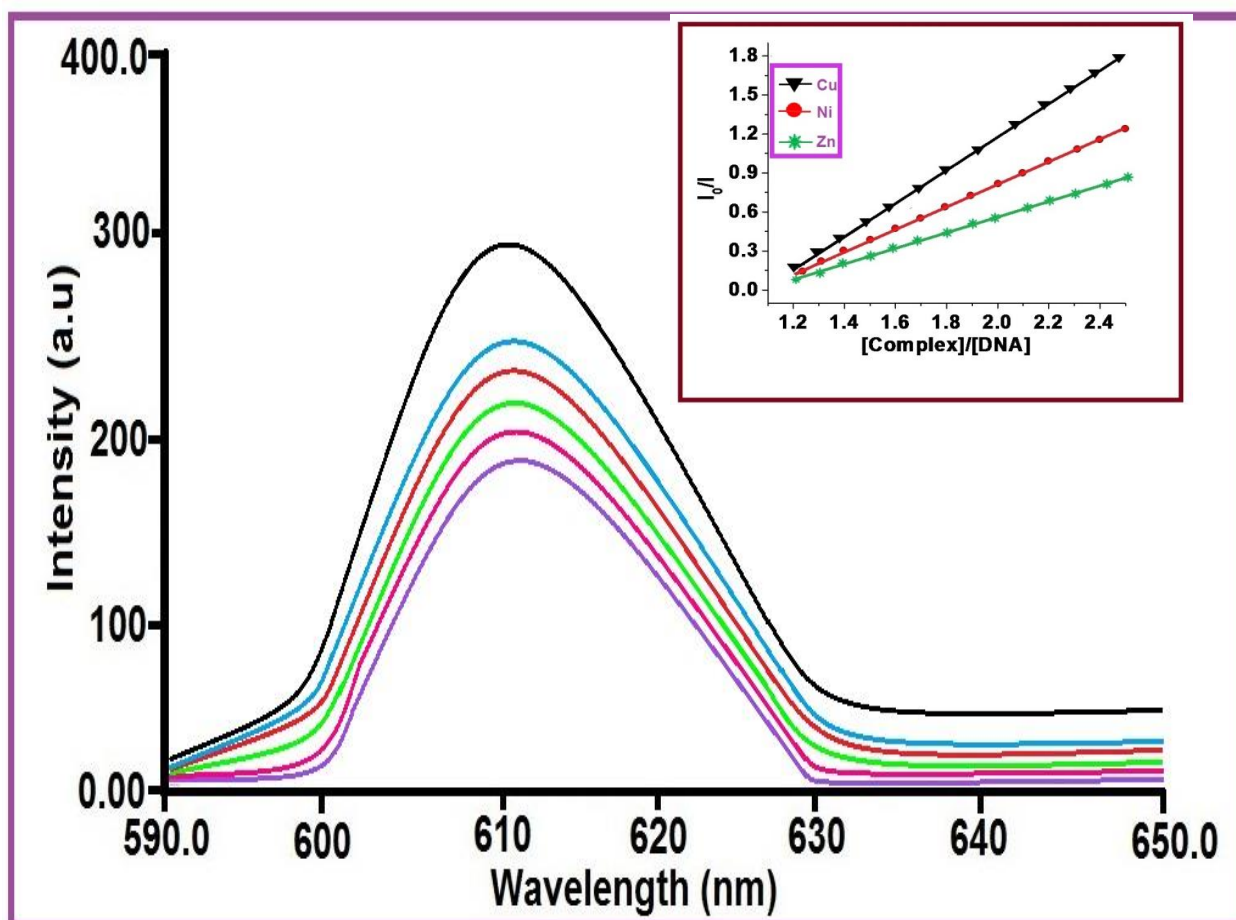


Fig 7: Emission spectrum of EB bound to DNA in the presence of Cu(II): ( $[EB] = 3.3 \mu\text{M}$ ,  $[DNA] = 40 \mu\text{M}$ ,  $[complex] = 0-25 \mu\text{M}$ ,  $\lambda_{ex} = 440 \text{ nm}$ ). Inset shows the plots of emission intensity  $I_0 / I$  vs  $[DNA] / [complex]$  for the titration of DNA with Cu(II), Ni(II) and Zn(II) complexes.

