

Research Article

Thermal, Spectral, Fluorescence, and Antimicrobial Studies of Cobalt, Nickel, Copper, and Zinc Complexes Derived from 4-[(5-Bromo-thiophen-2-ylmethylene)-amino]-3-mercapto-6-methyl-5-oxo-[1,2,4]triazine

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A new series of cobalt, nickel, copper, and zinc complexes of bidentate Schiff base derived from the condensation of 5-bromothiophene-2-carboxaldehyde with 4-amino-3-mercapto-6-methyl-5-oxo-[1,2,4]triazine were synthesized. Physical (magnetic measurements, molar conductance, TG), spectral (UV-Vis, IR, ¹HNMR, fluorescence, ESR), and analytical data have established the structures of synthesized Schiff base and its metal complexes. The presence of coordinated water in metal complexes was confirmed by IR and TG studies. The Schiff base exhibits a strong fluorescence emission, contrast to this partial fluorescence quenching phenomena is observed in its metal complexes. A square planar geometry for Cu(II) and octahedral geometry for Co(II), Ni(II) and Zn(II) complexes have been proposed. The Schiff base and its metal complexes have been screened for antibacterial (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*) and antifungal activities (*Aspergillus niger*, *A. flavus*).

1. Introduction

Triazine chemistry has attracted attention due to the utility of melamine derivatives as starting material for a variety of polymers [1], as scavenging resins in organic manipulations [2] and in medicinal chemistry [3]. They provide a wide range of applications in the field of optics [4], herbicides [5], and pharmaceuticals [6]. These are valuable bases for estrogen receptor modulators and are also used as bridging agents to synthesize herbicides and in the production of drugs or polymers [7, 8]. Many derivatives of 1,2,4-triazine form colored complexes with different metal ions and can be used as analytical reagent for their determination [9]. A range of polymer-supported triazines and their metal-bound analogues were screened for use in catalysis and organic synthesis [10, 11]. Goodman et al. reported that triazine-derived copper complexes have superoxide radical scavenging activity in pathological processes [12]. In our last publication, we have reported that Schiff base derived from

4-amino-3-mercapto-6-methyl-5-oxo-[1,2,4]triazine shows antibacterial and antifungal activity and enhanced antimicrobial activity is observed, when the Schiff base is combined to transition metal ions [13].

Encouraged by above-mentioned finding, we found it is to investigate the thermal, spectral, fluorescence, and antimicrobial studies of cobalt, nickel, copper, and zinc complexes derived from 4-[(5-bromo-thiophen-2-ylmethylene)-amino]-3-mercapto-6-methyl-5-oxo-[1,2,4]triazine.

2. Experimental

2.1. Materials. All the chemicals used in the present study were of analytical grade and used without further purification. 4-Amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine [14] was prepared by reported literature method.

2.2. Syntheses

2.2.1. 4-[(5-Bromo-thiophen-2-ylmethylene)-amino]-3-mercapto-6-methyl-5-oxo-[1,2,4]triazine [HL]. A solution of 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine (2.12 g, 13.5 mmol) in ethanol (40 mL) was treated with 5-bromothiophene-2-carboxaldehyde (2.69 g, 13.5 mmol). The reaction mixture was refluxed for 7 h and kept overnight at room temperature. The bluish white-colored solid crude so obtained was filtered off and washed with cold ethanol, dried, and recrystallized in ethanol.

Bluish white; yield (76%); m.p. 234–236°C; anal. calcd. for $C_9H_7BrN_4OS_2$: C, 32.64; H, 2.13; N, 16.92; found: C, 32.42; H, 2.08; N, 16.92%.

2.2.2. Metal Complexes of HL (1:1). The solid complexes were prepared by mixing hot ethanolic solutions of the HL (0.18 g, 0.54 mmol) with aqueous ethanolic solution of acetates of Co(II) (0.14 g, 0.54 mmol), Ni(II) (0.14 g, 0.54 mmol), Cu(II) (0.11 g, 0.54 mmol), and Zn(II) (0.12 g, 0.54 mmol), which resulted in the immediate precipitation of metal complexes. The solid complexes were filtered off, washed thoroughly with warm water, with aqueous ethanol to remove unreacted metal acetates or ligands, and finally with acetone, and vacuo dried.

Co(L)OAc·3H₂O: anal. calcd. for $C_{11}H_{15}BrCoN_4O_6S_2$: C, 26.31; H, 3.01; N, 11.16; Co, 11.73; found: C, 26.13; H, 2.85; N, 11.12; Co, 11.30%.

Ni(L)OAc·3H₂O: anal. calcd. for $C_{11}H_{15}BrNi_4NiO_6S_2$: C, 26.32; H, 3.01; N, 11.16; Ni, 11.69; found: C, 26.10; H, 3.00; N, 11.03; Ni, 11.22%.

