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New Bioactive Ligand and Its Ni(II) and Cu(II) Complexes: Synthetic Preparation, Spectroscopic Characterization and Biological Evaluation

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Abstract: Bioactive ligands are a large range of compounds that are identified by the involvement of a double bond between the C and N atoms. Their versatility stems from combining them with different aryl or alkyl substituents. These kinds of compounds are produced in the lab and occur naturally. For many years, chemists and biochemists have found great inspiration in bioactive ligands. In order to highlight the significance of bioactive ligands, this study offers a new perspective on this class of chemicals in this article. A new bioactive ligand 2-((5bromo-2-methoxybenzylidene) amino)-6-choloro-4-nitrophenol (L) were synthesized from 5-bromo-2methoxybenzaldehyde and 2-Amino-6-chloro-4-nitrophenol. Corresponding mononuclear Ni(II) and Cu(II) complexes were synthesized and spectrochemically characterized by FT-IR, NMR, UV-visible, ESI-MS, Thermogravimetric analysis (TGA) and cyclic voltammetry (CV). The band appearance at 1668 cm⁻¹ is assigned to azomethine v(C=N), which moves towards a lesser frequency region for both the metal complexes. The CV suggests a quasi-reversible one-electron transfer reaction. Metal complexes show six-coordinate distorted octahedral geometry. All the complexes are completely soluble in DMSO and DMF. Bioactive ligands and their metal complexes are biologically active. Metal complex Cu (II) shows better biomedical applications i.e., antifungal at 75% concentration and antibacterial at 50% concentration.

Keywords: Antibacterial, Antifungal, Bioactive ligand, Metal complexes, Thermal analysis.

1. Introduction

For over a century, since their initial elucidation within coordination chemistry, bioactive compounds, demonstrating diverse functionalities, have persistently played a significant role as ligands, particularly in medicinal applications. In coordination chemistry, bioactive ligands constitute a substantial class, exhibiting potential for targeted therapeutic and interventions catalytic processes, thus maintaining their crucial status in contemporary research ¹. Even though this subject has been thoroughly researched, produced via the condensation reaction of aromatic/aliphatic aldehydes and amines, forming stable complexes with various transition metal ions remains essential and of considerable interest in inorganic chemistry ^{2, 3}. Bioactive ligands and their metal complexes have been extensively studied because of their fantastic chemical characteristics and numerous applications, and their comparatively straightforward synthesis and structural variety have demonstrated that they are attractive leads for synthetic and structural research. The biological uses and chelating

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capabilities of metal complexes have garnered significant interest, and they can serve as models for species of biological significance ⁴⁻⁶. Many bioactive ligands with imino functionality have been demonstrated to possess a variety of biological properties, including antibacterial, antifungal, antidiabetic, DNA photocleavage, antitumor, antiproliferative, anticancer, anticorrosive, and anti-inflammatory properties. Metal-imine complexes were extensively studied because of their herbicidal and catalytic applications ⁷.

Due to the wide range of adaptable nitrogen and oxygen donor sets, they are employed as chelating ligands in producing metal complexes ⁸. Therapeutic uses and their highly desired ability to coordinate with transition metal ions, researchers have been focusing on the synthesis and characterization of transition metal complexes with bioactive ligands for several years ⁹. Because of their diverse structural makeup, ion-sensing capabilities, prospective biological implications, and various bonding mechanisms have drawn much attention ¹⁰⁻¹².

Received January 30, 2025 Accepted April 2, 2025 Published April 8, 2025 Herein, we have attempted the synthesis of bioactive ligand L and its metal (II) complexes of Nickel and Copper. The coordinated behavior of bioactive ligands and life-essential 3d-metal chelates have been characterized via elemental analysis, molar conductance, FT-IR, UV–Vis, Mass spectral analysis, TGA, and cyclic voltammetry (CV). The biological activity of these complexes with antifungal activity against *Fusarium Oxysporum* and *Aspergillus Niger* species and antibacterial agreement against grampositive bacteria *Staphylococcus aureus* and gramnegative bacteria *E.Coli* is described for the first time in the literature.

