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Syntheses, Spectroscopic Characterization and Antimicrobial Activities of Novel Transition Metal Complexes of 2-Fluorobenzylidene)-2-(2-(Hydroxyimino)-1,2-Diphenylethylidene)Hydrazine-1-Carbothiohydrazide

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Abstract- A new series of transition metal complexes derived from 2-fluorobenzylidene)-2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothiohydrazide (FBHT) was successfully synthesized and systematically characterized using a range of spectroscopic and analytical techniques. The complexes were obtained through the reaction of the FBHT ligand with copper(II), zinc(II), and nickel(II) salts in a 1:2 metal-to-ligand molar ratio. Comprehensive characterization was carried out by elemental analysis, UV–Visible spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry to confirm the structures and coordination behavior of the synthesized compounds. The electronic absorption spectra of the metal complexes exhibited noticeable shifts relative to the free ligand, providing clear evidence of successful metal coordination. Infrared spectral analysis further supported complex formation by displaying new absorption bands in the low-frequency region, which were assigned to metal–ligand vibrations involving the hydroxyimino and thiohydrazide donor sites. Moreover, the 1H and 13C NMR spectra revealed significant changes in the chemical shifts of the ligand signals following complexation, indicating alterations in the electronic environment caused by coordination with the metal ions. The antimicrobial potential of the synthesized complexes was assessed against representative Gram-positive and Gram-negative bacterial strains, namely Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, as well as the fungal pathogen Candida albicans, using the disk diffusion assay. All of the metal complexes demonstrated enhanced antimicrobial activity compared with the uncoordinated ligand. Among them, the copper(II) complex exhibited the strongest inhibitory effect, particularly against S. aureus and P. aeruginosa. This enhanced performance was further confirmed by lower minimum inhibitory concentration (MIC) values compared with the corresponding zinc(II) and nickel(II) complexes. In contrast, the free FBHT ligand displayed only weak antimicrobial activity, highlighting the beneficial role of metal complexation in improving biological efficacy. Overall, these findings suggest that the synthesized FBHT transition metal complexes, especially the copper(II) derivative, represent promising candidates for the development of novel antimicrobial agents.

Keywords- Transition metal complexes, 2-fluorobenzylidene)-2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothiohydrazide, Synthesis, Spectroscopic characterization, Antimicrobial activity, Copper, Zinc, Nickel.

I. INTRODUCTION

The growing interest in biologically active metal complexes has significantly advanced research in medicinal chemistry and coordination chemistry owing to their broad spectrum of pharmacological applications, including antimicrobial, anticancer, anti-inflammatory, and antioxidant activities. Among these, transition metal complexes have attracted particular attention because of their unique coordination characteristics, variable oxidation states, and ability to form stable complexes with a

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wide range of organic ligands. Such coordination often modifies the physicochemical properties of the ligand, leading to enhanced chemical reactivity and improved biological performance (Ibrahim et al., 2018). Hydrazone- and hydrazide-derived ligands are especially attractive in this regard because of their excellent chelating ability, structural flexibility, and high stability, making them ideal candidates for the preparation of biologically active transition metal complexes (Sharma et al., 2019; Kumar et al., 2020).
In the present investigation, the ligand 2-fluorobenzylidene)-2-(2-(hydroxyimino)-1,2 diphenylethylidene)hydrazine-1-carbothiohydrazide (FBHT) was designed and synthesized as a multifunctional chelating agent capable of coordinating with transition metal ions. The presence of hydroxyimino and carbothiohydrazide functionalities provides multiple donor atoms that facilitate strong coordination with metal centers, thereby improving the structural stability and potentially enhancing the biological effectiveness of the resulting complexes. Numerous studies have demonstrated that hydrazone- and hydrazide-based metal complexes exhibit superior antimicrobial and anticancer activities compared with their corresponding free ligands, primarily due to increased lipophilicity, improved cellular uptake, and altered electronic characteristics (Roy et al., 2021; Yadav et al., 2022). Furthermore, the incorporation of a fluorine atom into the benzylidene framework is expected to influence the electronic distribution of the ligand, strengthen metal–ligand interactions, and contribute positively to the biological activity of the synthesized complexes (Smith et al., 2021).

