Synthesis and Characterization of Novel Schiff base Cu(II) Complexes: Antimicrobial and Molecular Docking Studies

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Abstract N2O2 type complexes of Cu²⁺ ion have been synthesized by the reaction of Salicylaldehyde / 3,4-diaminobenzophenone / acetyl acetone and glutaric anhydride. The ligands and respective metal complexes was established through spectroscopic data (FT-IR, UV-Vis,¹H NMR and ¹³C NMR). They are non-electrolytic in nature as their molar conductivities (ΛM) in DMSO of 10⁻³ M solution from the EPR study the complexes proposed to be octahedral geometry. All the metal complexes have been screened for their antibacterial activity and the predicted binding affinity using molecular docking studies.

Keywords: EPR, 3,4-diaminobenzophenone, Antimicrobial Activity, Molecular Docking.

1. INTRODUCTION

Schiff base metal complexes have a huge number of synthetic uses in organic chemistry. Acylation of Schiff bases by acid anhydrides, acid chlorides and acyl cyanides is initiated by attack at the nitrogen atom and leads to net addition of the acylating agent to the C=N bond. Reactions of this type have been used for good purpose in natural product synthesis [1]. Copper is well known for bioessential element. Its complexes have proven to be an excellent for biological importance due to their binding ability and positive redox potential [2-5] Cu(II) metal complex exhibits the fastest water exchange rate for any transition metal hydro complexes. In recent years, many research had been carried out to study the uses of copper containing coordination complexes in various fields like medicinal, bioinorganic, catalytic and analytical chemistry.
This paper concentrates on the synthesis and biological activity of Schiff baseliganands and their copper (II) complexes.

2. EXPERIMENTAL

All the chemicals used were of analytic grade, and were purchased from Sigma-Aldrich. Metal salt was purchased from E. Merck and was used as received. All solvents used were of standard/spectroscopic grade.

2.1 Synthesis of Ligand L₁/L₂

Schiff base ligand was synthesized by a hot ethanolic solution of Acetyl acetone / Glutaric anhydride, Salicylaldehyde. They were mixed slowly with constant stirring. To the above mixture was added an ethanolic solution of 3,4-diaminobenzophenone. Their molar ratio is 1:1:1 temperature was maintained at 70°C for 2.30 hrs. in the presence of Concentrated Hydrochloric acid. On cooling the substance 24 hrs. at 0°C crystalline compound was separated out [8–10] This was filtered, washed with ethanol and then dried.

2.2 Synthesis of metal complexes (ML₁/ML₂)

Hot ethanolic solution of Schiff base ligand L₁/L₂ and copper nitrate hexahydrate in 1:1 molar ratio were mixed together. The reaction mixture was refluxed at 70°C for 2.30 hrs. The quantity of the reaction mixture was decreased to around 20-25%. The precipitate that formed was filtered off and washed with ethanol and dried using anhydrous CaCl₂.[8-10] Solubility’s of metal complexes were checked with various solvents these are insoluble in H₂O, CHCl₃, CCl₄, CH₃CN and partially soluble in ether, alcohol but freely soluble in DMF and DMSO.

2.3 Characterization Techniques

The IR spectrum was recorded using KBr pellets in the range of 4400-400 cm⁻¹. UV-Visible spectra were recorded on Perkin Elmer Lambda 3B UV-Visible Spectrophotometer in the range 200-900 nm. The molar conductance were measured using 10⁻³M solution of DMSO at 25°C using an Elico CM-180 Conductivity meter and Elico type CC-03 Conductivity cell of cell constant 1.05 cm⁻¹. The ¹H & ¹³C NMR spectra of the ligand was recorded in Joel 500 MHz NMR spectrometer using (CD₃)₂SO. The mass spectra of the complexes were recorded by JEOL GC mate Mass Spectrophotometer. Magnetic susceptibility was measured at room temperature on a Gouy balance using CuSO₄.5H₂O as a callibrant. The EPR spectra of the complex were recorded in DMF at room temperature on JEX-X3 Series of a system using the DPPH as the g-marker.
Antimicrobial activity were tested by using agar well diffusion method and molecular docking studies were recorded using AutoDockVinaPyRx software.

