

Synthesis, Characterization and Biological Studies of *Bis*-{ μ -2,2'-[ethane-1,3-diyl-*bis*(nitrilomethylidene)]diphenolato}dicopper(II) Using Triple Component Solvent System

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The *bis*-{ μ -2,2'-[ethane-1,3-diyl-*bis*(nitrilomethylidene)]diphenolato}dicopper(II) with the molecular formula $[\text{Cu}_2(\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_2)_2]$ shows the dimeric complex. Distorted square pyramidal geometry is formed in the community of CuII distinct by the two nitrogen atoms and two oxygen atoms of the [*N,N'*-*bis*-(salicylidene)-1,2-ethylene-diamine] (ligand) and one oxygen atom commencing both ligand performing as bridge between two ligand molecules, which are wrapped in parallel path among each other. In toluene the tilted complex showed crystalline structure but to acquire more apparent X-ray crystallographic data a combination of three solvent (toluene, ethanol, ethyl acetate) was used. The synthesized ligand was analyzed and characterized by mass spectrometry, Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy while the titled compound was characterized by X-ray crystallography and FTIR. The complex and ligand were also engaged for antifungal and antibacterial activities against fungal species *A. flavus*, *A. alternate* and *A. niger* and against bacterial species *Staphylococcus aureus*, *Escheria coli* and *Bacillus subtilis*. The results of these activities showed that the metal complex has extra antibacterial and antifungal actions than the precursor (ligand) against different culture media.

Key Words: Triple component solvent system, Schiff bases, Copper(II) complex. Salicylaldehyde, Ethylenediamine.

INTRODUCTION

The compounds which contains imine group (-CH=N) are identified as Schiff bases¹. Schiff bases can be synthesized by condensing compounds containing carbonyl and amine groups. These Schiff bases participate in variety of reactions to elucidate various types of mechanisms and also perform excellent role in different biological systems². Aldehydic Schiff bases of aromatic system have an effective conjugation character and form stable compounds than aliphatic one. Condensation reactions of ketones and carbonyl bearing compounds also form Schiff bases which also imparts significant responsibility to detect different groups *i.e.* amines and carbonyls during different reactions³. Other applications of these Schiff bases include analysis and characterization of ketones and aldehydes. Stable complexes are formed by transition metals with the Schiff bases⁴⁻⁹. Chelation of metal ions with Schiff bases ligands are used in the preparation of complexes. Different derivatives of Schiff bases acts as neutral ligands and form stable complexes with transition metals. For example a most stable metal complex is formed by Cu(II) with the Schiff base

formed by the condensation reaction of salicylaldehyde and ethylenediamine. These complexes and ligands are generally analyzed and characterized by mass spectrometry, FTIR, ¹H NMR, ¹³C NMR and X-ray crystallography and also engaged for antibacterial and antifungal activities by using different cultural strains.

EXPERIMENTAL

All solvents and reagents (chemicals) including ethylenediamine, copper acetate, ethanol, salicylaldehyde, acetic acid, toluene and ethyl acetate of AR grade were import from Sigma-Aldrich.

Synthesis of ligand: Condensation reaction of equimolar quantities of salicylaldehyde (24.4 g, 0.1 mol) and ethylenediamine (6 g, 0.1 mol) in 40 mL of ethanol was carried out for the synthesis of ligand. These reactants were continuously stirred and also engaged up to 8 h for reflux¹³.

Synthesis of complex: Metal complex was synthesized by reacting equimolar quantities of cupric acetate with ligand which was dissolved in ethanol. The whole material was continually stirred at room temperature and refluxed over night.

Crystallization of the metal complex was done by employing a combination of three solvents *i.e.* toluene, ethanol, ethyl acetate. Finally a product was obtained with fine crystals. Washing of product was done by mixing different solvents and then the product was dried.

Different spectroscopic techniques were used to get the spectroscopic data of compounds. Mass spectra of ligand was taken at 70 eV by electron ionization technique on Perkin-Elmer Clarus 680 GC-MS instrument in the R and D section of Zeta Chemical Co. Pvt. Ltd., Lahore, Pakistan. Fourier transform infrared spectra were taken on a Perkin Elmer FTIR and Thermo FTIR Nicolet IS 10. ^1H NMR and ^{13}C NMR were recorded *via* 60 MHz JNM-ECX60 FT-NMR system. Crystal data (X-ray crystallography) of titled complex was taken on the single crystal X-ray diffraction.

