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Der Pharma Chemica, 2014, 6(4):244-254
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Design, synthesis and spectral characterisation of novel acyclic ligand and their transition metal complexes as selective DNA binding agents

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ABSTRACT

Novel Co(II), Ni(II), Cu(II) and Zn(II) complexes have been designed and synthesized using the Schiff base ligand derived from 9,10-phenanthrenequinone and benzylamine. The ligand and its complexes have been characterized by analytical and spectral techniques. The higher conductance values in DMSO indicate that the complexes are 2:1 electrolytes. On the basis of electronic spectral investigations square planar geometry was assigned to Co(II), Ni((II) and Cu(II) complexes. The interaction of the complexes with CT-DNA has been explored by absorption, emission, circular dichoric and viscosity measurements. The complexes exhibited hypochromicity in absorption, which revealed that the complex bound to CT-DNA through an intercalation mode.

Keywords: 9,10-phenanthrenequinone, Schiff base complexes, DNA interaction, Intercalation, Hypochromism.

INTRODUCTION

The design of metal–drug complexes is of particular interest in the pharmacological research. Metal combinations with pharmaceutical agents are known to improve the activity of the drugs and decrease their toxicity [1]. Schiff base compounds are potential anticancer drugs and when administered as their metal complexes, the anticancer activity of these complexes has been enhanced in comparison to the free ligand. It has been suggested that the azomethine group in Schiff bases is responsible for the biological activities such as antitumor, antibacterial, antifungal and herbicidal activities [2]. Eversince the discovery of cisplatin [cis-diamminedichloroplatinum(II)], there has been a rapid expansion in research to find new and more efficacious metal-based anticancer drugs [3]. Schiff base complexes are considered to be the most important stereochemical models in transition metal coordination chemistry due to their preparative accessibility, structural variety, stability and wide application [4-6].

Investigations on DNA interactions with Schiff base transition metal complexes, especially for those containing multidentate aromatic ligands, have aroused considerable interests owing to their potential applications as new therapeutic agents which make them possible probes of DNA structure and conformation [7,8]. Binding of peptides, small organic and inorganic molecules to DNA will interfere with a number of processes like transcription and replication [9]. By considering this principle, various disorders like cancer, cystic fibrosis etc can be cured by using DNA as targets for drugs.. And with this emerges a whole new area of study called DNA drug interaction, which is of great topical importance since 1960 [10].

Among the host of DNA-binding agents reported so far, transition metal complexes are of relevance to the present work. Metal complexes have been found to be potential to bind DNA through multitude of interactions and to cleave the duplex by virtue of their intrinsic chemical, electrochemical and photochemical reactivities. The larger aromatic ring system was proved to account for the higher affinity for DNA and consequently for higher antitumor and photo

cleaving activities [11]. All the above facts encouraged us to synthesise the Schiff base ligand obtained from 9,10-Phenanthrenequinone and Benzylamine and its complexes with 3d-transition metals such as Co(II), Ni(II), Cu(II) & Zn(II). The characterisation of the synthesised compounds has been also reported. In addition the CT-DNA binding activities of the complexes have been studied by absorption, emission, circular dichoric spectral studies and viscosity measurements.

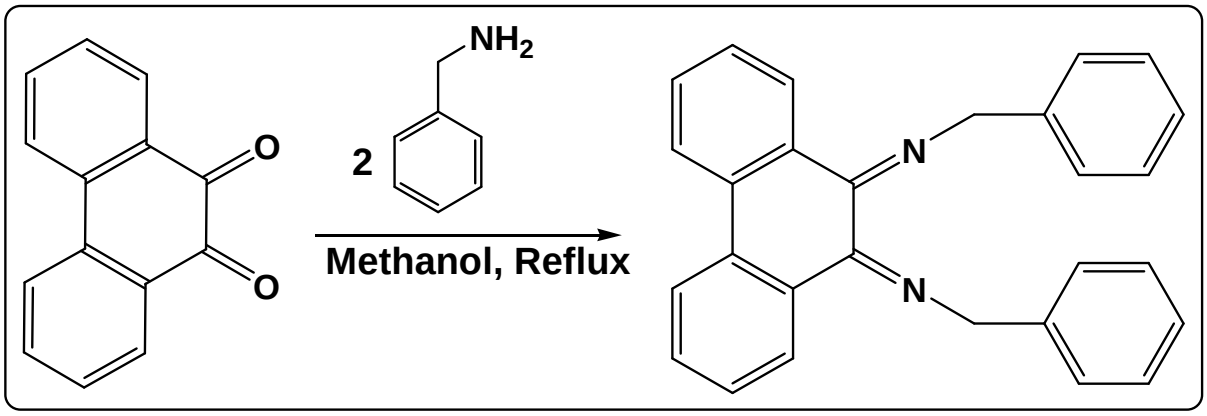
MATERIALS AND METHODS

2.1 Chemicals and methods

9,10-Phenanthrenequinone and benzylamine were purchased from Sigma Aldrich and used as such. Co(II), Ni(II), Cu(II) and Zn(II) metal salts were of analytical grade from Merck. All other reagents and solvents were purchased from commercial sources and were of analytical grade and were purified by distillation. Elemental analysis was recorded on a Carlo Erba model 1106 elemental analyzer. The infrared spectra of the solid samples were recorded in Perkin Elmer spectrometer in the range of 4000-400 cm^{-1}. Potassium bromide disc method was employed for sample preparation. Electronic spectra were recorded using Perkin Elmer Lambda-35 UV-Vis. spectrometer using DMSO as solvent in the range of 200-800 nm. The molar conductivity measurements of the metal complexes were carried out in ~10^{-3}M DMSO solutions using a Coronation digital conductivity meter. The ^{13}C NMR was recorded on a JEOL GSX-400 spectrometer employing $CDCl_3$ as solvent at ambient temperature. The mass spectral analysis was carried out using JEOL D-300 (EI) mass spectrometer. The emission spectra were recorded on a Perkin Elmer LS-45 fluorescence 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. Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution was prepared using deionized and sonicated triple distilled water. The Calf thymus (CT) DNA was procured from Bangalore Genie (India).

