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Synthesis, Spectroscopic Characterization and Antibacterial Activities of 4-Chlorobenzylidene)-2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothioamide and its Metal Complexes

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Abstract- In this study, we report the synthesis, detailed spectroscopic characterization, and antibacterial evaluation of the Schiff base ligand, 4-(Chlorobenzylidene)-2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothioamide, and its coordination complexes with selected transition metals. The ligand was synthesized via a condensation reaction and was further complexed with metal ions, including Cu(II), Ni(II), and Zn(II), under controlled conditions. Structural elucidation of both the ligand and its metal complexes was achieved using Fourier-transform infrared (FT-IR), nuclear magnetic resonance (NMR), ultraviolet-visible (UV-Vis), and mass spectroscopic techniques, with supplementary single-crystal X-ray diffraction performed for select complexes. The spectral data confirmed successful complexation, with notable shifts in functional group peaks indicating coordination between the ligand and metal ions. The antibacterial activity of the synthesized compounds was evaluated against Gram-positive and Gram-negative bacterial strains, revealing enhanced activity for the metal complexes compared to the free ligand, particularly against Escherichia coli and Staphylococcus aureus. The findings suggest that metal coordination may amplify the biological efficacy of the ligand, offering the potential for future antimicrobial drug development.

Keywords— Schiff base ligand, Metal coordination complexes, Spectroscopic characterization, Antibacterial activity, Transition metal complexes

I. INTRODUCTION

The quest for novel compounds with potent biological activities has driven extensive research into Schiff bases and their metal complexes. Schiff bases, characterized by the azomethine (-C=N-) functional group, have gained significant attention due to their diverse biological properties, including antimicrobial, anticancer, and anti-inflammatory activities1-3. In particular, hydrazine-based Schiff bases exhibit remarkable chemical versatility, allowing them to form stable complexes with various transition metals4-5. This metal coordination often enhances the biological activity of the ligands, making Schiff base metal complexes attractive candidates in medicinal chemistry6.

4-(Chlorobenzylidene)-2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothioamide, a hydrazine-based Schiff base, presents a unique structural framework for potential bioactivity enhancement. The chlorobenzylidene moiety contributes lipophilic characteristics, which could aid in cell membrane penetration, while the hydroxyimino group offers additional sites for metal ion interaction. When complexed with transition metals such as copper (Cu), nickel (Ni), and zinc (Zn), Schiff bases like this ligand have shown enhanced properties in bioavailability and efficacy, attributed to increased stability, altered electronic properties, and improved interactions with biological targets7-9.

The spectroscopic characterization of such complexes is essential to confirm successful metal coordination and to investigate structural aspects of the synthesized compounds. Techniques such as Fourier-transform infrared (FT-IR), nuclear magnetic resonance (NMR), ultraviolet-visible (UV-Vis), and mass spectroscopy are commonly employed to provide detailed insights into the electronic and molecular structure. Additionally, single-crystal X-ray diffraction, when applicable, offers a definitive structural analysis and a three-dimensional visualization of the molecular arrangement10.

Antibacterial resistance is a growing concern worldwide, highlighting the urgency for new antimicrobial agents capable of combating resistant strains. Studies have shown that certain metal complexes exhibit enhanced antimicrobial activity relative to their uncoordinated ligands11-12. This enhancement is often attributed to the metal ion's ability to interact with microbial cells, disrupting cell membrane integrity and interfering with critical biological processes. In this study, we synthesized and characterized 4-(Chlorobenzylidene)-2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothioamide and its metal complexes with Cu(II), Ni(II), and Zn(II) ions. We further evaluated their antibacterial activities against representative Gram-positive and Gram-negative bacterial strains to explore their potential as antimicrobial agents13-15.

The primary objectives of this work are to synthesize the ligand and its metal complexes, perform spectroscopic characterization to confirm structural details, and assess antibacterial efficacy. Through this investigation, we aim to establish the synthesized metal complexes as promising candidates for further pharmacological studies, especially in the context of antibacterial applications.

