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DESIGN, SYNTHESIS, CHEMICAL NUCLEASE ACTIVITY AND ANTIBACTERIAL EVALUATION OF Cu(II), Co(II), Ni(II) and Mn(II) SCHIFF BASE METAL COMPLEXES

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ABSTRACT

Four new Cu(II), Ni(II), Co(II) and Mn(II) metalloinsertors have been synthesized by using a Schiff base, formed by the condensation reaction of isatin, p-chloroaniline with 1, 10-phenanthroline. They have been structurally characterized by IR, UV–vis, NMR, EPR, elemental analyses, molar conductivity and magnetic susceptibility studies. The molar conductance data of the complexes indicate that they are non-electrolytes in nature. The spectral and analytical data reveal that the complexes take up distorted octahedral geometry. They have been examined for their antibacterial activities against few pathogens. The data reveal that the complexes exhibit better biocidal activities against bacteria than the free Schiff base ligand. The gel electrophoresis experiment reveals that the complexes are good chemical nucleases.

Keywords: Metal complexes, Antibacterial activity and DNA cleavage

INTRODUCTION

DNA binding of metal complexes has been widely investigated because they can be used as potential anticancer drugs, DNA structural probes, DNA foot printing agents, DNA dependent electron transfer probes, sequence-specific cleaving agents and so on **[1]**. DNA is the primary target molecule for mainly anticancer and antiviral therapies according to cell biologists. Investigations on the interaction of DNA with small molecules are significant in the aim of new types of pharmaceutical molecules. While the chemical nuclease activity of transition metal complexes was discovered in the year 1980, studying the interaction model and the mechanism of transition metal complexes with DNA moecule, and exploring the application of metal complexes in antineoplastic medication, molecular biology and bioengineering have turn out to be hotspots in recent years. Some kind of metal complexes when interacted with DNA might induce the breakage of DNA strands by appropriate methods **[2]**. Schiff bases form a vital separation of organic compounds in chemistry due to their useful physical and chemical properties and great number of reactions that they undergo. Schiff bases, derived from substituted aliphatic amines and aromatic aldehydes, have an extensive variety of applications in many fields, e.g. inorganic, biological, and analytical chemistry **[3-4]**.

In literature, several reports associate the consequence of the biological activity of metal complexes with the metal ions rather than the ligands. To our knowledge, no work has been reported on the condensed reaction of the isatin, p-chloroaniline with 1, 10-phenanthroline and its transition metal complexes as chemical nucleases. In continuation of our journey toward the exploration of novel potentially active drugs, we herein report the synthesis, characterization and DNA interaction of Schiff base transition metal(II) complexes.

EXPERIMENTAL

All reagents and chemicals used, isatin, p-chloroaniline with 1, 10-phenanthroline and various metal(II) chlorides, were Merck products and used as supplied. Commercial solvents

were distilled and then used for the preparation of ligand and its complexes.

Microanalyses (C, H and N) were performed in Carlo Erba1108 analyzer at Sophisticated Analytical Instrument Facility (SAIF). The electronic absorbance spectra in the 200–800 nm were recorded on Double beam spectrometer Cyber lab between 200 – 800 nm. Magnetic susceptibility measurements of the complexes were carried out by Gouy balance using copper sulfate pentahydrate as the calibrant. IR spectra were recorded with Perkin–Elmer FT-IR-8300 spectrophotometer in the 4000–400 cm^{-1}

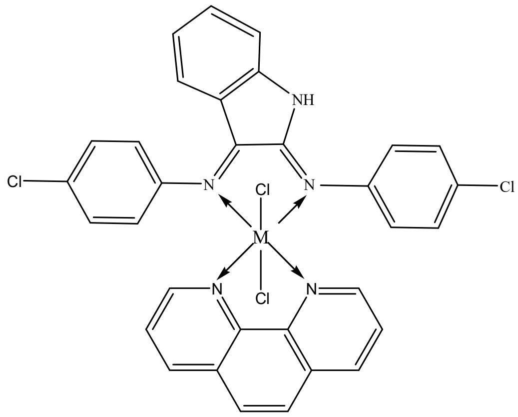
range using KBr pellets. NMR spectra were recorded on a Bruker Spectrometer at 400 MHZ in DMSO. Electron paramagnetic resonance spectra of the mixed ligand complexes of copper(II) were recorded on a Varian E 112 EPR spectrometer in DMSO solution both at room temperature and liquid nitrogen temperature (77 K) using TCNE (tetracyanoethylene) as the g-marker.