2.2 Synthesis of Schiff base ligand

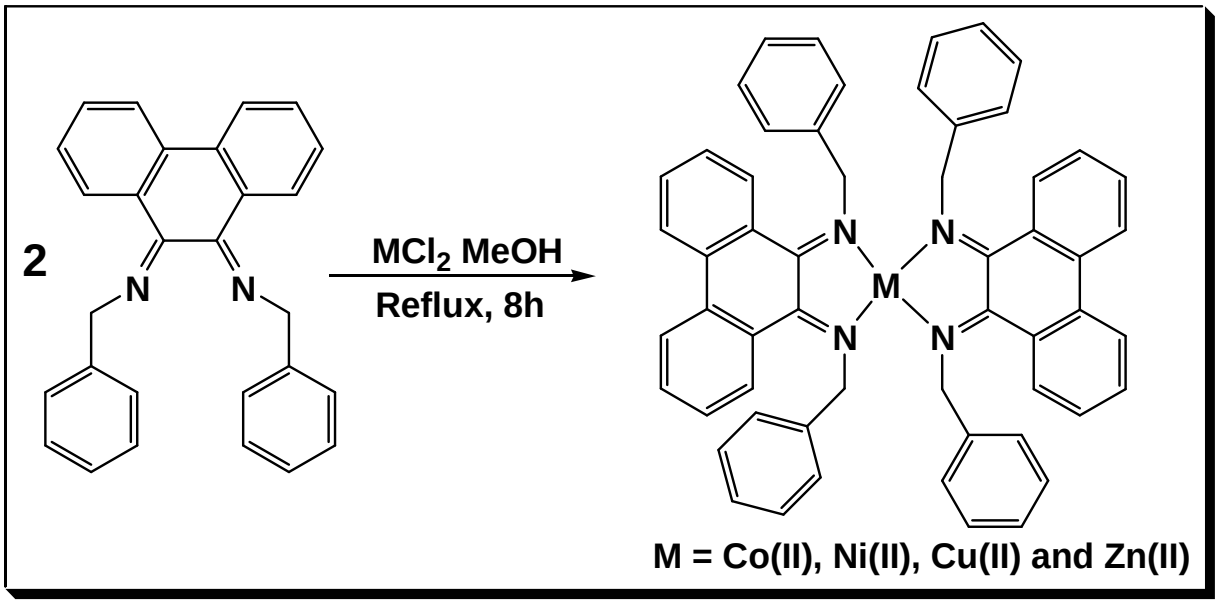
A methanolic solution of 9,10-Phenanthrenequinone (5 mmol, 1.04 gm) and Benzylamine (10 mmol, 1.07 gm) were mixed and gently heated for 2 hours with constant stirring. The characteristic pale yellow precipitate obtained was filtered out and recrystallized from methanol. Fine pale yellow product was obtained upon slow evaporation at room temperature. The obtained product was washed with alcohol, ether and dried in vacuum desiccator over anhydrous calcium chloride. The proposed structure of the obtained new Schiff base is depicted in Scheme 1.



Scheme. 1. Synthesis of Schiff base Ligand (L^1).

2.3 Synthesis of Schiff base metal complexes

To the yellowish solution of 2 mmol (0.77 **gm**) of the **(L^1)** ligand in 20 ml of methanol, a solution of metal(II) chloride (1 mmol) in 20 ml of aqueous methanol was added drop wise with constant stirring. The reaction mixture was refluxed for 2 h, at 50°C and the volume was reduced to half of the initial volume under reduced pressure. The precipitated metal complex was filtered, washed several times with cold ethanol, ether and then dried in *vacuum* over anhydrous $CaCl_2$. The structure of the obtained Schiff base metal complexes is given in Scheme 2.



Scheme 2. Synthesis of Schiff base metal complexes

2.4 DNA binding experiments

2.4.1 Absorption spectral studies

Electronic absorption spectrum of the complexes was recorded before and after addition of CT-DNA in the presence of 50 mM Tris-HCl buffer (pH 7.5). A fixed concentration of metal complexes (10 μM) was titrated with incremental amounts of CT-DNA over the range (0 – 200 μM). The equilibrium binding constant (K_b) values for the interaction of the complex with CT-DNA were obtained from absorption spectral titration data using the following equation **(1)** [12].

$$\mathbf{[DNA]/ (\varepsilon_a - \varepsilon_f) = [DNA]/ (\varepsilon_b - \varepsilon_f) + 1/Kb (\varepsilon_b - \varepsilon_f)} \quad \mathbf{(1)}$$

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.

2.4.2 Fluorescence emmission spectral studies

The fluorescence spectral method using ethidium bromide (EB) as a reference was used to determine the relative DNA binding properties of the **synthesized** complexes to calf thymus (CT) DNA in 50 mM Tris HCl / 1 mM NaCl buffer, pH 7.5). Fluorescence intensities of EB at 610 nm with an excitation wavelength of 510 nm were measured at different complex concentrations.

EB (weak fluorescent) + DNA (non-fluorescent) ⟶ EB-DNA (strong fluorescent)

The relative binding tendency of the complexes to CT DNA was determined from a comparison of the slopes of the lines in the fluorescence intensity versus complex concentration plot. The reduction of emission intensity gives a measure binding propensity of complex to CT-DNA. Stern-Volmer quenching constant Ksv of the complexes to CT-DNA was determined from the equation (2)

$$\mathbf{I_o/I=1 + Ksvr.} \quad \mathbf{(2)}$$

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) [13].

