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Spectroscopic, Thermal, Morphological, and Antimicrobial Studies of Cu (II), Co (II), Ni (II), and Zn (II) Complexes of (E)-2-[(4-Chlorophenyl)imino]methylphenol

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Abstract: Complexes were prepared using prepared ligand (E)-2-[(4-chlorophenyl)imino]methylphenol, by condensation and its interaction with Cu (II), Co (II), Ni (II), and Zn (II) ions. Prepared such complexes were characterized by Spectroscopic techniques using ATR-FTIR, UV–visible, 1H NMR, AAS, SEM, EDAX, and thermalanalysis, including TGA, DTG, and DTA. Spectral data confirmed bidentate coordination through azomethine nitrogen and phenolic oxygen. Thermal studies revealed a multi-step decomposition process, with final residues corresponding to the respective metal oxides. SEM images showed distinct morphologies ranging from rod-like Cu (II) crystals to cauliflower-type Ni (II) aggregates, while EDAX and AAS confirmed metal incorporation. Antimicrobial studies against Escherichia coli, Staphylococcus aureus, Aspergillus niger, and Alternaria alternata revealed selective activity for the ligand and Cu (II) complex, whereas Co (II), Ni (II), and Zn (II) complexes were largely inactive. Chelate stabilization, poor solubility, bulky coordination geometries, and lack of redox activity rationalize the limited activity of most complexes. The Correlation of structural, thermal, and biological data establishes that metal identity and electronic properties critically govern antimicrobial performance.

Keywords: Schiff base ligand, Transition metal complexes, Spectral characterization, Thermal analysis, SEM–EDAX, Antimicrobial activity, Structure–activity relationship, Copper(II) complex.

Introduction - Schiff bases, first reported by Hugo Schiff in 1864, are characterized by the azomethine (–CH=N–) functional group formed through condensation of primary amines with aldehydes or ketones (Schiff, 1864). These compounds possess versatile coordination behavior and biological relevance owing to the presence of donor atoms such as nitrogen and oxygen (Chandra & Kumar, 2004). Aromatic Schiff bases are generally more stable than aliphatic analogues due to extended π-conjugation and resonance stabilization, which enhances their chelating ability (Karthikeyan et al., 2006).

Substituent effects strongly influence Schiff base reactivity and biological properties. Electron-withdrawing groups such as chloro substituents decrease electron density on the azomethine nitrogen and modulate metal–ligand bonding strength (Chohan et al., 2005). 4-Chloroaniline is an aromatic amine with known pharmacological relevance, while 2-hydroxybenzaldehyde provides a phenolic oxygen atom positioned ortho to the imine linkage, enabling formation of stable five-membered chelate rings (Rehman et al., 2009).

Transition metal complexes of Schiff bases have attracted attention due to their catalytic, magnetic, and antimicrobial properties (Dharamraj et al., 2001). Among transition metals, Cu (II) is redox-active and capable of generating reactive oxygen species (ROS), whereas Zn (II), Ni (II), and Co (II) are comparatively redox-inert (Halliwell & Gutteridge, 2015). This difference is crucial in determining antimicrobial behavior.

Although many Schiff base complexes have been reported, most studies focus on either spectral characterization or biological screening without correlating coordination mode, morphology, thermal stability, and antimicrobial behavior in a unified manner (Chohan et al., 2005; Dharamraj et al., 2001). Therefore, a systematic correlation between structure, stability, surface morphology, and bioactivity remains underexplored. The present work includes the synthesis of derivatives of 4-chloroaniline and 2-hydroxybenzaldehyde, and their interactions with the metal ions like Cu (II), Co (II), Ni (II), and Zn (II). Their

physicochemical properties are correlated with morphology, thermal stability, and antimicrobial performance.

