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Synthesis, spectral characterization and biological evaluation of Co(II), Ni(II), Cu(II) and Mn(II) metal complexes of novel Isatin schiff base ligand

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ABSTRACT

A novel series of transition metal complexes of Co(II), Ni(II), Cu(II) and Mn(II) were synthesized from the Schiff base derived from isatin monohydrazone and P-Dimethylamino benzaldehyde. The mode of bonding and overall geometry of the complexes have been inferred through IR, EPR, electronic spectral studies, conductivity, magnetic, thermal, and electrochemical studies. The elemental analyses of the complexes confine to the stoichiometry of the type $ML_2 \cdot 2Cl$ [$M = Co(II), Ni(II), Cu(II)$ and $Mn(II)$]. The redox behavior of the Cu(II) complex was investigated using cyclic voltammetry. The Schiff base and its metal complexes have been screened for antibacterial (*Escherichia coli*, *Pseudomonas aerogenosa*, *Staphylococcus aureus*, *Klebsiella*), and antifungal activities (*Penicillium chrysogenum* and *Aspergillus niger*) by minimum inhibitory concentration method. The anthelmintic and insecticidal activity of the ligand and its metal complexes against earthworms and adult cockroaches (*P.Americana*) was investigated. The DNA cleavage study was done by agarose gel electrophoresis method. The complexes show activity against *Mycobacterium tuberculosis* strain H37Rv.

Keywords: Isatin; Spectral characterization; Antitubercular; DNA cleavage; cyclic voltammetry.

INTRODUCTION

Tuberculosis is an infectious bacterial disease caused by *Mycobacterium tuberculosis*, which most commonly affects the lungs[1]. It is transmitted from person to person via droplets from the throat and lungs of people with the active respiratory disease. It is termed as “ a global health emergency ” by world health organization (WHO) in 1993 as it affects 1.7 billion people per year that is equal to one-third of the entire world population. The first line of drugs used in the

treatment of tuberculosis (TB) is a combination of isoniazid, rifamycin, pyrazinamide and ethambutal. Thus the increasing clinical importance of tuberculosis has lent additional urgency to researchers to identify new effective antimycobacterial compounds[2]. Heterocycles bearing nitrogen, sulphur and oxygen atoms in their structure constitute the core structure of a number of biologically interesting compounds. Many indole derivatives reported in the literature are known to possess varied biological activities viz, antimalarial activity[3], antituberculosis activity and COX-2 inhibitors[4].

The isatin molecule (1H-indole-2, 3-Dione) is a versatile moiety that displays diverse biological activities [5-7]. The Isatin-thiosemicarbazone of Sn(IV) and Zr(IV) complexes of isatin Schiff bases are good antiviral drugs[8-9].

Although much attention has been directed to study the metal complexes of the Schiff base ligands derived from isatin[10]. No investigations have appeared in the literature to describe the metal complexes of the Schiff base derived from isatin monohydrazone and P-Dimethylamino benzaldehyde. Thus, the aim of present work is to synthesize and characterize Co(II), Ni(II), Cu(II), and Mn(II) metal complexes with newly synthesized Schiff base derived from isatin monohydrazone and P-Dimethylamino benzaldehyde, possessing donor sites of carbonyl oxygen and azomethine nitrogen.

