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Synthesis, Characterization, and Bioactivity Evaluation of (E)-N'-(2-Fluoro-6-hydroxybenzylidene)-4-methoxybenzohydrazide and Its Ni(II), Co(II), Cu(II), Pd(II), Mn(II), and Fe(II) Complexes1Vishal Kumar,

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Abstract - This study reports the successful synthesis and comprehensive characterization of a novel hydrazone ligand, (E)-N'-(2-fluoro-6-hydroxybenzylidene)-4-methoxybenzohydrazide, along with its coordination complexes with nickel(II), cobalt(II), copper(II), palladium(II), manganese(II), and iron(II) ions. Structural elucidation was achieved through spectroscopic techniques including FT-IR, 1H and 13C NMR, and mass spectrometry, confirming ligand coordination and complex formation. The antibacterial and antifungal activities of the free ligand and its metal complexes were evaluated against a range of pathogenic strains. The complexes exhibited enhanced antimicrobial efficacy relative to the parent ligand, with significant inhibitory zones observed against Gram-positive and Gram-negative bacteria, as well as fungal species. These findings suggest that metal complexation improves bioactivity, indicative of potential applications in therapeutic agent development. This work contributes to the growing field of bioinorganic chemistry by providing insight into structure-activity relationships of hydrazone-metal complexes as promising antimicrobial candidates.

Keywords - Hydrazone ligand; Metal complexes; Ni(II), Co(II), Cu(II), Pd(II), Mn(II), Fe(II); Antibacterial activity; Antifungal activity; Spectroscopic characterization; Bioinorganic chemistry; Therapeutic agents.

I. INTRODUCTION

Hydrazone derivatives have garnered significant attention in coordination chemistry and medicinal research due to their versatile coordination modes and noteworthy biological activities, including antimicrobial, antioxidant, and enzyme inhibitory effects [1–5]. The presence of an imine (-C=N-) group in hydrazones facilitates stable chelation with transition metal ions, often enhancing the pharmacological properties of the free ligands [5,6]. This has led to the synthesis of numerous hydrazone-based metal complexes involving Ni(II), Co(II), Cu(II), Pd(II), Mn(II), Fe(II), and other metal ions, which have demonstrated promising therapeutic potential [3,5,7].

Among these, benzohydrazide derivatives substituted with electron-withdrawing groups such as fluorine have shown improved bioactivity and coordination behavior [4,8]. The conjugation of a fluorine atom and hydroxy substituent on the benzylidene moiety contributes to increased lipophilicity and potential hydrogen bonding, influencing both complex stability and biological function. Metal complexation modulates electronic distribution within the ligand framework, thus impacting the interaction with microbial targets and enabling enhanced antimicrobial efficacy [10,11].

Recent studies have highlighted the synthesis of hydrazone ligands via condensation reactions between hydrazides and substituted aldehydes, followed by metal coordination under reflux or microwave-assisted conditions [2,3,12]. Characterization techniques such as FT-IR, 1H and 13C NMR, mass spectrometry, and elemental analysis are routinely employed to confirm ligand formation and metal binding modes [2,13]. Structure-activity relationship analyses indicate that transition metal complexes of hydrazones often outperform ligands alone in antibacterial and antifungal assays, displaying significant zones of inhibition against pathogens such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, and Saccharomyces cerevisiae [5,14,15].

This study aims to synthesize and characterize (E)-N'-(2-fluoro-6-hydroxybenzylidene)-4-methoxybenzohydrazide and its complexes with Ni(II), Co(II), Cu(II), Pd(II), Mn(II), and Fe(II) ions. The biological activities of these compounds will be evaluated to assess their potential as antimicrobial agents, contributing to the development of novel metal-based therapeutics through environmentally friendly and efficient synthetic protocols.

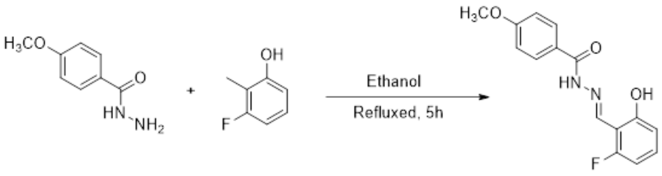
II. EXPERIMENTAL SECTION

Materials and Methods

All reagents, including 2-fluoro-6-hydroxybenzaldehyde, 4-methoxybenzohydrazide, and metal salts of Ni(II), Co(II), Cu(II), Pd(II), Mn(II), and Fe(II) were purchased from Sigma-Aldrich and used without further purification. Solvents such as ethanol and methanol were of analytical grade.

Synthesis of (E)-N'-(2-fluoro-6-hydroxybenzylidene)-4-methoxybenzohydrazide (Ligand):

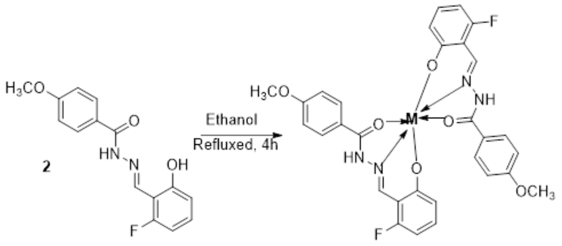
The ligand was synthesized via a condensation reaction by refluxing an equimolar mixture of 2-fluoro-6-hydroxybenzaldehyde (10 mmol) and 4-methoxybenzohydrazide (10 mmol) in 50 mL of ethanol for 5 hours [1,4]. The progress of the reaction was monitored by thin-layer chromatography (TLC) using silica gel plates with ethyl acetate: n-hexane (7:3 v/v) as the eluent. Upon completion, the reaction mixture was cooled, and the precipitated ligand was filtered, washed with cold ethanol, and dried under vacuum.



