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Research Article

Spectral Investigation and *In Vitro* Antibacterial Evaluation of Ni^{II} and Cu^{II} Complexes of Schiff Base Derived from Amoxicillin and α-Formylthiophene (αft)

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Two new metal complexes of general formula $M(Ha\alpha ft)_2$ [M = Ni^{II} and Cu^{II}] of asymmetrical Schiff base ligand (HL = Haαft) derived from amoxicillin and α-formylthiophene have been prepared and characterized by various physicochemical and spectral techniques. Molar conductance measurement indicates nonelectrolytic nature of the metal complexes. FT-IR spectral study reveals the ligation of metal ions at two different nitrogen [NN] donor sites of Haαft. FT-IR and electronic absorption spectral evidences suggest distorted tetrahedral and square planar geometry for Cu^{II} and Ni^{II} complexes, respectively. The structure optimization by molecular mechanics (MM) force field calculation through ArgusLab 4.0.1 version software also supports the concerned geometry of the complexes. The cell dimensions as suggested by XRPD study, a (6.753 Å), b (13.904 Å), c (20.122 Å), α (142.76°), β (106.580°), and γ (72.4343°) for Cu^{II} and a (24.2547 Å), b (6.6371 Å), c (5.5047 Å) ($\alpha = \beta = \gamma = 90°$) for Ni^{II} complexes, are in good agreement with their triclinic and orthorhombic crystal systems. Particle size calculation by Scherrer's formula indicates nanocrystalline nature of the complexes. The antibacterial sensitivity study suggests promising activities of Haαft (Ligand) and $M(Ha\alpha ft)_2$ complexes against four clinical pathogenic bacteria, namely, *E. coli*, *P. vulgaris*, *P. aeruginosa*, and *S. aureus*, though being less active than the standard drug amikacin.

1. Introduction

Increasing threat of drug resistance caused by several microorganisms is today's most debated public health concern. The development and modifications in the design of previous antibiotics, with new modes of action, requires intensive research to overcome these infections [1]. Schiff bases are the chemical compounds prepared by the condensation of active carbonyl groups with primary amines and have active azomethine linkage. Due to simplicity in preparation and wide range of applications in medical, biochemical, and industrial fields, Schiff bases are gaining much popularity in the recent years [2]. Presence of various coordination sites makes them as privileged ligands in the coordination chemistry of transition metals [3]. Schiff bases have important applications in the medical science as a potent antibacterial [4], antitumor [5], antiviral [6], and anti-inflammatory drugs [7]. The pharmacological activity of this class of compounds is often enhanced by complexation with metal ions. Serendipitous finding of cisplatin as a potent anticancer drug opened the gate of unexplored world of metal based chemotherapeutic agents [8, 9]. Recently, metal based drugs are gaining significant importance in medical sciences, due to their increased potentiality that actively interrupts the growth eager mechanism of microorganisms. The central metal ion in metal based drugs is usually the key feature of the mechanism of action. Several medical problems that arise due to free metal ion toxicity may be ameliorated by chelating agents like Schiff bases [10]. Amoxicillin is a very important class of β-lactamic antibiotic used in clinical therapy due to its specific

toxicity towards bacterial pathogens [11]. Broad spectrum activity of amoxicillin is related to better oral absorption and high concentrations in blood compared to ampicillin which effectively works against bacteria. However, the failure in its antibacterial activity has been attributed to the formation of β-lactamase enzyme which hydrolyzes the β-lactam ring of the molecule [12]. Several research findings in the medical science put amoxicillin as an abortive drug and require modifications, either by changing the ligand property or by complexation with metal ions. With this expectation, metal complexes of amoxicillin derived Schiff base will be the new hope for better drug substance in clinical practices. In the present investigation, we report here the synthesis, spectral characterization, coordination behavior, X-ray powder diffraction study, and antibacterial evaluation of Ni^{II} and Cu^{II} complexes of Schiff base ligand (Haαft) derived from amoxicillin and α-formylthiophene. The work has been extended to assure the correct geometry by MM2 calculations supported in CsChemOffice 3D ultra and ArgusLab 4.0.1 version software.

2. Experimental Section

2.1. Materials. All the chemicals and solvents used in the experiment were of analytical reagent grade (AR). They included amoxicillin trihydrate (Duchefa Biochemie, Netherland), α-formylthiophene (Spectrochem, Mumbai, India), and metal salts, namely, $NiCl_2{\cdot}6H_2O$ and $CuCl_2{\cdot}2H_2O$ (Merck). Distilled methanol (Qualigen) was used as solvent for the synthesis. All the glassware used in the research was of high grade borosilicate glass and was cleaned by triple distilled water. For the antibacterial sensitivity study, the bacterial cultures were prepared in Muller Hinton's nutrient agar media (Himedia Co.).

2.2. Measurements. The elemental microanalysis (C, H, and N) of the complexes was analyzed on Elementar vario EL III (Germany) model Analyzer. Double-check method was done to get the accuracy of the data. The pH measurement was done in Elico-16 pH meter. The electronic absorption spectra that reveal d-d and π-π and n-π transitions of the ligand and its complexes were recorded in single beam microprocessor Labtronics UV-Vis. spectrophotometer (LT-290 model) using DMSO as the solvent and internal reference chemical in 200–800 nm range. Characteristic shifts in IR band positions had been analyzed by intimate observations of infrared spectra of ligand and metal complexes that were run as KBr discs in the range 4000–400 cm^{-1} on a Perkin Elmer 783 FT-IR spectrophotometer. ^1H NMR spectra were recorded in DMSO-*d*6 solvent on a Bruker advance 400 MHz instrument using TMS as internal reference. ESI-MS spectra were accomplished on Agilent 6520 Q-TOF mass spectrometer equipped with an electron spray ionization source in the mass range of 200 to 1100. The XRD powder pattern was recorded on a vertical type Philips PW 1130/00 X-ray diffractometer, operated at 40 kV and 50 Ma generator using the monochromatised Cu-Kα line at wavelength 1.54056 Å as the radiation source and measurements were taken over the range of 2θ (10 to 80°). Crystallographic data were analyzed by X′Pert High Score, CRYSFIRE, and CHECKCELL software programme. The powder XRD pattern of the ligand and metal complexes was recorded in Xpowder software. The possible geometries of the metal complexes were evaluated by using the molecular modeling calculation, optimized by CsChem 3D Utra-11 programme and ArgusLab 4.0.1 version software.