### Viscosity studies

Hydrodynamic methods that are sensitive to length are regarded as one of the least confusing and most critical tests of a binding mode in solution in the absence of crystallographic structural data. Intercalating agents are expected to elongate the double helix to incorporate the ligands in between the base leading to an increase in the viscosity of DNA [37]. In contrast, complexes those bind individually in the DNA grooves by partial and/or nonclassical intercalation, under the same conditions, typically cause less executed (positive or negative) or no change in DNA solution viscosity [38].

The value of changes in the relative specific viscosities of CT-DNA in presence and absence of the complex respectively are plotted against  $R = [\text{complex}]/[\text{DNA}]$  versus  $(\eta/\eta^0)^{1/3}$ . The results indicate that the presence of the metal complex increases the viscosity of the DNA solution, as illustrated in Figure 8. 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 [39]. The results suggest that metal complexes bind DNA with an intercalative binding mode.

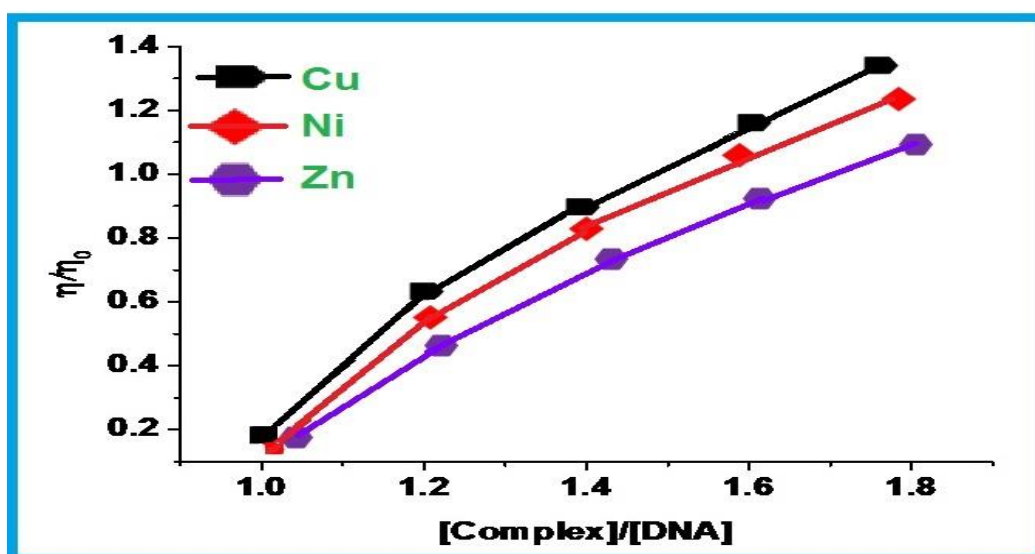


Fig 8: Viscosity measurements of the Cu(II), Ni(II) and Zn(II) complexes.