2. Experimental

2.1 Reagents and instruments. 5-bromo-2methoxybenzaldehyde, MW 215.05: 2-Amino-6g/mol: chloro-4-nitrophenol, MW 188.57 NiCl₂.6H₂O, MW 237.69 g/mol; CuCl₂.2H₂O, MW 170.48 g/mol, of analytical grade, by Sigma-Aldrich, India. FT-IR spectra were taken by BRUKER Alpha-II scientific, ranging between 4000 and 400 cm⁻¹. LABINDIA 3092 UV-VIS spectrophotometer has been utilized to record absorption spectra. 400 MHz JEOL Delta- 550 Spectrometer TMS as internal reference NMR spectra were analyzed in DMSO-d6. Molar conductance studied by EI Delux model-601 digital conductivity meter in DMSO (1×10^{-4}). (Centre for Advanced Research CAR), HSGSU, Sagar, Madhya Pradesh, India. FLASH 2000 Thermo Scientific Elemental Analyzer was used for elemental identification (C, H, and N) at Punjab University, Punjab. Mass spectra were recorded on Model-Maldi-TOF Synapt XS HD Mass

Spectrophotometer at SAIF, CSIR, Central Drug Research Institute Lucknow. Thermal data were recorded using the EXSTAR, SII 6300 EXSTAR instrument in an N2 environment at a 10 K min-1 heating rate of up to 900 K at room temperature at the Institute Instrumentation Centre Indian Institute of Technology Roorkee, India.

2.2 Synthesis

2.2.1 Synthesis of 2-((5-bromo-2methoxybenzylidene)amino)-6-choloro-4nitrophenol

(*L*): 5-bromo-2-methoxybenzaldehyde (2.15 g, 10 mM) and 2-Amino-6-chloro-4-nitrophenol (1.88 g, 10 mM) were dissolved independently in an appropriate amount of methanol while being stirred, and 2-4 drops of acetic acid catalyzed the reaction. The reaction mixture was refluxed for 7-8 hours at 50-70 °C. TLC tracked the reaction. The precipitated complex was filtered out and repeatedly cleaned using diethyl ether and methanol. The resulting solid was recrystallized and dried. It was kept in a vacuum environment for 6 hours. (Figure 1)

Chemical formula: $C_{14}H_{10}BrClN_2O_4$; MW: 385.60; Color: dirty green; Yield: 81%, m.p.: >430 °C; stable; highly soluble in DMSO and DMF. Molar conductance (DMSO) λ_m (cm² $\Omega^{-1}mol^{-1}$): 17.7.

UV–Vis (λ nm): 295, 372, 486;

FT-IR (vib, cm⁻¹): 1668(vC=N azomethine), 1173(vC-O), 3500–3700(vO-H);

Elemental Calcd. (%): C, 43.61; H, 2.61; Br, 20.27; Cl, 9.19; N, 7.27; O, 16.60; Found(%):C, 43.64; H, 2.60; Br, 20.76; Cl, 9.22; N, 7.24; O, 16.57.



2-((5-bromo-2-methoxybenzylidene)amino)-6-chloro-4-nitrophenol

Figure 1. Synthesis of L

2.2.2 Synthesis of the metal complexes [ML₂(H₂O)₂]; {M: Ni(II), Cu(II)}

Methanolic solution (1mM) of both the metal salt dissolved: Ni(II)(0.237g) and Cu(II)(0.170g). This was added with methanolic solution (2mM) of bioactive ligand [L (0.771g)] in an alkaline medium, continuously stirred, and heated ($40-60^{\circ}$ C) for 4-5 hours on a magnetic hot plate stirrer. TLC eluted with ethanol/toluene. The colored solid was washed with methanol and diethyl ether several times. The resulting solid was dried and recrystallized from methanol. It was stored in a vacuum environment. (Figure 2)

[NiL₂(H₂O)₂]: NiL₂

Mol. Formula: $C_{28}H_{22}Br_2Cl_2NiN_4O_{10}$; Mol. Wt.: 863.90; Color: Brown; Yield: 73%; M.P.: >350 °C; stable; soluble in DMSO and DMF; insoluble in non-polar solvents;

Molar conductance (DMSO) λm (cm² $\Omega^{-1}mol^{-1}$): 23.4.