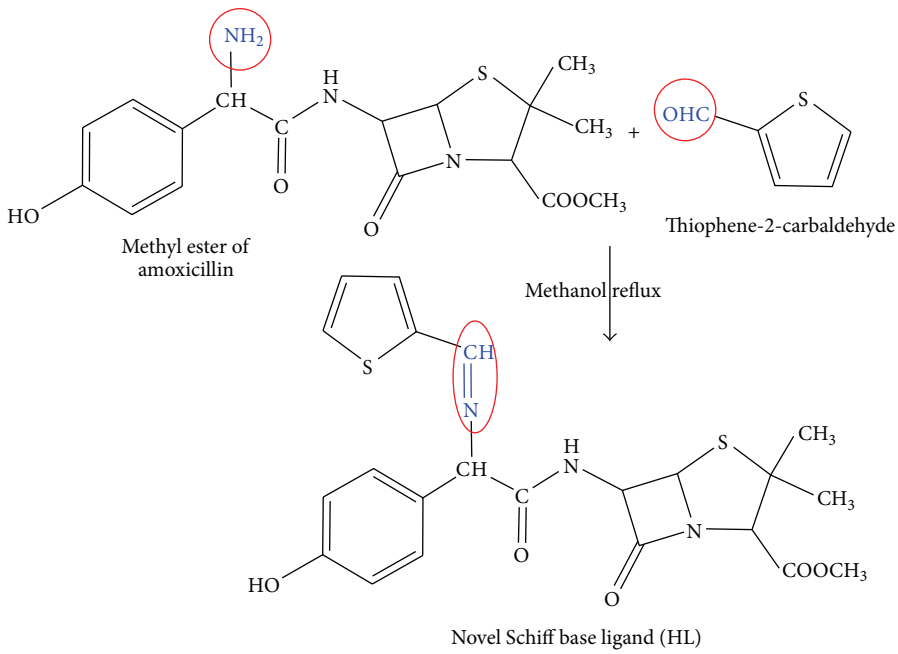
2.3. Synthesis of Ligand (HL = Haαft). To the hot and homogenously stirred solution of amoxicillin trihydrate (5 mmol, 2.097 gm) in distilled methanol (30 mL), α-formylthiophene (5 mmol, 0.47 mL) was added dropwise with constant stirring and the resulting solution was refluxed for about 6 h. The pH of the amoxicillin trihydrate solution had been adjusted to 8 by dropwise addition of alcoholic NaOH solution [13]. The solution was concentrated and allowed to cool. The bright yellow solid product was separated by slow diffusion process, purified by recrystallization from hot methanol and dried under vacuum. The synthetic design for the preparation of ligand (HL) is presented in Scheme 1.

2.4. Synthesis of Metal Complexes. In hot 30 mL methanolic solution of ligand (L) (4 mmol, 1.89 gm), 10 mL methanolic solution of the metal salts (2 mmol, 0.4752 gm $NiCl_2{\cdot}6H_2O$, and 0.34 gm $CuCl_2{\cdot}2H_2O$) was added dropwise under constant stirring over magnetic stirrer. The reaction mixture was refluxed for several hours under water bath and alternate stirring of the mixture was also done to facilitate the easy approach of ligand for coordination with metal ion. Colored solid complexes were formed on cooling the solution slowly at room temperature. The solid was filtered, washed with methanol, and dried under vacuum [14, 15]. The general structure of metal complex is presented in Figure 1:

$$MX_2 + 2HL \xrightarrow{\text{Reflux}} ML_2 \quad (1)$$

where MX_2 is Ni^{II} & Cu^{II} chloride salts. HL is Ligand. Ni^{II}-complex is $Ni(Ha\alpha ft)_2$. Cu^{II}-complex is $Cu(Ha\alpha ft)_2$.

2.5. Antibacterial Assay. The experimental portion of antibacterial study was carried out in microbiology laboratory of MMAM campus Biratnagar. The ligand (HL) and its metal complexes were screened *in vitro* for antibacterial sensitivity study against three Gram-negative and one Gram-positive bacterial strains using modified Kirby-Bauer paper disc diffusion method [16]. Mueller-Hinton's nutrient agar media were applied for the bacterial growth. Fresh cultures of standard bacteria, namely, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, were inoculated separately in five mL of tryptone soya broth, maintaining pH at 7.4 for better growth. The broth was incubated for 8 h at 37°C until visible growth changes to opaqueness. The broth was spread over nutrient agar media, prepared as usual in Petri plates using sterile stick swab and the well-sterilized paper discs of five mm diameter (Whatman number 1) impregnated with test compounds at two different concentrations, 1 and 0.1 $\mu g/\mu L$, in DMSO were stuck on the previously seeded bacterial culture [17]. The growth inhibition was compared with the standard drug amikacin of 30 μg per disc of six mm size (Himedia Co.). DMSO, which exhibited no antimicrobial activity against the test bacterial pathogens,



Scheme 1: Proposed route for the synthesis of ligand (HL).