3. RESULT AND DISCUSSION

Salicylaldehyde based metal complexes have a lot of uses including biological and analytical chemistry. The synthetic routes of the ligands and complexes are presented in scheme 1.

**Scheme 1**

**Step 1:**

**Schiff base ligands**

1. Salicylaldehyde + 3,4-diamino benzophenone + acetylacetone → $L_1$
2. Salicylaldehyde + 3,4-diamino benzophenone + glutaric anhydride → $L_2$

**Step 2:**

**Metal Complexes**

$$\text{Cu(NO}_3\text{)}_2 \cdot 6\text{H}_2\text{O} + L_1/L_2 \rightarrow ML_1/ML_2$$

**ESI Mass spectra**

The purity of the ligand ($L_1 / L_2$) 98.62% has been verified by HPLC. The ESI-mass spectrum (Fig. 1&2) of the ligand ($L_1 / L_2$) shows a parental ion

![Figure 1: ESI Mass spectrum of Ligand ($L_1$).](image-url)
peaks (M⁺) m/z = 398 and 412 respectively. A base peak is present at m/z = 315 (85%) for both the ligands. This peak corresponds to cationic species with three aromatic rings.

**IR spectra**

The IR spectra of Schiff base ligand and its metal complexes Table 1 (Fig. 3-6) shows that ν(C-O) and ν(C=N) modes appear at 1287-1327 cm⁻¹ and 1607-1618 cm⁻¹ respectively. The shifting of (C-O) to higher frequency as compared to the ligand (1287 cm⁻¹) is owing to the conversion of hydrogen bonded structure into a covalent metal bonded structure [11]. 2924-3070 cm⁻¹ are corresponding to C-H stretching of aromatic ring [12]. (M-L) bond is further confirmed by the appearance of a medium intensity band in the range 474-478 and 522-536 cm⁻¹ in the spectra of the complexes allotted to stretching.

**Table 1:** Molar conductance and Electronic Spectroscopic Data of the Schiff Base Ligands and its metal complexes.

| Compound            | Molar conductance Ω⁻¹ cm² mol⁻¹ | Colour       | M.P (°C) | Yield (%) | Amax (nm) |
|---------------------|---------------------------------|--------------|----------|-----------|-----------|
| Ligand(L₁)          | –                               | Off white    | 237      | 68        | 252,345   |
| Ligand(L₂)          | –                               | Off white    | 221      | 62        | 267,350   |
| [Cu(L₁)(NO₃)₂].xH₂O | 10.54                           | Dark brown   | 294      | 50        | 367,431,660,730 |
| [Cu(L₂)(NO₃)₂].xH₂O | 12.23                           | Dark brown   | >300     | 48        | 310,415,540,645 |

**Figure 2:** ESI Mass spectrum of Ligand (L₂).
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Figure 3: IR spectrum of L₁ Schiff base.

Figure 4: IR spectrum of L₂ Schiff base.
Figure 5: IR spectrum of [Cu(L₁)(NO₃)₂].xH₂O.

frequencies of (M-N) bond and (M-O) bond formation respectively [13]IR spectra of the nitrato complexes, display three medium intensity bands due to (N-O) stretching in the region ~1382–1384 cm⁻¹, suggesting that both the nitrate groups are coordinated to the central metal ion [14]

Figure 6: IR spectrum of [Cu(L₂)(NO₃)₂].xH₂O.
Electronic Spectra

The electronic spectrum of the Cu(II) complexes (Fig. 7-8) shows two absorption bands at 252, 345 nm and 267,350 nm for L₁ and L₂ respectively. The first band arise from π-π* transition with the azomethine chromospheres [11]. The second band is due to the n-π* transition. From the complex formation the absorption bands undergo a redshift compared to the free ligand as a result of coordination trough the nitrogen atoms of the C=N group. Electronic spectral data of ligand and metal complexes are shown in table 2.