Synthesis of Schiff base ligand (4-Chloro-phenyl)-(2-imino-1,2-dihydro-indol-3-ylidene)-amine)-1-chloro-4-methyl-benzene

The Schiff base ligand was synthesized by adding Isatin (1 mM) in 20 ml of ethanol, p-chloroaniline (2 mM) in 20 ml of ethanol. The mixture was refluxed for 2-3 hrs. Then solution of the ligand was kept for slow evaporation and yellow colored precipitate was collected and dried in air **[5]**.

Synthesis of Schiff base metal complexes

The ethanolic solution of synthesized ligand (1 mM) was added dropwise stirring to an ethanolic solution of the metal chloride (1 mM) (Metal = Cu(II), Co(II), Ni(II) and Mn(II)) with constant stirring and followed by an ethanolic solution of 1, 10-phenanthroline is added and the mixture was boiled under reflux for 3-5 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 vacuuo **[6-7]**.



M=Cu(II), Ni(II), Co(II) and Mn(II)
Figure 1: Structure of Mononuclear Schiff base Metal(II) complexes

RESULTS AND DISCUSSION

The Schiff base ligand and its Cu(II), Ni(II), Co(II) and Mn(II) complexes have been synthesized and characterized by spectral and elemental analytical data. They are found to be air stable. The ligand is soluble in common organic solvents and all the complexes are freely soluble in $CHCl_3$, DMF and DMSO but partially soluble in methanol and ethanol and insoluble in water.

Elemental analysis and molar conductivity measurements

The elemental analysis results for the metal complexes are in good agreement with that of the calculated values **(Table 1)** showing that the complexes have 1:1:1 metal–ligand stoichiometry of the type ML_1L_2, wherein L_1 is (4-Chloro-phenyl)-(2-imino-1,2-dihydro-indol-3-ylidene)-amine)-1-chloro-4-methyl-benzene and L_2

= 1, 10-phenanthroline. The metal(II) complexes were dissolved in DMF and the molar conductivities of 10^{-3} M of their solution at room temperaturewere measured. The lower conductance values 5.4–10.2 ohm^{-1} cm^{2} mol^{-1} of the complexes support their non-electrolytic nature of the compounds.

IR spectra

IR spectra of the complexes clearly point out the bonding association of the ligand with the metal ion. The spectrum of free ligand showed a band in the region 1616 cm^{-1} characteristics of the ν(C=N) (azomethine) stretching mode indicating the formation of the Schiff base product. This band was shifted toward lower frequencies in the spectrum of its metal complexes (1602–1610 cm^{-1}) compared with the above Schiff base indicating the involvement of the azomethine nitrogen in coordination with metal ion **[8]**. The coordination of nitrogen to the metal ion could be expected to reduce the electron density of the azomethine link and thus caused a shift in the ν(C=N) group. Conclusive evidence of the bonding was also shown by the observation that new bands in the spectra of all metal complexes appearing in the low frequency regions at 483–465 cm^{-1} characteristic to ν(M–N) stretching vibrations, respectively, that were not observed in the spectrum of free ligand. In addition, the appearance of new bands at 335.10-348.22 cm^{-1} showing the involvement of ν(M–Cl) bond in the complexes formation, supporting the IR spectral study **(Table 2)**.

^{1}H-NMR spectra

^{1}H NMR spectra of the ligand were recorded in DMSO- d_6 at room temperature. The azomethine proton (–CH=N) signal in the spectrum of the ligand was shifted down field (8.7 d) compared to the free ligand, suggesting deshielding of azomethine group due to the coordination with metal ion. And the other signals in the region 6.24–7.72 ppm exhibits due to aromatic protons. The peak observed at the range of 3.6 ppm due to -NH proton in ligand **[9]**.