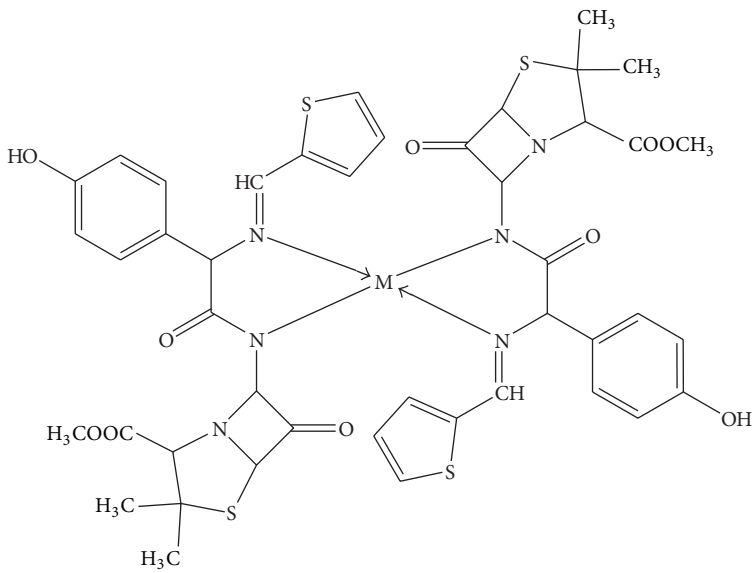


Figure 1: Structure of metal complex (M = Ni & Cu).

was used as negative control. Afterwards, the Petri plates were incubated at 37°C and the inhibition of the growth of organisms, evidenced by clearing zone surrounding each disc, was measured by using antibiogram zone measuring scale, after 36 h of incubation [18].

3. Results and Discussion

The carboxylic acid group (-COOH) of amoxicillin undergoes esterification with methanol. Esterified amoxicillin now interacts with α-formylthiophene for the formation of ligand. The ligand was soluble in methanol and polar solvents like DMSO and DMF. The melting points of ligand (195°C) and metal complexes (>210°C) suggested that the complexes are more stable than the ligand and all of them are air and moisture stable. IR spectral measurements are completely consistent with the proposed formulation of the ligand and complexes.

3.1. Physical Data and Microelemental Analysis. The Schiff base ligand and its metal complexes were subjected to elemental analysis. The results of elemental analysis with molecular formula and other physical data like melting points and color change of the compounds are presented in Table 1.

Table 1: Elemental analysis and physical properties measurement data.

Compound	Mol. formula	Mol. wt.	Colour	m. pt. (°C)	Calculated (found) (%)						pH
					C	H	N	O	S	M	
HL	$C_{22}H_{23}N_3O_5S_2$	473.57	Bright yellow	195	55.80 (55.68)	4.90 (4.91)	8.87 (8.82)	16.89 (16.75)	13.54 (13.59)	—	4.97
$Ni(Ha\alpha ft)_2$	$C_{44}H_{44}N_6NiO_{10}S_4$	1003.81	Brown	230	52.65 (52.67)	4.42 (4.421)	8.37 (8.29)	15.94 (15.87)	12.78 (12.75)	5.85 (5.94)	2.98
$Cu(Ha\alpha ft)_2$	$C_{44}H_{44}CuN_6O_{10}S_4$	1008.66	Black	220	52.39 (52.50)	4.45 (4.55)	8.33 (8.21)	15.86 (15.81)	12.72 (12.80)	6.30 (6.15)	2.5

Table 2: Characteristic IR band positions (cm^{-1}) of ligand and metal complexes.

Compound	ν(NH)	ν(C=N)	ν(C=O) lactam	$\nu(CH_3)$	ν(COO)	ν(M-N)
HL	3485.12 (br)	1661.56 (s)	1599.85	2965.69 (s)	1515.18	—
$Ni(Ha\alpha ft)_2$	3432.69 (br)	1651.25 (s)	1601.89	2963.51 (s)	1514.98	470.18
$Cu(Ha\alpha ft)_2$	3423.36 (br)	1652.09 (s)	1602.88	2961.96 (s)	1514.20	448.82

The results are in good agreement with suggested molecular formula. Sharp melting point of compounds indicates the purity of the prepared compounds [19].

The change in color of the ligand and its metal complexes from the parent compounds used for their preparation is the good indication of formation via chemical reaction [20]. Complexation with metal ions exhibited drop down of pH values due to deprotonation of ligands. In our work, decrease in pH values from ligand to metal complexes also confirms complexation [21].

3.2. Spectral Analysis

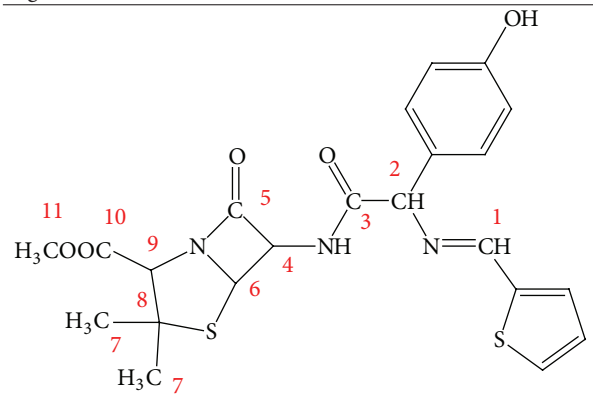
3.2.1. FT-IR Analysis. The most significant IR spectral bands are presented in Table 2. The IR spectral data comparison of the reported complexes with those of the uncomplexed ligand provides valuable information about the coordination sites of the ligand. Shift in the positions of strong intensity band at 1661.56 cm^{-1} characteristic for azomethine (HC=N) stretch for ligand to lower wave numbers 1652.09 and 1651.25 cm^{-1} for Cu^{II} and Ni^{II} complexes, respectively, support the coordination of the azomethine nitrogen to the metal centre [22]. This fact is further substantiated by the appearance of new bands below 500 cm^{-1} assignable for metal-nitrogen coordination. The medium intensity band at 448.82 cm^{-1} is assignable for Cu-N stretch and similar band for Ni^{II} complex appears at 470.18 cm^{-1}. There was no shift in band positions observed for the stretch of carboxylate group (-COO-), which appeared at 1515.18, 1514.20, and 1514.98 cm^{-1} characteristic for ligand, Cu^{II}, and Ni^{II} complexes, respectively [23]. This indicates no involvement of carboxylate group for the coordination with metal ions. The negative band shift from 3485.12 cm^{-1} assignable for N-H stretch in ligand provides good conformity for the coordination of metal ions with deprotonated nitrogen (NH) group of substituted amide [24]. M-N coordination mode is further ensured by the appearance of bands at 3423.36 and 3432.69 cm^{-1} for Cu^{II} and Ni^{II} complexes, respectively. A weak sharp band observed at 1599.85 cm^{-1} assignable for ν(C=O) lactam is restored in the metal complexes [25]. Lactam (C=O) stretch band in Cu^{II} and Ni^{II} complexes appeared at 1602.88 and 1601.89 cm^{-1}, respectively [26]. The bands in the range of 2961–2965 cm^{-1} in all the synthesized compounds could be assigned for the C-H stretch of methyl group [27].