Review of Related Literature: Schiff base metal complexes have attracted sustained attention in coordination and medicinal chemistry owing to their structural versatility, ease of synthesis, and wide spectrum of biological activities (Chandra & Kumar, 2004; Dharamraj et al., 2001). The defining azomethine (–CH=N–) functional group serves as an efficient donor site for transition metals and plays a decisive role in determining both coordination behavior and biological response (Karthikeyan et al., 2006). Schiff bases derived from salicylaldehyde analogues are particularly significant because the ortho-hydroxyl group enables bidentate N,O-chelation, resulting in stable five-membered chelate rings that strongly influence geometry and electronic structure of metal complexes (Rehman et al., 2009).

Chelation has often been correlated with enhanced lipophilicity and improved biological activity due to partial sharing of the metal positive charge with donor atoms and delocalization of π-electrons over the chelate ring (Tweedy, 1964; Chohan et al., 2005). This increase in lipophilicity can facilitate penetration of microbial membranes and improve intracellular accumulation. However, excessive chelation may also reduce biological activity by stabilizing the complex too strongly, thereby decreasing ligand flexibility and blocking reactive donor sites required for interaction with biomolecules (Chandra & Kumar, 2004; Mishra & Kaushik, 2007). Thus, chelation may either enhance or suppress antimicrobial activity depending on the balance between stability and reactivity.

Among transition metals, Cu(II) Schiff base complexes are frequently reported to exhibit superior antimicrobial activity compared with complexes of redox-inactive metals. This behavior has been attributed to the ability of copper to undergo Cu(II)/Cu(I) redox cycling under physiological conditions and generate reactive oxygen species (ROS), leading to oxidative damage of microbial proteins, membranes, and DNA (Borkow & Gabbay, 2005; Tardito et al., 2011). In contrast, Zn(II) complexes generally show weaker antimicrobial effects because Zn(II) is redox-inert and its biological activity mainly depends on specific enzyme inhibition or protein binding rather than oxidative stress pathways (Halliwell & Gutteridge, 2015; Rehman et al., 2009). Nickel(II) and cobalt (II) Schiff base complexes display variable biological behavior, which depends strongly on ligand substitution pattern, coordination geometry, and overall molecular size (Dharamraj et al., 2001; Karthikeyan et al., 2006).

Thermal stability studies of Schiff base metal complexes consistently show multistep decomposition patterns involving initial loss of organic moieties followed by formation of stable metal oxide residues (Halcrow, 2013; Lever, 1984). Such behavior reflects strong metal–ligand bonding and provides insight into coordination geometry and stoichiometry. Morphological investigations using scanning electron microscopy (SEM) have further demonstrated that crystal shape, surface roughness, and particle aggregation significantly influence interaction with microbial cells (Pelczar et al., 2008; Nikaido, 2003). Smooth, rod-like or faceted crystals tend to promote surface contact, whereas bulky, aggregated morphologies often restrict diffusion through microbial membranes.

Despite the large number of reports on Schiff base complexes, systematic correlation of morphology, thermal behavior, and antimicrobial activity remains limited. Most studies focus either on spectral characterization or biological screening without integrating physicochemical and biological data into a unified structure–property–activity relationship (Chohan et al., 2005; Dharamraj et al., 2001). This gap in the literature motivates the present investigation, which aims to correlate coordination mode, morphology, thermal stability, and antimicrobial performance for Schiff base complexes of Cu(II), Co(II), Ni(II), and Zn(II) derived from 4-chloroaniline and 2-hydroxybenzaldehyde.

Experimental Section:

Experimental:

Synthesis of Schiff Base Ligand (Compound B):

Equimolar quantities of 4-chloroaniline and 2-hydroxybenzaldehyde were refluxed in ethanol to obtain a yellow Schiff base ligand. The prepared product was dried after appropriate filtration and washing.

Synthesis of Metal Complexes (IB–IVB):

The ligand was reacted with Cu (II), Co (II), Ni (II), and Zn (II) salts in an ethanolic medium under reflux. The resulting complexes were isolated as coloured solids, washed, and dried.

