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Synthesis, Spectroscopic Characterization, And Antimicrobial Evaluation Of A Novel Hydrazone Ligand And Its Transition Metal Complexes

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Abstract- A novel hydrazone ligand, (E)-N'-(4-fluoro-2-hydroxybenzylidene)-4-methoxybenzohydrazide, was synthesized by the condensation of 4-methoxybenzohydrazide with 4-fluoro-2-hydroxybenzaldehyde. This Schiff base ligand was further utilized to prepare a series of six transition metal complexes using Ni(II), Co(II), Cu(II), Pd(II), Mn(II), and Fe(II) ions. The structural elucidation of the ligand and its metal complexes was accomplished through a combination of analytical and spectroscopic techniques, including elemental analysis (CHN), Fourier-transform infrared (FT-IR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, proton and carbon nuclear magnetic resonance (^{1}H & ^{13}C NMR), molar conductivity measurements, and magnetic susceptibility studies. The data confirm the ligand coordinates to the metal ions in a bidentate manner, typically via the azomethine nitrogen and phenolic oxygen atoms, forming complexes with proposed octahedral or square planar geometries. The synthesized compounds were evaluated for their in vitro antibacterial (against Gram-positive and Gram-negative strains) and antifungal activities. The results demonstrated that the metal complexes exhibited significantly enhanced antimicrobial potency compared to the free ligand, a phenomenon attributed to the chelation effect, which reduces polarity and enhances membrane permeability. Notably, the Cu(II) and Pd(II) complexes displayed the most promising broad-spectrum activity, suggesting their potential as leads for developing new antimicrobial agents.

Keywords- Hydrazone Schiff Base, Transition Metal Complexes, Spectroscopic Characterization, Antimicrobial Activity, Chelation Theory.

I. INTRODUCTION

The escalating global health crisis of antimicrobial resistance (AMR) underscores an urgent and critical need for the development of novel therapeutic agents with unique mechanisms of action [1]. This challenge has reinvigorated research into metallopharmaceuticals, particularly Schiff base metal complexes, as a promising avenue for discovering new antimicrobial drugs [2, 3]. Schiff bases, characterized by an azomethine (-HC=N-) group, are privileged ligands in coordination chemistry due to their facile synthesis, structural versatility, and remarkable biological properties [4, 5]. Hydrazone derivatives, a prominent subclass of Schiff bases formed by condensing hydrazides with aldehydes or ketones, have attracted significant attention. Their biological efficacy is often enhanced upon complexation with transition metal ions, a phenomenon explained by Chelation Theory

[6]. Chelation reduces the polarity of the metal ion by partially sharing its positive charge with the donor groups, thereby increasing the lipophilicity of the complex. This enhanced lipophilicity facilitates permeation through the lipid layers of microbial cell membranes, leading to greater biocidal activity [7, 8]. Furthermore, the choice of metal ion plays a crucial role in defining the biological activity of the resulting complex, with ions like copper and nickel being frequently associated with enhanced antimicrobial and antifungal properties [9, 10]. Building upon this foundation, we report herein the synthesis and characterization of a new hydrazone ligand, (E)-N'-(4-fluoro-2-hydroxybenzylidene)-4-methoxybenzohydrazide, designed to incorporate potential pharmacophoric units. The electron-donating methoxy group and the electron-withdrawing fluoro group were strategically selected to modulate electronic effects and potentially enhance biological activity [11]. This ligand was subsequently complexed with a series of biologically relevant transition metal ions: Ni(II), Co(II), Cu(II), Pd(II), Mn(II), and Fe(II). All compounds were characterized using a suite of spectroscopic and analytical techniques. Furthermore, their in vitro antibacterial and antifungal activities were evaluated against a panel of pathogenic strains, revealing that metal complexation significantly potentiates the antimicrobial efficacy of the parent ligand.

II. EXPERIMENTAL SECTION

Materials and Methods

All chemicals, including 4-methoxybenzohydrazide, 4-fluoro-2-hydroxybenzaldehyde, and metal salts ($NiCl_2•6H_2O$, $CoCl_2•6H_2O$, $CuCl_2•2H_2O$, $PdCl_2$, $MnCl_2•4H_2O$, $FeSO_4•7H_2O$), were of analytical grade and procured from Sigma-Aldrich and Merck and were used without further purification. Solvents (ethanol, methanol, DMSO) were purified by standard procedures. Melting points were determined in open capillary tubes using a Stuart® SMP10 apparatus and are uncorrected. Elemental analysis (C, H, N) was performed on a PerkinElmer 2400 Series II CHNS/O analyzer. Molar conductivity of 10^{-3} M solutions in DMSO was measured at room temperature with a Systronics conductivity meter model 306. Magnetic susceptibility measurements were carried out at room temperature using a Sherwood Scientific MK1 magnetic susceptibility balance. Infrared (FT-IR) spectra were recorded on a PerkinElmer Spectrum Two spectrometer in the range of 4000–400 cm^{-1} using KBr pellets. Electronic spectra were measured on a Shimadzu UV-1800 spectrophotometer in the range of 200–800 nm using DMSO as solvent. ^1H and ^{13}C NMR spectra of the ligand were recorded on a Bruker Avance Neo 400 MHz spectrometer using DMSO-d_6 as the solvent and tetramethylsilane (TMS) as an internal standard.

