Synthesis, Characterization and Biological Screening of Ni(II), Co(II) and Cu(II) Complexes with Schiff Base Ligand: *N,N′*-Bis[1-(4-chlorophenyl)ethylidene]ethane-1,2-diamine

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Summary: The Schiff base ligand (SBL), *N,N′*-Bis[1-(4-chlorophenyl)ethylidene]ethane-1,2-diamine **(L)**, was synthesized by condensation of 4-chloroacetophenone with ethylenediamine which was then employed for the synthesis of complexes **(1-3)** with transition metal(II) ions. The characterization of these compounds was performed by CHN analyses, spectroscopic (^{1}H-NMR, ^{13}C-NMR, IR and MS) and molar conductance data. The structure of **L** was further confirmed by using XRD. On the basis of electronic spectra and magnetic moment data, an octahedral geometry was proposed for all synthesized complexes **(1-3)**. The conductivity data was used for the confirmation of non-electrolytic nature of these complexes. The Schiff base ligand had a lower antibacterial activity and lethality to shrimp larvae than its complexes **(1-3)**.

Keywords*:* Schiff base ligands; Transition metals; X-ray crystallography; Antibacterial activity; Brine shrimp assay

Introduction

The outstanding ligands employed in coordination chemistry involve the use of Schiff base ligands [1], which can be smoothly synthesized by the condensation of aldehydes and ketones with amines. Moreover, these compounds are employed in metal complexes [2]. Enhancement in biological activity and decrease in cytotoxic effects on the host are achieved by incorporating metal ions into the Schiff base ligands [3]. The reactions of Schiff bases have obtained prime importance in organic chemistry because these reactions are utilized as a suitable route for making carbon-nitrogen bonds. The significance of such bonds can be considered by its use in biological systems for transamination and elucidation of racemization mechanisms [4]. Besides these facts, this bond is considered responsible for various biological activities, such as antimicrobial, anticonvulsant, anti-inflammatory, antitumor [5], herbicidal [6], antituberculosis and anti-HIV activities [7].

The above mentioned facts prompted the present study on the synthesis and characterization of a Schiff base ligand **(L)**, *N,N′*-bis[1-(4-chlorophenyl)ethylidene]ethane-1,2-diamine, obtained by refluxing of ethylenediamine with 4-chloroacetophenone and its subsequent complexation with nickel(II), cobalt(II) and copper(II) metals. The biological activities (*i.e.*, antibacterial and cytotoxicity) of the Schiff base ligand **(L)** and its synthesized complexes **(1-3)** were investigated and an effect of complexation on the activity of the ligand **(L)** was observed.

Experimental

Materials and methods

The chemicals employed for the synthesis were purchased from Merck and used without any further purification. The salts of metals employed for the synthesis were hydrated salts of the metal(II) acetates or chlorides [$Ni(OAc)_2.4H_2O$, $CoCl_2.6H_2O$, $CuCl_2.2H_2O$].

Physical measurements

Pre-coated silica gel G-25-UV$_{254}$ plates were used for conducting TLC of the synthesized compounds with detection made under a UV lamp at 254 and 366 nm. A Mettler Toledo electric balance was used for the purpose of weighing. Thermo Nicolet Avatar 320 FTIR spectrometer was used to take IR spectra of the compounds using KBr pellets. Gallenkamp apparatus was employed for determination of melting points, which are uncorrected. Perkin Elmer 2400 Series II elemental analyzer was used for elemental analyses and a Jenway 4010 was used for measuring the molar conductance of the compounds at room temperature

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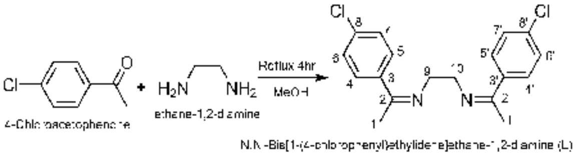
in DMSO solution (1×10^{-3} M). A Specord 200 UV-Vis spectrophotometer was used for recording the UV-Vis spectra. Moreover, a Guy-type magnetic balance (Hertz SG8SHJ) was employed for the measurement of the magnetic moment. A JEOL SX102/DA-6000 mass spectrometer was used for taking the mass spectra of these compounds by employing glycerol as matrix and *m/z* (%) is reported for the ions. The 1H-NMR spectra were recorded on a Bruker AMX-400 spectrometer in DMSO-d_6. The chemical shifts (δ) are given in ppm, relative to tetramethylsilane as an internal standard, and the scalar coupling constants (J) are reported in Hertz. Crystallographic measurements were carried out on Bruker SMART APEX II diffractometer equipped with CCD detector and Mo X-ray source.