Table 3: The ^{1}H NMR spectral data (δ, ppm) of ligand.

Compound	Chemical shift δ ppm	Assignment
HL	6.7–7.0	2d, 4H, aromatic protons
	9.95	s, 1H, azomethine protons
	7.1–8.0	m, 3H, thiophene ring protons
	0.9–1.4	s, 6H, methyl protons
	3.4–4.1	s, 3H, CH_3 of ester group
	8.5	s, 1H, NH proton

3.2.2. ^{1}H NMR Analysis. ^{1}H NMR spectral comparison of novel ligand and its metal complexes was made to confirm the binding mode of ligand with metal ions. The ^{1}H NMR spectra of the ligand are shown in Figure 2 and the spectral data are summarized in Table 3. The ^{1}H NMR spectral peak at 2.5 ppm in the spectra of ligand is assignable to DMSO solvent protons. The peaks in the region of 0.9–1.4 ppm are suggesting the peak for methyl protons present in the amoxicillin moiety, near the lactam ring [28]. Although Ni^{II} complex is diamagnetic, having square planar geometry, it offers nominal changes in the chemical shift values of different types of protons compared to the ligand. The azomethine proton appears as a singlet at 9.95 ppm for the ligand and at 10.038 ppm for Ni^{II} complex [29]. All four aromatic protons as a set of two doublets appear in the range of 6.6–7.3 ppm. In addition to this, methyl protons of ester group produced by esterification of carboxylic acid group of amoxicillin with methanol show singlet in the region of 3.3–4.7 ppm [30]. Thiophene ring has three protons which appear as a multiplet in the region of 7.1–8.7 ppm in the spectrum of the ligand. The ligand showed

Table 4: The ^{13}C NMR spectral data (δ, ppm) of the free ligand and metal complexes.

Ligand (HL)	Assignment	Chemical shift δ ppm
	C-1	157.594
	C-3	172.668
	C-5	170.8
	C-7	26.909
	C-8	26.909
	C-9	73.378
	C-10	178.399
	C-11	39.928
	Ar-C	115.652, 121.217, 128.318, 129.44, 136.56, 138.423, 145.635
	DMSO	39.65

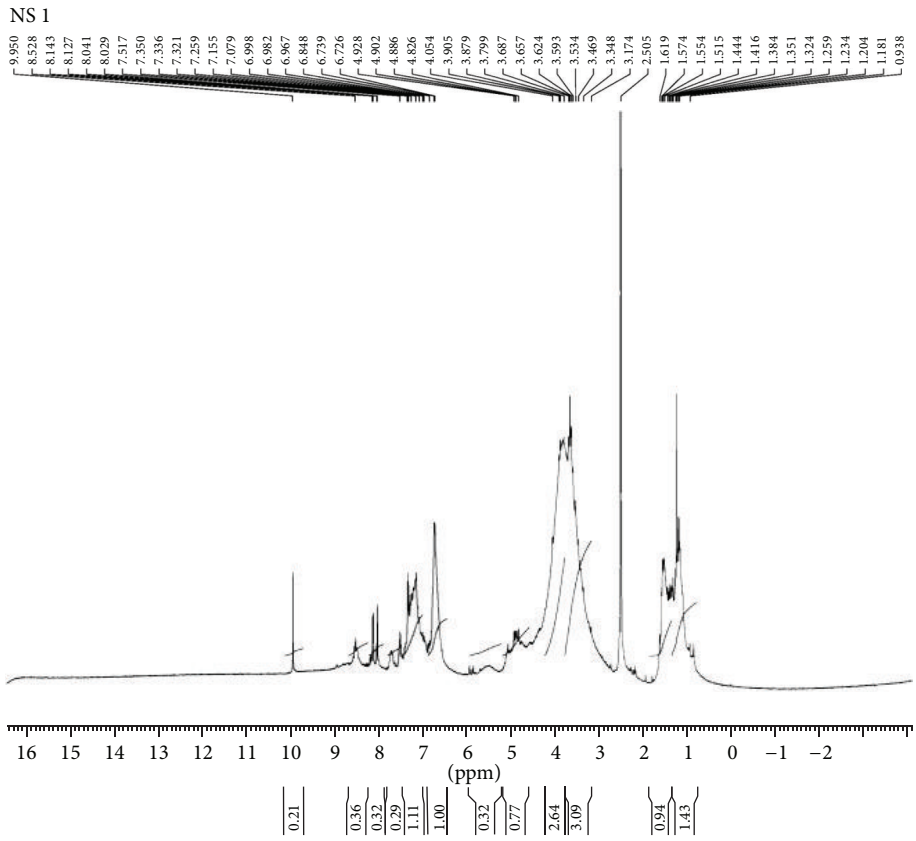


Figure 2: ^{1}H NMR spectrum of HL.

a singlet peak at 8.5 ppm assignable to NH proton [31]. It is important to mention here that no clear appreciable changes in ^{1}H NMR spectrum had been reported for Cu^{II} complex due to paramagnetic effects [32]. All these results indicated the participation of NH proton in the coordination process with metal ions.

