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PREPARATION, SPECTRAL ANALYSIS AND BIOLOGICAL ACTIVITY OF A BENZIMIDAZOLE-BASED SCHIFF BASE, 4-((1H-BENZIMIDAZOL-2-YLIMINO)METHYL)-2-METHOXY PHENOL, AND ITS Cu(II) AND Zn(II) COMPLEXES

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ABSTRACT
A benzimidazole-based Schiff base, 4-((1H-benzo[d]imidazol-2-ylimino)methyl)-2-methoxyphenol, was synthesised through a condensation reaction between the corresponding aldehyde and amine under controlled conditions. The ligand was then used to prepare its Cu(II) and Zn(II) metal complexes. The structural elucidation of the ligand and its metal complexes was carried out using FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopic techniques. The formation of the azomethine group was evident from characteristic IR bands along with the expected NMR signals. The antimicrobial potential of the synthesised compounds was assessed against a panel of selected pathogenic microorganisms, including Gram-positive and Gram-negative bacteria as well as fungal strains. Among the tested compounds, the Zn(II) complex showed comparatively better activity than both the free ligand and the Cu(II) complex. This enhanced activity may be related to metal coordination, which can improve lipophilicity and promote interaction with microbial cell membranes. These findings demonstrate that Schiff base metal complexes on a benzimidazole base are potential agents for further investigation as antimicrobial agents and provide useful information for the design of biologically active coordination compounds. The outcomes of this study support Goal 3: Good Health and Well-being of Sustainable Development by contributing to the ongoing search for new compounds with potential applications in the management of microbial infections.
Keywords: Benzimidazole, Zn(II), Antimicrobial, Schiff Bases, Cu(II).
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INTRODUCTION

Metal complexes based on Schiff bases have gained significant research interest due to their versatile structural frameworks and notable roles in biological systems.[1–4] Among transition metals, copper(II) is particularly important due to its flexible coordination behaviour and redox properties, often forming square-planar, square-pyramidal, or octahedral geometries that influence catalytic and antimicrobial activity.[5,6] In contrast, zinc(II) is a redox-inactive d^{10} metal ion that generally forms stable tetrahedral or octahedral complexes and is well known for its biological compatibility. It is frequently observed that coordination of Schiff bases with Cu(II) and Zn(II) enhances biological activity compared to free ligands, mainly due to improved interaction with biomolecules such as enzymes and DNA. Many studies have reported that they have been display multiple pharmacological properties such as antiviral, anti-inflammatory, antibacterial, anticancer, and antifungal activities.[7,8] These activities depend on structural factors such as heteroatoms, conjugation, and molecular planarity, which facilitate interaction with biological systems. The presence of hetero atom sites like nitrogen and oxygen in Schiff bases is particularly important in coordination chemistry, catalysis, and medicinal applications due to their tunable electronic properties. Chelation generally increases the stability of metal complexes and enhances biological activity, often attributed to increased lipophilicity and improved membrane permeability. Among heterocyclic compounds, benzimidazole is recognised as an important pharmacophore in medicinal chemistry because of its broad spectrum of biological activities and excellent coordination

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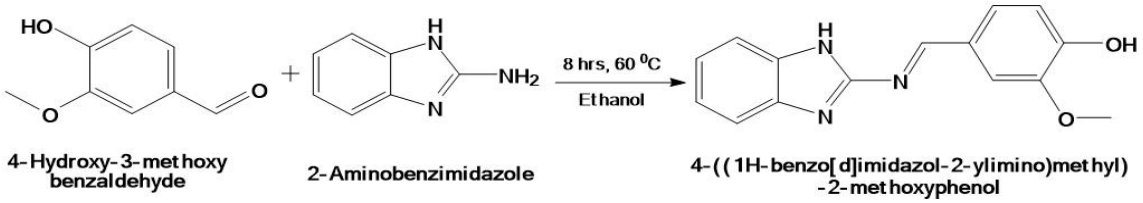
ability through nitrogen donor atoms. Benzimidazole-derived Schiff bases have attracted considerable attention owing to their antimicrobial, anticancer, antioxidant, and enzyme inhibitory properties. Likewise, vanillin-derived Schiff bases possess hydroxyl and methoxy functional groups that facilitate metal coordination and contribute to the formation of stable chelate complexes. Metal coordination generally enhances the physicochemical and biological properties of Schiff bases by improving their stability, lipophilicity, and interaction with biological targets. Consequently, benzimidazole–vanillin Schiff base metal complexes have emerged as promising candidates for the development of biologically active coordination compounds with potential pharmaceutical applications. Although numerous Schiff base metal complexes have been reported in recent years, studies describing the synthesis, structural characterisation, and comparative antimicrobial evaluation of 4-((1H-benzo[d]imidazol-2-ylimino)methyl)-2-methoxyphenol and its corresponding Cu(II) and Zn(II) complexes remain limited.[9,10] In particular, detailed investigations correlating their spectroscopic characteristics with antimicrobial performance are scarce. Therefore, the present study was undertaken to synthesize this benzimidazole-based Schiff base, prepare its Cu(II) and Zn(II) complexes, characterise the synthesized compounds using FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopy, and evaluate their antimicrobial activities against selected bacterial and fungal pathogens. The findings are expected to contribute to the understanding of structure–activity relationships in Schiff base metal complexes and to provide useful information for the development of new antimicrobial agents. This work is also aligned with the United Nations Sustainable Development Goal 3 (Good Health and Well-being) by supporting research directed toward the discovery of effective compounds for combating microbial infections.