Synthesis

Procedure of synthesis of ligand (L)

4-Chloroacetophenone (0.2 mol in 30 mL MeOH) and ethylenediamine (0.1 mol in 30 mL MeOH) were refluxed together at 90°C on water bath for 4 h. After refluxing, solvent was removed on a rotary evaporator down to one third of its initial volume, then acetone was added and the mixture was placed in an ice bath. Off white crystals of the Schiff base ligand **(L)** were obtained after keeping overnight at room temperature. Cold methanol (methanol at ambient temperature) was employed for filtering and washing the crystals, which were dried and recrystallized from methanol. Crystals of the Schiff base ligand thus obtained were dried under reduced pressure over anhydrous $CaCl_2$ for 12 h. A thin layer chromatographic method was used for checking the purity of the ligand (Scheme-1).



Scheme-1: Synthesis of Schiff base ligand (**L**).

General procedure for the synthesis of metal complexes **(1-3)**

The methanolic solutions of Ni, Co and Cu metal salts [$Ni(OAc)_2.4H_2O$, $CoCl_2.6H_2O$, $CuCl_2.2H_2O$] were added in 1:2 (M:L) molar ratio to a hot stirring methanolic solution of ligand **L** under reflux for 45 min. In order to get precipitate of complexes (**2** and **3**) of these metals except Ni(II), a methanolic solution of NaOH (1M) was added dropwise. In case of the complex of Ni(II) (**1**), reaction mixture was refluxed for further 30 min and solvent was evaporated to half of its initial volume whereby a purple material was obtained. Complexes **2** and **3** were washed with cold methanol after filtration while diethyl ether was used for washing the Ni(II) complex (**1**). All the complexes were dried under vacuum over anhydrous $CaCl_2$.

Biological studies

Antibacterial activity

The antibacterial activity of Schiff base ligand and its complexes was assessed using three strains of bacteria, *i.e.*, *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*. Agar as nutrient medium (10 mL) was poured into sterilized petri dishes and allowed to solidify. Spore suspensions of bacteria were used for the inoculation of plates. A sterilized cork borer was used for making wells (3 mm in diameter) in the center of plates. Solutions of the ligand (L) and complexes **1**–**3** (0.5 mL) were poured into these wells. The plates were incubated at 37°C for 24 h, after which the zone of inhibition produced in each plate around the respective wells was measured. Impenium was used as standard for comparison.

Brine shrimp assay

The artificial sea water was prepared by dissolving 3.8 g sea salt in double distilled and filtered water. The shrimp eggs (*i.e.* 1 mg *Artermia salina*) were added to the small tank of sea water. Partitions of the tank having these eggs were covered and darkened with aluminum foil. Illumination of perforation in the dam was employed to attract the shrimp larvae. Hatching and maturity of the shrimps was performed by allowing it to stand for 24 h at 25°C. The mature shrimp larvae were ready after a lapse of two days. The test compounds were dissolved in DMSO to prepare samples of different concentrations (*i.e.* 50, 100 and 150 mg mL^{-1}). As a result, 18 vials were prepared by making triplicate of each sample. The addition of 12 shrimps and 5 mL of sea water was done to each sample vial and then it was allowed to stay for 24 h under illumination. The data for shrimp lethality was analyzed by employing Finney computer programme and LD_{50} values were determined by counting the number of surviving shrimps [8].

Results and discussion

The ligand, *N,N'*-bis[1-(4-chlorophenyl) ethylidene]ethane-1,2-diamine **(L),** was obtained in the form of crystalline solid with off white colour. It was soluble in the aqueous medium and most of the common organic solvents apart from diethyl ether. Synthesized metal complexes **(1-3)** of the Schiff base ligand **(L)** were obtained in solid form and their decomposition temperatures reflect the fact that they are stable compounds. However, solubility behavior of these complexes was different from that of Schiff base ligand as these are insoluble in water and most common organic solvents, except DMF as well as DMSO.