2.4.3 CD spectral studies

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

2.4.4 Viscosity measurements

The binding mode of the complexes to 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_o) versus binding ratio of concentration of complex to that of concentration of CT-DNA , where η is the viscosity of DNA in the presence of complex and η_o is the viscosity of DNA alone. Viscosity values were calculated after correcting the flow-time of buffer alone (t_o), $\eta = (t-t_o)/t_o$ [14].

RESULTS AND DISCUSSION

3.1 Structural characterization of the Schiff base ligand

3.1.1 FT-IR spectral analysis

The infrared spectrum of the free 9,10-phenanthrenequinone shows a strong band at 1660 cm^{-1}, which corresponds to the $\upsilon_{(C=O)}$ and the infrared spectrum of benzylamine has strong bands at 3432 and 3328 cm^{-1} corresponding to the $-NH_2$ stretching frequency. On condensation these bands have disappeared and a new band has appeared at 1612 cm^{-1}, which is assigned to the $\upsilon_{(C=N)}$ as shown in Figure 1. This demonstrates the condensation between the 9,10-phenanthrenequinone and benzylamine resulting in the formation of the acyclic Schiff base ligand (L^1). The spectrum shows the medium intensity band at 1283 cm^{-1} which can be assigned to $\upsilon_{(C-N)}$, and the strong band in the 1589 cm^{-1} region is assigned to aromatic ring -C=C- stretching vibration. The other series of weak and strong bands between 3200 and 2850 cm^{-1} are related to (-C-H) modes of vibrations.

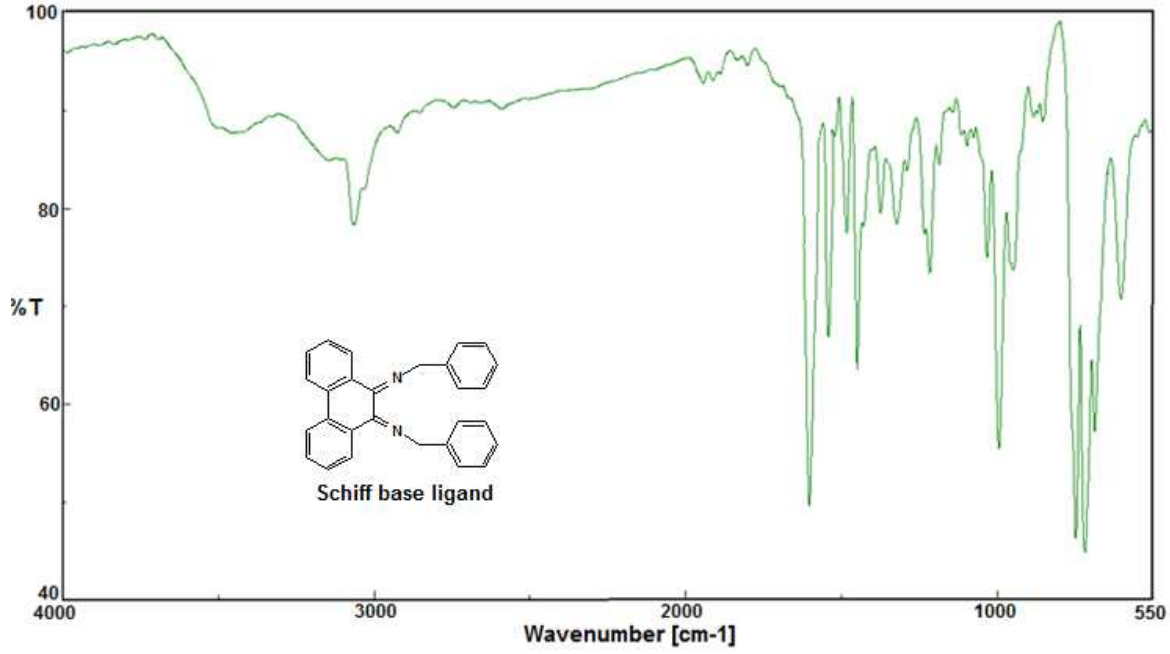


Figure 1. FT-IR spectrum of the Schiff base ligand (L^1)

In order to study the binding mode of the Schiff base to the metal in the complexes, the IR spectrum of the free ligand (L^1) was compared with those of the complexes as shown in Figure 2 and Figure S1, S2 and S3. The band at 1612 cm^{-1} for the imino group of the ligand (L^1) has been shifted to lower frequencies in the IR spectra of Co(II), Ni(II), Cu(II) and Zn(II) complexes (1599 cm^{-1}, 1601 cm^{-1}, 1605 cm^{-1} and 1596 cm^{-1}) indicating the coordination of the imino nitrogen to metal [15, 16]. The IR spectra of metal complexes also show some new bands in the region 423cm^{-1}, 418 cm^{-1}, 409 cm^{-1} and 467 cm^{-1} for Co(II), Ni(II), Cu(II) and Zn(II) complexes respectively which is due to the formation M-N bands [17].