MATERIALS AND METHODS

Experimental procedure

Physical Measurements

Carbon, hydrogen and nitrogen were estimated by using CHN Elemental Analyzer, Truespec LECO USA. The IR spectra of the Schiff base and its complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000-250 cm^{-1} region in KBr discs. The electronic spectra of the complexes were recorded in HPLC grade DMF on a VARIAN CARY 50-BIO UV-spectrophotometer in the region of 200-1100 nm. The $^1\text{H-NMR}$ spectra of ligand was recorded in $\text{D}_6\text{-DMSO}$ on a BRUKER 300 MHz spectrometer at room temperature using TMS as an internal reference. FAB-Mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6KV, 10Am) as the FAB gas. The accelerating voltage was 10 KV and the spectra were recorded at room temperature and *m*-Nitrobenzyl alcohol was used as the matrix. The mass spectrometer was operated in the +ve ion mode. The electrochemistry of the Cu(II) complex was recorded on CHI110A-electrochemical analyzer (Made in U.S.A) in dimethyl formamide (DMF) containing 0.05 M $n\text{-Bu}_4\text{NClO}_4$ as the supporting electrolyte. The EPR spectrum was recorded on Varian-E-4X-band EPR spectrometer and the field set is 3000 G at modulation frequency of 100 KHz under liquid nitrogen temperature using TCNE as "g" marker. Thermo gravimetric analyses data were measured from room temperature to 1000 $^\circ\text{C}$ at a heating rate of 10 $^\circ\text{C}/\text{min}$. The data were obtained by using a PERKIN-ELMER DIAMOND TG/DTA instrument. Molar conductivity measurements were recorded on ELICO-CM-82 T Conductivity Bridge with a cell having cell constant 0.51 and magnetic moment was carried out by using Faraday balance. The fluorescence studies of Schiff bases and their metal complexes were recorded on HITACHI F-7000 Fluorescence Spectrophotometer (made in Japan). The solutions of 10^{-3} M concentration were prepared in HPLC grade DMF and DMSO solvents.

Synthesis

Synthesis of isatin monohydrazone

Isatin (1.47 g, 10 mmol) was dissolved in ethanol (50 mL) and was added to a solution of hydrazine hydrate (0.05 g, 10 mmol) dissolved in hot ethanol (10 mL). The resulting reaction mixture was refluxed for 3 h on the water-bath. On cooling, the yellow compound formed was filtered, washed, dried, and recrystallized from ethanol. m.p. 224 °C and yield is 91%.

Syntheses of Schiff base

The Schiff base has been synthesized by refluxing the reaction mixture of hot ethanolic solution (30 mL) of P-dimethylamino benzaldehyde (0.01 mol) and hot ethanolic solution of (30 mL) of isatin monohydrazone (0.01 mol) for 4-5 h with 3-4 drops of hydrochloric acid. The product obtained after the evaporation of the solvent was filtered, washed with cold ethanol and recrystallized from ethanol. m.p is 285 °C (yield 75%) (Fig.1.).

Syntheses of metal complexes

Hot ethanolic solution (40 mL) of Schiff base (2 mmol) was mixed with hot ethanolic solution (15 mL) of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ / $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ / $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ / $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (1 mmol) and refluxed on water bath for 2 h. Then, to the reaction mixture 1 mmol of sodium acetate was added and reflux was continued for 3 h. The separated complexes were filtered, washed thoroughly with water, ethanol, ether, and finally dried in vacuum over fused CaCl_2 . Yield of the metal complexes lie in the range of 65-70 % (Table 1).

DNA cleavage experiment

Preparation of culture media

Nutrient broth (peptone, 10; Yeast extract, 5; NaCl, 10 in (g/l)) was used for culturing of *E. coli*. The 50 mL media was prepared and autoclaved for 15 min. at 121°C under 15 lb Pressure. The autoclaved media were inoculated with the seed culture and incubated at 37 °C for 24h.

Isolation of DNA

The fresh bacterial culture (1.5 mL) is centrifuged to obtain the pellet, which is then dissolved in 0.5 mL of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 10% SDS). To this 0.5 mL of saturated phenol was added and incubated at 55 °C for 10 min. Then it was centrifuged at 10,000 rpm for 10 min. Then equal volume of chloroform : Isoamyl alcohol (24:1) and 1/20th volume of 3M sodium acetate (pH 4.8) was added to this supernatant and centrifuged at 10,000 rpm for 10 min. To these supernatant 3 volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation. Dried the pellet and dissolve in TE buffer (10 mM tris pH 8.0, 1 mM EDTA) and stored in cold condition.

Agarose gel electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method. Test samples (1mg/mL) were prepared in DMF. The samples (100 µg) were added to the isolated DNA of *E. coli*. The samples were incubated for 2 hour at 37° C and then 20 µL of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) were loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 ltr) and finally loaded on agarose gel and pass the constant 50 V of electricity for

around 30 min. Then removed the gel and stained with 10.0 $\mu\text{g}/\text{mL}$ ethidium bromide for 10-15 min. the bands observed under UV transilluminator and photographed to determine the extent of DNA cleavage and the results were compared with standard DNA marker.