Electronic spectra and magnetic moment

The electronic spectral data of the metal(II) complexes in DMF solution are displayed in **Table 3**. The nature of the ligand field around the metal ion was deduced from the electronic spectra. The electronic spectrum of Co(II) complex exhibited three bands in the region of 650, 585 and 550 nm which were tentatively assigned to $^{4}T1g \rightarrow 4T_{2}g(F)$ (ν_1), $^{4}T_{1}g \rightarrow ^{4}A_{2}g(F)$ (ν_2) and $^{4}T_{1}g \rightarrow ^{4}T_{1}g(P)$ (ν_3) transitions, respectively. The value of magnetic moment was 5.10 B.M. which indicates the presence of Co(II) complex in octahedral geometry. The electronic spectrum of the Ni(II) complex showed three bands at 690, 545 and 570 nm assignable to $^3A_2g\rightarrow^3T_2g$ (F) (v_1), $^3A_2g\rightarrow^3T_1g$ (F) (v_2) and $^3A_2g\rightarrow^3T_1g$ (P) (v_3) transitions, respectively. The value of magnetic moment was 3.40 B.M; therefore octahedral geometry is suggested for this complex [10]. The ^2Eg and ^2T_2g states of the octahedral Cu(II) (d^9) split under the influence of the tetragonal distortion and the distortion can be such as to cause the three transitions $^2B_1g\rightarrow^2B_2g$; $^2B_1g\rightarrow^2Eg$ and $^2B_1g\rightarrow^2A_1g$ to remain unresolved in the spectra. It is concluded that, all three transitions 640, 555 and 520 nm lie within the single broad envelope centered at the same range previously mentioned. This assignment is in agreement with the general observation that Cu(II) d–d transitions are normally close in energy. The magnetic moment of 1.97 B.M. falls within the range normally observed for octahedral Cu(II) complexes. The electronic spectra of Mn(II) complexes show the absorption bands in the range 684, 545 and 520 nm. These absorption bands may be assigned to the $^6A_1g\rightarrow^4A_1g$, $^6A_1g\rightarrow^4A_2g$, and $^6A_1g\rightarrow^4Eg$, ^4A_1g transitions, respectively. These bands suggest that the complexes possess an octahedral geometry. The Mn(II) complex show magnetic moments is 5.92 B.M. at room temperature corresponding to five unpaired electrons which suggest octahedral geometry. In the spectra of the Schiff base ligand, the absorption band observed at 281-294 nm were assigned to intra-ligand $\pi\rightarrow\pi^*$ transition and the band at 342-390 nm were assigned due to $n\rightarrow\pi^*$ transition associated with the azomethine chromophore (-C=N).

EPR Studies

The EPR spectra of complexes provide information of importance in studying the metal ion environment. The ESR spectrum of Cu(II) complex were recorded on X-band at frequency 9.1 GHz at room temperature. The spectra exhibited a single anisotropic intense sharp signal at room temperature with no hyperfine splitting as shown in **Figure 2**. This suggests that copper ions are in mononuclear environment.

The EPR spectrum of the $[Cu(C_{20}H_{13}N_5Cl_4)]$ complexes show a broad signal with g_{iso} at 2.0001 which is consistent with an distorted octahedral geometry **[10]**.

Antibacterial activity

The synthesized ligand and the complexes were tested for their *in vitro* antibacterial activity. They were tested against the bacteria *S. aureus, B. subtilis, E. coli* and *K. pneumonia* and the values of the investigated compounds are summarized in **Tables 4**. A comparative study of values of ligand and the complexes indicates that the metal complexes exhibit higher antibacterial activity than the free ligand. Such increased activity of the complexes is able to be explained on the basis of the Tweedy's chelation theory **[11]**. Chelation reduces the polarity of the metal ion significantly because of the biased sharing of its positive charge with the donor group and also due to p-electron delocalization on the whole chelate ring. The lipids and polysaccharides are some significant constituents of the cell wall and membranes which are favoured for metal ion interaction. Apart from this, the cell walls also hold many phosphates, carbonyl and cystenyl ligands which keep up the integrity of the membrane by acting as a diffusion barrier and also give appropriate sites for binding. Furthermore, the reduction in polarity increases the lipophilic character of the chelates and an interaction among the metal ion and the lipid is favored. This may lead to the breakdown of the permeability barrier of the cell resulting in interfering with the normal cell processes. Besides this, the complexes might also indulge in the formation of hydrogen bonded interaction throughout the coordinated anions and azomethine group with the active centers of the cell constituents. Factors able of increasing lipophilic nature are expected to improve the antibacterial property. The synthesized Schiff base ligand has reasonable inhibitory effects on the growth of tested microorganism. This is owing to the presence of azomethine groups which contain chelating properties. These properties may be used in metal transport across the bacterial membranes or to connect to the bacterial cells at a specific site from which it can get in the way with their growth. The present investigations of antimicrobial activity data indicated that all of the newly synthesized complexes exhibited slightly different antibacterial activity as compared to that of the control drugs.