UV–Vis (λ nm): 371, 479;

FT-IR (vib, cm⁻¹): 1639(vC=N azomethine), 521(vM-O), 470 (M-N);

Elemental Calcd. (%): Elemental Analysis: C, 38.93; H, 2.57; Br, 18.50; Cl, 8.21; N, 6.49; Ni, 6.79; O, 18.51; Found(%):C, 38.96; H, 2.53; Br, 18.47; Cl, 8.24; N, 6.49; Ni, 6.76; O, 18.56.

[CuL₂(H₂O)₂]: CuL₂

Mol. Formula: $C_{28}H_{22}Br_2Cl_2CuN_4O_{10}$; Mol. Wt.: 868.75; Color: Blackish brown; Yield: 78%; M.P.: >365 °C; stable; soluble in DMSO and DMF; insoluble in non-polar solvents; **Molar conductance** (DMSO) λm (cm² Ω ⁻¹mol⁻¹): 26.5.

UV–Vis (λ nm): 379, 486;

FT-IR (vib, cm⁻¹): 1645(vC=N azomethine), 523(vM-O), 490 (M-N);

Elemental Calcd.(%): Elemental Analysis: C, 38.71; H, 2.55; Br, 18.40; Cl, 8.16; Cu, 7.31; N, 6.45; O, 18.42; Found(%):Elemental Analysis: C, 38.75; H, 2.50; Br, 18.45; Cl, 8.14; Cu, 7.34; N, 6.45; O, 18.39.





Figure 2. Synthesis of metal complexes

2.3. Biological applications

2.3.1. Antifungal assay

Using the poisoned food technique, the antifungal properties of tested compounds were studied against two fungal cultures, *Aspergillus niger*, and *Fusarium oxysporum*. Bavistin, the common fungicide, has been used ¹³. 2% Potato Dextrose Agar (PDA) media, autoclaved at 121°C for 20 minutes was used to assess the antifungal activity at different doses. Concentrations of 100, 75, 50, and 25 % of these substances (1mg/mL) were evaluated. A stock solution (1000 mg/mL) was prepared in DMSO. The control was 1 mL DMSO. Following their standards, these solutions were added to conical flasks (media

40°C) to achieve the required concentrations. In order to solidify the media, media of each concentration was transferred into a pair of Petri dishes (9 cm in diameter) under aseptic conditions ¹⁴. 5 mm (diameter) mycelia cut from an active, two-week-old colony of the target pathogenic fungus was positioned in the middle of each treatment plate once it had solidified. Until the fungus development in the control plates was nearly finished, the plates were incubated at 28°C. Every therapy against every fungus was tested in triplicate ¹⁵. To measure the growth inhibition percentage (*I*) utilizing the formula Equation 1.

% Growth inhibition (% I) =
$$\frac{D_0 - D_t}{D_0} X100$$

Where

 D_t = diameter of fungal colony at different treatments, D_o = diameter of fungal colony with control.

2.3.2. Antibacterial assay

Using the agar well-diffusion method, the antibacterial properties of tested compounds were studied against two bacterial strains: Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative). Antimicrobial sensitivity assays were conducted after antimicrobial susceptibility assays ¹⁶. A 14 cm sterile petri plate was filled with 75 mL of nutrient agar medium at 45°C, and a broth culture (0.75 mL) of the test strain was added. The mixture was thoroughly mixed. After the media had solidified,

sterile metallic borers were used to dig 8 mm wells. To each well, a 100 μ L DMSO solution of the test sample at a concentration of 1 mg/mL was applied. The negative control was DMSO, while the positive control was Streptomycin (1 mg/mL). Each bacterial strain triplicate plate was made and incubated aerobically for 24 hours at 37°C. In order to calculate the activity, the diameter of the zone exhibiting full inhibition (mm) was measured ¹⁷⁻¹⁹.