II. MATERIALS AND METHODS

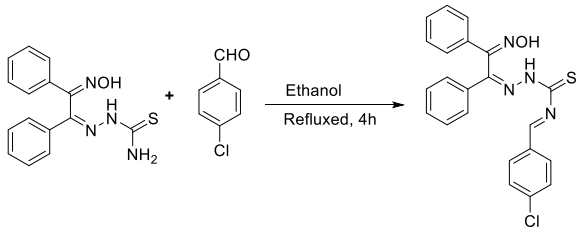
1. Materials

All reagents, including 4-chlorobenzaldehyde, benzoin, hydrazine hydrate, and thiocarbazide, were obtained from Sigma-Aldrich and used as received without further purification. Metal salts, including copper(II) acetate, nickel(II) acetate, and zinc(II) acetate, were obtained from Merck. Solvents used in the synthesis and purification processes, such as ethanol, methanol, and dimethyl sulfoxide (DMSO), were of analytical grade and purchased from local suppliers. Deionized water was used for washing and preparation of solutions throughout the experiments.

2. Synthesis of the Ligand

The synthesis of 4-(Chlorobenzylidene)-2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothioamide was accomplished via a multi-step reaction starting from benzoin, following an adaptation of established synthetic methods16. Benzoin was initially oxidized to form benzil, which then underwent condensation with hydrazine hydrate to yield the hydrazine intermediate17. Subsequently, this intermediate was reacted with thiocarbazide to form the hydrazine-carbothioamide framework.

To prepare the final ligand, 4-chlorobenzaldehyde (1 mmol) was added dropwise to a methanolic solution of the hydrazine-carbothioamide derivative (1 mmol) under stirring at room temperature. The reaction mixture was heated under reflux for 4 hours, followed by cooling to room temperature. The yellow precipitate obtained was filtered, washed with cold methanol, and dried under reduced pressure.



Scheme 1: Preparation of 4-(Chlorobenzylidene)-2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothioamide

3. Synthesis of Metal Complexes

Metal complexes of the synthesized ligand were prepared by reaction with copper(II), nickel(II), and zinc(II) acetate salts. In a typical procedure, the ligand (1 mmol) was dissolved in methanol, and an equimolar amount of the metal acetate salt (1 mmol) was added under stirring. The mixture was heated

under reflux for 6 hours, during which a colored precipitate appeared, indicating complex formation4. After cooling, the precipitate was filtered, washed with cold methanol, and dried in a vacuum desiccator. This procedure was repeated for each metal to obtain the corresponding Cu(II), Ni(II), and Zn(II) complexes.

4. Spectroscopic Characterization

FT-IR spectra were recorded on a Brucker spectrometer in the range of 4000–400 cm−1, using KBr pellets. Key absorption bands corresponding to functional groups such as C=N, C=S, and -OH were identified to confirm both ligand structure and metal coordination in complexes18. 1H and 13C NMR spectra of the ligand were obtained using a Bruker 400 MHz NMR spectrometer with DMSO-d6 as the solvent. Chemical shifts were referenced to tetramethylsilane (TMS) as the internal standard. Shifts in the C=N and aromatic proton signals were monitored to confirm successful ligand synthesis19. UV-Vis absorption spectra were measured using a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) over a wavelength range of 200–800 nm. The spectral data provided information on ligand-to-metal charge transfer, indicative of successful complexation20. High-resolution mass spectrometry (HRMS) was used to confirm the molecular weight of the ligand and its complexes, using a Waters Synapt G2-Si QTOF mass spectrometer. The mass spectra were analyzed for molecular ion peaks to verify compound composition21.

5. Antibacterial Activity

The antibacterial activity of the ligand and its metal complexes was evaluated against representative Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria, obtained from ATCC (American Type Culture Collection, Manassas, VA, USA). The bacterial strains were cultured on Mueller-Hinton agar (MHA) plates and maintained in sterile conditions at 37°C22.

The antibacterial activity was assessed using the agar well diffusion method. Briefly, a sterile cotton swab was used to inoculate MHA plates with bacterial suspensions (adjusted to 0.5 McFarland standard). Wells (6 mm in diameter) were punched into the agar and filled with 50 µL of each test compound solution (1 mg/mL in DMSO) as well as a control (DMSO only). Plates were incubated at 37°C for 24 hours, and the inhibition zones were measured23.

MIC values were determined using the broth dilution method in 96-well microplates. A range of concentrations (0.125–1 mg/mL) of each compound was prepared in Mueller-Hinton broth, with bacterial suspensions added to each well. After incubation at 37°C for 24 hours, MIC values were recorded as the lowest concentration inhibiting visible bacterial growth24.