Characterization Techniques:

UV–Visible, FTIR, and ^{1}H NMR spectroscopy were used to confirm structure and coordination. SEM, EDAX, and AAS provided morphological and elemental analysis. TG–DTG–DTA studies assessed thermal stability. Antimicrobial activity was evaluated by agar diffusion methods.

Result and Discussion:

Spectral Characterization: The FTIR spectrum of the Schiff base ligand exhibited a characteristic azomethine ν(C=N) stretching vibration in the region ~1615–1620 cm^{-1} and a broad phenolic ν(O–H) band around 3400 cm^{-1}, confirming the formation of the imine linkage and the presence of intramolecular hydrogen bonding. Upon coordination with metal ions, the disappearance of the phenolic ν(O–H) band and the systematic shift of the ν(C=N) band toward lower wavenumbers were observed, indicating deprotonation of the phenolic group and involvement of the azomethine nitrogen in coordination. These spectral changes confirm bidentate chelation through the N and O donor atoms of the ligand (Chandra & Kumar, 2004; Mishra & Kaushik, 2007).

Additional new bands appearing in the low-frequency region (450–600 cm^{-1}) were assigned to ν(M–N) and ν(M–

O) vibrations, further supporting metal–ligand bond formation (Dharamraj et al., 2001; Nakamoto, 2009).

Electronic spectra (UV-Visible) of the ligand showed intense absorption bands attributable to $\pi \rightarrow \pi^*$ transitions of the aromatic system and $n \rightarrow \pi^*$ transitions of the azomethine group. The spectra of the metal complexes displayed additional weak to moderate intensity bands in the visible region, assignable to d–d transitions or ligand-to-metal/metal-to-ligand charge transfer (LMCT/MLCT) transitions. These features provided information on coordination geometry: square planar or octahedral for Cu(II) and Ni(II), octahedral for Co(II), and tetrahedral or octahedral for Zn(II), consistent with reported Schiff base complexes (Lever, 1984; Halcrow, 2013).

The ^{1}H NMR spectra of the diamagnetic Zn(II) and Ni(II) complexes showed downfield shifts of the azomethine proton relative to the free ligand, reflecting deshielding due to coordination of the imine nitrogen to the metal center. The persistence or strong downfield displacement of the phenolic –OH signal further supported involvement of the oxygen atom in metal binding. These observations are in agreement with previously reported Schiff base complexes of transition metals (Chohan et al., 2005; Rehman et al., 2009).

These spectroscopic shifts arise from electron density withdrawal from donor atoms upon metal binding, validating chelation and explaining increased rigidity and stability of the complexes.

Thermal Analysis: Thermogravimetric analysis revealed no significant mass loss below 200°C for any of the complexes, confirming the absence of lattice or coordinated water molecules and supporting the FTIR results. Such thermal behavior is typical of strongly chelated Schiff base complexes in which metal–ligand bonding prevents incorporation of water molecules into the coordination sphere (Chandra & Kumar, 2004; Halcrow, 2013).

The complexes decomposed in successive stages, with initial weight loss corresponding to partial ligand degradation followed by complete breakdown of the organic moiety and formation of stable metal oxide residues (CuO, CoO, NiO, and ZnO). This multistep decomposition pattern reflects strong metal–ligand interactions and is characteristic of bidentate N,O-coordinated Schiff base complexes (Halcrow, 2013; Lever, 1984).

The observed thermal stability order Cu(II) > Ni(II) > Co(II) > Zn(II) can be attributed to differences in metal–ligand bond strength and ligand field stabilization energy (LFSE). Metals with greater LFSE and stronger covalent metal–ligand interactions yield complexes with higher thermal resistance, whereas redox-inert Zn(II) complexes generally display lower thermal stability due to weaker orbital overlap (Cotton et al., 1999; Miessler et al., 2014).

Higher thermal stability indicates stronger chelation and lattice energy, which correlates with reduced ligand lability and hence reduced biological interaction.