In vitro antibacterial and antifungal assay

The biological activities of synthesized Schiff base and its Co(II), Ni(II) Cu(II) and Mn(II) complexes have been studied for their antibacterial and antifungal activities by agar and potato dextrose agar diffusion methods respectively. The antibacterial and antifungal activities were done at 200 and 500 $\mu\text{g}/\text{mL}$ concentrations in DMF solvent by using six bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella*) and antifungal activities (*Penicillium chrysogenum* and *Aspergillus niger*) by minimum inhibitory concentration method. These bacterial strains were incubated for 24 h at 37 °C and fungal strains were incubated for 48 h at 37 °C. Standard antibacterial (Streptomycin) and antifungal drugs (Nystatin) were used for comparison under similar conditions.

Anthelmintic activity (In-vitro)

The anthelmintic assay was carried as per the method given in the literature [11] with minor modifications. The assay was performed on adult Indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [12]. The earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-4 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol.

All the synthesized compounds were subjected to anthelmintic activity studies against the earthworms at 2 and 10 mg mL^{-1} concentration and Albendazole used as a reference drug. The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50 °C) which stimulated the movement, if the worm was alive.

In Vitro anti-tuberculosis testing method

In vitro antituberculosis testing was carried out against the human virulent strain *Mycobacterium tuberculosis* (H37Rv) by reported method [13]. To sterile Kichner disperse medium (4.5 mL) dispersed in borosilicate test tube (150 x 20 mm), was added 0.5 mL of sterile normal bovine serum, inactivated by heating at 56 °C for 30 min. The Schiff base and its metal complexes under test were dissolved in DMF and added in the form of solution in such a way as to give final concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 mg per mL to the inoculums consisting of 0.1 mL of standard suspension of *M. tuberculosis* (H37Rv) containing 106 bacilli / mL. The tubes were incubated at 37 °C for eight days and then examined for the presence or absence of the growth of the test organism. The lowest concentration that showed no visible growth was taken as an end point. The minimum inhibition concentration for all the test compounds was measured and the results were compared with the standard drug tube with streptomycin and tube with DMF used as control.

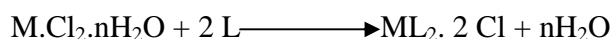
Insecticidal activity (in vitro)

The activity was carried out by the reported method [14]. Adult cockroaches (*P. americana*) were selected for the testing of in vitro insecticidal activity. 4% solutions of synthesized

compounds and standard drug cypermethrin (w/v), in acetone were used for experiment. The time of death of cockroaches was noted, on an average and denoted as KD (Knock Down) value in minutes. For each sample three replication were performed and same experiments were performed with standard drug.

RESULTS AND DISCUSSION

The formation of complexes of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ / $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ / $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ / $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ with Schiff base in ethanol is presented in the following reaction.



M = Co(II), Ni(II), Cu(II) and Mn(II)

All the metal complexes are stable and non-hygroscopic in nature. The complexes are insoluble in common organic solvents but soluble in DMF and DMSO. The elemental analyses shows that, the Co(II), Ni(II), Cu(II) and Mn(II) complexes have 1:2 stoichiometry of the type $\text{ML}_2 \cdot 2\text{Cl}$ Where L is acts as a bidentate ligand. The molar conductance values are too low to account for any dissociation of the complexes in DMF, indicating the nonelectrolytic nature of the complexes in DMF.

IR Spectra

The prominent infrared spectral data of Schiff base and its metal complexes are presented in Table 2. The IR spectra of the Schiff base exhibited characteristic band due to $\nu(\text{NH})$ and lactonyl carbon $\nu(\text{C}=\text{O})$ at 3140 cm^{-1} and 1750 cm^{-1} [15] respectively. In addition, the strong band at 1598 cm^{-1} and a characteristic high intensity band at 1621 cm^{-1} in the IR spectra of the Schiff base are assigned to $\nu(\text{C}=\text{N})$ and $(\text{HC}=\text{N})$ respectively.

