Mononuclear Zinc(II) Schiff base complex: Synthesis, Characterization, DNA Cleavage and Biogram assay of *N, N'*-(indoline-2, 3 –diylidene)bis(4-nitroaniline)

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ABSTRACT

Reaction of 1H-Indole-2, 3-dione and p-nitroaniline and M(II) salts in ethanol solution afforded homo mononuclear Schiff base complex, which was characterized by various spectroscopic methods. These studies revealed octahedral geometries for Zn(II) complex. The DNA cleavage efficiencies of the complex have been investigated by agarose gel electrophoresis method. The results suggest that mononuclear Schiff base complex can cleave pUC18DNA efficiently. In order to evaluate the biological activity, the Schiff base and their new metal complex have been screened for their antibacterial activity against bacterial species like- *Bacillus subtilis*, *Staphylococcus aureus* (as Gram-positive bacteria) and *Klebsilla pneumonia* and *Escherichia coli* (as Gram-negative bacteria).

Keywords: Antibacterial activity, Mononuclear metal complexes, DNA interaction, Schiff base.

INTRODUCTION

The development of biologically active mononuclear Schiff base metal complexes with identical ligating environments has undergone an inspiring development and important attention in recent years to study the inorganic perspectives of these metal centers for small molecule activation in biological process [1]. The virtual nature of the metal centers and the ligands environment are key issues that determine their physical and chemical behaviour of the complexes. The potential application has led to the formation of series of novel Schiff base compounds with a wide range of reactivity and stability, physical, chemical and biological properties [2]. Though anionic Schiff base ligands have been exploited for complexation, those with neutral Schiff base ligands have not been adequately studied. Several Schiff base ligands derived from p-nitroaniline derivatives and their Zinc(II) complexes have also been found to inhibit the growth of tumor cells[3]. The synthesis and spectral characterizations of mononuclear transition-metal complexes propagated by bridging atoms are of recent interest. The present work deals with the synthesis and characterization of Schiff-base ligand derived from 1H-Indole-2, 3-dione and p-nitroaniline to form the mononuclear complex.

Analytical and physical measurements

Elemental analyses for C, H and N were carried out using a Perkin Elmer elemental analyzer. FT-IR data were recorded as KBr disc using Thermo Nicolet, Avata 370 model spectrometer in the range 4000-400 cm⁻¹. UV Vis. Spectra was obtained in DMF on a

Perkin-Elmer Lambda 40(UV-Vis) spectrometer in the range 200-800 nm. Molar conductance of the complex in DMF was measured using an Elico model conductivity meter. Magnetic susceptibility measurements were carried out by employing the Gouy method at room temperature. NMR signals were obtained from Bruker Avance III, 400MHz model spectrometer.

Synthesis of *N, N'* - (indoline-2, 3 –diylidene)bis(4-nitroaniline)

The Schiff base ligand was synthesized by adding 1H-Indole-2, 3-dione (1 mM) in 20 ml of ethanol, and p-nitroaniline (2mM) in 20 ml of ethanol. The mixture was refluxed for 2 1/2 hrs. Then solution of the ligand was kept for slow evaporation and coloured precipitate was collected and dried in air [4].

Table 1: Synthesis data of *N, N'*-(indoline-2, 3 –diylidene)bis(4-nitroaniline)

Ligand	Compounds (g)		Time of refluxion (hours)	Yield (%)	Colour
C ₂₀ H ₁₃ N ₅ O ₄	Isatin 0.295	p-nitroaniline 0.552	2 ½	80	Yellowish Orange

Synthesis of mononuclear Schiff base metal complex

The ethanolic solution of synthesized ligand (2mM) was added drop wise to an ethanolic solution of the metal salt (1 mM) with constant stirring, and the mixture was boiled under reflux for 4 1/2 hrs. Then, the volume of the reaction mixture was reduced by evaporation. The precipitated complex were filtered off, washed with ethanol and then dried in vacuo [5].

Table 2: Synthesis data for Mononuclear Schiff base Zinc(II)complex.