**Figure 7:** Electronic spectrum of [Cu(L₁)(NO₃)₂]xH₂O.

**Figure 8:** Electronic spectrum of [Cu(L₂)(NO₃)₂]xH₂O.
Table 2: Infrared Spectroscopic Data of the Schiff Base Ligand and its metal complex.

| Compound                        | $\nu$ (C=N) | $\nu$ (C-O) | $\nu$ (M-N) | $\nu$ (M-O) | Ionic nitrate |
|---------------------------------|-------------|-------------|-------------|-------------|--------------|
| Ligand($L_1$)                   | 1607        | 1287        | -           | -           | -            |
| Ligand($L_2$)                   | 1618        | 1305        | -           | -           | -            |
| $[Cu(L_1)(NO_3)_2] \cdot xH_2O$ | 1610        | 1317        | 474         | 522         | 1384         |
| $[Cu(L_2)(NO_3)_2] \cdot xH_2O$ | 1612        | 1319        | 470         | 526         | 1384         |

NMR Spectra

$^1$H NMR spectrum of ligand ($L_1$ and $L_2$)(Fig. 9-10) shows signals at 8.10 ppm due to Ar-CH=N [15]A sharp multiplet signals 7.03 – 7.81 ppm due to Ar-H. A singlet corresponding to one proton observed at 12.80-13.20 ppm is due to Ar-OH. As well as in the $^{13}$C NMR spectrum of ligands (Fig. 11-12) indicated new resonance are 19.05(C-CH$_3$), 56 (C-CH$_2$-CO), 113-138(C=C), 158.45(C=N), 195.95(Ph-CO-Ph)[16].

Figure 9: $^1$H NMR spectrum of ligand ($L_1$).
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Molar conductance and EPR Study

On the basis of molar conductance measurements (Table 1) of the copper (II) complexes in DMSO corresponds to be non-electrolytic in nature of the complexes. So the complexes may be formulated as [M(L)_1(NO_3)]_x.xH_2Oand [Cu(L)_2(NO_3)_2].xH_2Owhere L_1 is (E)-4-((5-benzoyl-2-((E)-(2-hydroxy benzylidine) amino) phenyl)imino)pentan-2-one and L_2 is(E)-6-((4-benzoyl-2-((E)-(2-hydroxyl benzylidine)amino)phenyl)imino) tetrahydro-2H-pyran-2-one.

The magnetic moment measurement of both the complexes at room temperature lie in the range of 1.83 –1.85 B.M. [17,18] Electronic spectrum (Fig.7-8) of six coordinated copper complex display bands at 730,660 and 431nm for L_1 and 645,540 and 415 for L_2 corresponding to the following transitions 2B_1g \rightarrow 2B_2g; 2B_1g \rightarrow 2Eg and 2B_1g \rightarrow 2A_1g. The EPR spectral study (Fig.13-14) provides information of the metal ion environment. The spectrum of the complexes showed bands g|| > g ⊥ > gε, indicating that unpaired electron is localized in the dx^2-y^2 orbital [19]. In the Cu(II) complex G=(g||-2)/( g ⊥-2), which is more than 4 suggesting that there is no interaction between the copper centers [20] Thus the above results suggest that Cu(II) complexes possesses distorted octahedral structure.
Figure 11: $^{13}$C NMR spectrum of ligand ($L_1$).

Figure 12: $^{13}$C NMR spectrum of ligand ($L_2$).
Figure 13: EPR spectrum of $[\text{Cu}(L_1)(\text{NO}_3)_2] \cdot x\text{H}_2\text{O}$.

Figure 14: EPR spectrum of $[\text{Cu}(L_2)(\text{NO}_3)_2] \cdot x\text{H}_2\text{O}$.

4. ANTIMICROBIAL ACTIVITY

Antimicrobial activity was calculated as described by [21]. Surface of the Mueller Hinton Agar (MHA) plates. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested the zone
of inhibition was measured with a measuring scale. The antimicrobial activity of the ligand and its Cu(II) metal complexes were assayed against Gram-positive and Gram-negative bacteria. The result of antimicrobial activity are summarized in Table 3 and Fig. 15. From the data it is clear that the metal complex are effective against bacteria.