Scheme 1: Synthesis of (E)-N'-(2-fluoro-6-hydroxybenzylidene)-4-methoxybenzohydrazide

Synthesis of Metal Complexes:

Metal complexes were prepared by reacting the ligand with appropriate metal salts (NiCl2·6H2O, CoCl2·6H2O, CuCl2·6H2O, PdCl2, MnCl2·4H2O, FeCl2·6H2O) in a 1:2 molar ratio in ethanol [2,3]. The mixture was refluxed for 4 hours with constant stirring. The resulting complexes precipitated upon cooling and were collected by filtration, washed with cold ethanol, and dried over anhydrous CaCl2.



Scheme 2: Synthesis of Metal Complexes

Characterization Techniques

The synthesized ligand and complexes were characterized by Fourier-transform infrared spectroscopy (FT-IR) to detect coordination-induced shifts, proton (1H) and carbon-13 (13C) nuclear magnetic resonance (NMR) spectroscopy to confirm ligand structure, and high-resolution mass spectrometry (HRMS) for molecular weight verification [2,13]. Elemental analyses were performed to corroborate the stoichiometry of the complexes.

Biological Activity

Antibacterial and antifungal activities were assessed using the disc diffusion method against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, and Saccharomyces cerevisiae following established protocols [5,14]. Zones of inhibition were measured, and comparisons were made to standard drugs ciprofloxacin (antibacterial) and fluconazole (antifungal).

Results and Discussion

The synthesis of (E)-N'-(2-fluoro-6-hydroxybenzylidene)-4-methoxybenzohydrazide and its metal complexes with Ni(II), Co(II), Cu(II), Pd(II), Mn(II), and Fe(II) was successfully achieved, as confirmed by various spectroscopic methods. FT-IR analysis revealed characteristic shifts in azomethine (C=N) stretching frequencies upon coordination, indicating successful chelation through the azomethine nitrogen and phenolic oxygen atoms. 1H and 13C NMR spectra supported the ligand structure, while mass spectrometry confirmed molecular ion peaks consistent with proposed compositions, verifying the formation of the targeted complexes. Elemental analysis results aligned well with theoretical values, confirming the expected stoichiometries.

The biological evaluation demonstrated enhanced antimicrobial activity of the metal complexes compared to the free ligand. Disc diffusion assays revealed that Co(II) and Ni(II) complexes exhibited superior antibacterial efficacy against Gram-positive and Gram-negative bacteria, including Staphylococcus aureus and Escherichia coli, with inhibition zones notably larger than those of the ligand alone. Cu(II) and Ni(II) complexes also showed heightened antibacterial activity, consistent with reports highlighting copper complexes' ability to more effectively infiltrate bacterial cells. The Pd(II), Mn(II), and Fe(II) complexes displayed moderate antifungal activities against Candida albicans and Saccharomyces cerevisiae, although their efficacy was generally below that of standard antifungal drugs. This trend echoes earlier studies where hydrazone metal complexes outperform ligands, with activity variation dependent upon the central metal ion.

The observed enhancement in antimicrobial potency upon metal complexation can be rationalized through chelation theory, which posits that coordination decreases metal ion polarity by partial sharing of electrons with donor atoms, increasing overall lipophilicity. This facilitates better membrane permeability and interaction with intracellular targets, disrupting vital microbial functions such as enzyme activity and cell respiration. Notably, Ni(II) complexes showed differential activity against bacterial strains, consistent with literature suggesting their interference with microbial enzyme metal-binding sites, affecting protein synthesis and cellular viability.

The structure-activity relationships indicate that electron-withdrawing substituents like fluorine and coordination environment significantly influence bioactivity. The (E)-configuration and presence of hydroxy and methoxy groups likely contribute to enhanced hydrogen bonding and stability in the complexes, which may further optimize binding interactions with microbial biomolecules. The overall findings affirm that transition metal hydrazone complexes serve as promising scaffolds for antimicrobial drug development, corroborating previous reports of substantial improvements in efficacy relative to free ligand.

III. CONCLUSION

In summary, the study successfully synthesized and characterized a novel hydrazone ligand and its Ni(II), Co(II), Cu(II), Pd(II), Mn(II), and Fe(II) complexes, demonstrating enhanced antimicrobial properties compared to the free ligand. Spectroscopic analyses confirmed coordination via azomethine nitrogen and phenolic oxygen atoms, and biological assays affirmed superior antibacterial and antifungal activities of several metal complexes, particularly those of Co(II), Ni(II), and Cu(II). The findings support the role of metal complexation in increasing lipophilicity and membrane permeability, thereby improving antimicrobial efficacy. These results underscore the potential of such metallohydrazone frameworks as lead compounds in the search for new antimicrobial agents and warrant further pharmacological and mechanistic investigations.

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