Cu(L)OAc·H₂O: anal. calcd. for $C_{11}H_{11}BrCuN_4O_4S_2$: C, 28.06; H, 2.35; N, 11.90; Cu, 13.50; found: C, 28.00; H, 2.15; N, 11.82; Cu, 13.10%.

Zn(L)OAc·3H₂O: anal. calcd. for $C_{11}H_{15}BrZn_4O_6S_2Zn$: C, 25.97; H, 2.97; N, 11.01; Zn, 12.85; found: C, 25.65; H, 2.97; N, 11.01; Zn, 12.87%.

2.2.3. Metal Complexes of HL (1:2). The aqueous ethanolic solutions of acetates of Co(II) (0.17 g, 0.68 mmol), Ni(II) (0.17 g, 0.68 mmol), Cu(II) (0.14 g, 0.68 mmol), and Zn(II) (0.15 g, 0.68 mmol) were treated with the hot ethanolic solutions of the HL (0.45 g, 1.3 mmol). The products formed were filtered and purified by washing thoroughly with warm water, with aqueous ethanol to remove unreacted metal acetates or ligands, and finally with acetone and dried.

Co(L)₂·2H₂O: anal. calcd. for $C_{18}H_{16}Br_2CoN_8O_4S_4$: C, 28.62; H, 2.13; N, 14.83; Co, 7.80; found: C, 28.18; H, 2.01; N, 14.83; Co, 7.53%.

Ni(L)₂·2H₂O: anal. calcd. for $C_{18}H_{16}Br_2Ni_8NiO_4S_4$: C, 28.63; H, 2.14; N, 14.84; Ni, 7.77; found: C, 28.63; H, 2.12; N, 14.80; Ni, 7.72%.

Cu(L)₂: anal. calcd. for $C_{18}H_{12}Br_2CuN_8O_2S_4$: C, 29.86; H, 1.67; N, 15.48; Cu, 8.78; found: C, 29.85; H, 1.65; N, 15.80; Cu, 8.38%.

Zn(L)₂·2H₂O: anal. calcd. for $C_{18}H_{16}Br_2N_8O_4S_4Zn$: C, 28.38; H, 2.12; N, 14.71; Zn, 8.58; found: C, 28.37; H, 2.10; N, 14.53; Zn, 8.23%.

2.3. Analyses and Instrumentation. The elemental analyses (C, H, and N) were carried out at SAIF, Punjab University, Chandigarh, by using Perkin-Elmer 2400 Elemental Analyzer. The metal contents were determined gravimetrically by a literature procedure [15] after digesting the organic matter with aqua regia and evaporating the residue to dryness. IR spectra of neat samples of the ligand and its metal complexes were performed in the range 4000–250 cm⁻¹ on a MB-3000 ABB Spectrometer. The electronic spectra of solid complexes were recorded on T 90 (PG Instruments Ltd) UV/Vis spectrometer in the region 1100–200 nm. ¹H NMR spectra were recorded in DMSO-d₆ on a Bruker ACF 300 spectrometer at 300 MHz using tetramethylsilane as the internal standard. Magnetic moments were measured at Institute Instrumentation Centre, IIT Roorkee, on vibrating sample magnetometer (Model 155). The Perkin Elmer (Pyris Diamond) instrument was used to carry out thermal analysis of metal complex in atmospheric air (50–800°C) at a heating rate of 10°C Min⁻¹ using a reference to alumina powder. The fluorescence studies of Schiff base and its metal complexes were recorded on SHIMADZU RF-5301PC spectrophotometer. The solutions of 10⁻³ M concentration were prepared in HPLC grade DMF and the experiment was carried out at room temperature. ESR spectra were recorded on X-Band at frequency of 9.1 GHz under the magnetic field 3000 Gauss on a Varian E-112 ESR spectrometer at SAIF IIT Bombay.

2.4. Pharmacology

2.4.1. Antibacterial Activity (In Vitro). The newly synthesized Schiff base (HL) and its metal complexes were screened *in vitro* for their antibacterial activity against two Gram-positive (*Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121) and two Gram-negative (*Escherichia coli* MTCC 1652, *Pseudomonas aeruginosa* MTCC 741) bacterial strains by agar well-diffusion method as reported in our previous paper [13]. DMSO was used as a negative control whereas ciprofloxacin was used as positive control. All the bacterial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, and subcultured on nutrient agar.