EXPERIMENTAL

Synthesis of Schiff Base and its Metal Complexes

A modified procedure based on reported methods was employed for the synthesis of the Schiff base ligand.[11,12] In a typical preparation, an equimolar quantity of 4-hydroxy-3-methoxybenzaldehyde (0.01 mol) was dissolved in 20 mL of ethanol, resulting in a clear solution. To this, 2-aminobenzimidazole (0.01 mol) was added in portions with continuous agitation to ensure uniform mixing. The mixture was then subjected to reflux at 60 °C for 8 h, facilitating the condensation and formation of the –C=N– linkage characteristic of Schiff bases. Once the reaction was completed, as monitored by thin-layer chromatography, the mixture was allowed to cool to room temperature, resulting in the formation of a solid precipitate. The precipitated Schiff base was filtered, rinsed with a small quantity of cold ethanol to remove residual impurities, and subsequently recrystallised from methanol to improve purity. Finally, the purified Schiff base was allowed to dry using a hot-air oven to obtain the compound in solid form suitable for further characterisation and analysis. The metal complexes were prepared by reacting the ligand with suitable metal salts in a 1:2 molar ratio using methanol as the solvent. The metal salt solution (0.001 mmol) was prepared in methanol and heated. To this, the ligand solution (0.002 mmol) in methanol was added slowly with stirring. The reaction mixture was then kept under reflux at 60 °C for about 3 hours to form the complexes. After completion, the mixture was allowed to cool to room temperature and the solid complexes were formed. The products were separated by filtration, washed with ethanol to remove impurities, and then dried in a vacuum desiccator.



Scheme-1: Reaction Scheme for Preparation of Schiff Base Ligand, 4-((1H-benzo[d]imidazol-2-ylimino)methyl)-2-methoxyphenol

RESULTS AND DISCUSSION

FT-IR Spectra

The FT-IR spectral analysis of the produced Schiff base provides clear confirmation of imine formation and the existence of functional groups originating from both the benzimidazole and o-methoxyphenol

fragments (Fig.-1). A broad absorption band around 3500 cm^{-1} matches the phenolic O–H stretching, and its broadness suggests the involvement of the hydroxyl proton in intramolecular hydrogen bonding with the azomethine nitrogen, a phenomenon commonly observed in o-hydroxy Schiff bases. The peaks near 2930 cm^{-1} indicate aromatic C–H stretching, supporting the presence of conjugated aromatic rings belonging to both structural units. The most diagnostic feature of Schiff base formation, the azomethine (–C=N–) stretching vibration, appears prominently at 1585 cm^{-1}. The presence of the azomethine frequency together with the disappearance of the aldehyde C=O band confirms the Schiff base formation. This frequency aligns well with reported values for benzimidazole-based Schiff bases, typically observed in the 1570–1620 cm^{-1} region. Additionally, the characteristic aromatic C=C stretching appears around 1499 cm^{-1}, indicating the integrity of the conjugated aromatic framework. Vibrations in the 1200–1260 cm^{-1} region, especially near 1220 cm^{-1}, refer to C–O stretching of the methoxy substituent and phenolic oxygen, indicating the $–OCH_3$ group in the ligand. The benzimidazole moiety contributes additional ring vibrations in the 1450–1500 cm^{-1} and 750–850 cm^{-1} regions, characteristic of C–N stretching and aromatic C–H bending modes, respectively. The FT-IR spectra of the Cu(II) and Zn(II) complexes, illustrating the changes associated with metal coordination, are presented in Fig.-2 and Fig.-3, respectively. Overall, the FT-IR spectrum shows all expected vibrational signatures including phenolic O–H, aromatic C=C, imine C=N, aromatic C–H, methoxy C–O, and benzimidazole ring modes, providing clear confirmation for the successful synthesis of the Schiff base.[13]