In comparison with the spectra of the Schiff base, all the Co(II), Ni(II), Cu(II), and Mn(II) complexes exhibited the band of $\nu(\text{HC}=\text{N})$ in the region $1603 - 1615 \text{ cm}^{-1}$; showing the shift of band to lower wave numbers indicating that, the azomethine nitrogen is coordinated to the metal ion [16, 17]. The band of $\nu(\text{C}=\text{O})$ in the region $1698 - 1710 \text{ cm}^{-1}$ in the metal complexes showing the shift to lower wave numbers confirms that, the carbonyl oxygen is coordinated to the metal ion [18]. The unaltered position of a band due to $\nu(\text{NH})$ and $\nu(\text{C}=\text{N})$ in all the metal complexes indicates that, these groups are not involved in coordination. The new bands in the region of $450 - 470$ and $512 - 525 \text{ cm}^{-1}$ in the spectra of the complexes are assigned to stretching frequencies of (M-N) and (M-O) bonds respectively [19]. Another band in the region $335 - 350 \text{ cm}^{-1}$ is ascribed to (M-Cl) bond formation [20]. Thus the IR spectral results provide strong evidences for the complexation of Schiff base with metal ions in bidentate mode.

¹H NMR

In the ¹H-NMR spectrum of the Schiff base, the NH proton of isatin exhibited signal at 10.4 ppm (s, 1H) and the characteristic signal observed at 8.5 ppm (s, 1H) is due to azomethine proton. The multiplet signals around 6.3-7.3 ppm are ascribed to aromatic protons. The signals observed at 2.6 ppm are due to methyl protons. Thus, the NMR results further supports the I.R. inferences.

Electronic Spectral and Magnetic Studies

The Co(II) complexes exhibited bands in the region 8000–10,000 cm^{-1} and 18,000–20,000 cm^{-1} corresponding to ν_1 and ν_3 transitions attributed to ${}^4\text{T}_{1g}(\text{F}) \longrightarrow {}^4\text{T}_{2g}(\text{F})$, (ν_1) and ${}^4\text{T}_{1g}(\text{F}) \longrightarrow {}^4\text{T}_{2g}(\text{P})$ (ν_3). In the present investigation, the brownish Co(II) complexes showed absorption bands at 9256–9312 and 18,965–18,952 cm^{-1} , corresponding to ν_1 and ν_3 , characteristic of high spin octahedral Co(II) complexes [21]. However, ν_2 band is not observed because of its proximity to strong ν_3 transition. Magnetic measurements for the Co(II) complexes have magnetic moment values of 4.51–4.74 which agree with octahedral range [22] supporting the electronic spectral results.

The greenish Ni(II) complex exhibited three bands at 10415, 15645 and 26342 cm^{-1} which are attributed to the ${}^3\text{A}_{2g} \longrightarrow {}^3\text{T}_{2g}(\nu_1)$; ${}^3\text{A}_{2g} \longrightarrow {}^3\text{T}_{1g}(\text{F})$ (ν_2) and ${}^3\text{A}_{2g} \longrightarrow {}^3\text{T}_{1g}(\text{P})$ (ν_3) transitions respectively, indicating octahedral geometry around Ni(II) ion [23]. Ni(II) complex showed the magnetic moment value of 3.28 which is within the range of 2.7–3.3 BM suggesting [24] consistency with their octahedral environment.

The electronic spectra of Cu(II) complexes display two prominent bands. A low intensity broad band at 16,893 cm^{-1} is assignable to ${}^2\text{E}_g \longrightarrow {}^2\text{T}_{2g}$ transition and a high intensity band at 25,542 cm^{-1} is due to ligand \longrightarrow metal charge transfer. The electronic spectra suggest distorted octahedral geometry around Cu(II) ion [25]. The Cu(II) complexes showed magnetic moment values lie in the range 1.76–1.78 BM is consistent with octahedral geometry [26].

Electronic spectra of the Mn(II) complex display weak absorption bands at 18615 (ν_1), 23684 (ν_2), 27769 (ν_3) and 38890 cm^{-1} (ν_4) characteristic of octahedral geometry corresponding to ${}^6\text{A}_{1g} \longrightarrow {}^4\text{T}_{1g}({}^4\text{G})$, ${}^6\text{A}_{1g} \longrightarrow {}^4\text{E}_g({}^4\text{D})$, ${}^6\text{A}_{1g} \longrightarrow {}^4\text{T}_{1g}({}^4\text{P})$, ${}^6\text{A}_{1g} \longrightarrow {}^4\text{E}_g(\text{G})$ transitions, respectively. The complex shows magnetic moment in the range 5.42 – 5.48 B.M [27].

