



Synthesis, characterization and antimicrobial studies of a pentadentate Schiff base ligand and its Copper (II) complexes

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ABSTRACT

A pentadentate Schiff base ligand (H_3L) was synthesised from 1,3-diaminopropan-2-ol and 2'-hydroxy-5'-methylacetophenone and a series of copper(II) complexes were synthesised using H_3L and copper(II) precursor complexes of substituted benzoic acids. The synthesized complexes have been characterized on the basis of elemental analysis, molar conductance and spectroscopic studies such as UV-Visible and FT-IR. All the synthesized complexes and the Schiff base ligand were screened for their *in vitro* antibacterial activities against *Proteus mirabilis*, *Enterococcus faecalis*, and *Escherichia coli* and antifungal activities against *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* by well diffusion method. In addition, the antioxidant activities were also done for all the complexes.

Keywords: Schiff base, transition metal complexes, spectroscopic studies, antibacterial, antifungal and antioxidant activities.

INTRODUCTION

Schiff bases derived from an amine and a carbonyl compound are an important class of ligands that coordinate to metal ions via azomethine nitrogen and have been studied extensively [1-3]. Because of the versatility of the steric and electronic properties, which can be fine-tuned by choosing the appropriate amine precursors and ring substituents, metal complexes of Schiff-bases derived from aromatic carbonyl compounds like salicylaldehyde have received a great deal of attention in connection with studies on asymmetric catalysis and metalloprotein modelling. Transition metal complexes with their flexible coordination environments, varied spectral and redox properties provide us greater scope of designing new metal based potent agents. The complexes of copper with Schiff bases have wide applications in food industry, dye industry, analytical chemistry, catalysis, fungicidal, agrochemical, anti-inflammatory activity, antioxidant activities and biological activities [4-15]. Series of binuclear copper(II) complexes have been investigated extensively in recent years since such complexes mimic the aspects of bimetallic bio sites in various proteins and enzymes [16-18] and they also have plentiful applications including antibacterial, antifungal, antiviral and antioxidant activities.

Hence an attempt was made to synthesize a pentadentate Schiff base ligand H_3L derived from 1, 3-diaminopropan-2-ol and 2'-hydroxy-5'-methylacetophenone and a series of copper (II) complexes of this ligand. All the synthesised complexes and the Schiff base ligand have been characterized on the basis of elemental analysis, molar conductance and spectroscopic studies such as UV-Visible, FT-IR and were screened for their *in vitro* antibacterial, antifungal and the antioxidant activities.

EXPERIMENTAL SECTION

Materials and physical measurements

All the reagents and chemicals were procured from commercial sources and were used without purification. 1,3-diaminopropan-2-ol and 2'-hydroxy-5'-methylacetophenone were purchased from Sigma Aldrich, and all the other chemicals were purchased from S.D. fine Chemicals, Mumbai. Molar conductance of the complexes were measured in DMF (10^{-3} M) solution using a Guna digital conductivity meter. ^1H NMR spectra of the Schiff base was recorded on a Jeol WM 500 FT MHz NMR instrument using MeOD as solvent and TMS as internal reference. FT-IR spectra of the ligand and the solid complexes were recorded using KBr pellet in the region of $4000\text{-}400\text{ cm}^{-1}$ on Shimadzu FT-IR spectrophotometer. Electronic absorption spectra of the ligand and the complexes in DMF were recorded in the region 200-800 nm using a Systronics double beam UV-Visible spectrophotometer-2202.

Synthesis of Schiff base ligand

Schiff base ligand (H_3L) was synthesized by the reaction between 1, 3- diaminopropan-2-ol (1 mmol) and 2'-hydroxy-5'-methylacetophenone (2 mmol) in 20mL of ethanol. The mixture was stirred for 3 hours at room temperature. The pale yellow colour ligand was obtained. It was filtered washed with ethanol and dried.