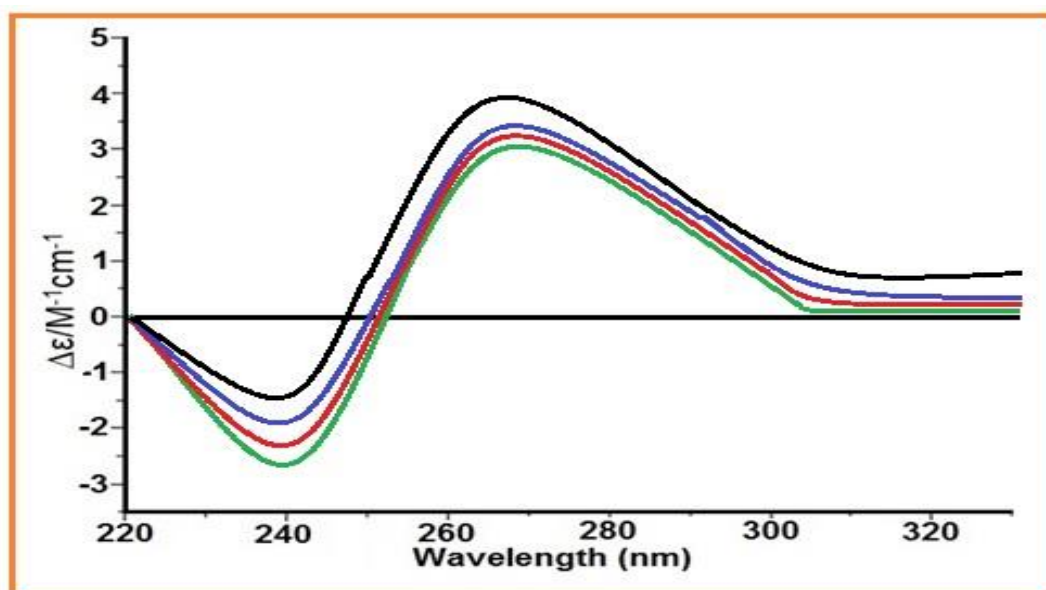


Fig 9: CD spectra recorded over the wavelength range 220-320 nm for solutions containing 2:1 ratio of CT-DNA (200 μM) and mononuclear Cu(II), Ni(II) and Zn(II) complexes (100 μM).

### Circular dichroic spectral studies

Circular dichroic spectroscopy is a technique useful in diagnosing changes in DNA morphology during drug–DNA interactions, as the band due to base stacking (275 nm) and that due to right handed helicity (248 nm) are quite sensitive to the mode of DNA interactions with small molecules [40]. As represented in Figure 9, the CD spectrum of DNA exhibits a positive absorption at 277nm due to the base stacking and a negative band at 240nm due to the helicity of B-DNA. In the presence of the complex, both the positive and negative peak intensities of the CD spectra of DNA were increased. The changes in the CD spectra in the presence of the complex show stabilization of the right handed B form of CT-DNA. These observations clearly indicate that the binding mode of the complexes should be intercalative, the stacking of the complex molecules between the DNA base pairs leads to an enhancement in the positive band and the partial unwinding of the helix decreases intensity of the negative band. So, the main interactions of the complexes with DNA can be ascribed to the intercalative mode.

### CONCLUSION:

In this report, coordination chemistry of a Schiff base ligand, obtained from the reaction of 1,2-Di(2-thienyl)-1,2-ethanedione (Thenil), hydrazine hydrate and 2-Hydroxy-5-methylbenzaldehyde is described. Cu(II), Ni(II), and Zn(II) complexes have been synthesized using the Schiff base ligand and characterized by using spectral, physicochemical and elemental analyses. The interaction of the synthesized complexes with CT-DNA was investigated by electronic absorption, competitive fluorescence titration, viscosity measurements and circular dichroic analyses. The results suggest that complexes interact with CT-DNA by intercalative modes. 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.