Complex	Zn(II) chloride (g)	Compounds (g)		Time of refluxion (hours)	Yield (%)	Colour
Zn(C ₄₀ H ₂₆ N ₁₀ O ₈ Cl ₂)	0.341	Isatin 0.295	p-nitroaniline 0.552	4 1/2	70	Black

Fig 1. Zn(II) Schiff base metal complex

Results and Discussion

The synthesized Zn(II) complex is coloured and it is stable in air. They are insoluble in water and sparingly soluble in organic solvents such as DMF, DMSO giving respective colours to the solutions. The analytical data shows that the complex is in ML_2X_2 type, where L = Schiff base ligand X = chloride ions and M = Zn(II). The structure of the ligand was elucidated by elemental analysis, IR, electronic and 1H NMR spectra.

Elemental analysis

The Schiff base and its Zn(II) complex is subjected to elemental analyses. The results of elemental analyses (C, H, N) with molecular formula and the melting point are presented in Table 3. From the analytical data, it was concluded that the complex is in 2:1 [L:M] stoichiometry ratio. The results obtained are in good agreement with those calculated for the suggested formulae and the melting point is sharp indicating the purity of the prepared Schiff base.

Table 3. Analytical and physical data of synthesized *N, N*-(indoline-2,3 diylidene)bis(4-nitroaniline) and its corresponding metal complex

Compound	Colour	Yield %	Melting point (°C)	% of Nitrogen		% of Metal	
				Cal	Exp	Cal	Exp
L	Yellowish orange	80	145	18.14	18.12	-	-
ZnL_2X_2	Black	70	<200	15.44	15.41	6.92	6.78

Molar conductivity measurements

The chelates were dissolved in DMF and the molar conductivities of 10^{-3} mol dm $^{-3}$ of their solutions at 29 °C were measured. It is concluded from the results that the chelates are found to have molar conductance value of 7.78 ohm $^{-1}$ cm $^{-1}$ mol $^{-1}$ indicating that these chelates are non-electrolytic in nature [6].

IR spectra and mode of bonding

The IR data of the spectra of Schiff base and its complex are listed in Table 4. The IR spectrum of the complex is compared with those of the free ligand in order to determine the coordination sites that may involve in chelation. There are some guide peaks, in the spectra of the ligand, which are of good help for achieving this goal. The position and the intensities of these peaks are expected to be changed upon chelation. Upon comparison it was found that the azomethine $\nu(\text{C}=\text{N})$ stretching vibration is found in the free ligand at 1613.42 cm $^{-1}$. This band is shifted to lower wavenumber (1608.18 cm $^{-1}$) in the complex indicating that amino group of diamine was completely condensed with isatin to give a Schiff base ligand [7]. The stretching vibration band; $\nu(\text{NH})$ of the isatin group, which found at 3410.18 cm $^{-1}$ in the free ligand. Metal- ligand bond is further confirmed by the appearance of a medium intensity band in the range 460.20 and 310.10 cm $^{-1}$ in the spectra of the complex assigned to stretching frequencies of $\nu(\text{M}-\text{N})$ bond and $\nu(\text{M}-\text{Cl})$ bond formation respectively [8].

Table 4. IR spectra (4000–200 cm $^{-1}$) of Schiff base and its metal complex

Compounds	$\nu(\text{C}=\text{N})$ (cm $^{-1}$)	$\nu(\text{M}-\text{Cl})$ (cm $^{-1}$)	$\nu(\text{M}-\text{N})$ (cm $^{-1}$)
C ₂₀ H ₁₃ N ₅ O ₄	1613.42	--	--
Zn(C ₄₀ H ₂₆ N ₁₀ O ₈ Cl ₂)	1608.18	310.10	460.20