Synthesis of precursor complexes

The precursor complexes were synthesized from $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{HOOC}_6\text{H}_4\text{-p-X}$ (where X = H, Cl, OH, NH_2 , NO_2). NaOH (2 mmol) was added to 20mL of ethanolic solution of appropriate substituted benzoic acid (2 mmol) and stirred for 30 min at room temperature. Then 5 mL of an aqueous solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1mmol) was added to the mixture, and stirred for another 30 min at room temperature. The resultant product was filtered, washed with ethanol and dried.

Synthesis of copper(II) complexes

An ethanolic solution of the Schiff base ligand (1 mmol) was treated with aqueous NaOH (3 mmol). To this solution the copper (II) precursor (1 mmol) was added and stirred for 3 hours at room temperature. The green copper(II) complexes obtained were dried.

RESULTS AND DISCUSSION

The Schiff base ligand (Figure 1) was stable at room temperature and soluble in ethyl alcohol and other organic solvents. The ^1H -NMR spectrum of the ligand was recorded in CH_3OD at 500 MHz spectrometer. The δ values for the protons in ppm are given in the Table 1.

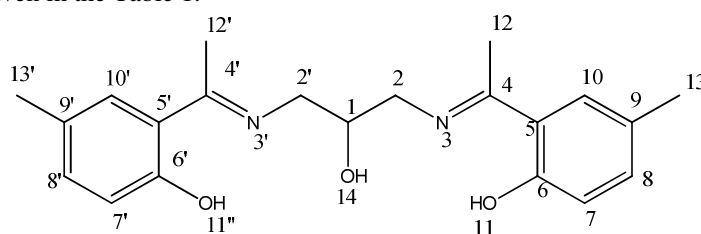


Figure 1. Structure of Schiff base ligand

The signals observed at δ 3.33 ppm and at δ 4.61 ppm corresponds to CH_3OD and solvent water respectively. Since spectrum was recorded in CH_3OD the OH protons got exchanged and hence were not observed in the spectrum.

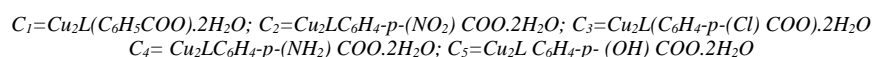
Table 1. NMR Spectral data of the ligand

Chemical Shift δ (ppm)	Multiplicity	Number of protons	Assignment
1.92	Singlet	6	12- & 12'-CH
2.26	Singlet	6	13- & 13'-CH
3.84-3.88	Multiplet	4	2- & 2'-CH
4.30-4.32	Multiplet	1	1-CH
6.72	Doublet	2	7- & 7'-CH
7.13	Doublet	2	8- & 8'-CH
7.45	Multiplet	2	10- & 10'-CH

All the synthesized copper(II) complexes were found to be freely soluble in DMSO, DMF and acetonitrile. The analytical data of the synthesized metal complexes are shown in Table 2.

Table 2. Analytical data of the metal complexes

Compound	Molecular Formula	Melting point (°C)	Elemental Analyses Found (Calculated)		
			C	H	N
H ₃ L	C ₂₁ H ₂₆ N ₂ O ₃	185	71.19 (71.10)	7.28 (7.34)	7.79 (7.9)
C1	C ₂₈ H ₃₂ Cu ₂ N ₂ O ₇	104	52.79 (52.86)	5.11 (5.03)	4.44 (4.4)
C2	C ₂₈ H ₃₁ Cu ₂ N ₃ O ₉	180	49.41 (49.36)	4.48 (4.55)	6.21 (6.17)
C3	C ₂₈ H ₃₁ Cu ₂ N ₂ O ₇ Cl	>200	50.19 (50.14)	4.54 (4.63)	4.11 (4.17)
C4	C ₂₈ H ₃₃ Cu ₂ N ₃ O ₇	199	51.58 (51.64)	5.15 (5.07)	6.51 (6.46)
C5	C ₂₈ H ₃₂ Cu ₂ N ₂ O ₈	86	51.61 (51.56)	4.89 (4.91)	4.28 (4.30)



The amount of copper in the complexes was estimated by complexometric titration with 0.01M EDTA solution using ammonia solution as the buffer solution and Fast Sulphone Black – F as the indicator. The percentage of copper in all the complexes agrees well with the calculated values that confirms the presence of two copper atoms in the complexes.