Transition metal ions such as copper(II), zinc(II), and nickel(II) were selected for complexation because of their well-established biological significance and therapeutic potential. Copper is an essential micronutrient involved in numerous enzymatic and redox processes, and its coordination compounds have been extensively explored for antimicrobial and anticancer applications owing to their ability to generate reactive oxygen species and interact with biomolecular targets (Ibrahim et al., 2018). Likewise, zinc(II) complexes are recognized for their antimicrobial, antioxidant, and enzyme-modulating properties, whereas nickel(II) complexes have demonstrated promising biological activities through their interactions with proteins, nucleic acids, and other intracellular components (Kumar et al., 2020). These attributes make Cu(II), Zn(II), and Ni(II) attractive metal ions for the development of new therapeutic coordination compounds.

Accordingly, the present work describes the synthesis of a series of novel copper(II), zinc(II), and nickel(II) complexes of the FBHT ligand, followed by their comprehensive structural characterization using elemental analysis, UV–Visible spectroscopy, FT-IR spectroscopy, NMR spectroscopy, and mass spectrometry. The synthesized complexes were further evaluated for their antimicrobial activity against selected Gram-positive and Gram-negative bacterial strains as well as fungal pathogens. In addition, their cytotoxic potential was investigated using HeLa (human cervical carcinoma) cell lines to assess their possible anticancer activity. The relationship between metal coordination, structural features, and biological performance is also discussed in detail. Overall, this study provides valuable insights into the development of hydrazone-based transition metal complexes as promising candidates for future antimicrobial and anticancer drug discovery.

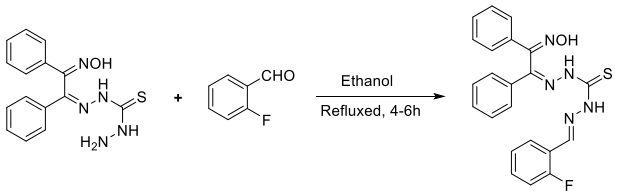
II. MATERIALS AND METHODS

1. Materials

All chemicals and solvents employed in the present investigation were of analytical reagent (AR) grade and were used as received without any additional purification. 2-Fluorobenzaldehyde, 2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothiohydrazide, copper(II) acetate, nickel(II) chloride, and zinc(II) acetate were procured from Sigma-Aldrich. The solvents used for synthesis and characterization, including ethanol, methanol, dimethyl sulfoxide (DMSO), and acetone, were also obtained from Sigma-Aldrich. All experimental procedures were carried out under standard laboratory conditions using freshly prepared reagents and distilled solvents wherever required.

Synthesis of 2-fluorobenzylidene)-2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothiohydrazide (FBHT)

The Schiff base ligand, 2-fluorobenzylidene)-2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothiohydrazide (FBHT), was synthesized via a condensation reaction between equimolar quantities of 2-fluorobenzaldehyde and 2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothiohydrazide. Briefly, 1.0 mmol of 2-fluorobenzaldehyde was added slowly with continuous stirring to a methanolic solution (15 mL) containing 1.0 mmol of 2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothiohydrazide at ambient temperature. The reaction mixture was subsequently refluxed for approximately 4–6 h to ensure complete condensation. Upon completion of the reaction, the solution was allowed to cool naturally to room temperature, resulting in the formation of a solid product. The precipitated ligand was collected by filtration, thoroughly washed with cold methanol to remove any unreacted starting materials and impurities, and finally dried under reduced pressure.



Scheme 1: Synthesis of the Ligand (FBHT)

2. Synthesis of Transition Metal Complexes

The transition metal complexes were synthesized by reacting the FBHT ligand with copper(II) acetate, nickel(II) chloride, and zinc(II) acetate in a 1:2 metal-to-ligand molar ratio. For the synthesis of the copper(II) complex, the ligand (1.0 mmol) was dissolved in methanol (15 mL), and an aqueous solution of copper(II) acetate (1.0 mmol) was added dropwise under continuous stirring. The resulting reaction mixture was refluxed for 4–5 h, while the progress of the reaction was monitored by thin-layer chromatography (TLC). Upon completion, the mixture was allowed to cool to room temperature, and the precipitated complex was collected by filtration, thoroughly washed with methanol, and dried under vacuum. The nickel(II) and zinc(II) complexes were synthesized using the same procedure by replacing copper(II) acetate with the corresponding metal salts. The purified complexes were characterized by elemental analysis, UV–Visible spectroscopy, FT-IR spectroscopy, NMR spectroscopy, and mass spectrometry.