SEM–EDAX and AAS: SEM micrographs revealed distinct metal-dependent morphologies: rod-like and prismatic crystals for the Cu(II) complex, flake-like particles for the Co(II) complex, block-shaped angular crystals for the Zn(II) complex, and cauliflower-like aggregated structures for the Ni(II) complex. Such morphological diversity reflects variations in nucleation and growth processes governed by coordination geometry and intermolecular packing forces (Nakamoto, 2009; Pelczar et al., 2008).

These morphological features are known to influence surface interaction with microorganisms by affecting surface area, roughness, and contact efficiency. Crystalline rod-like or faceted particles often promote localized interaction, whereas highly aggregated morphologies hinder diffusion and reduce biological accessibility (Nikaido, 2003; Mishra & Kaushik, 2007).

EDAX spectra confirmed the presence of only carbon, oxygen, chlorine, and the respective metal ions, with no detectable extraneous elements, demonstrating high purity of the synthesized complexes. The retention of chlorine verified that the para-chloro substituent remained intact after coordination. AAS measurements provided quantitative bulk metal content, which was consistently slightly higher than EDAX values owing to the difference between bulk (AAS) and surface-sensitive (EDAX) analytical techniques (Skoog et al., 2014; Willard et al., 1988).

Morphological compactness limits diffusion and surface contact with microbial cells, which explains the reduced biological activity of bulky complexes.

Antimicrobial activity: The antimicrobial activities of the Schiff base ligand derived from 4-chloroaniline and 2-hydroxybenzaldehyde (Compound B) and its Cu (II), Co (II), Ni (II), and Zn (II) complexes (IB–IVB) were evaluated against two bacterial strains, Escherichia coli (ATCC-25922) and Staphylococcus aureus (ATCC-25923), and two fungal strains, Aspergillus niger (ATCC-16888) and Alternaria alternata (ATCC-34957), using the agar diffusion method at different concentrations. The results are summarized in Table X.

The free Schiff base ligand exhibited moderate antibacterial activity against E. coli, with zones of inhibition of 18, 15, and 13 mm at 100, 50, and 25 mg mL^{-1}, respectively. This activity may be attributed to the presence of the azomethine (–CH=N–) group and the phenolic –OH functionality, which are known to interact with microbial enzymes through hydrogen bonding and π–π stacking interactions, thereby interfering with cellular metabolism (Chohan et al., 2005; Rehman et al., 2009). However, the ligand was inactive against S. aureus, A. niger, and A. alternata, indicating limited broad-spectrum efficacy.

Among the metal complexes, the Cu (II) complex (IB) showed selective activity. It retained moderate antibacterial activity against E. coli (17 mm at 100 mg mL^{-1}) and demonstrated antifungal activity against A. niger and A. alternata (20 and 14 mm at higher concentrations). This

behaviour can be rationalized by the redox-active nature of Cu (II), which can undergo Cu(II)/Cu(I) cycling in biological media and generate reactive oxygen species (ROS). These ROS induce oxidative stress, damaging microbial membranes, proteins, and DNA (Borkow and Gabbay, 2005; Tardito et al., 2011). SEM analysis revealed rod- and prism-shaped crystalline morphology with smooth surfaces for the Cu(II) complex, which may facilitate localized surface interactions with microbial cells, enhancing its biological response.

In contrast, the Co (II), Ni (II), and Zn (II) complexes exhibited negligible activity against both bacterial and fungal strains. The general inactivity of these complexes can be attributed to several interrelated physicochemical and biochemical factors. First, chelate stabilization plays a dominant role. Coordination of the Schiff base ligand through the azomethine nitrogen and phenolic oxygen results in the formation of stable five-membered chelate rings, significantly reducing ligand flexibility and blocking donor sites that would otherwise interact with biological targets such as enzymes and membrane proteins (Tweedy, 1964; Dharamraj et al., 2001). This stabilization suppresses further ligand exchange or redox processes that are often necessary for antimicrobial action.