3.2.3. ^{13}C NMR Analysis. The ^{13}C NMR spectral data for novel ligand has been recorded in DMSO. The measured chemical shifts of all the carbon atoms, as numbered in the structure of novel ligand, are listed in Table 4. As given in Figure 3, the azomethine carbon signal for the ligand appears at 157.594 ppm [33]. The signal for the carbon of carbonyl group (C-3) appears at 172.668 ppm in the spectrum of ligand [34]. Aromatic carbons showed signals in the range of 115–145 ppm. The carbon signal at 170.8 ppm is attributed to lactam carbonyl carbon. Due to resonance effect in diamagnetic and paramagnetic nature of the mentioned metal complexes, there was no marked change in their ^{13}C NMR spectra, compared to the ligand.

Table 5: Crystal data and structure refinement.

Compounds	HL	$Ni(Ha\alpha ft)_2$	$Cu(Ha\alpha ft)_2$
Formula	$C_{22}H_{23}N_3O_5S_2$	$C_{44}H_{44}N_6NiO_{10}S_4$	$C_{44}H_{44}CuN_6O_{10}S_4$
FW	473.57	1003.81	1008.66
Temp (K)	298	298	298
Wavelength	1.54056	1.54056	1.54056
Crystal system	Triclinic	Tetragonal	Triclinic
Space group	P1	P/4 mm	P1
		Unit cell dimension	
a (Å)	15.009	24.2547	6.753
b (Å)	20.077	6.6371	13.904
c (Å)	20.060	5.5047	20.122
α°	55.910	90	142.76
β°	65.330	90	106.580
γ°	59.920	90	72.4343
Volume (A^3)	4295.95	859.4463	1047.72
θ range (°)	5–75	5–75	5–75
Limiting indices	$-1 \le h \le 6$ $0 \le k \le 2$ $1 \le l \le 0$	$-2 \le h \le 3$ $0 \le k \le 3$ $1 \le l \le -2$	$1 \le h \le 0$ $0 \le k \le 2$ $-2 \le l \le 0$
Particle size (nm)	55.325	75.231	85.451

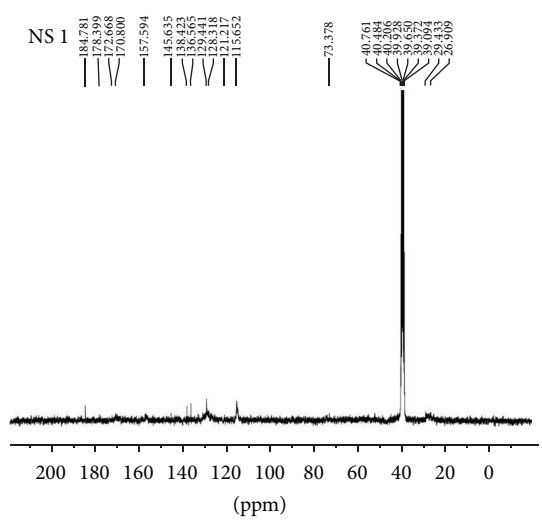


Figure 3: ^{13}C NMR spectrum of HL.

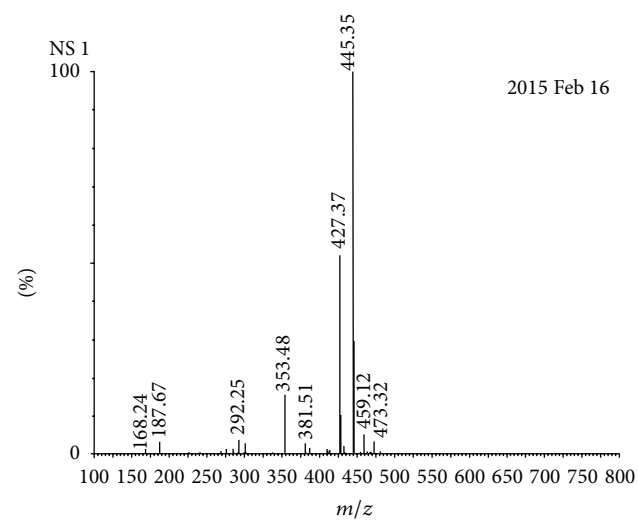


Figure 4: Mass spectrum of ligand (HL).

3.2.4. ESI-MS Analysis. The ESI mass spectrum of ligand (HL) offered a peak at m/z 473 amu corresponding to its molecular ion peak $[M^{\bullet+}]$ and it confirms the proposed molecular formula of the ligand. Other peaks in the mass spectrum of HL at m/z 459, 445, 353, 187, and 168 amu are the fragment peaks. Their intensities provide the idea of stability of the fragments. ESI mass spectrum of the complexes indicated the molecular ion peaks at m/z 1003 and 1008 amu characteristic for Ni^{II} and Cu^{II} complexes, respectively. The mass spectrum of ligand is presented in Figure 4.

3.2.5. X-Ray Powder Diffraction Study. The X-ray powder diffraction technique was carried to get useful crystal data information to deduce accurate cell parameters, crystal system, and the cell volume. Powder diffraction patterns of the ligand and metal complexes were recorded and crystallographic data are listed in Table 5. X-ray diffraction powder pattern of Cu^{II} complex is shown in Figure 5. The crystallite size of the ligand and its metal complexes was calculated from Scherrer's formula, $d_{XRD} = 0.9\lambda/\beta\cos\theta$, where λ is the wavelength, β is the full-width half maximum of the characteristic peak, and θ is the diffraction angle for the hkl plane [35, 36]. The diffractogram of ligand gave 13 reflection peaks with 100% intensity at $2\theta = 26.7349^\circ$. Associated with this, the interplanar distance was $d = 3.3348$ Å. The 26 reflections have been recorded for Cu^{II} complex with maxima at

Table 6: The relative peak positions (λ_{max}) of the free ligand and metal complexes.