The molar conductivity measurement of the complexes showed a lower molar conductivity value of 11 - 17 $\Omega^{-1}cm^2mol^{-1}$ in DMF indicating the non-electrolytic nature of the complexes [19, 20]. The magnetic susceptibility values of the copper (II) complexes were measured at room temperature with Guoy balance and the effective magnetic moment values are given in the Table 3. The magnetic moment values lie below the spin only value of 1.73 BM and were found to be in the range of 1.43-1.56 BM. This gives an indication of the magnetic interaction between the two copper (II) ions.

Table 3. Molar conductance and magnetic moment of the Cu(II) complexes

Compound	Molar Conductance $\Omega^{-1}cm^2mol^{-1}$	Magnetic moment BM/Cu
C1	16	1.56
C2	14	1.43
C3	12	1.50
C4	11	1.48
C5	17	1.43

UV-Visible Spectroscopy

The band appeared in UV-Visible spectrum of the ligand at 269 nm is associated with $\pi \rightarrow \pi^*$ transition of benzene ring and it shifted to 279-282 nm in the copper (II) complexes. The band at 324 nm is due to $\pi \rightarrow \pi^*$ transition of azomethine group and it shifted to 334-358 nm in the copper (II) complexes and the band at 346 nm of the ligand due to the $n \rightarrow \pi^*$ transition corresponds to imine moiety. The broad peak appeared at 610 -644 nm corresponds to d-d transition of Cu (II) ion in the complexes. The UV-Visible spectral data are given in the Table 4.

Table 4. UV-Visible Spectral data

Compound	$\pi \rightarrow \pi^*(nm)$	$n \rightarrow \pi^*(nm)$	d-d (nm)
H ₃ L	269, 324	346	-
C1	279, 344	387	639
C2	282, 334	387	620
C3	279, 358	387	624
C4	282, 339	387	644
C5	282, 358	387	610

FT-IR Spectra

The FT-IR spectral data of the compounds are given in the Table 5. The FT-IR spectra of the complexes when compared with that of the free ligand indicates the formation of complexes. Certain vibrational bands of the free ligands and their complexes, useful for determining the mode of coordination of the ligands, are given in Table 5. In general, they show intense bands between 1586–1603 cm^{-1} that corresponding to the imine (C=N) stretching vibrations. Vibrational frequency of the C=N group of the ligand at 1614 cm^{-1} is lowered by 11–28 cm^{-1} in the metal complexes, indicating coordination through the imine nitrogen [21-23].

The FTIR spectrum of free ligand shows characteristic band at 3394 cm^{-1} , assignable to the OH group which disappears in the metal complexes, indicating de-protonation of the OH group upon binding with metal ions. The appearance of the broad band between $3414\text{--}3446\text{ cm}^{-1}$ suggests the presence of co-ordinated water in Cu(II) complexes. For the complex H_3L the band at 3381 cm^{-1} is assigned to the OH group in the benzene ring. The FTIR spectra of the complexes show new bands in the $436\text{--}511\text{ cm}^{-1}$ and $525\text{--}599\text{ cm}^{-1}$ region, corresponding to $\nu(\text{M-O})$ and $\nu(\text{M-N})$ vibrations, respectively. The azomethine stretching frequencies of the free ligands were found to be decreased in the complexes indicating the participation of the azomethine nitrogen coordinating with metal ion.

Table 5. FTIR absorption bands of the Schiff base metal complexes in cm^{-1}

Compound	$\nu(\text{O-H})$	C=N	N-H	C-O	Cu-O	Cu-N	$\nu(\text{COO})$		
							(ν as)	(ν s)	$\Delta\nu = [\nu\text{as} - \nu\text{s}]$
H_3L	3394	1614	-	1303	-	-	-	-	-
C1	3426	1602	-	1225	472	561	1553	1398	155
C2	3446	1586	-	1225	511	599	1537	1390	147
C3	3441	1602	-	1221	469	525	1543	1400	143
C4	3414	1603	3327	1223	436	530	1518	1391	127
C5	3381	1603	-	1252	499	548	1537	1389	148