Second, poor aqueous solubility of the complexes limits their diffusion in biological media and reduces the effective concentration at the microbial cell surface. Chelation generally increases hydrophobicity and decreases ionization, restricting transport through aqueous environments and biological membranes (Chohan et al., 2005; Rehman et al., 2009). This effect is particularly significant for Gram-negative bacteria such as E. coli, which possess an outer lipopolysaccharide membrane that restricts the passage of bulky or poorly soluble molecules (Nikaido, 2003).

Third, steric hindrance and bulky coordination geometry further impede penetration through microbial cell walls. SEM observations showed compact, crystalline, and aggregated morphologies for the Co(II), Ni(II), and Zn(II) complexes, suggesting dense packing and limited surface reactivity. Such rigid three-dimensional structures hinder diffusion across bacterial peptidoglycan layers and fungal chitinous walls (Mishra and Kaushik, 2007; Pelczar et al., 2008).

Finally, the absence of redox activity in Zn(II), Ni(II), and Co(II) complexes plays a critical role in their biological inactivity. These metal ions are largely redox-inert under physiological conditions and therefore do not promote ROS generation. In contrast, Cu(II) complexes readily participate in redox cycling and induce oxidative stress, which constitutes a major antimicrobial mechanism (Halliwell and Gutteridge, 2015; Borkow and Gabbay, 2005). The superior performance of the Cu(II) complex in this study is therefore consistent with its unique electronic properties and coordination behavior.

Overall, these findings demonstrate that metal complexation does not universally enhance antimicrobial activity. Instead, biological efficacy depends on a delicate balance between stability and reactivity. Strong chelation may suppress ligand reactivity unless the metal ion contributes additional biochemical pathways such as redox cycling or membrane disruption (Chandra and Kumar, 2004; Karthikeyan et al., 2006). The observed inactivity of most complexes underscores the importance of rational ligand design and appropriate metal selection for the development of biologically active coordination compounds.

Table 1 (see in last page)

Conclusion :

1. A Schiff base ligand derived from 4-chloroaniline and 2-hydroxybenzaldehyde and its Cu(II), Co(II), Ni(II), and Zn(II) complexes were successfully synthesized and characterized using spectroscopic, thermal, and microscopic techniques.
2. FTIR and electronic spectral studies confirmed **bidentate N,O-chelation** through the azomethine nitrogen and phenolic oxygen atoms, forming stable five-membered chelate rings. This was evidenced by the downward shift of the ν(C=N) band, disappearance of the phenolic ν(O–H) vibration, and appearance of ν(M–N) and ν(M–O) bands in the low-frequency region (Chandra & Kumar, 2004; Lever, 1984).
3. Thermal analysis demonstrated that all complexes were stable up to approximately 200°C with **no low-temperature mass loss**, confirming the absence of lattice or coordinated water molecules. The multistep decomposition patterns terminating in metal oxide residues indicate strong metal–ligand interactions and chelate stabilization (Halcrow, 2013).
4. The observed thermal stability order, **Cu(II) > Ni(II) > Co(II) > Zn(II)**, reflects differences in metal–ligand bond strength and ligand field stabilization energy, highlighting the role of electronic configuration in determining complex stability (Lever, 1984; Mishra & Kaushik, 2007).
5. SEM analysis revealed distinct, metal-dependent morphologies: rod-like and prismatic crystals for Cu(II), block-shaped crystals for Zn(II), flake-like particles for Co(II), and cauliflower-like aggregates for Ni(II). Such variations in crystal habit and aggregation behavior significantly influence surface contact and diffusion processes at biological interfaces (Pelczar et al., 2008; Nikaido, 2003).
6. EDAX and AAS confirmed the presence of expected elements (C, O, Cl, and metal ions) without extraneous impurities and showed close agreement between surface and bulk metal contents, validating the purity and stoichiometry of the synthesized complexes (Dharamraj et al., 2001).
7. Antimicrobial studies showed **selective activity for the free ligand and the Cu(II) complex**, while Co(II), Ni(II), and Zn(II) complexes were largely inactive.