Chemical nuclease activity

The DNA cleavage efficacy of the complexes compared to that of the control is due to their efficient DNA-binding ability **[12]**. When super coiled pUC18DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact super coiled form (Form I). If scission occurs on one strand (nicking), the super coiled form will relax to make a slower moving nicked form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Forms I and II will be generated. In the current study, DNA cleavage was analyzed by monitor the conversion of super coiled DNA (Form I) to nicked DNA (Form II) in the presence of oxidant H_2O_2. From **Figure**

3 (lanes 3–6), it is evident that the complexes cleave DNA more efficiently in the presence of an oxidant (H_2O_2). This may be attributed to the formation of hydroxyl free radicals. The OH^o free radicals participate in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of a sugar phosphate back bone. All the complexes showed prominent nuclease activity in the presence of oxidant H_2O_2 which may be due to the increased production of hydroxyl radicals. Control experiments by means of DNA alone (lane 1) did not show any significant cleavage of pUC18DNA even on longer exposure time. From the observed results, it is concluded that all the complexes efficiently cleave the DNA as compared to control DNA.

Table 1: Analytical data of the Schiff base ligand and its mononuclear metal complexes

Compound	Molecular Formula	color	Yield %	Melting Point (°)	% of Nitrogen		% of Metal		Molar conductance Λ_m (ohm^{-1} cm^2 mol^{-1})
					Cal	Exp	Cal	Exp	
L	$C_{20}H_{13}N_3Cl_2$	Yellowish orange	80	140	9.88	9.86	-	-	-
$[CuL_1L_2]$	$[Cu(C_{20}H_{13}N_5Cl_4)]$	Green	70	>200	11.15	11.14	12.64	12.63	5.4
$[NiL_1L_2]$	$[Ni(C_{20}H_{13}N_5Cl_4)]$	Brown	75	>200	11.26	11.23	11.80	11.79	8.2
$[CoL_1L_2]$	$[Co(C_{20}H_{13}N_5Cl_4)]$	Brown	70	>200	11.25	11.23	11.83	11.82	7.9
$[MnL_1L_2]$	$[Mn(C_{20}H_{13}N_5Cl_4)]$	Black	75	>200	11.34	11.33	11.12	11.10	10.2

Table 2: Infrared Spectroscopic Data of the Schiff Base Ligand and its mononuclear metal complex

Compounds	(C=N) (cm^{-1})	(C-O) (cm^{-1})	(M−N) (cm^{-1})	(M−Cl) (cm^{-1})
$C_{20}H_{13}N_3Cl_2$	1616	1255	--	--
$[Cu(C_{20}H_{13}N_5Cl_4)]$	1608	1270	465	338.12
$[Ni(C_{20}H_{13}N_5Cl_4)]$	1610	1310	470	335.10
$[Co(C_{20}H_{13}N_5Cl_4)]$	1615	1330	483	348.22
$[Mn(C_{20}H_{13}N_5Cl_4)]$	1602	1325	480	345.36

Table 3: Electronic Spectral data of Schiff base ligand and its complexes

Compound	Electronic spectra (nm)				Geometry of the complex
	$\pi\rightarrow\pi^*$	$n\rightarrow\pi^*$	L→M	d-d	
$C_{20}H_{13}N_3Cl_2$	295	374	-	-	-
$[Cu(C_{20}H_{13}N_5Cl_4)]$	280	375	450	640, 555, 520	Distorted Octahedral
$[Ni(C_{20}H_{13}N_5Cl_4)]$	290	395	445	690, 545, 570	
$[Co(C_{20}H_{13}N_5Cl_4)]$	285	340	455	650, 585, 550	
$[Mn(C_{20}H_{13}N_5Cl_4)]$	290	344	435	684, 545, 520	

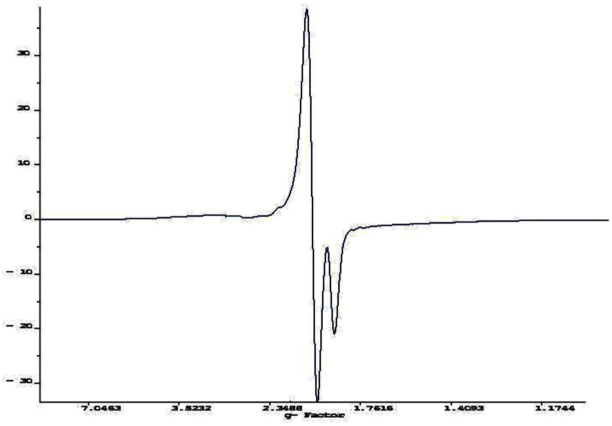


Figure 3: EPR spectra of $[Cu(C_{20}H_{13}N_5Cl_4)]$ complex

Table 4: Antibacterial activity for heterocyclic Schiff base ligands and its mononuclear metal complexes