*Physical, analytical and spectral data for L and the complexes **1–3***

N,N'-Bis[1-(4-chlorophenyl)ethylidene]ethane-1,2-diamine **(L):** Off white crystals; Yield: 75 %; m.p. 168 °C; Anal. Calcd. for $C_{18}H_{18}Cl_2N_2$: C, 64.91; H, 5.58; N, 8.52 %. Found: C, 64.87; H, 5.44; N, 8.41 %. IR (KBr, cm^{-1}): 2850 (C–H), 1625 (C=N), 1473, 1082, 720 (benzene ring), 827 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6, δ / ppm): 7.81 (4H, *d, J* = 8.7 Hz, H-4, H-5, H-4', H-5'), 7.43 (4H, d, *J* = 8.7 Hz, H-6, H-7, H-6', H-7'), 3.79 (4H, s, H-9, H-10), 2.22 (6H, s, H-1, H-1'); ^{13}C-NMR (100 MHz, DMSO-d_6, δ / ppm): 163.7 (C-2, C-2'), 139.2 (C-3, C-3'), 134.1 (C-8, C-8'), 128.1 (C-6, C-7, C-6', C-7'), 128.0 (C-4, C-5, C-4', C-5'), 52.7 (C-9, C-10), 15.1 (C-1, C-1'); MS (*m/z)*: 334.25 $[M+1]^+$.

[$NiL_2(OAc)_2$] **(1).** Purple solid; Yield: 71 %; m.p. 228 °C (decomp.); Anal. Calcd. for $C_{40}H_{42}Cl_4N_4O_4Ni$: C, 57.01; H, 5.10; N, 6.71; Ni, 6.84 %. Found: C, 56.97; H, 5.02; N, 6.64; Ni, 6.96 %. IR (KBr, cm^{-1}): 2920 (C–H), 1681 $(C=O)_{as}$, 1556 (C=N), 1400, 1099, 730 (benzene ring), 1281 $(C–O)_s$, 628 (C–Cl), 409 (Ni–N); Molar conductance (DMSO) λm (Ohm^{-1} cm^2 mol^{-1}): 4.8; MS (*m/z*): 844.29 $[M+1]^+$; Magnetic moment, $\boldsymbol{\mu}_{\text{eff}}$: 3.12 $\boldsymbol{\mu}_{\text{B}}$.

[CoL_2Cl_2] **(2).** Fawn solid; Yield: 60%; m.p. 230°C (decomp.); Anal. Calcd. for $C_{36}H_{36}Cl_6N_4Co$: C, 54.32; H, 4.61; N, 7.11; Co, 7.42 %. Found: C, 54.30; H, 4.56; N, 7.04; Co, 7.40 %. IR (KBr, cm^{-1}): 3087 (C–H), 1567 (C=N), 1462, 1054, 780 (benzene ring), 568 (C–Cl), 429 (Co–N), 264 (Co–Cl); Molar conductance (DMSO) λm (Ohm^{-1} cm^2 mol^{-1}): 3.5; MS (*m/z*): 797.03 $[M+1]^+$; Magnetic moment, $\boldsymbol{\mu}$eff: 4.95 μ_{B}.

[CuL_2Cl_2] **(3).** Deep blue stone solid; Yield: 65 %; m.p. 263 °C (decomp.); Anal. Calcd. for $C_{36}H_{36}Cl_6N_4Cu$: C, 54.02; H, 4.60; N, 6.91; Cu, 7.94 %. Found: C, 53.98; H, 4.53; N, 6.99; Cu, 7.93 %. IR (KBr, cm^{-1}): 2943 (C–H), 1569 (C=N), 1454, 1031, 743 (benzene ring), 679 (C–Cl), 469 (Cu–N), 289 (Cu–Cl); Molar conductance (DMSO) λm (Ohm^{-1} cm^2 mol^{-1}): 5.2; MS (*m/z*): 801.04 $[M+1]^+$; Magnetic moment, $\boldsymbol{\mu}_{\text{eff}}$: 1.81 μ_{B}.

FAB mass spectra

The FAB (positive) mass spectra were used to analyze the stoichiometric composition of the free ligand (**L**) and complexes (**1-3**). The molecular ion peak $[M+1]^+$ at m/z 334.25, indicated free ligand and confirmed the theoretical molecular weight *i.e.* 333. The stoichiometry of metal to ligand in the ratio 1:2 was supported by the observed molecular ion peaks in the spectra of complexes. The data of mass spectra was found to be in complete agreement to the data obtained *via* elemental analysis.