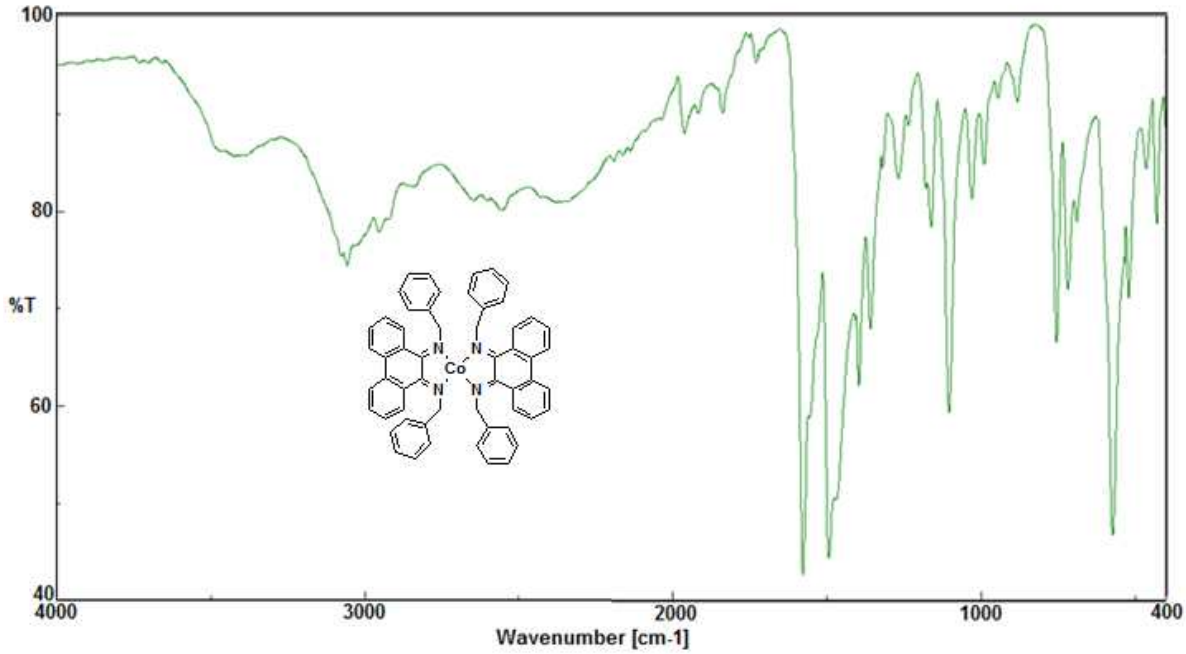


Figure 2. FT-IR spectrum of Co(II) complex

3.1.2 Mass spectra

The molecular ion peak [M^+] at m/z = 385 confirms the molecular weight of the acyclic Schiff base ligand. The various peaks at m/z = 356, 330, 282, 194 and 107 corresponds to the various fragments $C_{26}H_{20}N_2$, $C_{24}H_{18}N_2$, $C_{20}H_{14}N_2$, $C_{14}H_{11}N$ and C_7H_7N respectively as shown in Figure 3. This confirms the molecular structure of the ligand.

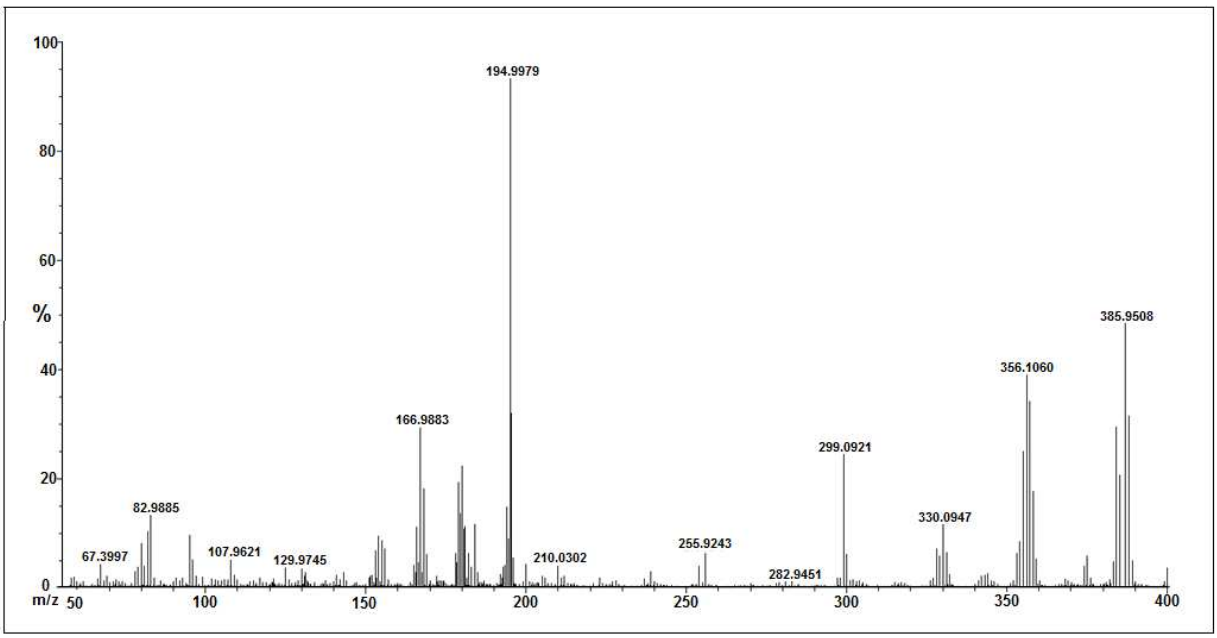


Figure. 3 EI-Mass spectrum of Schiff base ligand (L^1)

3.1.3 ^{13}C NMR spectral analysis

The ^{13}C NMR signal at δ 169.57 corresponds to the most important imino (C=N) carbon. The signal at δ 123.9, 129.93, 130.2, 131.02, 133.9, 135.86, 143.11 and 141.28 corresponds to aromatic (Ar-C) carbons. The signal at δ 58.6 corresponds to the CH_2 carbon. The absence of C=O signal confirms the formation of acyclic Schiff base ligand (L) as represented in Figure 4.