Compounds	Zone of inhibition (mm)															
	Gram positive bacteria								Gram negative bacteria							
	Staphylococcus aureus				*Bacillus subtilis*				*Escherichia coli*				*Klebsiella pneumoniae*			
	Concentration (µg/mL)															
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
$C_{20}H_{13}N_3Cl_2$	7	7	8	9	7	7	9	9	6	6	7	8	7	8	8	9
$[Cu(C_{20}H_{13}N_5Cl_4)]$	12	13	14	15	12	10	13	14	10	10	12	13	11	12	15	17
$[Ni(C_{20}H_{13}N_5Cl_4)]$	9	8	10	12	9	8	11	12	6	7	7	9	9	8	9	10
$[Co(C_{20}H_{13}N_5Cl_4)]$	10	9	12	13	10	11	12	14	8	9	9	11	9	10	11	12
$[Mn(C_{20}H_{13}N_5Cl_4)]$	8	9	9	10	8	9	9	10	7	8	9	9	7	8	9	10
Streptomycin	16	17	20	22	14	15	17	20	12	13	16	20	13	14	16	18

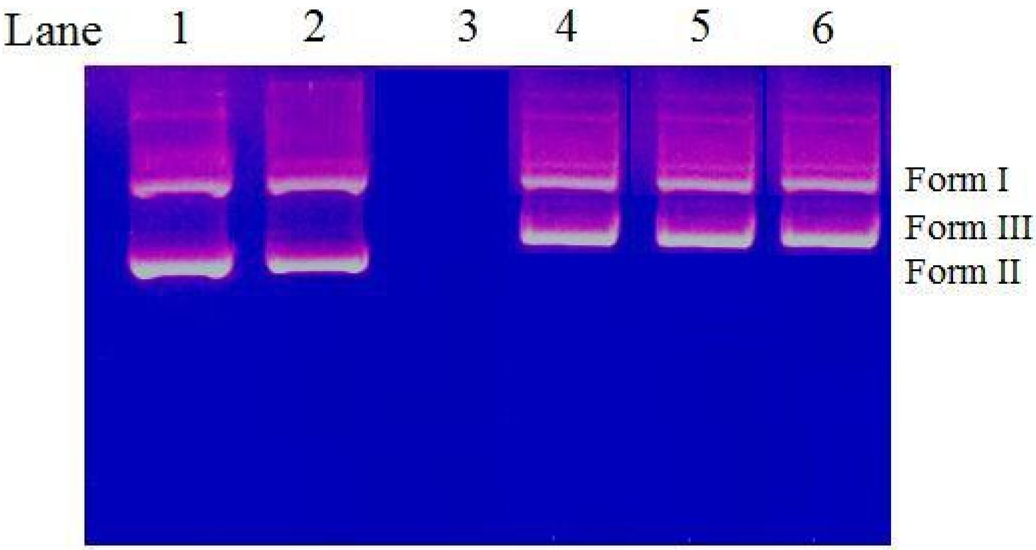


Figure 3: Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by H_2O_2 and metal complexes. Lane 1-DNA alone; Lane 2- DNA alone + H_2O_2; Lane 3-DNA + $[Cu(C_{20}H_{13}N_5Cl_4)]$ + H_2O_2; Lane 4-DNA + $[Ni(C_{20}H_{13}N_5Cl_4)]$ + H_2O_2; Lane5-DNA + $[Co(C_{20}H_{13}N_5Cl_4)]$; Lane 6-DNA+ $[Mn(C_{20}H_{13}N_5Cl_4)]$+ H_2O_2.

CONCLUSIONS

From the elemental analysis, molar conductivity, UV–Vis, magnetic, IR and ^{1}H NMR spectral data, it is possible to decide the type of coordination of the ligand in their metal complexes. Based on these data, a distorted octahedral geometry is assigned to these complexes. Chemical nuclease activity of these metal complexes with super coiled pUC18DNA was investigated by gel electrophoresis. From the results, it is found that the metal complexes cleaved DNA effectively in the presence of H_2O_2 as compared to the control DNA. Further, the promising results have been observed for the antimicrobial screening particularly for the metal complexes against bacteria and what may be attributed to the fact that the metal complexes are potentially active against bacterial cells.

DECLARATIONS

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Conflict of interests

The authors declare that they have no conflict of interest

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