### Supplementary Figure captions.

**Figure S1.** FT-IR spectrum of Ni(II) complex.

**Figure S2.** FT-IR spectrum of Zn(II) complex.

**Figure S3.** Mass spectrum of Ni(II) complex.

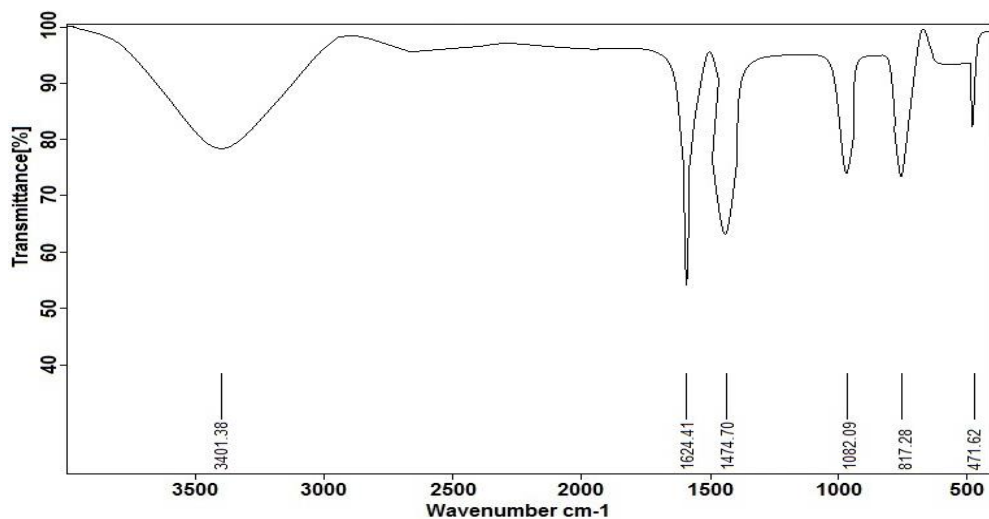
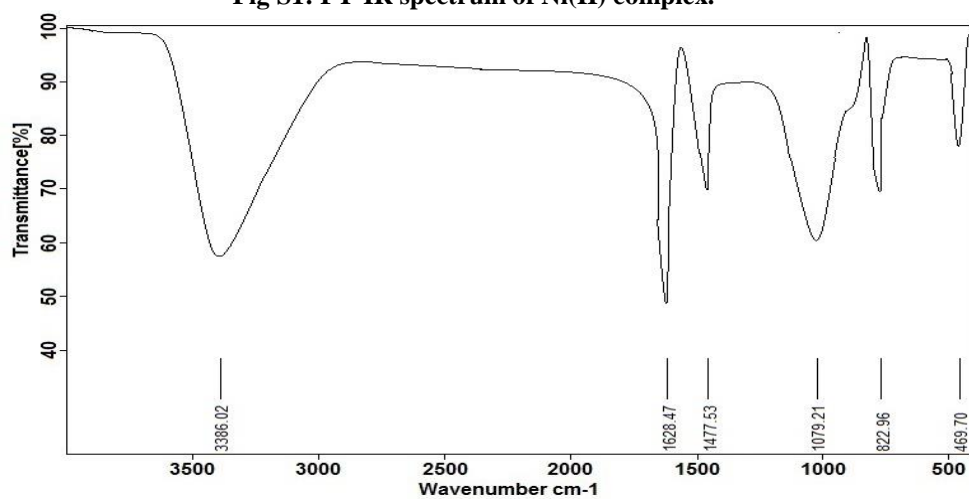
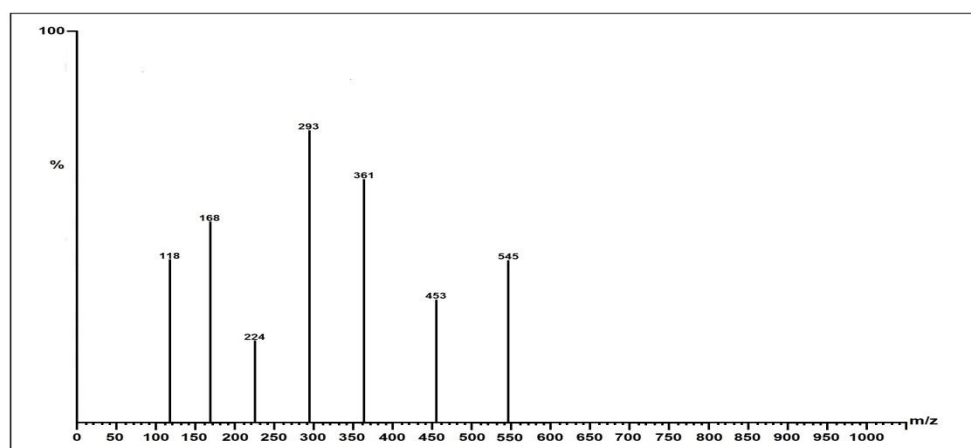
**Figure S4.** Mass spectrum of Zn(II) complex.

**Figure S5.** Absorption spectra of complexes Ni(II), ( $1 \times 10^{-5}$  M) in the absence and presence of increasing amounts of CT-DNA ( $0-2.5 \times 10^{-3}$  M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5).

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

**Figure S7.** Emission spectrum of EB bound to DNA in the presence of Ni(II); ([EB] = 3.3  $\mu$ M, [DNA] = 40  $\mu$ M, [complex] = 0-25  $\mu$ M,  $\lambda_{ex}$  = 440 nm).

**Figure S8.** Emission spectrum of EB bound to DNA in the presence of Zn(II); ([EB] = 3.3  $\mu$ M, [DNA] = 40  $\mu$ M, [complex] = 0-25  $\mu$ M,  $\lambda_{ex}$  = 440 nm).