8. The inactivity of Co(II), Ni(II), and Zn(II) complexes is attributed to strong chelate stabilization that blocks reactive donor sites, reduced ligand flexibility, poor aqueous solubility limiting diffusion, and bulky coordination geometries hindering penetration through microbial cell walls (Tweedy, 1964; Chohan et al., 2005; Nikaido, 2003).
9. The enhanced activity of the Cu(II) complex is rationalized by its redox-active nature, which enables Cu(II)/Cu(I) cycling and generation of reactive oxygen species that induce oxidative stress in microbial membranes, proteins, and DNA (Borkow & Gabbay, 2005; Tardito et al., 2011).
10. These findings demonstrate that **metal complexation does not inherently enhance antimicrobial activity**; instead, biological performance depends critically on the balance between chelate stability and metal-centered electronic properties, underscoring the importance of rational metal selection in Schiff base complex design (Chandra & Kumar, 2004; Karthikeyan et al., 2006).

Recommendations:

1. Structural modification of the Schiff base ligand by introducing electron-donating or electron-withdrawing substituents should be explored to fine-tune lipophilicity, coordination strength, and redox behavior, thereby optimizing biological performance.
2. Incorporation of redox-active metal ions such as Fe(III), Mn(II/III), or mixed-metal systems is recommended, as these metals can introduce additional oxidative pathways and potentially enhance antimicrobial or catalytic activity *(Halliwell & Gutteridge, 2015).*
3. Advanced mechanistic investigations, including DNA-binding assays, reactive oxygen species (ROS) quantification, and molecular docking studies, should be conducted to elucidate the precise mode of biological action of the complexes.
4. Cytotoxicity and selectivity studies against mammalian cell lines are essential to assess therapeutic safety and pharmacological relevance of the synthesized compounds.
5. Systematic correlation of antimicrobial activity with crystal morphology, thermal stability, and electronic structure should be explored to guide the rational design of Schiff base metal complexes as functional bioinorganic and antimicrobial agents.

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References:-

1. **Borkow, G., & Gabbay, J.** (2005). Copper as a biocidal tool. *Current Medicinal Chemistry*, **12**(18), 2163–2175.
2. **Chandra, S., & Kumar, R.** (2004). Synthesis and biological evaluation of Schiff base metal complexes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **60**, 2825–2829.
3. **Chohan, Z. H., Supuran, C. T., & Scozzafava, A.** (2005). Metal binding and antibacterial activity of Schiff bases. *Journal of Enzyme Inhibition and Medicinal Chemistry*, **20**(2), 183–188.
4. **Cotton, F. A., Wilkinson, G., Murillo, C. A., & Bochmann, M.** (1999). *Advanced Inorganic Chemistry* (6th ed.). New York: Wiley.
5. **Dharamraj, N., Viswanathamurthi, P., & Natarajan, K.** (2001). Ruthenium(II) Schiff base complexes: antimicrobial activity. *Transition Metal Chemistry*, **26**, 105–109.
6. **Halcrow, M. A.** (2013). Spin-crossover materials: Properties and applications. *Chemical Society Reviews*, **42**, 1784–1795.
7. **Halliwell, B., & Gutteridge, J. M. C.** (2015). *Free radicals in biology and medicine* (5th ed.). Oxford: Oxford University Press.
8. **Karthikeyan, M. S., et al.** (2006). Antimicrobial activity of metal chelates. *Bioorganic & Medicinal Chemistry*, **14**, 7482–7489.
9. **Lever, A. B. P.** (1984). *Inorganic Electronic Spectroscopy* (2nd ed.). Amsterdam: Elsevier.
10. **Miessler, G. L., Fischer, P. J., & Tarr, D. A.** (2014). *Inorganic Chemistry* (5th ed.). New York: Pearson.
11. **Mishra, A. P., & Kaushik, N. K.** (2007). Biological significance of Schiff bases and metal complexes. *E-Journal of Chemistry*, **4**, 483–489.
12. **Nakamoto, K.** (2009). *Infrared and Raman spectra of inorganic and coordination compounds* (6th ed.). New York: Wiley.
13. **Nikaido, H.** (2003). Molecular basis of bacterial outer-membrane permeability. *Microbiology and Molecular Biology Reviews*, **67**(4), 593–656.
14. **Pelczar, M. J., Chan, E. C. S., & Krieg, N. R.** (2008). *Microbiology* (5th ed.). New York: McGraw-Hill.
15. **Rehman, W., Saman, F., & Ahmad, I.** (2009). Synthesis and antimicrobial studies of Schiff base metal complexes. *Journal of the Serbian Chemical Society*, **74**, 635–642.
16. **Schiff, H.** (1864). Mittheilungen aus dem Universitätslaboratorium in Pisa. *Justus Liebigs Annalen der Chemie*, **131**, 118–119.
17. **Skoog, D. A., Holler, F. J., & Crouch, S. R.** (2014).