Infrared spectra

The binding approach of ligand **(L)** to metal ions in the complexes **(1-3)** was studied by comparison of IR spectra of free Schiff base ligand **(L)** and synthesized metal complexes. The disappearance of characteristic bands of carbonyl group, ν(C=O), for free 4-chloroacetophenone and the peaks for N–H stretching ν($-NH_2$) of free ethylenediamine suggested that reaction went to completion and that no unreacted ketone or amine remained [9]. Moreover, the azomethine band of the free ligand at 1625 cm^{-1} underwent a shift towards the lower frequency region at 1556-1569 cm^{-1} due to complexation of the ligand with the metal ion. This observation further indicated that nitrogen atom of azomethine group was involved in coordination of ligand to metal atom and it can be described by fact that empty d-orbitals of the metal atom accept the electron pair available on nitrogen atom of azomethine group [10]. A medium intensity band at 409-491 cm^{-1} assignable to ν(M–N) [11] in the spectra of all the complexes further strengthened this fact.

For aromatic rings, the out of plane bending ν(C–H) was observed at 720-780 cm^{-1} and the absorption bands appeared in the regions of 1400-1480 cm^{-1} and 1031-1099 cm^{-1} ν(C=C) were assigned to stretching vibrations of aromatic ring system. The acetate complex **(1)** showed absorption band at 1681 cm^{-1}, which was assigned to the $\nu(C=O)_{as}$ (*i.e.,* asymmetric stretching vibrations of carbonyl of acetate ion) and the band observed at 1281 cm^{-1} in acetate complexes was assigned to symmetric stretching vibration $\nu(C–O)_s$ of acetate ion, which indicated that the coordination of acetate group in a unidentate manner to central metal ion (Fig. 1).

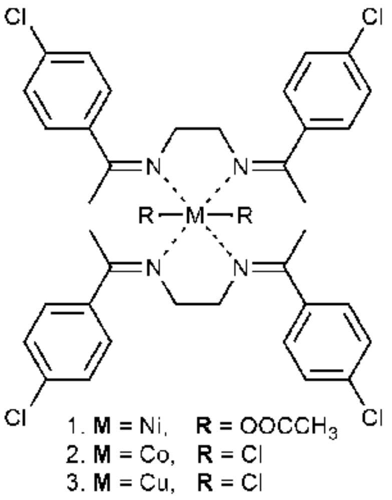


Fig. 1: Proposed structures of complexes (**1-3**).

The bands for ν(M–Cl) metal-halogen bond were observed at 264 and at 289 cm^{-1} for complexes **(2-3)** (Fig. 1) [12]. The addition of Ag^{+} and Pb^{2+} solutions did not result in precipitation of chloride ions, which confirmed that the chlorides were inside the coordination sphere. The stretching vibration ν(C–H) of the methyl group of the ligand **(L)** and complexes **(1-3)** were assigned to the bands in the region of 2850-3087 cm^{-1}.

Crystallographic studies

Crystal data: **(L)**, $C_{18}H_{18}Cl_2N_2$, $M_r = 33.24$ g mol^{-1}, white rod like, 0.31, 0.11, 0.08 mm^3, monoclinic, space group $P2_1/c$, $a = 4.3427(2)$, $b = 10.1489(5)$, $c = 18.9213(8)$ Å, $\beta = 91.458(1)^{o}$, $V = 833.67(5)$, $Z = 2$, $Dc = 1.328$ g cm^{-3}, $F(000) = 348$, MoKα radition, $\lambda = 0.71073$ Å, $T = 296$ (2) K, 2θmax = 54.0°, 7719 reflection collected, 2071 unique ($R_{\text{int}} = 0.021$), Final GooF = 1.083, $R_1 = 0.042$, $wR_2 = 1.03$, R indices based on 1405 reflections with $I > 2\sigma(I)$ (refinement on $F2$) 101 parameters, Lp and absorption correction applied, $\mu = 0.39$ mm^{-1}.

Refinement: A Kappa Apex II diffractometer (Bruker) was used to collect the data under the mentioned conditioned. Cell refinement and data reduction was carried out using SAINT [13], while SHELXS-97 was used for structure solution and refinement [14]. Molecular graphics were refined by PLATON [15] and WinGX [16]. All the non-hydrogen atoms were refined with anisotropic displacement parameters. C–H hydrogen atoms (aromatic and aliphatic) were positioned geometrically and refined as riding atom over their parent carbon atoms.

The ligand was crystalized with a centro-symmetric space group to study the three dimensional behavior of the molecule. The crystal structure reveals that the molecule gained a crystallographic inversion symmetry position in the unit cell. The central backbone of molecule was oriented at a dihedral angle of 19.26 (12)° with respect to the aromatic ring (Fig. 2). A *trans* conformation was observed around the two C=N double bonds. The root mean square deviation values of the atoms C7, C8, N1 and C9 from the plane generated through the C1—C6 are 0.072 (3)°, -0.326 (4)°, 0.465 (3)° and 0.562 (4)° respectively. The molecule showed no type of hydrogen bonding or π-π interaction.