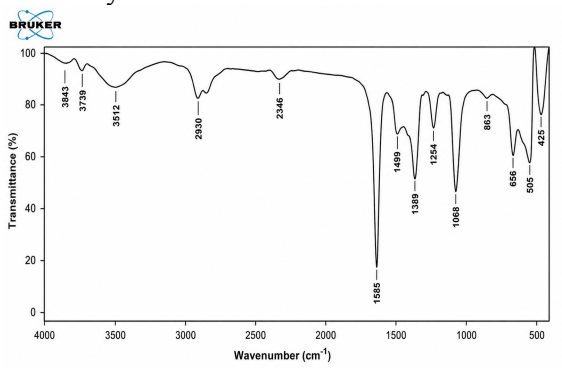


Fig.-1: FT-IR Spectrum of 4-((1H-benzo[d] imidazole-2-ylimino)methyl)-2-methoxyphenol

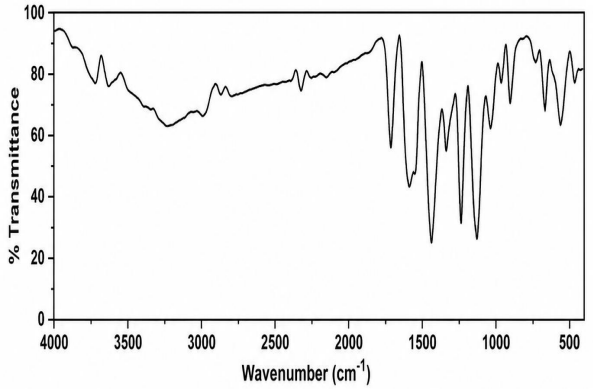


Fig.-2: FT-IR Spectrum of Cu(II) Complex

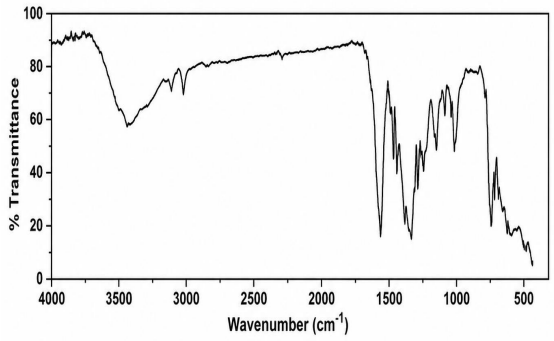


Fig.-3: FT-IR Spectrum of Zn(II) Complex

^{1}H NMR Spectra

The ^{1}H NMR spectral analysis shows all the expected proton resonances of the designed Schiff base ligand (Fig.-4). The chemical shift values closely match those reported in the literature for related benzimidazole Schiff base derivatives. A singlet appearing at δ 3.8 ppm corresponds to the methoxy protons. This falls within the typical literature range of δ 3.70–3.90 ppm reported for o-vanillin-derived Schiff bases. A broad downfield signal at δ 5.4 ppm is assigned to the phenolic –OH proton. Hydrogen-bonded phenolic protons in o-hydroxy Schiff bases generally appear between δ 4.8–6.0 ppm. The signal at δ 5.1 ppm is attributed to the benzimidazole N–H proton. Benzimidazole N–H resonances are

commonly reported in the δ 5.0–5.4 ppm region. Multiplets between δ 6.8–7.7 ppm represent aromatic protons from both the methoxyphenyl and benzimidazole rings. Literature for related benzimidazole-imine Schiff bases reports values between δ 6.7–8.0 ppm. The azomethine proton typically appears downfield at δ 8.2–8.7 ppm in Schiff bases.[14] Although overlapping in some spectra, its presence is confirmed by ^{13}C NMR. Published benzimidazole Schiff bases consistently show –CH=N– protons at δ 8.20–8.80 ppm.