Compound	Peak positions (λ_{max}) in nm			
	$\pi \rightarrow \pi^*$ transitions	n $\rightarrow \pi^*$ transitions	LMCT band	d-d transition
HL	310–268	391, 361	—	—
Ni(Haαft)$_2$	307, 272	391, 370	465	520
Cu(Haαft)$_2$	285	370	450	—

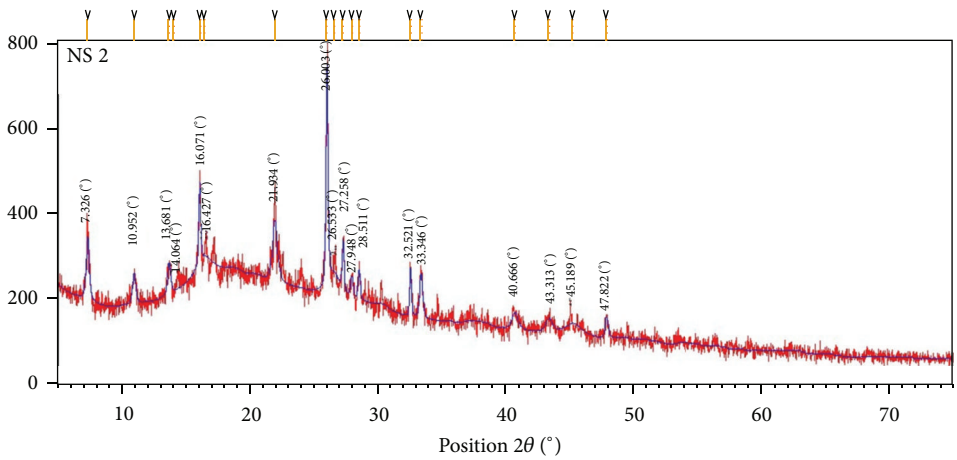


Figure 5: Powder XRD pattern of Cu(Haαft)$_2$.

$2\theta = 26.0048°$ and interplanar distance $d = 3.4265$ Å. Unit cell dimensions of ligand a (15.009 Å), b (20.077 Å), c (20.060 Å), α (55.910°), β (65.330°), and γ (59.920°) confirm its triclinic crystal system. a (6.753 Å), b (13.904 Å), c (20.122 Å), α (*142.76*°), β (106.580°), and γ (72.4343°) data for the Cu^{II} complex also confirms triclinic crystal system. Ni^{II} complex has unit cell dimensions a (24.2547 Å), b (6.6371 Å), and c (5.5047 Å) ($\alpha = \beta = \gamma = 90°$) and this confirms orthorhombic crystal system (Table 5). Particle size calculation data, 55.325, 85.451, and 75.231 nm for the ligand, Cu^{II}, and Ni^{II} complexes, respectively, revealed their nanocrystalline nature.

3.2.6. Molar Conductance and Magnetic Properties of the Complexes. Molar conductance values of the soluble complexes in DMSO (1×10^{-3} M) at room temperature vary from 5.1 to 20.1 Ω^{-1} cm^2 mol^{-1} revealing their nonelectrolytic nature. The observed range is below 50 Ω^{-1} cm^2 mol^{-1} characteristic for nonelectrolytes [37]. The room temperature magnetic moments for tetra coordinated Ni^{II} and Cu^{II} complexes indicate their respective diamagnetic and paramagnetic nature. Ni^{II} complex has observed magnetic moment value 0 BM, which is consistent with its square planar geometry, using dsp^2 hybridization in the coordination process. Cu^{II} complex in a distorted tetrahedral geometry has observed magnetic moment value μ_{eff} = 1.84 BM, which is greater than the theoretical spin only magnetic moment value, μ_{SO} = 1.73 BM. This is considered possibly due to orbital contribution that sum up in the spin interaction magnetic momentum [38]. The Jahn-Teller distortion effect in the Cu^{II} complex forced it to acquire distorted tetrahedral geometry.

3.2.7. Electronic Absorption Spectra. The electronic spectra of the Schiff base ligand and its metal complexes were recorded in DMSO solution and are presented in Table 6. The absorption peaks at the region of 361 and 391 nm of high intensity for ligand are attributable to intraligand n $\rightarrow \pi^*$ transitions of azomethine and lactam carbonyl group [39, 40]. Similar peaks at 268 and 310 nm for the ligand are assignable to intraligand $\pi \rightarrow \pi^*$ transitions. These peaks are still present in Ni^{II} and Cu^{II} complexes, but slightly shifted from the ligand peaks. The complexes of Ni^{II} and Cu^{II} show the low intensity bands in the visible region at 465 and 450 nm, respectively, which are assignable to the $^1A_{1g} \rightarrow {}^1B_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions for ligand metal charge transfer band [41]. The electronic spectrum of the Ni^{II} complex exhibits a single band at 520 nm which may be assignable to the $^1A_{1g} \rightarrow {}^1B_{2g}$, d-d transition typical for the square planar geometry [42]. As this d-d transition is a high energy process, it may probably confirm its square planar geometry. The lack of any electronic transitions at higher wavelengths indicates large crystal field splitting and is consistent with the said geometry for the Ni^{II} complex [43]. Absence of d-d transition band in the spectra of Cu^{II} complex probably indicates distorted tetrahedral geometry, which is also supported by other spectral studies.

3.3. Antibacterial Sensitivity Study. The pure metals are considered biologically inactive. However, the activity of metal cations depends on their bioavailability and the presence of bioligands [44]. It was, therefore, of great interest to investigate antibacterial activity of Schiff base and its transition metal complexes against some bacterial pathogens. Here, four

Table 7: Antibacterial sensitivity of synthesized compounds.

