Synthesis, Spectroscopic, Coordination, DNA cleavage and Antibacterial activities of Mn(II) complex derived from 1H-Indole-2, 3-dione

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ABSTRACT

A new Schiff base ligand was derived from 1H-Indole-2, 3-dione and p-nitroaniline and then it is allowed to react with metal salts to form mononuclear Schiff base metal complex. The synthesized complex was characterized based on elemental analyses, IR, UV, ¹H NMR, molar conductance, and magnetic moment studies. The molar conductance data reveal that the chelates are non electrolytes. An IR spectrum shows that the Schiff bases are coordinated to the metal ions in a manner with NN donor sites of p-nitroaniline. The formation of ligand was also ascertained by ¹H NMR spectra. The electronic spectra show that Mn(II) complex has octahedral geometry. The synthesized ligand, in comparison to their metal complex was screened for their antibacterial activity against bacterial species *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsilla pneumonia*. The activity data show that the metal complex is more potent antibacterial than the parent organic ligand against one or more bacterial species. DNA cleavage ability of the synthesized mononuclear Schiff base metal complex was also performed by gel electrophoresis.

Keywords: 1H-Indole-2, 3-dione, mononuclear metal complex, Antibacterial activity, DNA cleavage.

INTRODUCTION

Designing and synthesis of a new ligand was possibly the most important step in the development of metal complexes which exhibit exclusive properties and novel reactivity due to electron donor, structural, electron acceptor properties, functional groups and the position of the ligand in the coordination sphere. This may be the factor for different studies [1, 2]. A large number of Schiff base compounds are often used as ligands in coordination chemistry by considering their metal binding ability. Schiff bases have gained value because of pharmacological and physiological activities associated with them. Schiff base metal complexes have ability to reversibly bind oxygen in epoxidation reactions [3], biological activity [4, 5], catalytic activity in hydrogenation of olefins [6,7] photochromic properties [8] and nonlinear optical materials [9]. The Schiff base complexes was also used as drugs and they possess a wide variety of antimicrobial activity against bacteria, fungi and it also inhibits the growth of certain type of tumors [10-13].

In view of these finding, this part of work has devoted with the aim to synthesize transition Mn(II) complex with Schiff bases ligand, *N, N'*-(indoline-2,3 –diylidene)bis(4-nitroaniline) and to examine their physical properties involving spectral behaviours, the electrical conductance values and to determine the efficiency of the synthesized complex against pathogenic bacteria.

MATERIALS AND METHODS

All the chemicals were purchased from commercial sources and used without any further purification.

Physical measurements

Elemental analysis (C, H and N) was obtained using Perkin Elmer elemental analyzer. The molar conductance of the complexes in DMF (10^{-3}) solution was measured at 27 ± 3 °C with an Elico model conductivity meter. Infrared spectra were recorded on the Thermo Nicolet, Avatar 370 model FT-IR spectrometer using KBr disc in the range of 4000- 400 cm^{-1} . Electronic spectra were recorded at 300 K on a Perkin-Elmer Lambda 40(UV-Vis) spectrometer using DMF in the range 200-800 nm. 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 prepared by adding 1H-Indole-2, 3-dione (0.2942g) (1 mM) in 20 ml of ethanol, p-nitroaniline (2mM) (0.5526g) in 20 ml of ethanol. The mixture was refluxed for 2 ½ hrs. Then the solution of the ligand was kept for slow evaporation and Yellowish orange colored precipitate was collected and dried in air [14].

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 (1mM) (0.5526) with constant stirring, and the mixture was boiled under reflux for 4 ½ hrs. Then, the volume of the reaction mixture was reduced by evaporation. The precipitated complexes were filtered off, washed with ethanol and then dried in *vacuo*.

Fig 1. Synthesis of *N, N'*-(indoline-2,3 –diylidene)bis(4-nitroaniline)

Fig 2. Synthesis of Mononuclear Schiff base Manganese(II) complex

Anti-bacterial activity

Synthesised Schiff base and its Mn(II) complex were screened for their biological activities against four bacterial strains namely *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. The bacterial strains were sub cultured individually in nutrient agar medium [15]. Wells were dug in the agar media using sterile metallic borer. The well was filled with the test solution at different concentrations such as 25, 50, 75 and 100 $\mu\text{g}/\text{ml}$ and the plates were incubated for 24 h at 37 $^{\circ}\text{C}$. During the incubation period the test solution diffused and the growth of the inoculated microorganism was affected. The inhibition zone was noted. The standard *Streptomycin* was compared with our synthesized compounds.