2.4.2. Antifungal Activity (In Vitro). The newly synthesized Schiff base (HL) and its metal complexes were screened *in vitro* for their antifungal activity against two fungi, *Aspergillus niger* and *A. flavus*, the ear pathogens isolated from the patients of Kurukshetra [16] and evaluated by poisoned food technique [13]. The molds were grown on Sabouraud dextrose agar (SDA) at 25°C for 7 days and used as inocula. DMSO was used as the negative control whereas fluconazole was used as the positive control. The experiments

were performed in triplicates. Diameters of fungal colonies were measured and expressed as percent mycelial inhibition.

$$\text{Percent inhibition of mycelial growth} = (d_c - d_t)/d_c \times 100, \quad (1)$$

where d_c average diameter of fungal colony in negative control sets and d_t average diameter fungal colony in experimental sets.

2.4.3. Determination of Minimum Inhibitory Concentration (MIC). MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of the various compounds against bacterial strains was tested through a modified agar well-diffusion method [17]. In this method, a twofold serial dilution of each synthesized compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range 512 to $1 \mu\text{g mL}^{-1}$. A $100 \mu\text{L}$ volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with $100 \mu\text{L}$ of standardized inoculum (10^6 cfu mL^{-1}) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 h and observed for the inhibition zones. Ciprofloxacin was used as positive control.

3. Results and Discussion

The synthesis of Schiff base (HL) is schematically represented in Figure 1. Elemental analyses indicated the formation of 1:1 and 1:2 metal complexes of HL with Co(II), Ni(II), Cu(II), and Zn(II) metal ions. All the complexes are dark green, brown to dark brown in color and nonhygroscopic in nature. The molar conductance values of the complexes (measured in 10^{-3}M DMF) are in the range $8.1\text{--}10.3 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$ indicating the nonelectrolytic nature [18, 19]. The low conductivity values are in agreement with low solubility of metal complexes in water, ethanol, chloroform, acetone, and most organic solvents. On the other hand, they are soluble in DMSO and DMF and decomposed at higher temperature. The purity of ligands and their metal complexes has been checked by TLC.

3.1. IR Spectra. The comparative analysis of the IR spectra of the complexes and of the free ligand (Table 1) revealed that the absorption band characteristic of the stretching vibrations of -C=N- (azomethine group) is shifted towards lower wave number from 1599 cm^{-1} in the spectrum of the ligand to $1575\text{--}1589 \text{ cm}^{-1}$ in the complexes [19, 20]. This indicates the coordination of the nitrogen of this group with the metal ions. Further coordination of metal to azomethine nitrogen is confirmed by a new band at $488\text{--}498 \text{ cm}^{-1}$ due to $\nu(\text{M-N})$. The $\nu(\text{C=O})$ stretching frequency of the ligand at 1690 cm^{-1} is not altered in almost all of the complexes, indicating that the oxygen of C=O is not involved in bonding with metal ion [13]. A characteristic strong band at 2476 cm^{-1} is ascribed to $\nu(\text{SH})$, which disappeared in the spectra of metal complexes, confirming deprotonation and coordination of thiol group

[13, 19]. This is further supported by the lower frequency band appeared at $733\text{--}756 \text{ cm}^{-1}$ in the metal complexes due to $\nu(\text{C-S})$. The new band in the region of $371\text{--}382 \text{ cm}^{-1}$ in the metal complexes is assigned to vibrations of $\nu(\text{M-S})$ bond [21, 22]. The broad band around 3305 cm^{-1} , corresponding to $\nu(\text{O-H})$ indicates the presence of coordinated water molecules. In addition, a strong band around 1745 cm^{-1} has been assigned to $\nu(\text{OOCCH}_3)$ in 1:1 metal complexes. Further, the absorptions corresponding to asymmetric and symmetric stretching of the acetate groups at ~ 1617 and 1420 cm^{-1} , respectively confirms the monodentate nature of the acetate ion in complexes [23, 24].

3.2. ^1H NMR Spectra. In the ^1H -NMR spectra (Table 2), the signal of the azomethine proton is shifted from 8.52 ppm in the spectrum of the ligand to 8.80 and 8.73 ppm, in the spectra of 1:1 and 1:2 metal complexes, respectively. This shows that the most probable binding of the ligand with the metal ion is realized through the nitrogen atom of the azomethine group [13, 17]. The peak corresponding to -SH at 10.30 ppm in the free ligand is not observed in the spectra of the metal complexes. This clearly shows deprotonation of -SH during complex formation. In the spectrum of the ligand, signals due to aromatic protons appeared as two doublets at δ 7.16 and 7.33 ppm. These resonance signals remain almost unaltered in the spectra of the complexes. The methyl group protons of triazine appeared as singlet at δ 2.35 ppm in the spectrum of ligand and slightly shielded in the spectra of metal complexes. In the spectra of 1:1 Zn complex singlet at δ 1.85 is due to methyl group of acetate ion [20].