Magnetic susceptibility and electronic spectra measurements

The UV–vis spectra of the ligand and its complex were recorded in DMF solution in the wavelength range from 200 to 800 nm. The electronic absorption data of the ligand and the metal complex are as shown in Table 5. The nature of the ligand field around the metal ion was deduced from the electronic spectra. In the spectra, the bands were assigned to 335 and 295 nm attributed to $\pi \rightarrow \pi^*$ transition and the band at 360 and 300 nm were assigned due to $n \rightarrow \pi^*$ transition associated with the C=N stretching of the azomethine group. The bands were observed to have undergone bathochromic shift in the metal complex due to complexation. The spectra of the complex further display bands in the range 445 nm which might be assigned to charge transfer transition from the ligand to metal ions (L $\rightarrow$ M) [9]. The zinc complex gives only, a high-intensity band at 300 nm, due to the absence of d–d transition, which is assigned to a ligand–metal charge transfer besides the characteristic transition of ligand [10]. The zinc(II) complex is found to be diamagnetic as expected for d¹⁰ configuration.

Table 5. Electronic spectra data of the Schiff base ligand and its Zn(II) complex

Compounds	Electronic spectra (nm)		
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	$L \rightarrow M$
$C_{20}H_{13}N_5O_4$	335	360	-
$Zn(C_{40}H_{26}N_{10}O_8Cl_2)$	295	300	445

 1H – NMR spectra

1H NMR spectroscopy has been proved useful in establishing the nature and structure of many Schiff bases as well as their complexes in solutions. The 1H NMR spectrum of Schiff base was recorded in dimethylsulfoxide ($DMSO-d_6$) solution using tetramethylsilane (TMS) as internal standard.

Three different types of protons were identified.

- The multiplets around 6.6-7.9 ppm are assigned to aromatic protons.
- The azomethine group in the Schiff base appears at 8.68 ppm was observed and
- The N-H protons appear at 10.99 ppm [11].

Fig 2. 1H NMR spectrum of $C_{20}H_{13}N_5O_4$ **Fig 3. 1H NMR spectrum of aromatic protons**

Antibacterial screening

In testing the antibacterial activity of these compounds more than one test organism to increase the possibility of detecting antibiotic principles in tested materials. The sensitivity of a microorganism to antibiotics and other antimicrobial agents was screened by the assay plates which incubated at 37° C for 24 hours for bacteria [12]. The results of antibacterial activity of the ligand and the metal complex against gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and gram negative (*Escherichia coli*, *Klebsiella pneumonia*) by disc diffusion method is shown in the figure 4- 7. From the above it was concluded that the metal complex show more activity than the ligand against the four bacteria in the same experimental conditions.

Fig 4. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against *Staphylococcus aureus*

Fig 5. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against *Bacillus subtilis*

Fig 6. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against *Escherichia coli*

Fig 7. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against *Klebsiella pneumoniae*

DNA cleavage study

Gel electrophoresis experiments were performed using pUC19DNA in presence of H_2O_2 . When pUC19 DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact super coil form (Form I) if scission occurs on one strand (nicking), the super coil will relax to generate a slower moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Forms I and II will be generated. From figure the complex shows more activity in the presence of oxidant which may be due to the reaction of hydroxyl radical with DNA. The mononuclear Zn(II) complex was found to be

highly active in cleaving DNA than the ligand and control DNA in the presence of hydrogen peroxide [13].

Conclusion

The new Schiff base ligand and its mononuclear Zinc(II) complex were synthesized. The coordination and the type of geometry of mononuclear Zinc(II) complex were determined by IR, electronic, magnetic and molar conductivity studies. Lower molar conductivity value obtained for the complex indicates that it is a non-electrolyte. The DNA cleavage activity of the newly synthesized *N, N'*-(indoline-2, 3-diylidene)bis(4-nitroaniline) and $\text{Zn}(\text{C}_{40}\text{H}_{26}\text{N}_{10}\text{O}_8\text{Cl}_2)$ was examined by gel electrophoresis technique. The result indicates that the $\text{Zn}(\text{C}_{40}\text{H}_{26}\text{N}_{10}\text{O}_8\text{Cl}_2)$ complex cleave DNA more efficiently when compared to newly synthesized Schiff base ligand $\text{C}_{20}\text{H}_{13}\text{N}_5\text{O}_4$. The antibacterial activity of the metal complex was found to be more than the Schiff base ligand.

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