DNA Cleavage Experiments

The DNA cleavage of super coiled pUC19 DNA promoted by metal complex was proceeded by addition of reaction mixture (20 μ l). The reaction mixture 50 μ M containing pUC19 DNA, 50mM, Tris-HCl, pH 7.4, 50mM NaCl, 10mM H₂O₂ added in a followed by adding Millipore water for final volume. Then the mixed solutions were incubated at 37 °C for 1 hr. They were checked by agarose electrophoresis method.

RESULTS AND DISCUSSION

Manganese (II) complex was synthesized on reaction between metal ions and ligand at 1:2 [M: L] ratio. The prepared complex is very stable at room temperature. The ligand and its metal complex are coloured and insoluble in water and many common organic solvents but soluble in DMF and DMSO, decomposed at very higher temperature. These compounds were characterized by elemental analysis, IR, UV-Vis spectral studies, NMR and magnetic moment studies. The ligand and metal complex were evaluated for their biological activities.

Elemental analysis

Carbon, hydrogen and nitrogen content in the Schiff base ligand and their mononuclear metal complex have been calculated. The elemental analysis data were compared with that of the molecular formula. The formula of the ligand is ML₂X₂ it can be derived from analytical data, Where L = Schiff base X = chloride ions and M = Mn(II). The difference between the two was greater than the acceptable limits of experimental errors. The elemental analytical data is in good agreement with those calculated formula. The physical properties and microanalysis of each component C, H and N present in the synthesized compounds are listed in the Table 1.

Table 1. Analytical data of the Schiff base ligand and its mononuclear metal complex

Compounds	Molecular Formula	Color	Yield %	Melting Point (°C)	%of Nitrogen		% of Metal	
					Cal	Exp	Cal	Exp
L	C ₂₀ H ₁₃ N ₅ O ₄	Yellowish Orange	85	156	19.1	19.0		-
MnL ₂ X ₂	Mn(C ₄₀ H ₂₆ N ₁₀ O ₈ Cl ₂)	Black	75	>200	16.4	16.2	7.4	7.2

Molar conductivity and magnetic moment

The metal complex was dissolved in DMF and the molar conductivities of their 10⁻³ M solutions at room temperature were measured to establish the charge of the metal complex. The conductance value of metal complex is deduced in Table 7, which indicates that the metal complex shows non-electrolytic nature due to no counter ions in the proposed structure [16].

IR spectral studies

The IR frequencies and their tentative results for the synthesized ligand and its complex were attained by comparison with the vibrational frequencies of the free ligand and its complex. The IR spectra assignments provide valuable information regarding the

coordinating sites of synthesized Schiff base ligand with the metal ion. The shift of the stretching frequencies of the azomethine $\nu(\text{C}=\text{N})$ group of the complex to lower frequencies which lie in the range of 1607.28 cm^{-1} , compared with the free ligand band at 1611.22 cm^{-1} , may be due to the coordination of the two azomethine groups to metal ions [17]. Also, the stretching frequency of the $\nu(\text{NH})$ for the NH groups in the ligand and metal complex were observed as band at 3410.78 cm^{-1} and 3380.54 cm^{-1} . Metal-ligand bond is further confirmed by the appearance of a medium intensity band in the range 480.28 and 340.30 cm^{-1} in the spectra of the complex assigned to stretching frequencies of (M-N) bond and (M-Cl) bond formation respectively [18].

Table 2. IR spectral frequencies (cm^{-1}) of the Schiff base ligand and its metal complex and their assignments

Compounds	$\nu(\text{C}=\text{N})$ (cm^{-1})	$\nu(\text{C}-\text{C})$ (cm^{-1})	$\nu(\text{C}=\text{C})$ (cm^{-1})	$\nu(\text{N}-\text{H})$ (cm^{-1})	$\nu(\text{M}-\text{Cl})$ (cm^{-1})	$\nu(\text{M}-\text{N})$ (cm^{-1})
Ligand	1611.22	1289.80	1591.27	3410.78	--	--
Mn(II) complex	1607.28	1310.40	1520.40	3380.54	340.30	480.28

Electronic spectral and magnetic moment measurements

Table 3 lists the characteristic electronic absorption bands of the ligand and its metal complex in DMF solution. The electronic transitions due to the organic ligand in the metal complex, showed the absorption bands of the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions results from the $-\text{C}=\text{N}-$ group appeared at 290 and 340 nm regions, respectively. These values are lower than the corresponding absorption bands for the ligand, which observed at 330 and 360 nm, respectively. This may be due to the coordination of the nitrogen atom of the ligand to the metal ion. The complex shows intense bands in the region of 440 nm, which can be assigned as L-M transition [19].