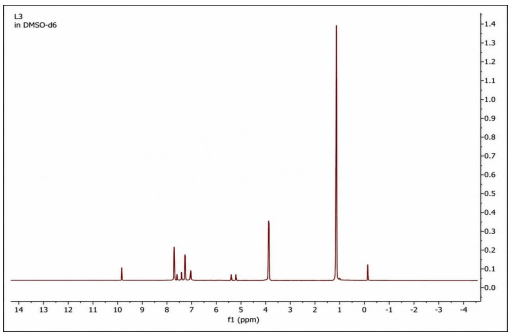


Fig.-4: ^{1}H NMR Spectral Image of 4-((1H-benzo[d] imidazole-2-ylimino)methyl)-2-methoxyphenol

^{13}C NMR Spectra

The ^{13}C NMR spectrum further validates the structure through chemical shifts entirely consistent with those reported for o-vanillin and benzimidazole Schiff bases (Fig.-5). A signal at δ 57 ppm corresponds to the methoxy carbon. This value matches literature reports of δ 55–60 ppm for vanillin-derived methoxy groups. Multiple resonances appear between δ 112–152 ppm, representing aromatic carbons. Similar Schiff bases commonly exhibit aromatic carbon signals in the δ 110–160 ppm range. A strong signal at δ160 ppm is attributed to the imine carbon. This is a key diagnostic peak; imine carbons generally resonate at δ158–165 ppm for benzimidazole Schiff bases.[15] A deshielded carbon signal near δ 159 ppm corresponds to the imidazole carbon. Benzimidazole carbons typically appear between δ 155–160 ppm.

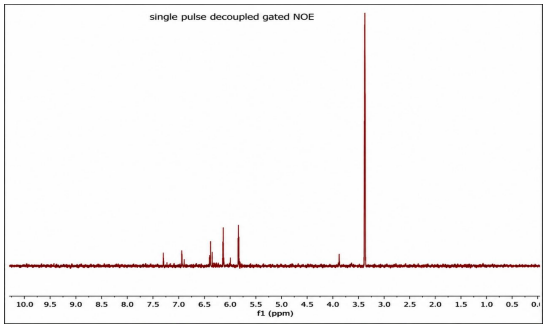


Fig.-5: ^{13}C NMR Spectrum of the Synthesized Schiff Base, 4-((1H-benzo[d] imidazol-2-ylimino)methyl)-2-methoxyphenol

Biological Evaluation

The antimicrobial properties of the prepared compounds were assessed by the agar well diffusion technique. The agar diffusion technique is widely used to determine the antibacterial potential of plant or microbial extracts.[16] In a manner similar to that employed in the disc diffusion approach, the specific volume of microbial inoculum was spread evenly across the agar plate. A sterile cork borer or pick was used to drill a hole of 6 to 8 mm in diameter, and a predefined amount of the required concentration of antibacterial or extraction solution was put into the hole. Depending on the microorganisms to be investigated, the agar plates were incubated under appropriate conditions. Consequently, the antimicrobial agent spreads through the agar medium, preventing the growth of the tested microorganism. The wells were loaded with the test solution and followed incubation at 35 °C for 24 hours to evaluate microbial growth inhibition. The growth of the inoculated microorganisms is influenced by the diffusion of the solution to be tested. A zone of inhibition is created, and the concentration was recorded. The zone of inhibition was calculated using the standard drug, tetracycline and solutions of all compounds were

prepared using dimethyl sulfoxide (DMSO) as the solvent. Antimicrobial evaluation was carried out against Gram-positive bacteria, namely *Staphylococcus aureus (SA)* and *Bacillus subtilis (BS)*, together with Gram-negative bacteria, including *Escherichia coli (EC)* and *Pseudomonas aeruginosa (PA)*. The antifungal potential was assessed against *Candida albicans (CA)* and *Candida parapsilosis (CP)* by the agar disc diffusion technique. Their activities were compared with tetracycline and fluconazole, respectively. The effectiveness of the compounds was evaluated by recording the diameter of the zone of inhibition in millimetres. The experimental findings indicate a clear trend that the metal complexes possess significantly greater activities compared to the free Schiff base ligand. This enhancement is consistent with the classical chelation theory, which states that metal coordination enhances the lipophilicity of a ligand, enabling easier diffusion through the lipid membranes of microorganisms and thereby improving biological efficacy. The free Schiff base showed only mild antibacterial activity, producing relatively small inhibition zones of 6 mm for all tested bacteria. In contrast, both the metal complexes [Cu(II) and Zn(II)] exhibited notable antibacterial potency, with inhibition zones ranging from 10–14 mm, depending on the microorganism. Of the synthesised compounds, the Zn(II) complex consistently exhibited the highest antibacterial activity, especially against *S. aureus* (14 mm) and *B. subtilis* (13 mm). A slightly lower zone of inhibition (12 mm) was observed against *Escherichia coli*, although this value still surpasses that of the free ligand (Table-1). The antifungal activities followed a similar pattern that the free Schiff base exhibited only modest antifungal effects, whereas the metal complexes produced higher inhibition zones, particularly against *Candida albicans* (Table-2). The corresponding metal complexes showed inhibition zones of 7 mm, compared to 4–6 mm for the free ligand. In the case of *Candida parapsilosis*, the Zn(II) complex showed superior activity (6 mm) relative to the Cu(II) complex (5 mm).