Compounds	Conc. (μg/μL)	Diameter of zone of inhibition in (mm)			
		E. coli	*P. vulgaris*	*P. aeruginosa*	*S. aureus*
L	1	9	10	10	12
	0.5	8	8	9	10
Ni(Haαft)$_2$	1	12	12	14	15
	0.5	9	10	11	12
Cu(Haαft)$_2$	1	11	14	13	14
	0.5	9	12	11	11
DMSO		0	0	0	0
Standard		19	18	18	20

clinical bacterial pathogens, namely, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* (Gram-negative), and *Staphylococcus aureus* (Gram-positive), have been focused on for the microbiological interaction with the synthesized compounds.

In the present work, the antibacterial activity results presented in Table 7 show that the ligand (HL) and its two metal complexes exhibit potential biological activity. Two different concentrations of the compounds have been selected for the biological interactions with bacterial pathogens. They show increased bactericidal effect at higher concentrations. The graphical charts of the comparable antibacterial sensitivity study of the synthesized compounds are presented in Figures 6 and 7. The results demonstrate better antibacterial activity of metal complexes compared to the free ligand. The azomethine group present in the ligand may be the cause for its remarkable activity. The enhancement in the activity of metal complexes is considered due to coordination of metal ions with the donor sites of ligand that might inhibit the enzyme production, which is suitable for the bacterial growth. The chelation makes the compound lipophilic due to partial sharing of positive charge and π-electrons delocalization within the whole chelate ring [45–47]. This increased lipophilicity enhances the ability of complexes for permeation through the lipid layer of the cell membrane and blocks the metal binding sites in enzymes of the microorganisms. These complexes also disturb the respiration process of the cell and this freezes the synthesis of proteins, which restricts further growth of the organisms [48]. The data also reveals that the activity of free ligand and metal complexes is lesser than standard drug amikacin. Overall comparison of observed data gave information that metal complexes are more active than the free ligand against all bacteria. However, they show better inhibitory effect with *S. aureus* compared to other bacterial pathogens. The variation in the effectiveness of different compounds against different bacterial pathogens depends on the impermeability through the cell membrane of microorganisms.

3.4. Molecular Modeling Analysis. To provide clear and deep perception of the geometry of the ligand and metal complexes, molecular modeling has been carried out by using molecular mechanics and Hartree-Fock (HF) quantum mechanics calculations. The geometry optimization was

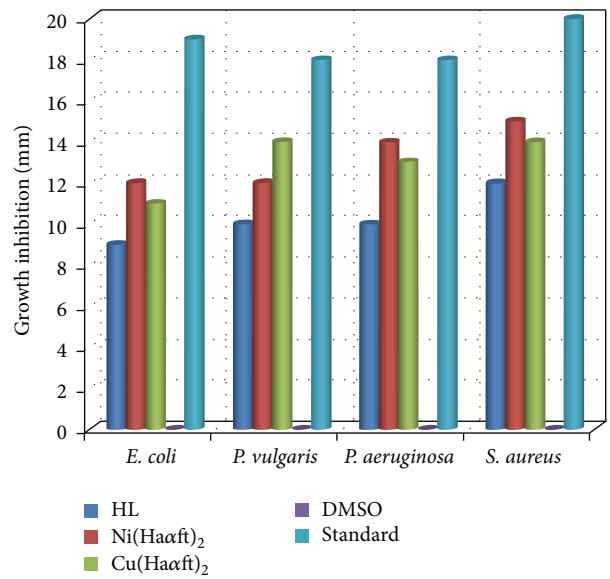


Figure 6: Antibacterial sensitivity at the concentration of 1 μg/μL.

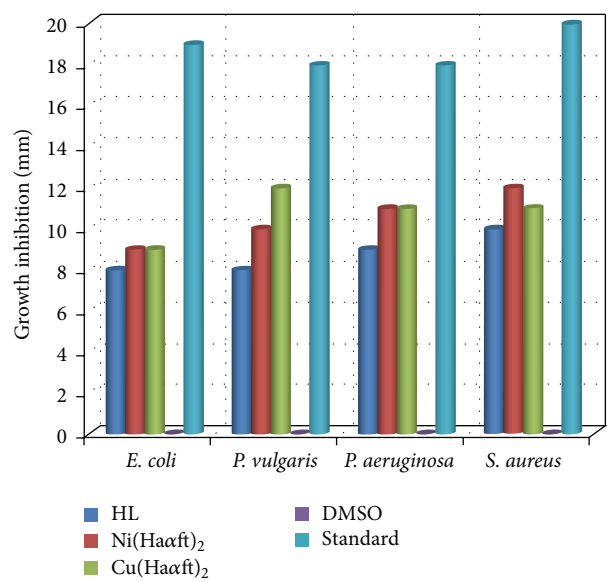


Figure 7: Antibacterial sensitivity at the concentration of 0.5 μg/μL.

done by Universal Force Field (UFF) with minimum RMS gradient 0.100, supported in ArgusLab programme [49]. Single point energy calculation of the ligand through Hartree-Fock SCF computation with maximum iteration 200 and convergence rate 10^{-10} kcal/mol reveals the final SCF energy −121796.5370 kcal/mol and heat of formation −78.0985 kcal/mol. After the geometry optimization by UFF (molecular mechanics) technique, the final geometrical energy of the ligand has been computed to 161.2699 kcal/mol and this provides stability of the molecule [50, 51]. The surface structure of the ligand has been computed through HOMO and LUMO plot. Similar study with the Ni^{II} and Cu^{II} complexes has assured their final geometrical energy 449.0564 and 752.1825 kcal/mol, respectively. The optimized structures

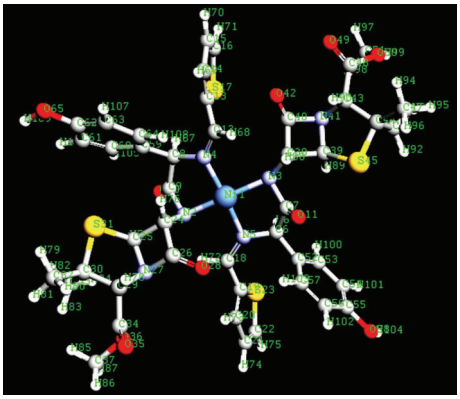


Figure 8: Molecular modeling structure of Ni(Haαft)$_2$.