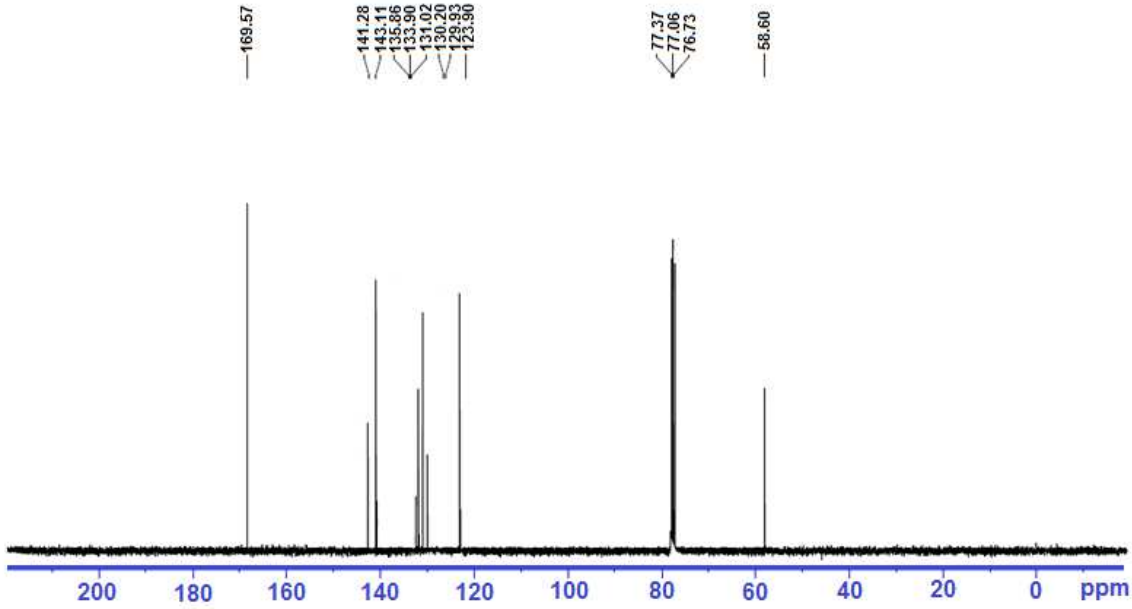


Figure. 4 ^{13}C-NMR spectrum of Schiff base ligand (L)

3.1.4 Structural characterization of the metal complexes.

The molar conductance data of Co(II), Ni(II), Cu(II) and Zn(II) complexes are provided in **Table 1**. The conductance data indicate that all the metal complexes synthesized are 2:1 electrolytes. The electrolytic nature of the metal complexes suggests that the anions of the salts present outside the coordination sphere in the formation of metal complexes.

Table 1. Molar conductance data

Complexes	Molar conductance ($Ohm^{-1} cm^2 mol^{-1}$)	Nature
CoL^1	71.60	2:1 electrolyte
NiL^1	76.58	2:1 electrolyte
CuL^1	82.94	2:1 electrolyte
ZnL^1	72.47	2:1 electrolyte

3.1.5 Electronic spectral analysis of the metal complexes

The electronic spectra of the complexes have been measured in the range 200-800 nm in DMSO as shown in Figure 5. The absorption spectra of the Co(II) complex consists three bands. A low intensity band at 710 nm is indicative of square planar geometry and is assigned to the $^2A_{1g} \rightarrow {}^2B_{1g}$ transition. A shoulder in the region 400 nm is attributed to the charge transfer band. The third one and more intense peak below 300 nm can be assigned to the intraligand charge-transfer π-π* transitions of the ligand. These are the characteristic of Co^{2+} in the four coordination environment.

The absorption spectra of the Ni(II) complex displays three bands. A low intensity band at 640 nm is indicative of square planar geometry and is assigned to the $^1A_{1g} \rightarrow {}^1B_{1g}$ transition. A shoulder in the region 400 nm is attributed to the charge transfer band. The third one and more intense peaks below 300 nm assigned to the intraligand charge-transfer π-π* transitions of the ligand. These are the characteristic of Ni^{2+} in the four coordination environment.

The absorption spectra of the Cu(II) complex also exhibits three bands. A low intensity band at 625 nm is indicative of square planar geometry and is assigned to the $^2B_{1g} \rightarrow {}^2A_{1g}$ [18] transition. A shoulder in the region 400 nm is attributed to the charge transfer band. The third one or more intense peaks below 300 nm assigned to the intraligand charge-transfer π-π* transitions of the ligand. These are the characteristic of Cu^{2+} in the four coordination environment. Zn (II) ion which has a completely filled d^{10} electronic configuration is not expected to show any d-d electronic transition.