3. Results and Discussion

3.1. Molar conductivity

The non-electrolytic nature was validated by the λm values, which ranged between 17.30 to 27.40 (cm² Ω ⁻¹mol⁻¹). The molar conductance of the substances under test was determined in DMSO at a concentration of 5 × 10⁻⁴ M at 25°C²⁰. (Table 1)

Samples	Color	Yield (%)	$\begin{array}{c} Molar\\ Conductance\\ (\Omega^{-1}cm^2 \end{array}$	M.P. or Dec.	M. W. %	Molecular weight Found %						
			mol ⁻¹)	Tem. (°C)		М	С	Н	Br	Cl	N	0
L	Dirty green	81%	17.7	>430	385.60	_	43.64	2.60	20.76	9.22	7.24	16.57
NiL ₂	Brown	73%	23.4	>350	863.90	6.76	38.96	2.53	18.47	8.24	6.49	18.56
CuL ₂	Blackish brown	78%	26.5	>365	868.75	7.34	38.75	2.50	18.45	8.14	6.45	18.39

Table 1. Elemental analysis of L and their metal (II) Complexes and molar conductance.



Figure 3. Absorption spectra of L and its metal complexes.

3.2. Electronic absorption spectroscopy

The UV-Vis spectra of all compounds show a considerable shift in electronic transition (Figure 3).

At 295 nm, 372 nm, and 486 nm, the ligand produced an absorption band attributed to $\pi \to \pi^*$ transition (aromatic ring) and non-bonding electrons of azomethine nitrogen (C=N) group $n \rightarrow \pi^*$ transition. The observed bands in all complexes move towards higher wavelength regions. Ni(II) and Cu(II) metal complexes show two significant shifting at 371 nm (26954 cm⁻¹), 479 nm (20877 cm⁻¹) and 379 nm (26385 cm⁻¹), 486 nm (20576 cm⁻¹) respectively. At 642 nm (15576 cm⁻¹), both metal complexes exhibited weak d-d transition bands. All the electronic spectra were performed in DMSO ²¹⁻²³.

3.3. Infrared spectral studies of L and its metal complexes

The IR spectra of L are compared with its metal complexes. All spectra have been taken in DMSO.

Some important characteristic IR bands are assigned and identified to specific functional groups. The band appearance at 1668 cm⁻¹ assigned to azomethine v(C=N) confirmed the synthesis of targeted ligand, 1173 cm⁻¹ v(C-O) and a broadband 3500-3700 cm⁻¹ v(O-H). In comparison, the azomethine bond moves towards a lesser frequency region for Ni(II) and Cu(II) metal complexes at 1639 cm⁻¹ and 1645 cm⁻¹, respectively. The new M-O band appears at 521 cm⁻¹ (for Ni) and 523 cm⁻¹ (for Cu)²⁴. Fortunately, the M-N stretching vibrations observed at 470-490 cm⁻¹ confirmed metal complexes formation. Figure 4 was the graphical representation of all tested compounds ^{25, 26}.



Figure 4. FT-IR spectra of L and its metal complexes.

3.4. ¹H NMR studies of L

¹H NMR spectrum of L represented in Figure 5, proton signals at 9.74 ppm (s, 1H) and 8.03 ppm (s, 1H) are assigned to phenolic –OH and –CH=N respectively ²⁷. Signals at 7.61- 6.66 ppm (m, 5H) and 3.39 ppm (s, 3H) are due to the aromatic proton and methoxy group, respectively ^{28, 29}.



Figure 5. ¹H NMR specrum of L.

3.5. Mass spectral studies of L and its metal complexes

There was a molecular ion peak at m/z 386.7 (calc. 385.60 amu) in the mass spectrum of L. Accordingly, its chemical formula is $C_{14}H_{10}BrCIN_2O_4$. ESI-MS data have verified the molecular formula of the ligand and its metal complexes. The $[M+1]^+$ molecular ion peak is given by ligand ³⁰. Ni and Cu metal complexes, with molecular weights of m/z 863.1 (calc. 863.90 amu) and m/z 867.3 (calc. 868.75 amu), which correspond to its m/z or $[M+1]^+$ ³¹. The mass spectra of the ligand and its metal complexes are

shown in supplementary data in Figure S1 (a-c) below.

3.6. Thermal analysis

The TGA/DTA of metal complexes has been performed within the temperature range from 25° C to 995°C, with a heating rate of 10° /min. in N₂ atmosphere ³². TGA data were obtained from the analysis of their thermogram (Figure 6), which also provided insight into the presence of chelating components and water molecules inside and outside the coordination sphere ³³. The decomposition manner of both the metal complexes is mentioned in Table 2.

