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Schiff Base Metal Complexes: A New Frontier in the Search for Effective Antiseptic Agents

*Dueke-Eze, C. U. and Fasina, T. M.
Department of Chemistry, Faculty of Physical and Earth Sciences, University of Lagos, Nigeria

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Abstract

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The global increase in antimicrobial resistance has emphasized the urgent need for novel and effective antiseptic agents. This challenge has spurred interest in developing new chemical entities, including coordination compounds that can enhance antimicrobial efficacy and offer alternative strategies for infection control. In view of this growing antimicrobial challenge, isoniazid Schiff bases, namely N-(2-hydroxy-5-bromobenzylidene)isonictinoylhydrazide(L1) and N-(2-hydroxy methoxybenzylidene) isonictinoylhydrazide (L2), and their Cu(II) and Co(II) complexes are reported. The compounds were characterized based on melting point, FTIR, 1H and 13C NMR and elemental analysis. The Schiff bases act as tridentate ligands bonding through azomethine nitrogen, phenolic, and carbonyl oxygen atoms. The in vitro antibacterial study was performed on the compounds against *Staphylococcus aureus* ATCC 25923, *Enterococcus feacalis* ATCC 29212, *Escherichia coli 2592* and *Pseudomonas aeruginosa 27853*. The Schiff base ligands and their complexes were observed to exhibit a strong inhibitory effect on *S. aureus* bacteria, with the cobalt complexes exhibiting the greatest effect. This implies that these compounds may be considered promising candidates for further exploration as potential antiseptic agents subject to future toxicological evaluation and formulation studies.

***Corresponding Author:** Dueke-Eze, C. U.**;** *cdueke-eze@unilag.edu.ng*

Introduction

Antibacterial resistance is a growing global health concern, particularly in warm climates and within less economically empowered regions of the world (WHO, 2025[a]). The World Health Organisation estimates that a significant proportion of the global population is at risk of bacterial infections, especially in developing countries with limited access to effective healthcare and sanitation systems (WHO, 2025[b]). These infections are caused by a wide range of pathogenic bacteria that can be transmitted through contaminated food, water, air, or direct contact, and in many cases, through vector-related or environmental exposure (Ashbolt, 2015; WHO, 2015; CDC, 2024).

Some common examples of bacteria that cause infectious diseases in humans are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. In many regions, immunity against bacterial infections is not fully protective and repeated exposure may still result in recurrent infections, particularly in immunocompromised individuals. In Nigeria, bacterial infections remain a major primary healthcare challenge, especially among children and the elderly (Jonah *et al.,* 2024; The Guardian, 2024; Adewumi, 2025). They contribute significantly to childhood morbidity and mortality, as well as maternal health complications. This situation is worsened by the increasing resistance of bacteria to commonly used antibiotics, leading to high rates of treatment failure. Clinical resistance to multiple antibiotic classes has been reported in several regions worldwide, indicating that many bacterial strains have developed mechanisms to survive exposure to conventional antimicrobial agents (WHO, 2025[a]).

This strongly highlights the urgent need for continued research into new antibacterial agents with improved efficacy and novel mechanisms of action. The applications of Schiff base compounds and their complexes have gained a lot of attention because of their promise as new antibacterial compounds. The reason for their popularity is their structural diversity, together with their facile modification by coordination with a variety of metal ions (Malay and Ray, 2025).

Schiff bases, obtained by condensation of primary amines with aldehydes or ketones, contain the azomethine (C=N) function, which makes them

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biologically active molecules. They can vary greatly in their electronic, steric, and lipophilic characteristics due to their structural diversity.

Reports of Schiff bases with much greater antimicrobial effects when complexed with metal ions than as free ligands are well documented (Paswan *et al.,* 2026; Jorge *et al.,* 2024). According to chelation theory, metal complexation decreases the polarity of the metal ion and enhances lipophilicity. Consequently, it becomes easier for these complexes to enter bacterial cells, thus increasing their biological efficiency. At the same time, the presence of nitrogen of azomethine and other donating atoms (oxygen, sulfur) allows effective interaction with metals, resulting in the formation of chelates with a higher degree of stability and reactivity. Their transition metal complexes are especially useful because of the ability of many metal ions to exist in more than one oxidation state (for example, copper, cobalt, nickel, iron, and zinc). The redox behaviour of these complexes makes it possible for them to be involved in electron transfer reactions that interfere with biochemical reactions within bacteria. Moreover, they show great structural versatility in terms of tetrahedral, square planar, and octahedral geometry, which may affect their biological behaviour. The capacity of these complexes to adopt different coordination modes allows the adjustment of their pharmacological characteristics, like their solubility, stability, and specificity. Furthermore, the synthetic convenience, economic aspect, and the use of pharmacophoric substitution to increase antibacterial activity are some additional benefits.