Antibacterial activity: To check antibacterial activity various strains were used. Strains used were *Staphylococcus aureus*, *Escheria coli* and *Bacillus subtilis*¹⁰. The metal complex and ligand were employed for antimicrobial activity by using Disc diffusion method CLSI, 2007. The main procedure used to check the antibacterial activity includes mixing of agar in water *i.e.* distilled water was taken in which nutrient agar was dissolved, which was uniformly dispersed. Then the material set aside in autoclave for sterilization for 20 min at 121 °C. The medium then transferred to Petri plates but before Petri plates it was treated with inoculums. Henceforth, parallel discs of filter papers containing 100 μL (10 %) of ligand and metal complex were employed on the medium. The medium in the Petri plates was left at 37 °C for 24 h for the growth of bacteria. Clear zones were formed by ligand and metal complex, which inhibited the bacterial growth. Inhibition zones were measured in millimeters by employing zone reader¹¹. The standard drug employed was rifampicin.

Antifungal activity: To check antifungal activity different strains were employed. *A. alternate*, *A. flavus* and *A. niger*¹⁰ were the used strains. Synthesis of growth medium was done and then engaged to Petri dishes after sterilization. Incubation for growth of fungus was carried at 28 °C for 48 h. Growth medium was employed to filter paper discs for fungus growth. Ligand and its metal complexes were applied up to 100 μL (10 %) on each disc and incubation started. Clear zones were formed by the ligand and metal complex, which showed antifungal activity¹¹. Fluconazol was the standard drug used for antifungal activity of complex and ligand¹².

RESULTS AND DISCUSSION

Crystallization in triple solvent system: Three independent solvents were checked for crystallization of the obtained complex. Even so, the dissolution of the titled complex in single solvent was not successfully done and hinder the proper crystal formation. Solubility of the titled complex by using 0.5 g in three separate solvents *i.e.* in ethyl acetate, toluene and ethanol was 1.2, 0.5 and 0.1 % correspondingly. On the basis of previously reported results^{4,13-15}, the solubility of the metal complex was increased successfully by employing a combination of three solvents and the results showed that solubility of the metal complex was up to 11 % for 0.5 g of the complex dissolved. This was due to the intermolecular bonding and bridging of the used solvents.

Characterization of ligand

Fourier transform infrared spectroscopic analysis:

FTIR was used to confirm the ligand's functional groups. The range used for the FTIR spectra was 4000-400 cm^{-1} as KBr pellets. On the basis of previously work this assignment was made¹⁶. The bending vibrations for imine group were observed at 1625-1608.9 cm^{-1} and for phenolic-OH group bending vibrations were observed at 1370 and 1198 cm^{-1} for ligand. Data shown in Tables 1 and 2.

TABLE-1
BENDING VIBRATIONAL IR FREQUENCIES
IN cm^{-1} FOR IMINE GROUP

Compound	Observed value	Standard value
Ligand	1625-1609 cm^{-1}	1630-1575 cm^{-1}
Complex	1625-1597 cm^{-1}	1630-1575 cm^{-1}

TABLE-2
BENDING VIBRATIONAL IR FREQUENCIES
IN cm^{-1} FOR PHENOLIC GROUP

Compound	Observed value (cm^{-1})	Standard value (cm^{-1})
Ligand	1370 and 1198	1410-1310 s and <i>ca.</i> 1200
Complex	1386 and 1190	1410-1310 s and <i>ca.</i> 1200

^1H NMR: NMR spectra were recorded by using ECX60 FT NMR (60 MHz) and solvent used was CDCl_3 . Reference standard used was TMS. The singlet peak at 3.95 ppm showed by protons of ethylene. Each aryl group has four protons in the molecule. Different environment of these protons was found and data of these shown in the Table-3 with labeling from C₁, C₄, C₁, C₂, C₃ and C₄ protons showed doublet, triplet, triplet and doublet at 7.02, 7.52, 7.08 and 7.66 correspondingly. Imines protons showed singlet at 8.54 ppm. Imine group have π electrons, which were deshielded and showed high value. A singlet at 11.26 ppm showed by phenolic protons which was probably due to the strong effect of deshielded oxygen atom.

TABLE-3
 ^1H NMR DATA OF LIGAND

Position of protons	Type of peak	Value (ppm)
C ₁	Doublet	7.02
C ₂	Triplet	7.08
C ₃	Triplet	7.52
C ₄	Doublet	7.66

^{13}C NMR: The simple and same environment of the ligand molecule containing different atoms permit it easy ^{13}C NMR spectrum. The data shown in the Table-4, two carbon atoms corresponds to each peak in the molecule. First peak at 61.9 ppm is due to the $\text{CH}_2\text{-CH}_2$ group. The signals of sp^3 hybridized carbon atom present in the middle of the molecule mostly appears at about 30 ppm but due to the presence of electronegative atom (N) and the presence of π bonds adjacent to $\text{CH}_2\text{-CH}_2$ group caused such downfield from 30 ppm (base value) to the 61.9 ppm (found value). The CH carbon exhibit sp^2 hybridization, which is not terminal commonly found at 140 ppm with found value 157.5. The presence of electronegative nitrogen (N) was correlated with the observed value of carbon which showed some deviation. Also the aryl group contains

carbon atoms, which were observed from 120-30 ppm whereas the phenolic carbons of the molecule showed peak at 161.1 ppm. This showed the presence of more electronegative oxygen atom, which is bonded to carbon and described the downfield shift. On the contrary oxygen atoms also contain lone pair, which was correlated with the *ortho* and *para* carbons of the phenols which gave up field shift by mesomeric effect. The *meta* carbon of phenol remained unchanged as in the Table-4.