DTG Samples Temp. Wt. loss (%) Lost fragments (no. of molecules) Residues (%) range(°C) peak By products мо (°C) Found Cal. Found Cal. 29-294 191.77 11.88 12.00 Two molecules of lattice water NiO 15.5 15.10 Two molecules of coordinated water NiL₂ 294-648 20.50 438.65 20.61 Non-chelating part of ligand 648-980 875.91 43.00 43.66 Chelating part of ligand 30-179 87.54 Two molecules of lattice water CuO 4.8 4.35 23.88 23.78 CuL₂ 179-351 206.52 16.47 16.74 Two molecules of coordinated water Non-chelating part of ligand 351-988 734.7 46.00 46.00 Chelating part of ligand

Table 2. Decomposition manner of Ni and Cu complexes.



Figure 6. Thermogram of metal complexes (a) NiL₂ (b) CuL₂.

3.7. Cyclic voltammetry (redox properties)

In order to observe the structural and spectral changes associated with electron transfer, the redox properties of the compounds tested were examined. The vertex potential parameters range from -2.0 to +2.0 V at 100mV s⁻¹ scan rate. 0.1M TBAP is a supporting electrolyte, with DMSO as a solvent. The cyclic voltamogram of all three compounds has been performed in 6 stop crossings, as shown in Figure 7 (a-c). The cyclic voltamogram of L shows the electronic potential at anode = 0.329V and the electronic potential at cathode = -1.350V, indicating the redox properties. The cyclic voltametric data of metal complexes are represented in Table 3. Electrochemical data confirmed the quasi-reversible process and the possibility of a one-electron transfer reaction. The constant value of the square root of the scan rate indicates a diffusion-controlled process ³⁵.



Figure 7. Cyclic voltamogram (a) L (b) NiL₂ (c) CuL₂.

Table 3. Electrochemical data of bioactive ligand and its metal complexes.

Sample	Epa(V)	Epc(V)	$\Delta E(\mathbf{V})^{a}$	Ia	Ic	Ia/Ic	$\Delta E_{1/2}(V)$
L	0.329	-1.350	-1.021	5.632	-6.433	0.880	0.839
NiL ₂	0.361	-1.384	-1.023	8.015	-9.021	0.888	0.872
CuL ₂	0.336	-1.228	-0.892	4.983	-5.694	0.885	0.800

^a $\Delta \mathbf{E}(\mathbf{V}) = \mathbf{Epa-Epc}, \ ^{\mathbf{b}}\mathbf{E}_{1/2}(\mathbf{V}) = \frac{\mathbf{Epa+Epc}}{2}$

3.8. Bio-assay investigations

3.8.1 Antifungal activity

The antifungal activity is based on the % growth inhibition technique, also known as the poisoned food technique, and is measured under four criteria: significant (>70%), good (60-70%), moderate (50-60%), and non-significant (<50%) growth inhibition, as mentioned in Table 4. For each sample, three measurements (M1, M2, and M3) of growth diameter were taken after 7 days. Figure 8 (a) illustrates these triplicated measurements. The average growth diameter and standard deviation were then calculated from these three values and are presented in Table 4. The error values (standard deviations) indicate the

variability within each set of measurements. The results proved that the Cu(II) complex showed better antifungal activity against both fungi. The better biological activities of metal complexes are explained by Tweedy chelation theory ³⁶. When orbitals of the metal ion and ligand overlap, the polarity of the metal ion is reduced; hence, metal complexes penetrate easily into the lipid membranes. Complete activity was performed at a 75% concentration; the minimum growth was observed (MIC) (Figure 8) ³⁷. This value (75%) indicates that the microorganism is susceptible to the antifungal agent at concentrations of 0.75 mg/mL or higher.

Table 4. Growth inhibition % of ligand and its metal complexes against *Fusarium oxysporum* and *Aspergillus niger*.