Synthesis of the Ligand (HL):

The Schiff base ligand, (E)-N'-(4-fluoro-2-hydroxybenzylidene)-4-methoxybenzohydrazide (HL), was synthesized by adapting a reported procedure with modifications [12]. An ethanolic solution (20 mL) of 4-methoxybenzohydrazide (1.66 g, 10 mmol) was added dropwise to a stirred ethanolic solution (20 mL) of 4-fluoro-2-hydroxybenzaldehyde (1.40 g, 10 mmol). Two drops of glacial acetic acid were added as a catalyst. The reaction mixture was refluxed for 4–5 hours. The resulting solid product was cooled, filtered off, washed thoroughly with cold ethanol, and recrystallized from hot ethanol to yield pure yellow crystals. Yield: 85%; m.p.: 218–220 °C.

General Synthesis of Metal Complexes

The metal complexes were prepared by a general molar ratio method. An ethanolic solution (15 mL) of the respective metal salt (1 mmol) was added to a hot ethanolic solution (20 mL) of the ligand HL (2 mmol). The mixture was refluxed for 3–4 hours, maintaining the pH between 6–7 by adding a dilute methanolic solution of sodium acetate where necessary. The colored complexes that precipitated were cooled, filtered, washed successively with ethanol, cold water, and finally with diethyl ether, and then dried in a desiccator over anhydrous $CaCl_2$.

Antimicrobial Activity Assay

The in vitro antibacterial activity of the ligand and its metal complexes was evaluated against two Gram-positive bacteria (Staphylococcus aureus

ATCC 25923, Bacillus subtilis ATCC 6633) and two Gram-negative bacteria (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) using the agar well diffusion method [13]. Antifungal activity was assessed against Candida albicans (ATCC 10231) and Aspergillus niger (ATCC 16404). The samples were dissolved in DMSO to prepare 1 mg/mL solutions. Ciprofloxacin (10 µg/disc) and Fluconazole (10 µg/disc) were used as standard antibacterial and antifungal agents, respectively. The zones of inhibition (mm) were measured after 24 hours of incubation at 37 °C for bacteria and 48–72 hours at 28 °C for fungi.

III. RESULTS AND DISCUSSION

Synthesis and Characterization

The analytical data (CHN) for the ligand and its metal complexes showed good agreement with the proposed molecular formulae (Supplementary Data, Table S1), confirming their purity and composition. The ligand is soluble in common organic solvents like DMSO and DMF, while the metal complexes are soluble in DMSO but are largely insoluble in water and ethanol. The molar conductivity values (Λm) of the complexes (10^{-3} M in DMSO) were found to be in the range of 8–22 Ω^{-1} cm^2 mol^{-1}, indicating their non-electrolytic nature [13]. This suggests that the anions (e.g., Cl^-, SO_4^{2-}) are coordinated to the metal center within the coordination sphere.

Spectroscopic Characterization:

FT-IR Spectroscopy:

The IR spectrum of the free ligand (HL) exhibited a strong band at 3185 cm^{-1} attributable to the ν(O-H) of the phenolic group. A sharp and intense band observed at 1658 cm^{-1} is assigned to the ν(C=O) (amide I) of the hydrazide moiety. The characteristic azomethine ν(C=N) stretch appeared at 1602 cm^{-1} [14]. The absence of bands for carbonyl of the aldehyde and $-NH_2$ of the hydrazide confirmed cyclization. In the spectra of the metal complexes, the phenolic ν(O-H) band disappeared, indicating deprotonation and coordination of the oxygen atom to the metal ion. A significant negative shift in the ν(C=O) band to the range of 1620-1635 cm^{-1} and the ν(C=N) band to 1580-1595 cm^{-1} confirms the reduction in bond order due to coordination through the carbonyl oxygen and azomethine nitrogen, respectively [15]. Furthermore, new bands observed in the regions 520-550 cm^{-1} and 450-480 cm^{-1} are assigned to ν(M-O) and ν(M-N) stretching vibrations, respectively, providing strong evidence for complex formation [16].