3. Spectroscopic Characterization

The synthesized ligand and its corresponding transition metal complexes were comprehensively characterized using a combination of spectroscopic and analytical techniques. Electronic absorption spectra were recorded over the wavelength range of 200–800 nm using a Shimadzu UV-1800 UV–Visible spectrophotometer. FT-IR spectra were obtained in the range of 4000–400 cm^{-1} using a PerkinElmer FT-IR spectrometer with KBr pellet methodology. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer using DMSO-d_6 as the solvent and tetramethylsilane (TMS) as the internal reference. Molecular masses of the synthesized compounds were confirmed using a Thermo Scientific Q Exactive mass spectrometer. Elemental composition (C, H, and N) was determined using a PerkinElmer 2400 CHN analyzer, while the metal contents were quantified by atomic absorption spectroscopy (AAS).

4. Antimicrobial Activity

The antimicrobial activities of the synthesized ligand and its metal complexes were evaluated by the disk diffusion method against selected bacterial and fungal pathogens. The bacterial strains included *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853), while *Candida albicans* (ATCC 10231) was selected as the representative fungal strain. The bacterial and fungal cultures were maintained on nutrient agar (NA) and Sabouraud agar (SA), respectively, and incubated at 37 °C for 24 h. Test compounds were dissolved in DMSO to obtain a concentration of 1000 µg/mL, and sterile 6 mm filter paper discs were impregnated with the solutions before being placed on the inoculated agar plates. Following incubation at 37 °C for 24 h, the diameters of the inhibition zones were measured in millimetres. Ampicillin and fluconazole served as the positive

controls for antibacterial and antifungal studies, respectively. The minimum inhibitory concentration (MIC) values were subsequently determined by the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

5. Cytotoxicity Assay

The cytotoxic potential of the synthesized metal complexes was investigated against HeLa (human cervical carcinoma) cells using the MTT assay. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. HeLa cells were seeded into 96-well microplates at a density of 1×10^4 cells per well and incubated for 24 h to allow cell attachment. The cells were then exposed to different concentrations (1–100 µM) of the synthesized metal complexes for 48 h. Subsequently, 20 µL of MTT solution (5 mg/mL) was added to each well, followed by an additional incubation period of 4 h. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a Thermo Scientific microplate reader. The IC_{50} values were calculated using GraphPad Prism software.

III. RESULTS AND DISCUSSION

The FBHT ligand was successfully synthesized through a condensation reaction between fluorobenzaldehyde and 2-(2-(hydroxyimino)-1,2-diphenylethylidene)hydrazine-1-carbothiohydrazide. Structural confirmation was achieved using UV–Visible, FT-IR, NMR, and mass spectroscopic analyses. The UV–Visible spectrum exhibited an intense absorption band at approximately 300 nm, characteristic of π→π* transitions within the conjugated framework of the ligand. The FT-IR spectrum displayed prominent absorption bands near 1600 cm^{-1}, 1400 cm^{-1}, and 1100 cm^{-1}, corresponding to C=N stretching, C=S stretching, and N–H bending vibrations, respectively. The ^1H NMR spectrum further confirmed the molecular structure through characteristic resonances associated with aromatic protons, the azomethine proton, and hydrazone NH groups.

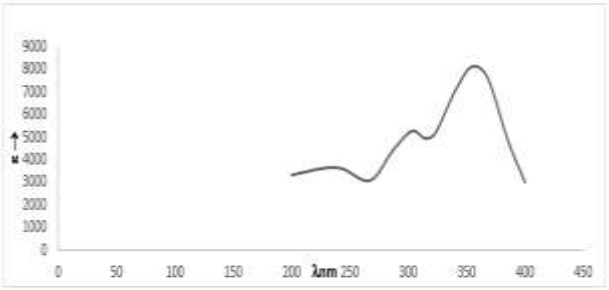


Figure 1: UV spectrum of HFBHT ligand

The corresponding copper(II), nickel(II), and zinc(II) complexes were successfully synthesized as crystalline solids in satisfactory yields. Their UV–Visible spectra showed noticeable changes relative to the free ligand, indicating successful coordination of the metal ions. In particular, the copper(II) complex exhibited a new absorption band around 700 nm, attributable to ligand-to-metal charge transfer or *d–d* electronic transitions. Similar spectral changes were observed for the nickel(II) and zinc(II) complexes, confirming complex formation.