The electronic spectrum of the brownish black Mn(II) complex in DMF solution showed that the complex shows an octahedral geometry. The electronic spectra of Mn(II) complexes show the absorption bands in the range 615, 545, 530 nm. These absorption bands may be assigned to the ${}^6\text{A}_{1g} \rightarrow {}^4\text{A}_{1g}$, ${}^6\text{A}_{1g} \rightarrow {}^4\text{A}_{2g}$, and ${}^6\text{A}_{1g} \rightarrow {}^4\text{E}_g$, ${}^4\text{A}_{1g}$ transitions, respectively. These bands suggest that the complex possess an octahedral geometry. The Mn(II) complex show magnetic moments is 5.90 B.M. at room temperature corresponding to five unpaired electrons which suggest octahedral geometry [20].

Table 3. Electronic spectral data of Schiff base ligand and its Mn(II) complex.

Compounds	Absorbance (nm)				Geometry of the complex
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	L $\rightarrow$ M	d-d	
Ligand	330	360	-	-	-
Mn(II) complex	290	340	440	615, 545, 530	Distorted Octahedral

^1H NMR spectra Fig 3. ^1H NMR spectrum of $\text{C}_{20}\text{H}_{13}\text{N}_5\text{O}_4$

The ^1H NMR Schiff base was recorded in DMSO at room temperature. Three different type of protons were identified i) resonance exhibits due to phenolic $-\text{OH}$ protons around 10.90 ppm [21], ii) characteristic resonance due to azomethine proton in the Schiff base appears at 8.60 ppm was observed and iii) the other signals in the region 6.6-7.8 ppm exhibits due to aromatic protons.

Antibacterial activity

N,N' -(indoline-2,3-diylidene)bis(4-nitroaniline) and its $\text{Mn}(\text{II})$ metal complex were evaluated for antimicrobial activity against two strain Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*). The Schiff base ligand was found to be biologically active. The results shows that the metal complex exhibit antimicrobial activity and enhanced it comparing with the parent Schiff base ligand [22].

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 pneumonia*

DNA cleavage by agarose gel electrophoresis

The ligand and its Mn(II) complex were studied for their DNA cleavage activity by agarose gel electrophoresis method against supercoiled pUC19 DNA as a target molecule. DNA-cleavage studies are used for the rational design and to construct new and more capable drugs that are targeted to DNA [23]. The DNA cleavage study by gel electrophoresis analysis clearly shows that the lanes of Mn(II) complex showed complete cleavage of supercoiled DNA whereas ligand showed partial cleavage of supercoiled DNA. These results indicate that the important role of coordination of nitrogen to the metal ion in these isolated DNA cleavage reactions. On the basis of the cleavage of DNA observed in case of ligand and its Mn(II) complex, it can be concluded that the compounds under present study inhibited the growth of pathogenic organism by DNA cleavage as has been observed on the DNA cleavage of supercoiled plasmid pUC19 DNA.

CONCLUSION

Based on the spectroscopic studies, the coordinating ability of the ligand has been proved in complexation reaction with Mn(II) ion. The conductivity measurement of the metal complex in 10^{-3} M DMF solutions at room temperature indicating that the complex is non-electrolytic in nature. From the infrared spectra, it is cleared that the metal ion is coordinated through azomethine nitrogen and chloride ions of MnCl_2 . UV-visible and magnetic measurements show that Mn(II) complex possesses distorted octahedral geometry respectively. The antimicrobial activity results showed that the $\text{Mn}(\text{C}_{40}\text{H}_{26}\text{N}_{10}\text{O}_8\text{Cl}_2)$ complex has exhibited higher activity when compared to their respective ligand. The DNA cleavage studies revealed that $\text{Mn}(\text{C}_{40}\text{H}_{26}\text{N}_{10}\text{O}_8\text{Cl}_2)$ showed good efficiency towards DNA cleavage.

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