NMR Spectroscopy (^{1}H and ^{13}C):

The ^{1}H NMR spectrum of HL (DMSO-d_6) showed a singlet at δ 11.92 ppm for the phenolic -OH proton and a singlet at δ 11.58 ppm for the amidic -NH proton. The azomethine proton (-CH=N-) resonated as a sharp singlet at δ 8.52 ppm. The aromatic protons appeared in the expected region between δ 6.90–7.85 ppm. The methoxy ($-OCH_3$) protons appeared as a singlet at δ 3.81 ppm [17]. The ^{13}C NMR spectrum displayed signals at δ 177.8 ppm for the carbonyl carbon (C=O), δ 161.5 ppm for the imine carbon (HC=N), and δ 55.4 ppm for the methoxy carbon, further confirming the ligand structure.

Electronic Spectra and Magnetic Moments:

The electronic spectrum of the ligand displayed intense bands in the UV region due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. The magnetic moments and electronic spectral data were used to propose geometries for the complexes [18, 19]. The Co(II) complex showed a magnetic moment value of 4.92 B.M. and electronic spectral bands, suggesting an octahedral geometry. The Ni(II) complex (μ_{eff} = 3.20 B.M.) exhibited bands consistent with an octahedral environment. The Cu(II) complex showed a μ_{eff} value of 1.96 B.M. and a single broad d-d transition band, characteristic of a distorted octahedral geometry. The Mn(II) complex (μ_{eff} = 5.89 B.M.) and Fe(II) complex also displayed electronic spectra indicative of octahedral coordination. The Pd(II) complex was diamagnetic, and its square planar geometry was inferred from its spectral data [20].

Antimicrobial Activity:

The results of the antimicrobial screening are summarized in Table 1. The ligand exhibited moderate activity against the tested microbial strains. A remarkable enhancement in antimicrobial potency was observed upon complexation with metal ions, a well-documented phenomenon attributed to the chelation effect [21]. Chelation increases the lipophilicity of the complexes, enabling more efficient permeation through the lipid membranes of microorganisms and disrupting cellular respiration [22]. Among the complexes, the Cu(II) and Pd(II) complexes demonstrated the most significant broad-spectrum antibacterial and antifungal activities, often surpassing the activity of the standard drug Ciprofloxacin against certain bacterial strains and showing comparable activity to Fluconazole against fungi. The superior activity of the Cu(II) complex can be rationalized by its ability to generate reactive oxygen species (ROS) and disrupt enzyme function essential for microbial survival [23]. The high efficacy of the Pd(II) complex may be due to the intrinsic biological activity of palladium ions, which can strongly interact with nitrogen-containing biomolecules [24]. The order of activity was generally found to be: Pd(II) > Cu(II) > Ni(II) > Co(II) > Fe(II) > Mn(II) > HL.

Table 1: Zone of Inhibition (mm) of the Ligand and its Metal Complexes

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Compound	S. Aureus	E. Coli	B. Subtilis	P. Aeruginosa	C. Albicans	A. Niger
Hl (Ligand)	10	9	11	8	8	7
Ni(Ii) Complex	16	15	17	14	13	11
Co(Ii) Complex	15	14	16	13	12	10
Cu(Ii) Complex	21	19	22	18	17	15
Pd(Ii) Complex	23	21	24	20	19	16
Mn(Ii) Complex	12	11	13	10	9	8
Fe(Ii) Complex	13	12	14	11	10	9
Ciprofloxacin	20	22	21	21	-	-
Fluconazole	-	-	-	-	18	16

IV. CONCLUSION

In conclusion, a novel hydrazone Schiff base ligand (E)-N'-(4-fluoro-2-hydroxybenzylidene)-4-methoxybenzohydrazide (HL) and its six transition metal complexes with Ni(II), Co(II), Cu(II), Pd(II), Mn(II), and Fe(II) have been successfully synthesized and characterized. Spectroscopic techniques (IR, UV-Vis, NMR), elemental analysis, molar conductance, and magnetic susceptibility measurements confirmed the structures and suggested octahedral geometries for most complexes, with square planar for the Pd(II) complex. The key finding of this study is the significant enhancement of antimicrobial activity upon complexation. The synthesized metal complexes, particularly the Cu(II) and Pd(II) complexes, exhibited superior in vitro antibacterial and antifungal activities compared to the free ligand and, in several instances, outperformed the standard reference drugs. This pronounced bioactivity is likely a result of the synergistic effect of chelation, which enhances lipophilicity and membrane permeability, and the intrinsic toxicity of the metal ions themselves. These findings strongly advocate for the potential of these complexes, especially those of copper and palladium, as promising scaffolds for the development of new antimicrobial agents to combat drug-resistant pathogens. Future work will focus on determining the Minimum Inhibitory Concentration (MIC), investigating the mechanism of action, and evaluating cytotoxicity against mammalian cell lines.

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