FAB-mass Spectral Studies of Schiff base and Its Co(II) complex(1)

The FAB mass spectrum of Schiff base showed molecular ion peak at m/z 308 equivalent to its molecular weight. The fragments in the spectrum leading to the formation of the species $[\text{C}_{17}\text{H}_{16}\text{N}_4\text{O}]^+$. The FAB mass spectrum of Co(II) complex (1) showed a molecular ion peaks $[\text{M}]^+$ at m/z 744, $746[\text{M}+2]^+$ and $748[\text{M}+4]^+$ which are equivalent to its molecular weight of the Co(II) complex (1). Some other peaks appeared at m/z 478 and 564 corresponds to the $[\text{Co}(\text{C}_{16}\text{H}_{22}\text{N}_6\text{O})\text{Cl}]^+$ and $[\text{Co}(\text{C}_{26}\text{H}_{12}\text{N}_6\text{O})\text{Cl}]^+$ species which are resulted from the lost of $\text{C}_8\text{H}_5\text{N}_2\text{OCl}$ and $\text{C}_{18}\text{H}_{22}\text{N}_2$ fragments from the parent compound. The two peaks appeared at m/z 674 and 709 are due to loss of two chlorine atoms and one chlorine atom respectively. All these fragmentation patterns are well observed in the FAB mass spectrum.

ESR Studies of Cu(II) Complex(3)

The ESR spectrum of Cu(II) complex(3) at RT shows one intense absorption band in the high field region is due to tumbling of the molecules. However, at LNT four well-resolved peaks in low field region are observed. The g_{\parallel} and g_{\perp} values have been found to be 2.1009 and 2.011, respectively. The trend $g_{\parallel} > g_{\perp} > 2.0023$ indicates that the unpaired electron is localized in the $d_{x^2-y^2}$ orbital of Cu(II) and is characteristic for axial symmetry [28]. Thus, the ESR spectral data confirm that the Cu(II) complex possess distorted octahedral geometry.

Electrochemical Studies

A cyclic voltammogram of Cu(II) complex(3) (Fig.2.) displays a reduction peak at $E_{pc} = -1.4V$ with an associated oxidation peak at $E_{pa} = -0.69V$ at a scan rate of 50mV/s. The peak separation of this couple (ΔE_p) is 0.71V and increases with scan rate. The most significant feature of the Cu(II) complex is the Cu(II)/Cu(I) couple which is a quasi-reversible one electron oxidation. The ratio of cathodic to anodic peak height was less than one; however, the peak current increases with increase of the square root of the scan rate, establishing diffusion controlled electrode process [30]. The separation in peak potentials increases at higher scan rates consistent with quasi-reversibility of the Cu(II)/Cu(I) couple.

Thermogravimetric Analyses

The thermogravimetric analysis were performed for some of the complexes to assist in predicting the molecular structures, the weight losses were measured from the ambient temperature up to 800 °C using a heating rate of 10 °C / min. These complexes decompose gradually with the formation of respective metal oxide above 710°C. All the metal complexes showed only a single decomposition curve between 340 °C and 360 °C corresponding to the loss of organic moiety. Above 710 °C all metal (II) complexes were decomposed leading to the formation of their respective metal oxides.

Pharmacological Results

In-vitro Antimicrobial assay

The antimicrobial results are systematized in Table 3. From the antibacterial studies it is inferred that, the Schiff base was found to be potentially inactive compared to its metal complexes. Some of the complexes showed high antibacterial activity against *S. aureus* and *Klebsilla*. In case of antifungal activity, the Schiff base and their complexes were found to be less active compared to the standard drug. It is evident from the results that, the biological activity of some of the metal complexes is higher than the ligands. This enhancement in the activity of the metal complexes can be explained on the basis of chelation theory [29-30]. It is, however, known that the chelating tends to make the Schiff base act as more powerful and potent bacteriostatic agents, thus inhibiting the growth of bacteria and fungi more than the parent Schiff base.

In-Vitro Antituberculosis Activity

The Antituberculosis activity results are systematized in Table 5. From the Antituberculosis activity studies it is inferred that, on complexation enhances the Antitubercular activity. The results showed that the compounds Mn(II) and Cu(II) inhibited the growth of *M. tuberculosis* at concentration 12.5 mg/ml. The Co(II) and Ni(II) complexes exhibited moderate activity when compared to standard drug streptomycin against *M. tuberculosis* which showed minimum inhibitory concentration at 50 mg/ml. Schiff base showed activity at higher concentration when compared to standard drug Streptomycin against the microorganism tested. Under these conditions standard drug streptomycin was sensitive at concentration 7 mg/ml and control DMF did not show any antituberculosis activity.