The pyridine ring is one of the essential heterocyclic compounds that exhibit many biological activities; antibacterial activity is prominent among them (Marinescu & Popa, 2022; Islam *et al.,* 2023; Mattew *et al.,* 2023). Isoniazid (isonicotinic acid hydrazide), having a pyridine ring as its base structure, is known as an active biomolecule having remarkable complex formation properties owing to the presence of nitrogen-coordinating atoms. Thus, Schiff bases/metal complexes of Isoniazid have significance in antimicrobial applications (Hossain *et al.,* 2017; Camellia *et al.,* 2022; Jorge *et al.,* 2024; Suganya and Puthilibia, 2024; Islam *et al.,* 2026).

The coordination sites of the metal ions are enhanced due to the presence of the azomethine (-C=N-) group and the nitrogen atom of the pyridine ring from isoniazid in the Schiff base, thus facilitating chelation. This will be effective for enhancing the antibacterial action of the complexed metal ions by increasing their lipophilicity and membrane penetration ability. Considering these favourable attributes, the current paper describes the synthesis and antibacterial action of Isoniazid-based Schiff bases and their complexes with copper(II) and cobalt(II).

Materials and Methods

The highest purity chemicals were employed and obtained from Sigma Aldrich Chemicals Ltd, Germany. Solvents employed were of analytical grade without further purification.

Physical data and characterisation studies:

Uncorrected melting points (ºC) were determined by using the Stuart SMP3 melting point apparatus. Elements present in compounds were analysed by using a Perkin–Elmer 2400 CHNS/O elemental analyser. Electronic absorption spectra were recorded in DMF using a Cecil Super Aquarius 9000 series UV-vis spectrophotometer. Infrared spectra of complexes were obtained using the FTS 7000 series Digilab Win-IR Pro spectrometer attached with diamond ATR accessories in the range 4000 to 400 cm-1. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d6 solvent by using a Varian Mercury 300 MHz NMR spectrometer, and chemical shifts are reported relative to the solvent. Conductivities were measured in DMF solvent by using the DDS-307 conductivity meter.

General Synthesis of Schiff bases

To an agitated mixture of isonicotinic acid hydrazide (0.30mmol) in hot absolute ethanol (50ml) was added a mixture of the desired 5-substituted 2-hydroxybenzaldehyde in hot absolute ethanol (30ml). 0.5ml of glacial acetic acid was added to the above mixture and allowed to reflux for 8 hours. Slow evaporation of the reaction mixture yielded a white solid, which was collected using filtration. Recrystallisation from ethanol afforded pure samples of the Schiff bases.

N'-(2-hydroxy-5-bromobenzylidene)isonicotinoylhydrazide) (L1)

Yield: 9.13mg (95%); mp: 261-264°C; Rf: 0.78. IR(cm^{-1}): 3252, 3068, 2957, 1669, 1616, 1550, 1473, 1407, 1376, 1339, 1286, 1264, 1194, 1158, 1062, 997, 926, 853, 833, 795; ^{1}HNMR(300MHz, DMSO-d6) δH: 6.93(d, J=8.7Hz, 1H), 7.45 (dd, *J* 11.7Hz, 1H), 7.85 (m, 3H), 8.65 (s, 1H), 8.81 (d, *J* 5.4Hz, 2H), 11.14 (s, 1H); 12.36 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C: 111.09, 119.23, 121.84, 122.07, 130.60, 134.44, 140.41, 146.81, 150.91, 156.98, 162.02.