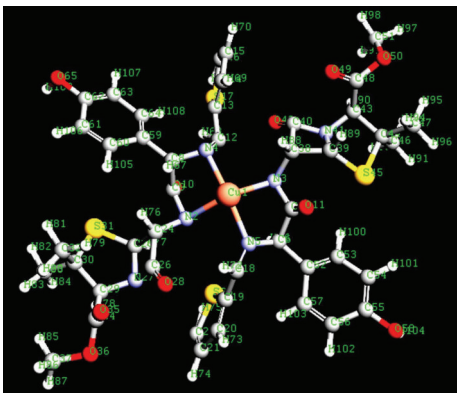


Figure 9: Molecular modeling structure of Cu(Haαft)$_2$.

of the metal complexes are presented in Figures 8 and 9 and they reveal square planar geometry for Ni^{II} and tetrahedral geometry for Cu^{II} complexes. The computational findings are in good support of the proposed structures of the complexes. The selected bond lengths, bond angles, and bond energies of the ligand and metal complexes are given in Tables 8(a)–8(f).

4. Conclusion

The present study focuses the microbiological interaction of prepared compounds against clinically important bacterial pathogens. The antibacterial sensitivity data of the compounds presented in this report allowed us to state that the metal complexes have generally better antibacterial activity than uncomplexed ligand, but less active than control drug. The electronic absorption spectral study reveals d-d transition band at 520 nm for Ni^{II} complex and this high energy process suggests its square planar geometry. The charge transfer band for Cu^{II} complex at 450 nm indicates its tetrahedral geometry. X-ray powder diffraction study reveals triclinic crystal system for the ligand and Cu^{II} complex, while it is trigonal crystal system for Ni^{II} complex. The particle size calculation by Scherrer's formula indicates their nanocrystalline nature. The ESI-MS study that provided molecular mass of the ligand and metal complexes truly confirms the

Table 8: (a) Selected bond lengths and bond energies of ligand (HL). (b) Selected bond angles and bond energies of ligand (HL). (c) Selected bond lengths and bond energies of Ni^{II}-complex. (d) Selected bond angles and bond energies of Ni^{II}-complex. (e) Selected bond lengths and bond energies of Cu^{II}-complex. (f) Selected bond angles and bond energies of Cu^{II}-complex.

(a)

Bond type	Bond lengths (Å)	Bond energy (kcal/mol)
C_8-N_{15}	1.447	532.154
N_{15}-C_{16}	1.291	749.036
C_{16}-C_{17}	1.464	386.878
C_8-C_{22}	1.489	367.716
C_{22}-N_{23}	1.437	543.390
N_{23}-C_{24}	1.462	515.889

(b)

Bond type	Bond angle (°)	Bond energy (kcal/mol)
C_8-N_{15}-C_{16}	120.00	226.230
N_{15}-C_8-C_{22}	109.47	312.267
N_{15}-C_{16}-C_{17}	120.00	295.502
N_{15}-C_8-H_{14}	109.47	169.990
C_{22}-N_{23}-C_{24}	106.70	261.205
N_{23}-C_{22}-O_{25}	120.00	378.598

(c)

Bond type	Bond lengths (Å)	Bond energy (kcal/mol)
Ni_1-N_2	1.885	306.275
Ni_1-N_3	1.885	306.275
Ni_1-N_4	1.870	313.824
Ni_1-N_5	1.870	313.824

(d)

Bond type	Bond angle (°)	Bond energy (kcal/mol)
N_2-Ni_1-N_3	90.00	340.090
N_2-Ni_1-N_4	90.00	344.228
N_2-Ni_1-N_5	90.00	344.228
Ni_1-N_2-C_9	106.00	212.735
Ni_1-N_2-C_{24}	106.00	208.934
N_3-Ni_1-N_4	90.00	344.228
N_3-Ni_1-N_5	90.00	344.228
N_4-Ni_1-N_5	90.00	348.473

(e)

Bond type	Bond lengths (Å)	Bond energy (kcal/mol)
Cu_1-N_2	2.031	176.938
Cu_1-N_3	2.031	176.938
Cu_1-N_4	2.016	181.007
Cu_1-N_5	2.016	181.007

(f)

Bond type	Bond angle (°)	Bond energy (kcal/mol)
N_2-Cu_1-N_3	109.47	156.977
N_2-Cu_1-N_4	109.47	158.764
N_2-Cu_1-N_5	109.47	158.764
Cu_1-N_2-C_9	106.70	132.072
Cu_1-N_2-C_{24}	106.70	129.991
N_3-Cu_1-N_4	109.47	158.764
N_3-Cu_1-N_5	109.47	158.764
N_4-Cu_1-N_5	109.47	160.587

ML_2 type of complexes. The FT-IR, ^{1}H NMR, and ^{13}C NMR spectral results are also in good agreement with the proposed molecular and structural formula of the ligand and its metal complexes. The change in pH value occurring in the reaction process greatly supports the deprotonation during complex formation which is further supported by the change in their colors. The sharp melting point of the compounds gives the evidence of purity of the synthesized compounds.

Conflict of Interests

The authors declare no conflict of interests.

Authors' Contribution

The author Narendra Kumar Chaudhary prepared the paper and designed the whole experimental analysis. The author Parashuram Mishra performed molecular modeling study and proofreading of the final paper.

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