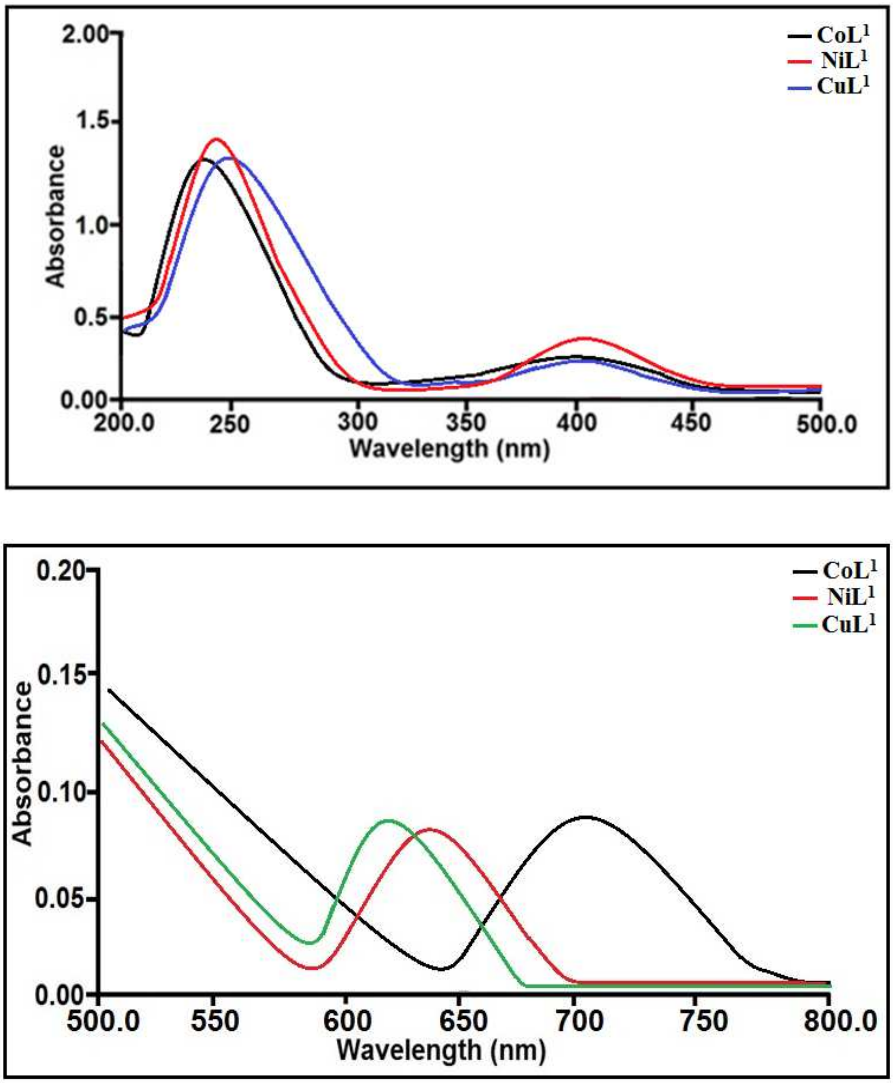


Figure. 5 Electronic spectrum of the CoL^1, NiL^1 & CuL^1 complexes

3.2 DNA binding experiments

3.2.1 Absorption spectral studies

The binding of metal complexes to DNA was monitored classically through absorption titration method. Metal complexes bound to DNA through intercalation is characterized by the change in absorbance (hypochromism) and red shift in wavelength, due to the intercalative binding mode involving a stacking interaction between the DNA pairs [19]. The electronic absorption spectra of the complexes were significantly perturbed by the addition of increasing amounts of DNA. With increasing concentration of CT-DNA (0 – 200 µM), hypochromism in the absorption bands around about 220-260nm for the Co(II), Ni(II), Cu(II) and Zn(II) complexes was observed accompanied by a red shift of not more than 5-12 nm, suggesting of stabilation of the DNA Helix as shown in Figure. 6 and Figure S4, S5 and S6.

In order to compare quantitatively the binding affinity of complexes with CT-DNA the intrinsic binding constants K_b of the complexes were determined and listed in Table 2. These results suggest that upon addition of DNA to complexes, Cu(II) complex has marginally higher binding affinities than that of Ni(II), Co(II) and Zn(II) complex. The significant difference in DNA binding affinity of the four metal (II) complexes could be understood as a result of the fact that the complex with higher number of metal (II) chelates showed stronger binding affinity with CT-DNA.

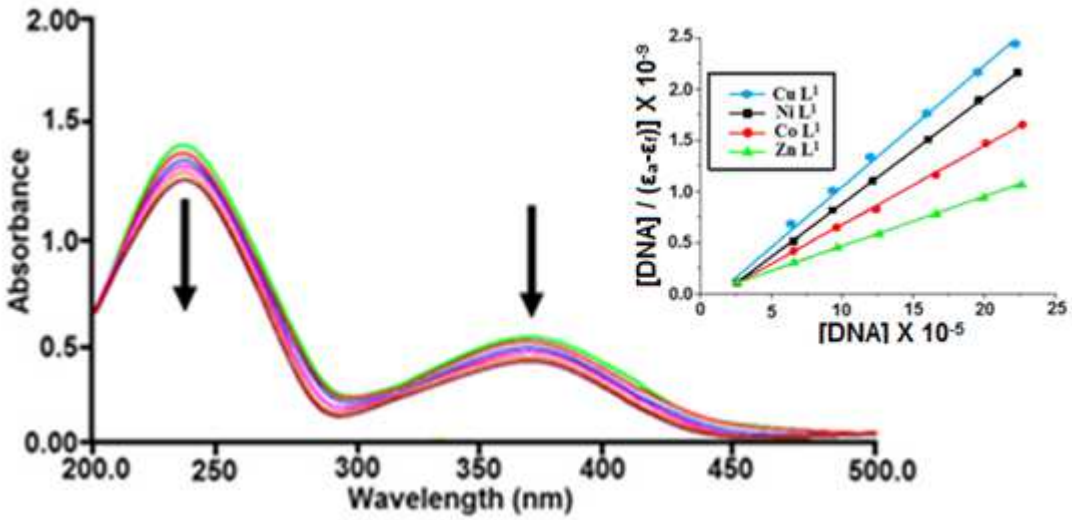


Figure. 6. Absorption spectra of complex CoL^1 (1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-2.5 x 10^{-3} M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5). Arrow shows the absorbance changing upon increasing DNA concentrations. The Inset shows the plots of [DNA] / (ϵ_a-ϵ_f) versus [DNA] for the titration of DNA with CoL^1, NiL^1, CuL^1 and ZnL^1 complexes