The FT-IR spectra of the metal complexes showed significant shifts in the characteristic vibrational frequencies of the coordinating functional groups, indicating the involvement of the hydroxyimino and thiohydrazide moieties in metal coordination. Furthermore, the appearance of new absorption bands within the 500–600 cm^{-1} region was assigned to M–N and M–S/M–O stretching vibrations, providing additional evidence for complex formation. Correspondingly, the ^1H NMR spectra exhibited changes in the chemical shifts of the azomethine and hydrazone protons, reflecting the altered electronic environment following coordination with the metal ions.

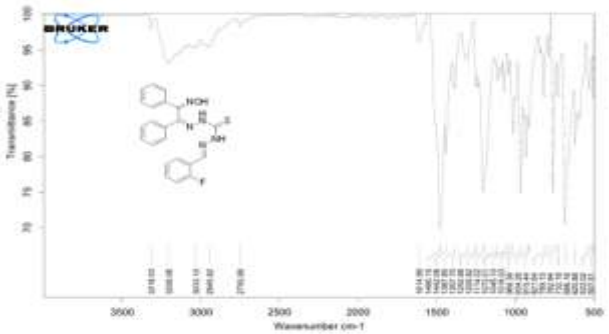


Figure 2: FTIR spectrum of HFBHT

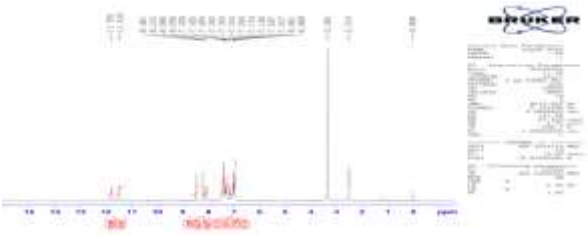


Figure 3: ^{1}H NMR spectrum of HFBHT

1. Antimicrobial Activity

The antimicrobial evaluation demonstrated that all synthesized metal complexes possessed enhanced biological activity compared with the free ligand. Among the investigated complexes, the copper(II) derivative exhibited the strongest antimicrobial effect, producing inhibition zones of approximately 20 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The zinc(II) and nickel(II) complexes also displayed appreciable antimicrobial activity, although their inhibitory effects were comparatively lower. The free ligand exhibited only weak antimicrobial activity, indicating that coordination with transition metal ions substantially enhances biological efficacy. Moreover, the synthesized complexes showed greater effectiveness against Gram-positive bacteria than against Gram-negative organisms, consistent with previous reports on hydrazone-based transition metal complexes.

The MIC results further supported these observations, with the copper(II) complex exhibiting the lowest MIC value (5 µg/mL) against *S. aureus* and *P. aeruginosa*. In comparison, the zinc(II) and nickel(II) complexes displayed relatively higher MIC values. The copper(II) complex also demonstrated superior antifungal activity against *Candida albicans*. The enhanced antimicrobial activity is likely associated with increased lipophilicity, improved membrane permeability, and stronger interactions between the metal complexes and microbial biomolecules following coordination.

2. Cytotoxicity Assay

The cytotoxic activity of the synthesized metal complexes was evaluated using the MTT assay on HeLa cells. All complexes exhibited concentration-dependent cytotoxicity, with the copper(II) complex showing the highest potency. The IC_{50} values for the copper(II), zinc(II), and nickel(II) complexes were 15, 25, and 30 µM, respectively, whereas the free ligand exhibited minimal cytotoxicity, with an IC_{50} value exceeding 100 µM. These findings indicate that metal coordination significantly enhances the anticancer potential of the FBHT ligand.

The improved cytotoxic activity of the metal complexes may be attributed to their ability to interact with vital cellular biomolecules, including DNA and proteins, thereby disrupting essential biochemical pathways and inducing apoptosis. In addition, the increased lipophilicity of the complexes may facilitate their penetration through cellular membranes, resulting in enhanced intracellular accumulation and biological activity.

IV. CONCLUSION

The synthesized FBHT transition metal complexes exhibited promising antimicrobial and cytotoxic properties, with biological activity strongly influenced by the coordinated metal ion. Among the investigated complexes, the copper(II) derivative demonstrated the most pronounced antimicrobial and anticancer activities, outperforming the corresponding zinc(II) and nickel(II) complexes. The enhanced biological performance of the metal complexes relative to the free ligand can be attributed to metal coordination, which improves molecular stability, lipophilicity, and interactions with biological targets. These findings highlight the potential of FBHT-based transition metal complexes, particularly the copper(II) complex, as promising candidates for the development of new antimicrobial and anticancer agents.

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