**Fig S1: FT-IR spectrum of Ni(II) complex.****Fig S2: FT-IR spectrum of Zn(II) complex.****Fig S3: Mass spectrum of Ni(II) complex.**

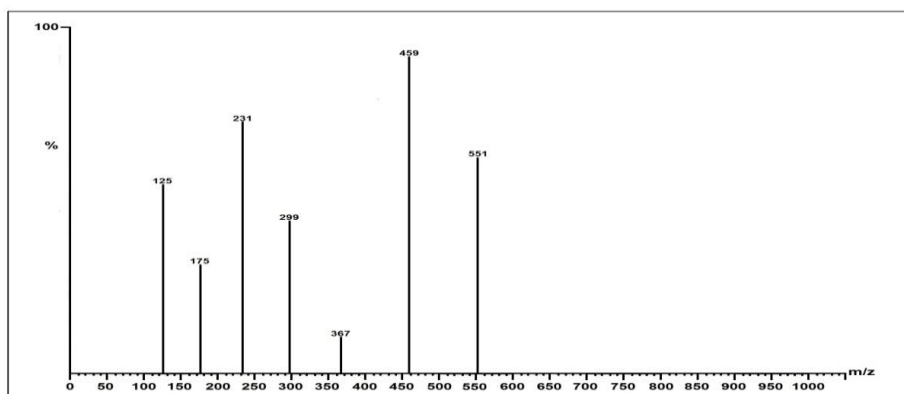


Fig S4: Mass spectrum of Zn(II) complex.

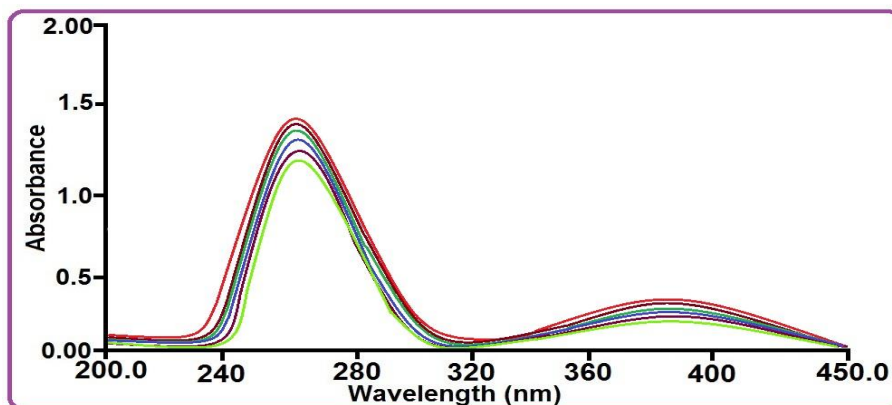


Fig S5: Absorption spectra of complexes Ni(II), ( $1 \times 10^{-5}$  M ) in the absence and presence of increasing amounts of CT-DNA ( $0-2.5 \times 10^{-3}$  M ) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5).

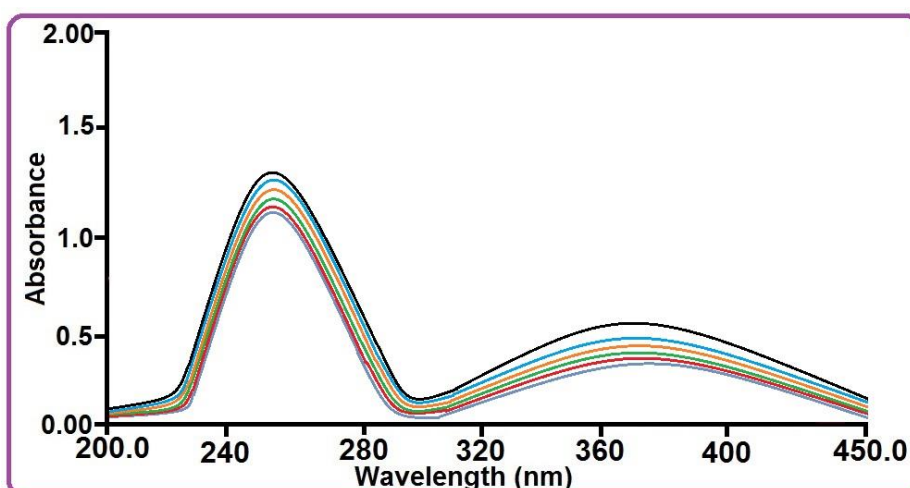


Fig S6: Absorption spectra of complexes Zn(II), ( $1 \times 10^{-5}$  M ) in the absence and presence of increasing amounts of CT-DNA ( $0-2.5 \times 10^{-3}$  M ) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5).