Anthelmintic Activity (in vitro)

The anthelmintic activity was tested on earthworms (*Pheretima Posthuma*). Amongst the complexes Cu(II), Co(II), Ni(II) and Mn(II) the Cu(II) and Co(II) complexes showed more activity than the standard and the remaining complexes are less active. Based on the above

results, it may be stated that the activity of the Schiff base is enhanced on complexation with Cu(II) and Co(II) ions Table 4.

In vitro Insecticidal Activity

The insecticidal activity results are systematized in Table 5. In vitro Insecticidal activity was tested on Adult cockroaches (*P. americana*). Amongst the complexes Cu(II) and Mn(II) showed more activity than the standard drug Cypermethrin and rest of the compounds are less active compared to the standard drug. Based on the above results, it may be stated that the activity of the Schiff base is enhanced on complexation with Cu (II) and Mn(II) ions.

Electrophoretic Analysis

Co(II), Ni(II) and Cu(II) complexes were studied for their DNA cleavage activity by Agarose gel electrophoresis method (Figure 3). DNA cleavage reactions generally proceed via two major pathways (1) Oxidative cleavage of the sugar and / or nucleobase moiety and (2) hydrolytic pathway involving the phosphate group. Iron and copper complexes are known to be useful for oxidative cleavage of DNA involving nucleobase oxidation and / or degradation of sugar by abstraction of deoxyribose hydrogen atoms while complexes containing strong Lewis acids like copper (II) and Zinc (II) are suitable for hydrolytic cleavage of DNA. Sigman *et al* have reported *bis*(phen)copper (I) complex as first “copper based chemical nuclease” that cleaves the DNA in presence of H₂O₂ and thiol. Similarly, the anticancer antibiotic bleomycins containing iron cleave DNA in an oxidative manner.

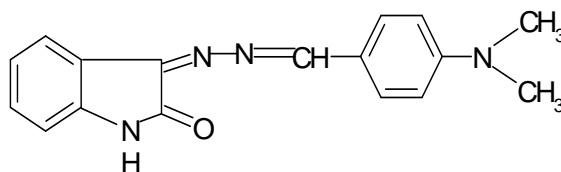


Figure 1. Synthesized Schiff base.

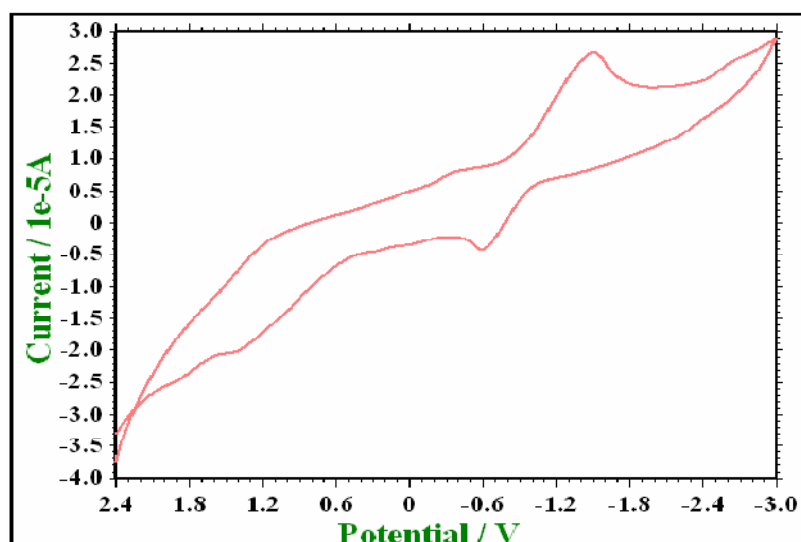


Figure 2. Cyclic voltammogram of Cu(II) complex(3).