3.2.2 Fluorescence spectral studies

The binding of complexes **CoL^1, NiL^1, CuL^1 and ZnL^1** to the CT-DNA has been examined by fluorescence spectral method. EB emits intense fluorescent light in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs of the CT-DNA and the complexes [20-22]. The emission spectra of EB-DNA in the absence and presence of complexes are presented in Figure 7. The emission band around 612 nm of the DNA-EB system decreased in intensity with the increasing concentration of the complexes as represented in Figure 7 and Figure S7, S8 and S9. It has been reported that the enhanced fluorescence emission intensity can be quenched by the addition of another molecule [23]. The observed quenching of the DNA-EB fluorescence intensity for the complexes suggested that they can display EB from the DNA- EB system and interact with DNA probably *via* the intercalative mode.

The extent of reduction in the emission intensity gives a measure of the binding propensity of the complexes towards DNA. The apparent binding constants (K_{app}) were tabulated in table 2. The maximum value obtained for copper complex is in consistent with the absorption spectral measurements.

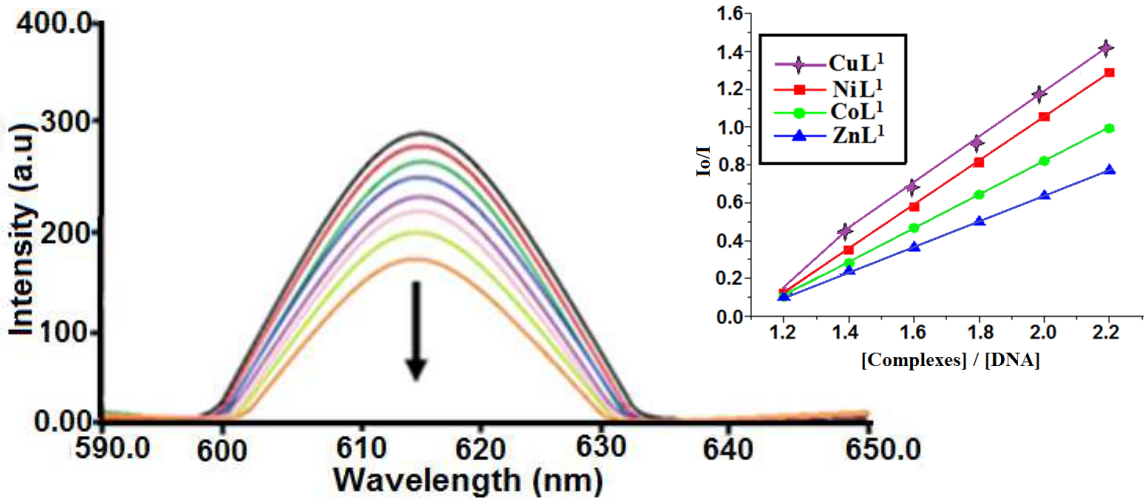


Figure. 7. Emission spectrum of EB bound to DNA in the presence of CoL^1: ([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-25 µM, λex= 430 nm). Arrow shows the absorbance changing upon increasing complex concentrations. Inset shows the plots of emission intensity I_o / I *vs* [DNA] / [complex]

Table 2: Binding constant (K_b) and Apparent binding constant (K_{app}) calculated for acyclic mononuclear [Co(II), Cu(II), Ni(II) and Zn(II)] complexes

S. No	Complex	Binding Constant (K_b)	Apparent Binding constant (K_{app})
1	CoL^1	5.83×10^4	$1.3 \times 10^5\ M^{-1}$
2	NiL^1	2.36×10^5	$4.4 \times 10^5\ M^{-1}$
3	CuL^1	1.02×10^6	$8.3 \times 10^5\ M^{-1}$
4	ZnL^1	3.08×10^4	$2.5 \times 10^4\ M^{-1}$

3.2.3 Circular dichoric spectral studies

Circular dichoric (CD) spectroscopy is useful in diagnosing changes in DNA morphology during drug–DNA interactions [24], since the positive band due to base stacking (275 nm) and the negative one due to right handed helicity (245 nm) are quite sensitive to the mode of DNA interactions with small molecules. The changes in CD signals of DNA observed on interaction with drugs may often be assigned to the corresponding changes in DNA structure [25]. CD spectra of CT-DNA in presence and absence of the complexes are shown in Figure 8. Those significant changes indicate conformational changes and unwinding of DNA base pairs with destabilization of the DNA double helix, which is consistent with DNA intercalation binding mode suggested above [26]. The interaction of CT-DNA with the ligand has also been checked by CD spectroscopy. The nature of CD spectra of free CT-DNA and that of CT-DNA complexed with the ligand are very similar, and also the changes are less notable than the complexes.