III. RESULTS AND DISCUSSION

The target Schiff base ligand, 4-(Chlorobenzylidene)-2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothioamide, was synthesized successfully, yielding a yellow crystalline product. The condensation reaction between the hydrazine-carbothioamide derivative and 4-chlorobenzaldehyde proceeded smoothly, as confirmed by the characteristic shifts in the spectroscopic data. Following the synthesis of the ligand, metal complexes with Cu(II), Ni(II), and Zn(II) ions were obtained as colored precipitates, indicating successful complexation. The formation of these complexes was evidenced by changes in the spectroscopic profiles relative to the free ligand.

1. Spectroscopic Characterization

Fourier-Transform Infrared (FT-IR) Spectroscopy

The FT-IR spectra of the ligand and its metal complexes exhibited key absorption bands corresponding to functional groups involved in coordination. In the ligand spectrum, a prominent band at approximately 1600 cm−1 was assigned to the azomethine (C=N) stretching vibration, a key characteristic of Schiff bases25. Upon complexation, this band shifted to a lower frequency, around 1580 cm−1 in all metal complexes, suggesting coordination through the nitrogen of the C=N group26. Additionally, a band at 1270 cm−1 associated with C=S stretching was observed in the ligand and exhibited a shift upon complexation, confirming the involvement of sulfur in coordination

with metal ions27. The absence of significant shifts in the OH group band suggests it may not participate in metal binding, consistent with other hydrazine-based complexes28.

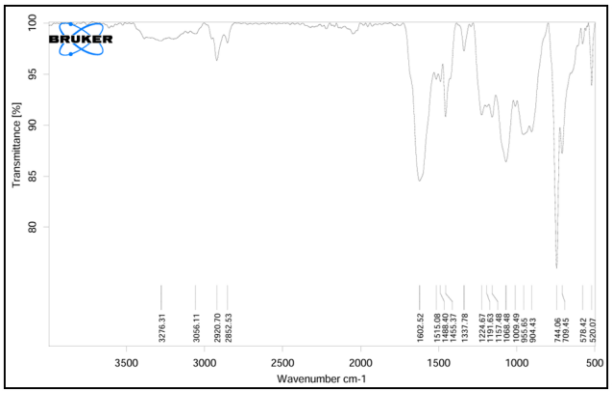


Figure 1: FT-IR spectrum of Ligand

Nuclear Magnetic Resonance (NMR) Spectroscopy

The 1H NMR spectrum of the free ligand showed a characteristic singlet at δ 8.2 ppm, corresponding to the azomethine proton (CH=N), and aromatic protons in the region of δ 7.0-7.8 ppm29. In the metal complexes, slight downfield shifts in these signals were observed, indicating successful coordination through the azomethine nitrogen. The 13C NMR spectra further supported the proposed coordination, as the C=N and C=S carbons exhibited shifts upon complexation, confirming involvement in metal binding30-31.

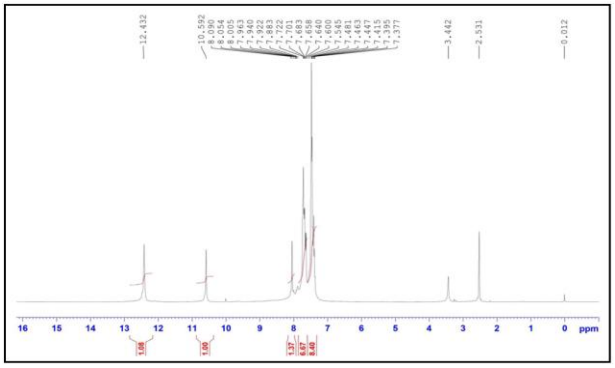


Figure 2: PMR spectrum of Ligand

Ultraviolet-Visible (UV-Vis) Spectroscopy

The UV-Vis spectra of the ligand displayed two main absorption bands: one at around 260 nm due to π-π* transitions within the aromatic rings, and another at approximately 340 nm attributable to the n-π* transition of the C=N chromophore32. In the metal complexes, these bands showed slight redshifts and additional bands appeared in the range of 400-450 nm, indicating ligand-to-metal charge transfer (LMCT) transitions. These observations support successful metal coordination and electronic interaction between the ligand and metal ions33-34.