Principles of instrumental analysis (6th ed.). Boston: Cengage.

18. **Tardito, S., et al.** (2011). Copper complexes as antimicrobial agents. *Journal of Medicinal Chemistry*, **54**, 5527–5538.
19. **Tweedy, B. G.** (1964). Plant extracts with metal ions as antimicrobial agents. *Phytopathology*, **55**, 910–914.
20. **Willard, H. H., Merritt, L. L., Dean, J. A., & Settle, F. A.** (1988). *Instrumental methods of analysis* (7th ed.). New Delhi: CBS.

Table 1 . Antimicrobial activity of the Schiff base ligand and its Cu(II), Co(II), Ni(II), and Zn(II) complexes against selected bacterial and fungal strains, with proposed mechanistic explanations based on coordination chemistry and morphology.

S. No.	Synthesised Compound	Indicator strains	Inhibitory Activity (in mm)	Level of efficacy	Mode of action	References
1	Ligand (Compound B)	*Escherichia coli* (ATCC-25922)	18 (100 mg mL^{-1}), 15 (50mg mL^{-1}), 13 (25mg mL^{-1})	Moderate	Azomethine (–CH=N–) and phenolic –OH groups interact with microbial enzymes via hydrogen bonding and π–π stacking, interfering with metabolism	Chohan et al. (2005); Rehman et al. (2009)
2	Ligand (Compound B)	*Staphylococcus aureus* (ATCC-25923), *Aspergillus niger* (ATCC-16888) *Alternaria alternata* (ATCC-34957)	Nil	Inactive	Limited lipophilicity and absence of metal-assisted pathways restrict broad-spectrum activity	Chohan et al. (2005); Karthikeyan et al. (2006)
3	Cu(II) complex (IB)	*Escherichia coli* (ATCC-25922)	17 (100 mg mL^{-1})	Moderate	Cu(II)/Cu(I) redox cycling generates ROS causing oxidative stress to proteins, membranes, and DNA	Borkow & Gabbay (2005); Tardito et al. (2011)
4	Cu(II) complex (IB)	*Aspergillus niger* (ATCC-16888) *Alternaria alternata* (ATCC-34957)	20, 14 (high conc.)	Moderate (fungal)	Smooth rod/prism morphology promotes surface interaction; ROS-mediated damage enhances antifungal response	Borkow & Gabbay (2005); Pelczar et al. (2008)
5	Co(II) complex (IIB)	All strains	Nil	Inactive	Strong chelate stabilization blocks reactive donor sites and suppresses ligand exchange	Tweedy (1964); Dharamraj et al. (2001)
6	Ni(II) complex (IVB)	All strains	Nil	Inactive	Poor aqueous solubility and compact aggregate morphology limit diffusion and cellular penetration	Nikaido (2003); Mishra & Kaushik (2007)
7	Zn(II) complex (IIIB)	All strains	Nil	Inactive	Bulky coordination sphere + redox-inert Zn(II) prevents ROS generation and biochemical disruption	Halliwell & Gutteridge (2015); Pelczar et al. (2008)

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