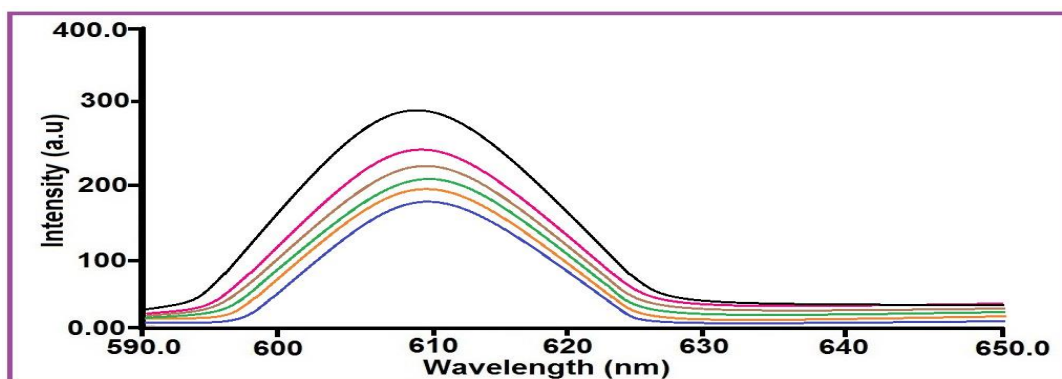


Fig S7: Emission spectrum of EB bound to DNA in the presence of Ni(II); ([EB] = 3.3  $\mu$ M, [DNA] = 40  $\mu$ M, [complex] = 0-25  $\mu$ M,  $\lambda_{ex}$  = 440 nm).

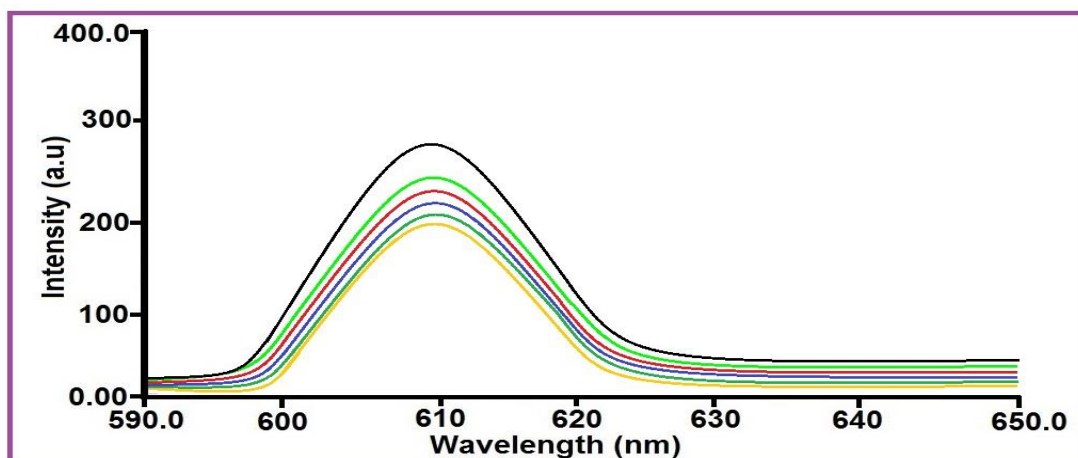


Fig S8: Emission spectrum of EB bound to DNA in the presence of Zn(II); ([EB] = 3.3  $\mu$ M, [DNA] = 40  $\mu$ M, [complex] = 0-25  $\mu$ M,  $\lambda_{ex}$  = 440 nm).

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