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Anal. calcd. for $C_{13}H_{10}BrN_3O_2$: C, 48.77, H, 3.15, N, 13.13. Found: C, 48.75 H, 2.79, N, 13.15.*(E)-N'-(2-hydroxy-5-methoxybenzylidene)isonicotinoylhydrazide) (L2)*
Yield: 9.62 mg (86%); mp: 205–207 ºC; R_f: 0.45. IR (cm^{-1}): 3188, 3053, 2835, 1649, 1613, 1580, 1543, 1490, 1366, 1332, 1299, 1268, 1206, 1177, 1116, 1085, 1035, 991, 842, 823, 782, 753, 727; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H: 3.72 (s, 3H), 6.95 (m, 2H), 7.18 (d, *J* 3.3Hz, 1H), 7.86 (d, *J* 5.7Hz, 2H), 8.68 (s, 1H), 8.81 (d, *J* 4.2Hz, 2H), 10.53 (s, 1H); 12.29 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C: 56.00, 112.22, 117.89, 119.21, 119.50, 122.08, 140.59, 148.77, 150.91, 152.09, 152.71, 161.93. Anal. calcd. for $C_{14}H_{13}N_3O_3$: C, 61.99, H, 4.83, N, 15.49. Found: C, 61.82, H, 4.87, N, 15.67.

General Procedure for the Preparation of Schiff Bases Metal Complexes (CuL1-CuL2 & CoL1-CoL2)

The solution of $CuCl_2 \cdot 2H_2O$ or $CoCl_2 \cdot 6H_2O$ (1.10 mmol) in ethanol: water (1:1, 5 mL) was added slowly to the hot solution of the respective ligand (1.00 mmol) in absolute ethanol (15 mL) with continuous stirring. The reaction mixture was stirred at room temperature for 1 h, followed by refluxing for 3 h. The resultant precipitate was filtered, washed thoroughly with a mixture of cold ethanol-water (1:1) and dried under vacuum over silica gel.

Cu-N'-(2-hydroxy-5-bromobenzylidene)isonicotinoylhydrazide) (Cu-L1)

Yield: 4.49 mg (58%); mp: 313-317 ºC. IR (cm^{-1}): 3049, 1597, 1499, 1448, 1415, 1379, 1352, 1285, 1176, 1030, 856, 816, 727, 690, 663, 645, 591, 541, 483.
Anal. calcd. for $C_{26}H_{26}Br_2CuN_6O_8$: C, 40.35, H, 3.39, N, 10.86, Cu, 8.21. Found: C, 40.00, H, 2.43, N, 10.57, Cu, 8.81.
Cu-N'-(2-hydroxy-5-methoxybenzylidene)isonicotinoylhydrazide) (Cu-L2)
Yield: 2.22 mg (33%); mp: >349.1 ºC. IR (cm^{-1}): 3246, 3054, 2834, 1677, 1616, 1539, 1489, 1419, 1344, 1287, 1259, 1145, 1030, 967, 762, 705, 601, 488.
Anal. calcd. for $C_{28}H_{32}CuN_6O_{10}$: C, 49.74, H, 4.77, N, 12.43, Cu, 9.40. Found: C, 49.68, H, 3.96, N, 12.20, Cu, 8.89.
Co-N'-(2-hydroxy-5-bromobenzylidene)isonicotinoylhydrazide) (Co-L1)
Yield: 4.16 mg (60 %); mp: 254-257 ºC. IR (cm^{-1}): 3015, 1598, 1539, 1436, 1419, 1288, 1201, 1153, 1066, 713, 688, 585, 508, 464.
Anal. calcd. for $C_{26}H_{18}Br_2CoN_6O_4$: C, 45.69, H, 3.54, N, 16.40, Co, 8.62. Found: C, 45.70, H, 3.12, N, 16.00, Co, 8.72.
Co-N'-(2-hydroxy-5-methoxybenzylidene)isonicotinoylhydrazide) (Co-L2)
Yield: 2.81 mg (68 %); mp: 268.8 ºC (dec.). IR (cm^{-1}): 3247, 1677, 1615, 1574, 1538, 1489, 1373, 1278, 1257, 1029, 966, 929,895,879, 763, 680, 597, 498.
Anal. calcd. for $C_{14}H_{14}ClCoN_3O_4$: C, 43.94, H, 3.69, N, 10.98, Co, 15.40. Found: C, 43.88, H, 3.88, N, 10.91, Co, 15.12.

Antibacterial Studies: The antibacterial properties of the ligand and metal complexes were evaluated using the agar well diffusion method (Dueke-eze *et al.,* 2023; cited from Valgas *et al.,* 2007). Stock solutions were obtained by dissolving 5 mg of each sample in 1 mL of N, N-dimethylformamide (DMF). The prepared samples were tested against *Staphylococcus aureus, Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The inoculated Mueller-Hinton agar plates were allowed to stand for about 2 minutes following the seeding of standardized bacterial cultures. Sterile holes were made on the inoculated plates with a sterile cork borer and subsequently filled with the test samples using a micropipette. Incubation was done at 37 °C for 24 hours, allowing diffusion of the samples and inhibiting bacterial growth. The diameters of the zone of inhibition around each well were measured and documented. Control tests were also carried out where only the DMF solvent was placed in the wells.