The bands observed at $1518\text{--}1553\text{ cm}^{-1}$ and $1389\text{--}1400\text{ cm}^{-1}$, corresponds to $\nu\text{as}(\text{COO}^-)$ and $\nu\text{s}(\text{COO}^-)$, respectively. The value of $\Delta\nu$ ($\Delta\nu = \nu\text{as} - \nu\text{s}$) ranging from $127\text{--}155\text{ cm}^{-1}$ which is lower than that of the free carboxylate anion (190 cm^{-1}) confirmed a bidentate coordination nature of the carboxylate group present in the complexes [24,25].

Based on the physico-chemical and spectral studies the proposed structure of the complexes is given as in Figure 2.

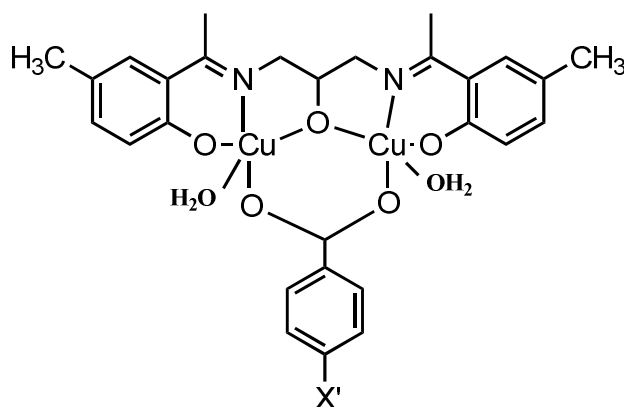


Figure 2. Proposed structure of Schiff base Cu(II) complexes
 $X = \text{H}, \text{NO}_2, \text{Cl}, \text{NH}_2, \text{OH}$

Antibacterial Assay

Metal ion plays a vital role in a number of different biological processes [26,27]. The interaction of these ions with binuclear Schiff base complexes is of great interest. The Schiff base ligand and the five metal complexes produced different inhibition zones against the tested bacterial species *Proteus mirabilis*, *Escherichia coli* and *Enterococcus faecalis*. (Table 6).

Table 6. Antibacterial Activity

MICROORGANISMS	Control	H_3L	C ₁	C ₂	C ₃	C ₄	C ₅	Ciprofloxacin (5mcg)
<i>Proteus mirabilis</i>	-	-	6	5	5	8	5	29
<i>Escherichia coli</i>	-	8	5	5	7	6	5	30
<i>Enterococcus faecalis</i>	-	10	6	6	8	7	5	30

For the bacteria *Proteus mirabilis* the complexation increases the antibacterial activity. The antibacterial activity of Schiff base metal complexes in DMF against bacterial species is shown in Figure 4.

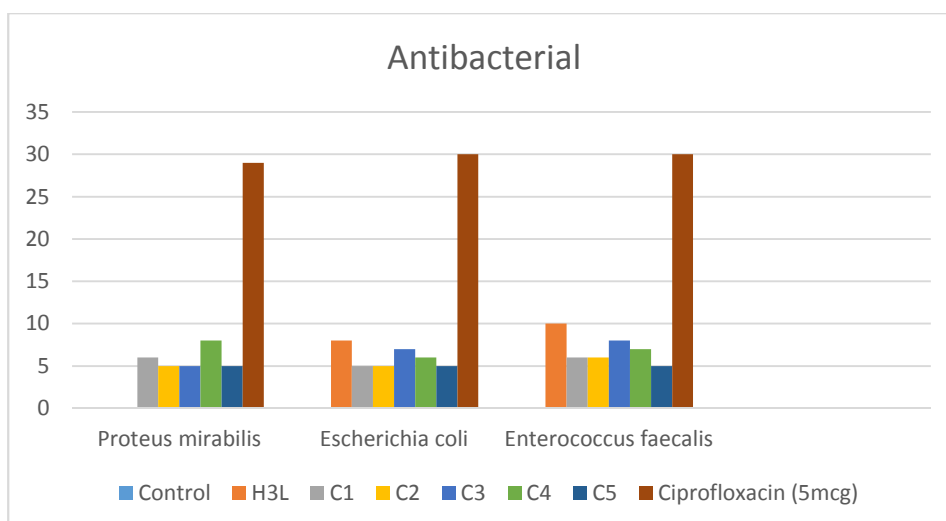


Figure 4. Antibacterial activity

Antifungal Activity

The Schiff base and their binuclear copper complexes were used for their antimicrobial studies by means of fungi, such as *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* (Table 7).