TABLE-4
 ^{13}C NMR DATA OF LIGAND

Position of Carbon	Observed value (ppm)	Peak area equivalent to No. of carbon
1 and 1'	61.9	Two
2 and 2'	157.5	Two
3 and 3'	124.6	Two
4 and 4'	161.1	Two
5 and 5'	117.8	Two
6 and 6'	132.4	Two
7 and 7'	121.4	Two
8 and 8'	132.1	Two

Mass spectrometry analysis: The ligand was analyzed for molecular mass. On the basis of previous reported results the data was elucidated and confirmed⁵. The ligand showed peak at 268 as a base peak, which also a molecular ion peak. The OH group of the phenol cleaved out and gave peak at 251 as fragment peak. The compound also showed two fragments by cleavage of ethylene-nitrogen bond with molecular mass 120 and 148 correspondingly. Fragment No. 2 containing molecular mass at 120 combined with nitrogen resulted in the peak at 134. Fragment No. 2 having molecular weight 120 further eliminated nitrogen atom resulted the fragment observed at 106. The molecule also showed isotopic peak at 269 and have intensity of 1 % of the molecular ion peak. Data shown in Table-5.

TABLE-5
MASS SPECTROMETRY DATA OF LIGAND

Compound	Calculated M.W	Molecular ion peak
Ligand	268	268

Characterizations of complex

Fourier transform infrared spectroscopy analysis: The metal complex was analyzed by FTIR. The range used for FTIR spectra of the titled complex was from 4000-400 cm^{-1} as KBr pallets. On the bases of previously reported work this assignment was made⁷. The FTIR spectrum of the titled metal complex showed bending vibrations of Imine group at 1625-1597 cm^{-1} and OH group of phenol showed bending vibrations at 1386 and 1190 cm^{-1} for ligand. Data for these vibrations had shown in Tables 1 and 2.

XRD analysis: X-ray crystallography was used to establish the structure of titled compound $[\text{Cu}_2(\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_2)_2]$. Table-6 showed the atom numbering scheme and crystal data of structure (Fig. 1). The angles, bond lengths and structure of the molecule are shown in Fig. 2 and Table-7. From the justification of the crystal structure it is clear that dimeric structure of the metal complex has formed¹⁷ with 2 copper

metals Cu(1) and Cu(1)^o. Schiff base ligand (L) contains 2 nitrogen atoms and 2 oxygen atoms, which are bonded to 2 copper metals *i.e.* each nitrogen and oxygen form bond with each metal correspondingly. Furthermore, a distorted square pyramidal geometry is formed by oxygen atom of the adjacent ligand. Cu(1)-O(6) and Cu(1)-O(6)^o bond distances are 1.937 (6) nm and 2.424 (7) nm correspondingly. The bond length of Cu(1)-O(6)^o is greater than Cu(1)-O(6). This difference of 0.487 nm indicates that Cu(1)-O(6) is covalent and Cu(1)-O(6)^o is coordinate one. The bond distance of Cu(1)-O(17) is 1.897(7) nm and it is smaller than Cu(1)-O(6)^o nevertheless greater than Cu(1)-O(6) showing that Cu(1)-O(17) is a covalent bond. This difference in bond lengths of both the covalent bonds is due to the coordinate covalent bonds of Cu(1)-O(6) and Cu(1)-O(6)^o. Moreover the metal and nitrogen bond distances *i.e.* Cu(1)-N(2) and Cu(1)-N(13) are same *i.e.* 1.950(8) nm showing that both are coordinate covalent bonds. The bond angles connecting Cu center and ligand for N(2)-Cu(1)-O(6), O(6)-Cu(1)-O(6)^o, N(2)-Cu(1)-N(13), O(6)-Cu(1)-N(13), N(2)-Cu(1)-O(17), O(6)-Cu(1)-O(17), N(13)-Cu(1)-O(17), O(6)^o-Cu(1)-N(13) and O(6)^o-Cu(1)-N(17) are 95.7(3)^o, 86.6(3)^o, 83.6 (3)^o, 170.1(3)^o, 170.3(3)^o, 93.7(3)^o, 91.8(3)^o, 102.3(3)^o and 92.0 (3)^o correspondingly¹⁸.