Results and Discussion: Synthesis:
The condensation reaction of INH with the respective 5-substituted-2-hydroxybenzaldehydes led to the formation of two Schiff bases, which included *N'--(2-hydroxy-5-bromobenzylidene)isonicotinoylhydrazide (L1) and N-(2-hydroxy-5-methoxybenzylidene)isonicotinoylhydrazide (L2).*

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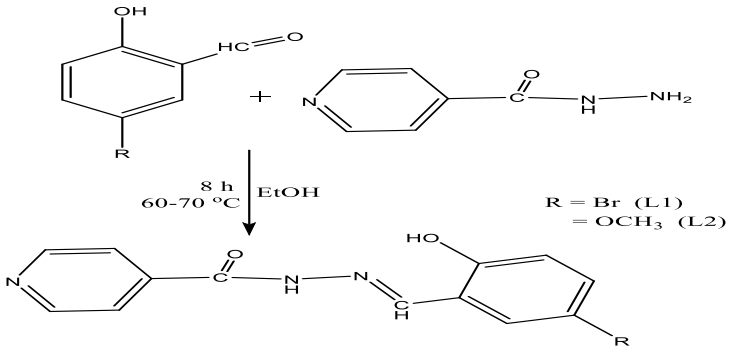

Scheme 1: Synthetic Route to Schiff Base Formation

The compounds were obtained in moderate to high yield in the range 45-95%. The chemical formula of the compounds was ascertained according to their elemental (CHNM) composition, which was consistent with the theoretical prediction. It is worth mentioning that the melting points of all the synthesized Schiff bases and metal complexes were sharp, indicating good quality and purity of the products. Table 1 summarises the physical and analytical data of the synthesized compounds

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Table 1: Physical and analytical data for the ligands and their metal complexes

Compound code	Molecular formular (M.wt. (g/mol))	mp: (°C)	Yield(%)	Microanalysis: % Calculated (Found)			
				C	H	N	M
L1	$C_{13}H_{10}BrN_3O_2$ (320)	261-264	95	48.77 (48.75)	3.15 (2.79)	13.13 (13.15)	=
Cu-L1	$C_{26}H_{26}Br_2CuN_6O_8$ (774)	313-317	58	40.35 (40.00)	3.39 (2.43)	10.86 (10.57)	8.21 (8.81)
Co-L1	$C_{26}H_{18}Br_2CoN_6O_4$ (697)	254 -257	60	44.79 (45.63)	2.60 (3.57)	12.05 (12.54)	8.45 (8.51)
L2	$C_{14}H_{13}N_3O_3$ (271)	205-207	86	61.99 (61.82)	4.83 (4.87)	15.49 (15.67)	-
Cu-L2	$C_{28}H_{32}CuN_6O_{10}$ (675)	>349	45	49.74 (49.68)	4.77 (3.96)	12.43 (12.20)	9.40 (8.89)
Co-L2	$C_{14}H_{14}ClCoN_3O_4$ (382)	>349	68	43.94 (43.88)	3.69 (3.88)	10.98 (10.91)	15.40 (15.12)

M.wt = molecular weight; mp = Melting point

Singlet peaks were observed in 1Hand 13NMR spectra for the ligands corresponding to HC=N protons and OH protons at δH 8.65 and 12.36 ppm for L1 and δH 8.68 and 12.29 ppm for L2. Signals due to all carbon atoms have been resolved in the 13C NMR spectra. Therefore, the structure of the ligand is confirmed from the above NMR spectral data.