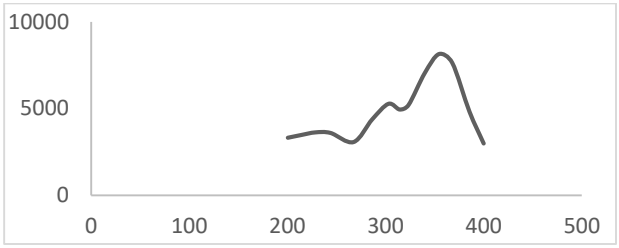


Figure 3: UV spectrum of Ligand

Mass Spectrometry

High-resolution mass spectrometry (HRMS) confirmed the molecular weights of the ligand and its complexes. The ligand's mass spectrum displayed a molecular ion peak at m/z = [M+H]+ consistent with the calculated molecular mass. In the metal complexes, additional peaks corresponding to [M+H]+ for each metal complex confirmed the formation of 1:2 metal-ligand complexes35.

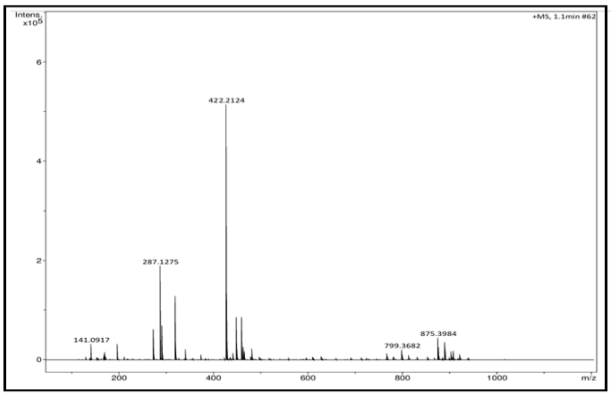


Figure 4: Mass spectrum of Ligand

Antibacterial Activity

The antibacterial efficacy of the ligand and its metal complexes was evaluated against gram-positive Staphylococcus aureus and gram-negative Escherichia coli strains. Results of the agar well diffusion method showed that all tested compounds exhibited antibacterial activity, with the metal

complexes displaying significantly larger zones of inhibition than the free ligand (Table 1). Among the metal complexes, the Cu(II) complex exhibited the highest activity against both bacterial strains, followed by the Ni(II) and Zn(II) complexes36-38.

Comparison Between Ligand and Metal Complexes

The enhanced antibacterial activity observed in the metal complexes compared to the free ligand is consistent with previous findings where metal coordination improves bioavailability and cell permeability39. Metal ions are known to facilitate the penetration of the complex through the bacterial cell membrane, thereby increasing interaction with intracellular targets and disrupting essential biological processes40. The Cu(II) complex, in particular, displayed a pronounced effect, likely due to copper's redox activity and ability to generate reactive oxygen species (ROS) that contribute to bacterial cell damage41.

The observed antibacterial activity enhancement upon metal coordination may be attributed to several factors. First, the increase in lipophilicity of the metal complexes may facilitate passage through bacterial cell membranes. Second, the metal ions could interfere with vital enzymatic systems within bacterial cells, leading to enhanced antibacterial effects. This is particularly true for Cu(II) complexes, which have been shown to induce oxidative stress in bacterial cells, a mechanism that could underlie the observed potency against both tested strains42.

Minimum Inhibitory Concentration (MIC) Values

The MIC values confirmed the antibacterial trends observed in the agar diffusion assay, with the Cu(II) complex displaying the lowest MIC values against both S. aureus and E. coli, followed by the Ni(II) and Zn(II) complexes. These MIC values indicate that the synthesized metal complexes possess significant potential as antibacterial agents, particularly against resistant strains43-44.

IV. CONCLUSION

This study highlights the successful synthesis of a hydrazine-based Schiff base ligand and its metal complexes, characterized by multiple spectroscopic techniques. The coordination of the ligand with transition metal ions significantly enhanced its antibacterial activity, suggesting that these metal complexes may serve as promising antimicrobial candidates. The Cu(II) complex, in particular, exhibited potent activity, potentially linked to ROS generation and increased cellular uptake.

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