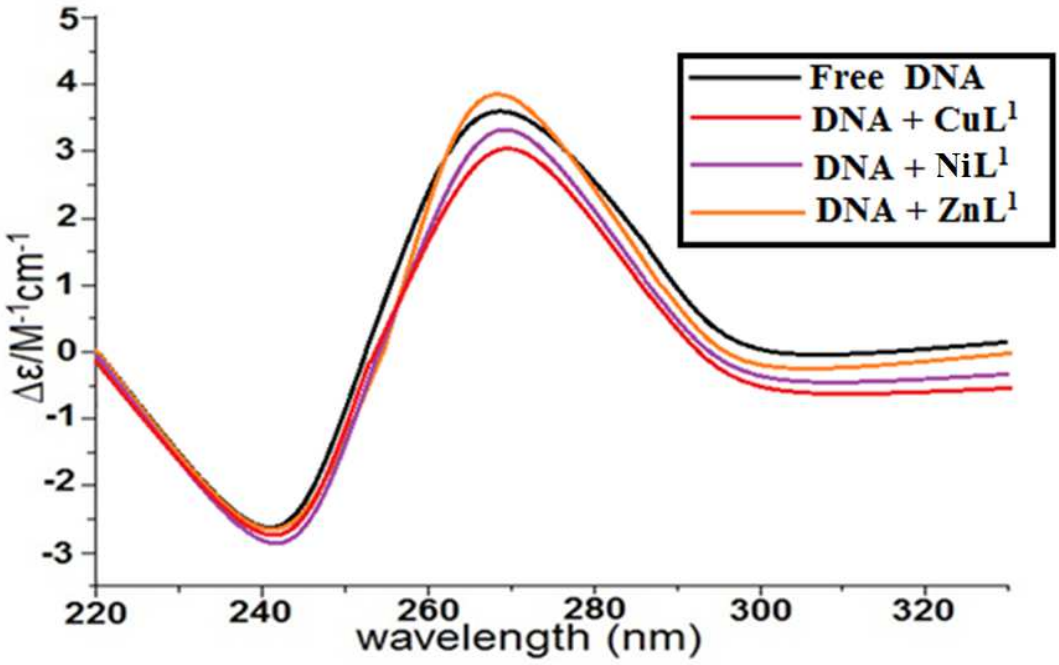


Figure. 8 CD spectra recorded over the wavelength range 220-320 nm for solutions containing 2:1 ratio of CT-DNA (200 µM) and mononuclear NiL^1, CuL^1 and ZnL^1 complexes (100 µM)

3.2.4 Viscosity measurements

Viscosity, sensitive to length increase is regarded as the one of the least ambiguous and the most relevant tests of binding model with CT-DNA in solution in the absence of crystallographic structural data [27].Viscosity studies of CT-DNA have been carried out with the metal complexes of different concentrations for further identification and affirmation of interaction mode between complex and DNA. Viscosity of DNA with complex of particular concentration has been calculated on the basis of DNA flow rate through a capillary viscometer. From the obtained flow rates, the specific viscosity contribution due to the DNA in the presence of metal complexes (binding agent) was calculated.

In general when the intercalative mode of interaction plays between complex and DNA, DNA helix gets prolonged by separating the base pairs to accommodate the binding of ligand which further results in the enhancement of DNA viscosity. From Figure 9, it can be noted that the viscosity of DNA is found to be increased when it interacts with metal complexes. These observations clearly rule out the effect of strong intercalation of the complexes on the base

stacking and decreased right-handedness of CT-DNA as well as also evidenced by UV–Vis., fluorescence, and circular dichoric spectroscopic results.

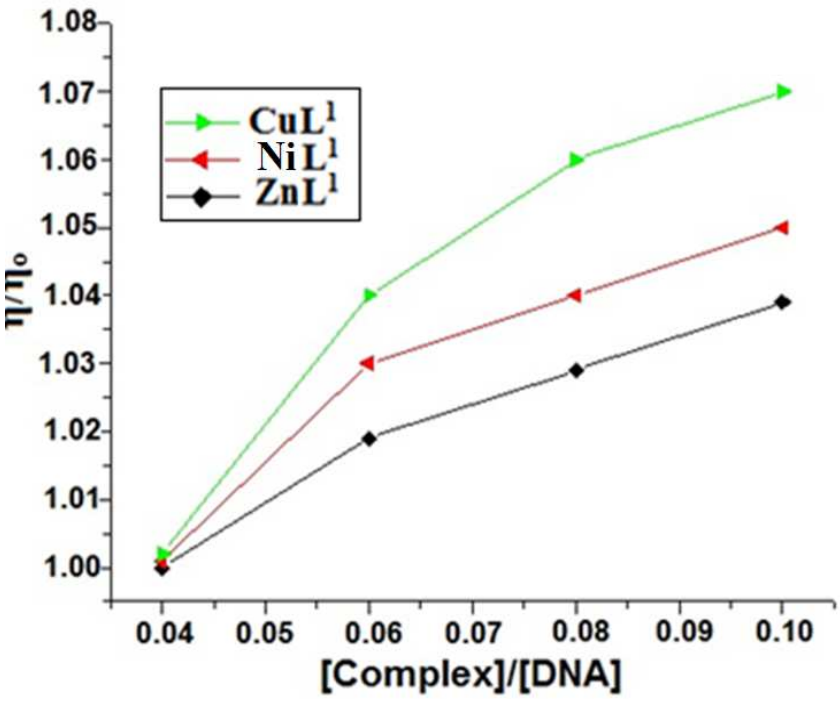


Figure. 9 Viscosity measurements of the NiL^1, CuL^1 & ZnL^1 complexes

CONCLUSION

The 9,10-phenanthrenequinone and benzylamine based acyclic Schiff base ligand ($\mathbf{L^1}$) and its transition metal complexes (**CoL^1, NiL^1, CuL^1 and ZnL^1**) have been prepared and fully characterized. In agreement with UV–vis absorption, fluorescence, CD spectra and viscosity measurements, all the complexes exhibited strong intercalation binding affinity, following the order of **CuL^1> NiL^1> CoL^1> ZnL^1**. The better binding properties of the complexes should be attributed to the good coplanarity of the ligand after coordination with metal ions. Meanwhile, nature of the central metal ions also affected the intercalative ability. These results indicate that DNA might also serve as the primary target of these compounds; in addition, they should have many potential practical applications, just like the promising therapeutic drug candidates. Therefore, understanding the modes of the binding of metal complexes to DNA and the factors that can affect the binding is of fundamental importance in understanding DNA binding in general.

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