	Fusarium	oxysporum	Aspergillus niger			
Sample	Growth in cm	Growth Inhibition %	Growth in cm	Growth Inhibition %		
Control	7.87 ± 0.3	0	8.04 ± 0.3	0		
Bavistin	0.18 ± 0.04	97.52	0.25 ± 0.05	96.87		
DMSO	4.62 ± 0.3	40.64	4.70 ± 0.4	42.23		
L	2.18 ± 0.2	74.21	2.11 ± 0.07	75.54		
NiL ₂	1.78 ± 0.06	76.94	1.73 ± 0.3	78.13		
CuL ₂	1.60 ± 0.07	79.02	1.67 ± 0.1	81.34		





Figure 8. Antifungal activity (a) Statistical representation (b) % Growth inhibition (c) Pictorial representation of ligand and its metal complexes.

3.8.2 Antibacterial activity

The in vitro antibacterial activity of the tested samples was evaluated against two bacteria, Staphylococcus aureus (Gram-positive) and Escherichia coli (Gramnegative), using Streptomycin as the standard drug (Figure 9) $^{38-40}$. The agar well-diffusion method was employed, and each test was performed three times. The average growth diameter and standard deviation were calculated from these measurements (M1, M2, and M3) and presented in Table 5. The error values (standard deviations) reflect the variability within each set of measurements. An antibacterial susceptibility assay was conducted, followed by an antimicrobial sensitivity assay (MIC). The antibacterial susceptibility was tested at 100% concentration, while the minimum inhibitory concentration (MIC) was determined at 50% concentration, corresponding to a MIC value of 0.5 mg/mL, as shown in Table 5. The copper complex exhibited better antibacterial activity against both bacteria. A key factor influencing the inhibition zone is the lipophilicity of metal complexes ⁴¹. Although the exact mechanism of antibacterial activity of these organic molecules is not fully understood, studies suggest that hydrogen bonding with the bacterial cell wall may lead to cell damage. Additionally, electron-withdrawing groups are known to enhance antimicrobial activity ⁴².

Table 5. Zone of inhibition of bioactive ligand and its metal complexes against *Staphylococcus aureus* and *Escherichia coli*.

Compounds	Zone of inhibition (mm)						
	Staphyloco	occus aureus	Escherichia coli				
	Susceptibility (100% conc)	MIC (50%)	Susceptibility (100% conc)	MIC (50%)			
L	16	10 ± 2	15	10 ± 1.2			
NiL ₂	19	11 ± 2.5	17	13 ± 0.4			
CuL ₂	19	12 ± 0.5	20	14 ± 1.7			
Streptomycin	26	21 ± 1	28	20 ± 1			





Figure 9. (a) Statistical representation of inhibition zones, (b) Pictorial representation of ligand and its metal complexes against two bacterial strains: *Staphylococcus aureus* and *Escherichia coli*.

4. Conclusion

This work presents the spectroscopic and biomedical studies of a novel ligand and its (Ni(II), Cu(II)) metal complexes. From the spectral studies, it has been observed that the distorted octahedral geometry of the Ni and Cu complexes. Thermal studies confirmed the thermal stability and the presence of two water molecules in their coordination sphere. The electrochemical analysis concluded that it was quasireversible, with the possibility of one electron transfer reaction being controlled by diffusion. The antimicrobial potency of all tested compounds was confirmed by antimicrobial screening. The interaction of Cu complex with Aspergillus niger and Escherichia coli is highly noticeable. The enhanced bioactivity of the Cu complex is likely due to the ability of copper ions to disrupt cellular function through interactions with enzymes and nucleic acids, displacing essential metal ions and inhibiting enzyme activity. Descriptive statistics were used for the statistical analysis. These findings would be the new hope for developing more potent drugs in the biomedical field.

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Abbreviations

L 2-((5-bromo-2-methoxybenzylidene)amino)-6-choloro-4-nitrophenol Fourier Transform Infrared FT-IR Spectroscopy NMR Nuclear Magnetic Resonance UV-visible Ultraviolet-Visible Spectroscopy ESI-MS Electrospray Ionization Mass Spectrometry Thermogravimetric analysis TGA cyclic voltammetry CV DMSO Dimethyl Sulfoxide Dimethylformamide DMF Molecular Weight MW Tetramethylsilane TMS millimolar mΜ Thin Layer Chromatography TLC Calcd. Calculated M.P. Melting Point Dec. Temp. Degradation temperature

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Supplementary Data









Figures S1. Mass spectra of bioactive ligand and its metal complexes (a-c).