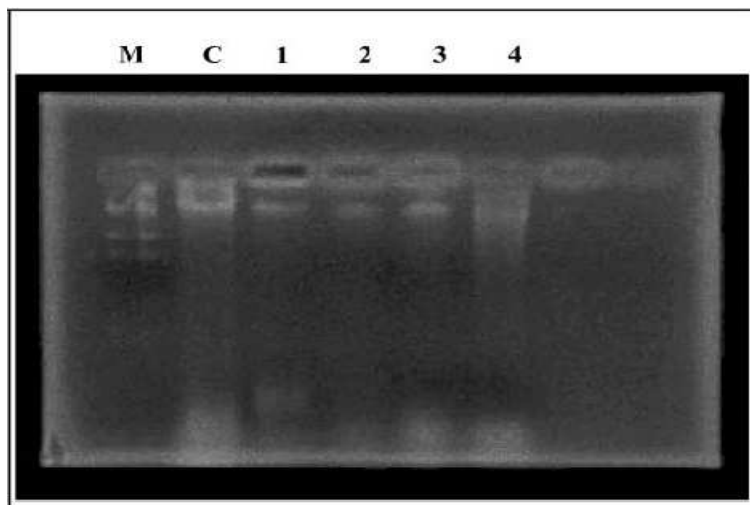


Figure 3. M: Standard Molecular weight Marker; C-*E. coli*- Control DNA of *E. coli*; Lane 1- 4: *E.coli* DNA treated with Co(II), Cu(II) ,Ni(II) complexes.

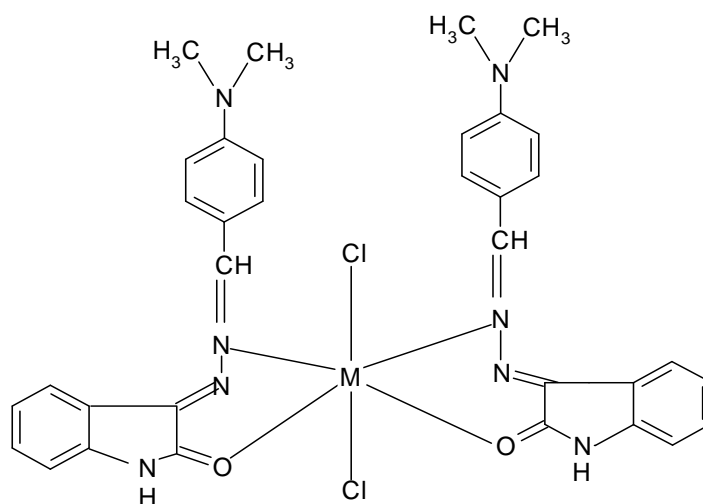


Figure 4. Structure for metal complexes.
M = Co, Ni, Cu & Mn.

The gel after the electrophoresis clearly revealed that, the gel shows that all compounds have the cleavage activity. Complexes 1, 2 and 3 have acted on DNA as there was molecular weight difference between the control and the treated DNA samples. The difference was observed in the bands (Lane 1, 2 and 3) compared to the control DNA of *E. coli*. Whereas compound 4 shown such difference along with a streak, indicating unspecific cleavage too. This shows that, the control DNA alone does not show any apparent cleavage whereas Cu(II) complexes shown. However, the nature of reactive intermediates involved in the DNA cleavage by the complexes has not been clear. The results indicated the important role of metal in these isolated DNA cleavage reactions. As the compound was observed to cleave the DNA, it can be concluded that, the compounds inhibit the growth of the pathogenic organism by cleaving the genome.

Table 1. Elemental analyses of Schiff base and its metal complexes along with molar conductance and magnetic moment data

S.No.	Empirical Formula	Color/ yield	M%		C%		H%		N%		Molar conductance Ohm ⁻¹ cm ² mole ⁻¹	μ_{eff} (BM)
			Obsd.	Cald.	Obsd.	Cald.	Obsd.	Cald.	Obsd.	Cald.		
1	C ₁₇ H ₁₆ N ₄ O	Colorless solid 75%	--	--	69.45	69.86	5.46	5.48	19.11	19.17	--	--
2	Co[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	Brown / 68%	8.19	8.25	57.04	57.14	4.37	4.48	15.55	15.68	19.20	4.75
3	Ni[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	Yellowish Green /70%	8.20	8.22	57.06	57.17	4.42	4.48	15.56	15.69	22.26	3.27
4	Cu[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	Dark Green / 67%	8.87	8.87	56.66	56.78	4.43	4.43	15.55	15.58	23.16	1.75
5	Mn[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	Yellow/ 65%	7.70	7.75	57.25	57.46	4.49	4.50	15.67	15.77	23.65	5.05

Table 2. The important infrared frequencies (in cm-1) of Schiff base and its metal complexes.