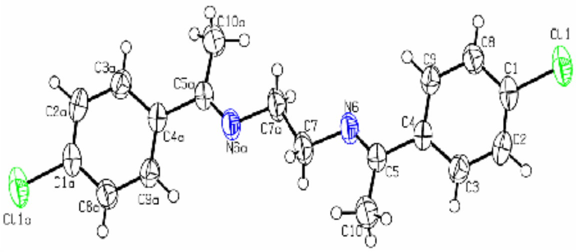


Fig. 2: Labelled diagram of (**L**) with thermal ellipsoids drawn at 50% probability.

Electronic spectra and magnetic moments

The position, number of d-d transitions and charge transfer formed the basis for stereochemistry of metal ions in complexes **(1-3)** and the stereochemistry was assigned by observing electronic spectra. Octahedral geometry was suggested for all complexes [17] by observing electronic spectra and magnetic moment values (Table 1), which was further strengthened by the low value of molar conductance, indicating the complexes had a non-electrolytic nature.

Table-1: Electronic and magnetic moment data of complexes **(1-3)**; octahedral geometry.

Compound	Electronic spectra wavelength(cm^{-1})	Magnetic moment (B.M.)	Geometry
1	**10100, 15500, 22300**	**3.12**	**Octahedral**
2	**12350, 17200**	**4.95**	**Octahedral**
3	**11400, 18400**	**1.81**	**Octahedral**

Molar conductance

The molar conductivities of the complexes **(1-3)** were measured as 1×10^{-3} M solutions in DMSO at ambient temperature. The complexes **(1-3)** were found to have molar conductance values in the range of 3.1-5.2 ohm^{-1} cm^{2} mol^{-1}, which was also in agreement with the nature of complexes (i.e. non electrolytic) [18].

The tentative proposed structures of the complexes **(1-3)** are illustrated in Fig. 1, which can be easily analyzed in the light of analytical and spectral data.

Biological activities

Antibacterial activity

The agar plate technique was employed to study the antibacterial activity of Schiff base ligand **(L)** and its complexes **(1-3)**. The studies were conducted by using 1% solution of the compounds. It was found that free ligand was less active than the metal chelates **(1-3),** as shown by the results given in Table-2. The Tweedy chelation theory can explain these facts [19]. The polarity of the metal ion was reduced by the chelation of metal ion and the sharing of its electrons with a donor atom. The reduction of polarity causes blockage of the binding sites in the enzymes of pathogens and enhances penetration of the complexes into the lipid membrane. The phenomenon of protein synthesis is blocked by interference of complexes with the respiration processes of the cell and, as a result further growth of pathogens is restricted.

Table-2: Antibacterial activity of ligand **(L)** and complexes **(1-3)**.

Compounds	**Diameter of inhibition zone (mm)**		
	S. aureus	***E. coli***	***B. subtilis***
L	**2**	**1**	**2**
1	**4**	**3**	**20**
2	**6**	**5**	**9**
3	**3**	**5**	**5**
Impenium (standard)	**8**	**7**	**11**

Brine shrimp assay

The convenient way to evaluate new bioactive synthetic products is to monitor *in vivo* lethality to shrimp larvae because new bioactive compounds are often cytotoxic to shrimp larvae. Such a type of bioassay is quick and economical in general and further *in vivo* animal experiments on a larger scale are based on this intermediary test. The brine shrimp lethality test was used to determine the cytotoxicity of the Schiff base ligand and its complexes. Considerable activity against brine shrimp nauplii was observed for the Schiff base ligand and its complexes. The LD_{50} value (the amount that caused the death of 50 % of the shrimp larvae) of 0.053 μg mL^{-1} for complex **3** showing that the copper complex was more active than the others. The complexes **1** and **2** were found to also have good activity with LD_{50} values of 1.26 and 45.02 μg mL^{-1}, respectively. The LD_{50} value for the free Schiff base ligand was 61.77 μg mL^{-1}.

Conclusion

This work presents metal (II) complexes with a Schiff base ligand having two binding sites, obtained by the condensation reaction of ethylenediamine with 4-chloroacetophenone and their characterization with the aid of various analytical and spectroscopic techniques. The metal complexes **(1-3)** were found to have greater antibacterial and brine shrimp lethality than the free Schiff base ligand **(L)**.

Supplementary material

The cif file for ligand was submitted to the Cambridge Crystallographic Data Centre and obtained the CCDC No. 878412. The crystallographic supplementary material is available free of cost from same databank.

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