Table 7. Antifungal Activity

Microorganisms	Control	H ₃ L	C ₁	C ₂	C ₃	C ₄	C ₅	Amphotericin-B (50 mcg)
<i>Candida albicans</i>	-	-	5	6	13	6	6	13
<i>Aspergillus niger</i>	-	-	10	7	12	-	-	10
<i>Aspergillus flavus</i>	-	5	7	5	10	-	8	13

For the fungus *Candida albicans* and *Aspergillus niger* the complexes showed a good activity against the fungi whereas for the ligand there is no activity. The complex with chlorine atom substituted in the benzoic acid moiety showed very good activity against all the three fungi. Antifungal activity of ligand and its metal chelates were shown in Fig.5.

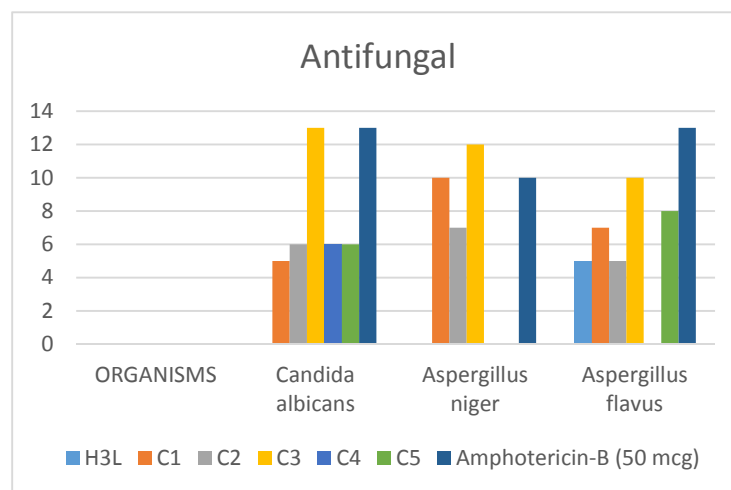


Figure 5. Antifungal activity

Antioxidant Studies

The scavenging of DPPH radicals is simple and convenient method which is extensively used to evaluate the antioxidant properties of the Schiff and its metal complexes [28-30]. The DPPH radicals are stable but in the presence of synthesized compounds capable the consuming the stable free radical the radical property is destroyed resulting in a colour change from purple to yellow. It was observed that the Schiff base and their complexes can indeed reduce the concentration of the initial DPPH radical in solution and this is taken as evidence of their antioxidant capabilities. The inhibitory effect on DPPH radical is shown in table 8.

$$\% \text{ inhibition} = \left[1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

The antioxidant activity of the amino and hydroxyl substituted complexes are comparatively good among all the other complexes.

Table 8

Compound	Percentage of inhibition
H ₃ L	46.70
C ₁	36.76
C ₂	44.85
C ₃	41.54
C ₄	61.76
C ₅	53.31

CONCLUSION

In the present study a pentadentate Schiff base ligand was synthesised and was characterised by NMR, UV-Visible and FT-IR Spectroscopic techniques. The formation of azomethine linkage was confirmed by FT-IR Spectroscopy. A series of binuclear copper(II) complexes have been synthesized and characterized by various physico-chemical and spectral analyses. The molar conductance of all the complexes suggested their nonelectrolytic nature. The coordinating mode of the donor atoms of the ligand was confirmed by FT-IR spectral details. In vitro antimicrobial and antioxidant studies revealed that the hydroxy and amino substituted complexes were found to exhibit good antioxidant property and the chlorine atom substituted complex showed a very good antifungal activity against the fungi under study.

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