Compound	Indol ring $\nu(\text{NH})$	Lactonyl $\nu(\text{C=O})$	$\nu(\text{HC=N})$	$\nu(\text{C=N})$	$\nu(\text{M-N})$	$\nu(\text{M-O})$	$\nu(\text{M-Cl})$
C ₁₇ H ₁₆ N ₄ O	3140	1750	1621	1598	--	--	--
Co[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	3142	1712	1603	1590	450	520	340
Ni[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	3139	1706	1615	1592	455	517	335
Cu[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	3132	1698	1610	1591	460	525	342
Mn[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	3130	1710	1615	1592	470	512	350

Table 3. Antimicrobial and Antituberculosis activities of synthesized compounds.

Compound	Conc. mg/mL	*Growth Inhibition against Bacteria in mm				*Growth Inhibition against Fungi in mm		Antituberculosis activity in MIC (mg / mL)
		<i>E. coli</i>	<i>P. auregenosa</i>	<i>Klebsilla</i>	<i>S.aureus</i>	<i>P.chrysogenum</i>	<i>A.Niger</i>	
C ₁₇ H ₁₆ N ₄ O	200	7	6	9	10	6	10	100
	500	9	7	9	11	12	11	
Co[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	200	11	9	7	10	9	9	50
	500	13	12	9	12	11	13	
Ni[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	200	7	9	11	7	6	8	50
	500	8	9	12	11	16	11	
Cu[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	200	11	14	15	11	12	10	12.0
	500	16	19	18	16	17	16	
Mn[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	200	7	10	8	13	14	12	12.0
	500	18	19	15	21	18	16	
Streptomycin	500	22	23	27	28	--	--	7
Nystatin	500	--	--	--	--	22	20	--
Control (DMF)	--	--	--	--	--	--	--	--

*Less than 13 mm - inactive; 13–17mm - moderately active; above 17mm - highly active.

Table 4. Anthelmintic activity of compounds (in minutes).

S. No.	Compounds	Concentration, %			
		2%		4%	
		Paralytic time	Lethal time	Paralytic time	Lethal time
1	C ₁₇ H ₁₆ N ₄ O	9	17	10	18
2	Co[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	5	11	6	12
3	Ni[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	8	15	10	18
4	Cu[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	7	12	5	13
5	Mn[C ₁₇ H ₁₆ N ₄ O] ₂ .2Cl	9	15	10	20
	Std. drug	6	13	6	14

Table 5. Insecticidal activity of compounds (in minutes).

S.No.	Compounds	K.D. Value, min
1	C ₁₇ H ₁₆ N ₄ O	11
2	Co[C ₁₇ H ₁₆ N ₄ O] ₂ . 2Cl	9
3	Ni[C ₁₇ H ₁₆ N ₄ O] ₂ . 2Cl	10
4	Cu[C ₁₇ H ₁₆ N ₄ O] ₂ . 2Cl	8
5	Mn[C ₁₇ H ₁₆ N ₄ O] ₂ . 2Cl	7
6	Std.drug Cypermethrin	8

CONCLUSION

The synthesized Schiff base act as bidentate ligand through the coordination of azomethine nitrogen and carbonyl oxygen atom to the metal ion. The bonding of ligand to metal ion was confirmed by the analytical, IR, electronic, magnetic, EPR, FAB-mass, thermal and electrochemical studies. In biological results it confirms that, the Schiff base is biologically active and their metal (II) complexes have shown more promising activities than the Schiff base. All the newly synthesized compounds were tested for their antituberculosis activity against *M. tuberculosis*. Compounds Cu(II) and Mn(II) showed good activity compared with standard drug streptomycin. The interaction of Co(II), Ni(II), Cu(II) and Mn(II) complexes with DNA was investigated by gel electrophoresis technique. From the observation, it was found that all metal(II) complexes cleave DNA more efficiently. All these observations put together lead us to propose the structure shown in figure 4 in which, the complex having the stoichiometry of the type [ML₂. 2Cl] (M=Co (II), Ni(II), Cu(II) and Mn(II))

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