Table 3: Diameter of zone inhibition (mm) for the Schiff base ligand and its complexes.

| Microorganism   | Escherichia coli | Staphylococcus | Enterococci | Pseudomonas |
|-----------------|------------------|----------------|-------------|-------------|
| Ligand(L₁)      | – 5mm 9mm 8mm 10mm 11mm | – – – – – – – | – 5mm 13mm | – – – – – – |
| [Cu(L₁)(NO₃)₂].xH₂O | – – – – – – – – | – – – – – – | – – – – | – – – – |
| Ligand(L₂)      | – 5mm 7mm – – – – – | – – – – – – | – 4mm 6mm | – – – – |
| [Cu(L₂)(NO₃)₂].xH₂O | – 5mm 9mm – – – – | – – – – – – | – 3mm | – – – – |

Figure 15: Comparison of MIC (mg/ml) of macrocyclic ligand and its Cu(II) Complexes against E.Coli.

5. MOLECULAR DOCKING STUDY

The biological importance of the ligands are assessed by performing docking studies using AutoDockVinaPyRx software[22]The retrieved pdb file (4s1y) is given as input in AutoDockVina and assigned as macromolecule that adds charges and hydrogen bonds to the atoms thus preparing the protein. Ligand preparation including the generation of various tautomers, assigning bond orders, ring conformations and stereo chemistries of the ligand were carried
out. All the conformations generated were further used for docking study. A receptor grid was generated around the protein active site by selecting the active residues (His 288, Met 298, Met 329, Met 548) and Run autogrid option. The docking calculations were performed using Run Vina and the Binding affinity was used to determine the best docked structure from the output. The predicted binding affinity is in kcal/mol. The pdb structure 4s1y [23] of human serum albumin is used for docking studies which plays a key role in increasing the growth and productivity of cells and increases overall cell health. The best docked complex selected has a binding score of -10.7 for Cu(II) complex of ligand 2 which predicts a good inhibition. The pdb structure 4sy1 of human serum albumin is used for the docking studies with the Copper complexes. The following table shows the binding affinity of ligand with 4sy1.

Docking score using Autodock Vina with the macromolecule 4s1y

| Ligands /Complexes          | Binding Affinity (kcal/mol) |
|----------------------------|-----------------------------|
| Ligand(L1)                 | -7.9                        |
| Copper (II) complex of L1  | -9.0                        |
| Ligand (L1)                | -9.2                        |
| Copper (II) complex of L2  | -10.7                       |

The docked ligand (L1) interacts with the protein by forming three H bonds with the residues Ser192 and Glu292 with bond distances 3.33Å and 3.53Å respectively (Fig. 16). Similarly Copper (II) complex (ML1) also forms 3 H-bonds with the protein in residues Glu292, Gln196, Lys 199 with bond distances 3.43 Å, 3.09Å and 3.21Å respectively (Fig. 17).

**Figure 16:** Ligand (L1) docked with 4s1y showing formation of hydrogen bond and distances.
Figure 17: Copper (II) complex($ML_1$) docked with 4s1y showing formation of hydrogen bond and distances.

The docked ligand ($L_2$) interacts with the protein by forming four H bonds with the residues Lys195 Å, Asp451 Å, Arg222 Å and Lys 92 Å with bond distances 3.12 Å, 3.49 Å, 3.21 Å and 3.36 Å respectively (Fig. 18). Copper (II) complex ($ML_2$) forms 3 H-bonds with Asp108, Asn429 and His146 residues with bond distances 3.22 Å, 3.11 Å and 3.22 Å respectively. (Fig. 19).

Figure 18: Ligand ($L_2$) docked with 4s1y showing formation of hydrogen bond and distances.

Figure 19: Copper (II) complex($ML_2$) docked with 4s1y showing formation of hydrogen bond and distances.
CONCLUSIONS

The formation of Copper (II) complexes are thermally stable. The complexes were characterized by spectral and analytical data. Based on the spectral data anCu(II) complexes assigned to the distorted octahedral geometry, based on the biological study the complexes are good antimicrobial agent. Binding affinity and inhibition level were determined using molecular docking study.

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