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Table 2: Characteristic IR (cm^{-1}) and NMR bands of Schiff bases and their metal complexes

	IR bands (cm^{-1})					Chemical shift (ppm)			
						HC=N		C-OH	
Code	*ν*OH	*ν*C=N	*ν*C=O	*ν*(M-O)	*ν*(M-N)	δ_H	δ_C	δ_H	δ_C
L1	3252	1616	1669	-	-	8.65	140.41	12.36	156.98
Cu-L1	-	1597	-	541	483	-		-	
Co-L1		1598	-	508	464	-		-	
L2	3188	1613	1649	-	-	8.68	140.59	12.29	152.71
Cu-L2		1616	1677	601	488	-		-	
Co-L2		1615	1677	597	498	-		-	

The reaction of the Schiff bases with copper (II) and cobalt (II) dichloride in ethanol gave rise to the required metal complexes. Comparison of the infrared spectrum of the Schiff base ligands with that of the metal complexes was used to establish the way of bonding the Schiff bases in the complexes. The shifting of the azomethine (C=N) nitrogen absorption at 1616 and 1613 cm-1, as well as the absence of νOH in the complexes, shows that the azomethine nitrogen and phenolic oxygen atoms participate in the metal-ligand complex formation (Suganya and Puthilibia, 2024). Furthermore, the carbonyl absorption disappears or shifts to shorter or longer wave numbers. All this suggests that Schiff bases act as tridentate ligands bonding through azomethine nitrogen, phenolic, and carbonyl oxygen atoms (Table 2). This is also supported by the presence of new peaks at 508–601 cm-1, 464–498 cm-1, attributed to M-O and M-N, respectively.

Antibacterial Activity: The results obtained after the antibacterial testing of the synthesized compounds were analyzed. Table-3 gives details of the assay done for the test of antibacterial activity on various compounds at different concentrations (0.500,0.250, 0.125 and 0.625 mg/ml) against various Gram-positive and Gram–negative bacteria strains, including *S. aureus, E.feacalis*, *E. coli* and *P. aeruginosa*. The results showed that there is a notable difference in the antimicrobial activity based on the structure of the compound, the type of metal ion, and the bacteria. In particular, it is worth noting the high level of activity against the *Staphylococcus aureus* bacteria for almost all the compounds tested, exhibiting 3+ activity. This suggests that the compounds have high inhibitory activity against Gram-positive bacteria, making them promising candidates for antiseptic development. On the other hand, the action against Gram-negative bacteria like *E. coli* and *P. aeruginosa* was generally poor or not observed at all. The reason for this is probably because of the complicated nature of the Gram-negative bacteria, especially because they possess an outer lipopolysaccharide layer.

There were a few instances where there was an increase in the antibacterial activity upon the complexation with metal, which proves the chelation theory that states that chelation makes the metal less polar and more lipophilic (Tarallo *et al.,* 2010; Joseph *et al.,* 2012). Moreover, the presence of an electron-donating methoxy group in the ligands is also believed to play an important role in increasing the electron density on the aromatic ring of L2, which increases its interaction with the biological targets, thus explaining the difference in activities between the L1 and L2 series.

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Table 3: Antibacterial Activity of L1-CoL2

Compound	Conc (mg/ml)	*S.aureus*	*E.feacalis*	*E.coli*	*P.aeruginosa*
LI	0.500	3+	2+	0	0
	0.250	3+	1+	0	0
	0.125	2+	0	0	0
	0.625	0	0	0	0
Cu-L1	0.500	3+	0	1+	0
	0.250	3+	0	0	0
	0.125	1+	0	0	0
	0.625	1+	0	0	0
Co-L1	0.500	3+	3+	3+	0
	0.250	3+	0	0	0
	0.125	3+	0	0	0
	0.625	1+	0	0	0
L2	0.500	3+	0	3+	1+
	0.250	3+	0	0	0
	0.125	1+	0	0	0
	0.625	1+	0	0	0
Cu-L2	0.500	3+	0	0	0
	0.250	3+	0	0	0
	0.125	2+	0	0	0
	0.625	1+	0	0	0
Co-L2	0.500	3+	0	0	0
	0.250	3+	0	0	0
	0.125	3+	0	0	0
	0.625	1+	0	0	0
Control	0.500-0.625	0	0	0	0

Inhibition values = 1 - 5 mm = 1+ (less active); 6 - 11 mm = 2+ (moderate active); >12 mm = 3+ (highly active) , 0 = not detected

Conclusion

Two Schiff bases and their corresponding Cu(II) and Co(II) complexes were successfully synthesized and evaluated for antibacterial activity against selected bacterial strains. The results indicate that metal complexation enhanced the antibacterial performance of the ligands, while the electron-donating methoxy (-OCH_3 for L2) substituent appeared to contribute positively to biological activity. Notably, the compounds showed pronounced effectiveness against *S. aureus*, suggesting their potential for further investigation as antiseptic agents.

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