Table-1: Zone of Inhibition (mm) of Synthesised Compounds against Bacterial Strains

	Gram (+) bacteria		Gram (–) bacteria	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
Schiff base	06 mm	06 mm	06 mm	06 mm
Cu(II) complex	12 mm	11 mm	11 mm	10 mm
Zn(II) complex	14 mm	13 mm	12 mm	12 mm
Tetracycline	15 mm	15 mm	15 mm	15 mm

Table-2: Zone of Inhibition (mm) of Synthesized Compounds against Fungal Strains

	Candida albicans	*Candida parapsilosis*
Schiff base	04 mm	06 mm
Cu(II) complex	07 mm	05 mm
Zn(II) complex	07 mm	06 mm
Fluconazole	05 mm	05 mm

Comparative Literature Survey

The antimicrobial potential of the synthesised compounds was compared with some recent literature reports on similar vanillin and benzimidazole-based systems (Table-3).[17-21] In the present work, the free ligand showed only low antibacterial activity. But after coordination with metal ions, the activity increased clearly. Among them, the Zn(II) complex showed better inhibition (12–14 mm) compared to Cu(II). So, the order observed is Zn(II) > Cu(II) > ligand.

Table-3: Comparison of Antimicrobial Potential of the Present Study with Recent Literature Reports

No.	Author(s) / Year	Ligand Type / System	Tested Organisms	ZOI (mm)
1	Present study	Benzimidazole–vanillin Schiff base (ligand)	*SA, BS, EC, PA*	4–6
2	Present study	Cu(II) complex	*SA, BS, EC, PA*	10–12
3	Present study	Zn(II) complex	*SA, BS, EC, PA*	12–14
4	Khan et al. (2024)	Vanillin-derived Schiff bases	*EC, KP, PA*	15.0–16.6
5	Demirbağ *et al.* (2025)	Vanillin-derived Schiff bases	*SA, EC, EF, PA*	12–18
6	Mitu *et al.* (2025)	Mn(II), Ni(II) and Cu(II) complexes of o-vanillin Schiff base	*SA, BS, EC, PA, CA*	11–20
7	Singh *et al.* (2025)	o-Vanillin-derived transition metal Schiff base complexes	*SA, EC, PA*	10–19

8	Pinheiro *et al.* (2025)	Zn(II) complex with vanillin-derived Schiff base	*SA, EC, CA*	12–20

CONCLUSION

In the present investigation, benzimidazole Schiff base and its corresponding metal complexes of Cu(II) and Zn(II) were successfully synthesised through a conventional condensation method and subsequent complexation reactions. The structural features of the synthesised compounds were thoroughly validated using ^{13}C NMR, ^{1}H NMR, and FT-IR spectroscopy, all of which confirmed the formation of the >C=N– (azomethine) group and its coordination to the metal centres. The synthesised compounds were assessed for antimicrobial activity against different pathogenic microorganisms. The results of the biological assessment concluded that the Schiff base possessed limited antimicrobial activity, while the complexes displayed considerably higher antibacterial and antifungal effectiveness. Overall, the findings of this research demonstrate that coordination with metal ions markedly enhances the biological potential of the parent Schiff base ligand. The Zn(II) complex, in particular, emerges as a promising candidate for further pharmacological investigation due to its broad-spectrum antimicrobial activity. These results contribute meaningful insight into the design of biologically active Schiff base metal complexes and highlight their potential applications in medicinal chemistry, antimicrobial drug development, and bioinorganic research. The present study also supports Good Health and Well-being (UN Sustainable Development Goal 3) by contributing to the development of new antimicrobial compounds that may help address the growing challenge of infectious diseases and antimicrobial resistance.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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