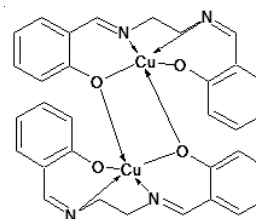


Fig. 1. Configuration of complex

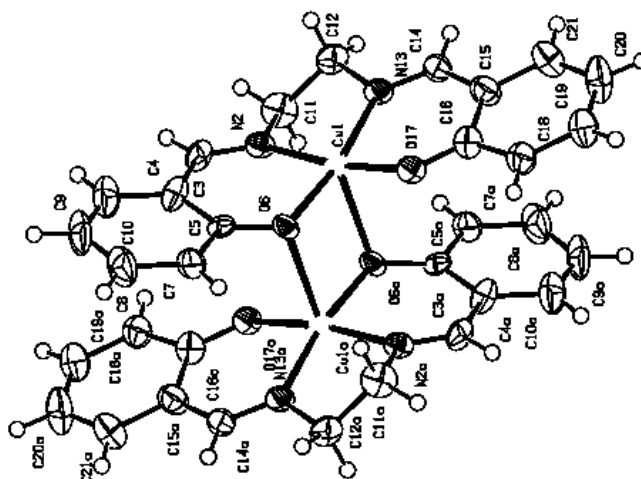


Fig. 2. Ellipsoid displacement view for atomic numbering scheme of Fig. 1 at 50 % probability level

Biological activity: Different varieties of organisms were used to test the biological activity of the metal complex and ligand. The purpose of different varieties of organisms is to increase the capability for the antifungal and antibacterial ability shown by ligand and metal complex. These activities were tested by using disc diffusion method. Several cultural strains both fungal and bacterial strains were employed in order to test the bacterial activity¹⁹.

TABLE-6
CRYSTAL DATA FOR I

Formula	C ₃₂ H ₂₈ N ₄ O ₄ Cu ₂
F. Wt.	659.7
Crystal class	Monoclinic
Space group C	2/c
a	26.632(5)
b	6.9755(10)
c	14.708(2)
α	90
β	97.471(5)
γ	90
Colour	Brown
Shape	Needle
Volume	2709.0(7)
Z	4
Radiation type	MoK _α
Wavelength	0.710730
Dx	1.62
R _{int}	0.0006
θ _{max}	28.32
H _{min} , H _{max}	-35, 35
K _{min} , K _{max}	-9, 8
L _{min} , L _{max}	-19, 19
R-factor	0.05
Max shift/su	0.0003
Weighted R-factor	0.08
δ _{Rho min}	-1.69
δ _{Rho max}	2.92
Reflections used	3328
Sigma (I) limit	-10.00
Number of parameters	191
Goodness of fit	1.096

TABLE-7
SELECTED BOND LENGTHS (nm) AND ANGLES (°) FOR I

Cu(1)-N(2)	1.950(8)
Cu(1)-O(6)	1.937(6)
Cu(1)-O(6) ^o	2.424(7)
Cu(1)-N(13)	1.950(8)
Cu(1)-O(17)	1.897(7)
N(2)-Cu(1)-O(6)	95.7(3)
O(6)-Cu(1)-O(6) ^o	86.6(3)
N(2)-Cu(1)-N(13)	83.6(3)
O(6)-Cu(1)-N(13)	170.1(3)
N(2)-Cu(1)-O(17)	170.3(3)
O(6)-Cu(1)-O(17)	93.7(3)
N(3)-Cu(1)-O(17)	91.8(3)
O(6) ^o -Cu(1)-N(13)	102.3(3)
O(6) ^o -Cu(1)-O(17)	92.0(3)

Antibacterial activity: Disc diffusion method was performed to check the antibacterial activities of ligand and metal complex. *Staphylococcus aureus*, *Escheria coli* and *Bacillus subtilis* were the strains used^{2,10,19}. The antibacterial activity of the metal complex was more than parent ligand. *S.*

aureus showed maximum activity of ligand with zone 8.25 mm and the antibacterial activities of ligand against *E. coli* and *B. subtilis* were insignificant with zone 5.25 mm and 3.25 mm correspondingly. The metal complex showed maximum antibacterial activity against *E. coli* with zone 11 mm and activities of metal complex against *B. subtilis* and *S. aureus* were with zone 5.75 mm and 6 mm correspondingly.

Antifungal activity: The antifungal activities of ligand and complex were tested against variety of fungus by using disc diffusion method. Different strain *i.e.* *A. alternate*, *A. flavis* and *A. niger* were used^{2,10,19}. Antifungal activity of ligand was less than the metal complex. Maximum ligand antifungal activity against *A. alternata* was with zone 4.25 mm and activities against *A. niger* and *A. flavis* was 4 mm and 6 mm correspondingly. Metal complex showed highest antifungal activity against *A. flavis* with zone 14.5 mm and against *A. niger* and *A. alternata* were with zones 11 mm and 10.25 mm respectively.

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