3.3. Electronic and Magnetic Moments Measurements. In order to obtain further structural information, the electronic spectra and magnetic moments of the complexes have been measured (Table 3). At room temperature Co(II) complexes show magnetic moments 4.13 and 4.20 BM, indicating three unpaired electrons. The electronic spectra of Co(II) complexes exhibit absorption in the region $11560\text{--}12004$, and $22258\text{--}22779 \text{ cm}^{-1}$. These bands may be assigned to $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{2g}(\text{F})$ (ν_1) and $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{1g}(\text{P})$ (ν_3) transitions respectively. The position of these bands is consistent with octahedral geometry around the Co(II) ion [25]. With these values the ligand field parameters (Dq), the Racah parameter (B) and nephelauxetic ratio (β) have been calculated [26]. The value of B, which is lower than the free ion value of 971 cm^{-1} , indicates overlapping of ligand-metal orbitals.

The Ni(II) complexes reported herein are found to have room temperature magnetic moment values 3.78 and 3.76 BM, corresponding to two unpaired electrons. The electronic spectra of Ni(II) complexes display three absorption bands in the range $11261\text{--}11376$, $16200\text{--}16345$, and $26115\text{--}26238 \text{ cm}^{-1}$. These bands may be assigned to three spin allowed transitions $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{2g}(\text{F})$ (ν_1), $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{1g}(\text{F})$ (ν_2), and $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{1g}(\text{P})$ (ν_3), respectively. The position of these bands indicates that the complexes have an octahedral geometry around the Ni(II) complexes [13, 19, 27–31]. The ligand field parameters (Dq,

TABLE 1: IR spectral data of the ligand and its metal complexes.

Compound	$\nu(\text{C}=\text{O})$	$\nu(\text{N}=\text{CH})$	$\nu(\text{C}-\text{S})$	$\nu(\text{S}-\text{H})$	$\nu(\text{OOCCH}_3)$	$\nu(\text{H}_2\text{O}/\text{OH})$	$\nu(\text{M}-\text{S})$	$\nu(\text{M}-\text{N})$
HL	1690	1599	—	2476	—	—	—	—
$\text{Co}(\text{L})(\text{OAc}) \cdot 3\text{H}_2\text{O}$	1685	1582	748	—	1744	3310	376	498
$\text{Co}(\text{L})_2 \cdot 2\text{H}_2\text{O}$	1688	1575	750	—	—	3318	378	493
$\text{Ni}(\text{L})(\text{OAc}) \cdot 3\text{H}_2\text{O}$	1686	1587	756	—	1744	3298	382	488
$\text{Ni}(\text{L})_2 \cdot 2\text{H}_2\text{O}$	1686	1589	756	—	—	3298	381	488
$\text{Cu}(\text{L})(\text{OAc}) \cdot \text{H}_2\text{O}$	1687	1582	748	—	1745	3307	371	490
$\text{Cu}(\text{L})_2$	1686	1583	752	—	—	—	373	495
$\text{Zn}(\text{L})(\text{OAc}) \cdot 3\text{H}_2\text{O}$	1689	1580	737	—	1743	3313	372	492
$\text{Zn}(\text{L})_2 \cdot 2\text{H}_2\text{O}$	1690	1582	733	—	—	3315	373	490

TABLE 2: ^1H NMR spectral data of Schiff base and Zn(II) metal complexes.

Compound	^1H NMR (ppm)
HL [$\text{C}_9\text{H}_7\text{BrN}_4\text{OS}_2$]	CDCl_3 , 2.35 (s, 3H, $-\text{CH}_3$), 7.16 (d, 1H, Ar-H), 7.33 (d, 1H, Ar-H), 8.52 (s, 1H, $-\text{N}=\text{CH}-$), 10.30 (s, 1H, $-\text{SH}$)
$\text{Zn}(\text{L})(\text{OAc}) \cdot 3\text{H}_2\text{O}$ [$\text{C}_{11}\text{H}_{15}\text{BrN}_4\text{O}_6\text{S}_2\text{Zn}$]	$\text{DMSO } d_6$, 2.18 (s, 3H, $-\text{CH}_3$), 7.22 (d, 1H, Ar-H), 7.38 (d, 1H, Ar-H), 8.80 (s, 1H, $-\text{N}=\text{CH}-$), 1.85 (s, 3H, CH_3COO)
$\text{Zn}(\text{L})_2 \cdot 2\text{H}_2\text{O}$ [$\text{C}_{18}\text{H}_{16}\text{Br}_2\text{N}_8\text{O}_4\text{S}_4\text{Zn}$]	$\text{DMSO } d_6$, 2.20 (s, 6H, $-\text{CH}_3$), 7.38 (d, 2H, Ar-H), 7.59 (d, 2H, Ar-H), 8.73 (s, 2H, $-\text{N}=\text{CH}-$)

TABLE 3: Electronic spectra, magnetic data, and ligand field parameters of metal complexes.

Compound	Transitions (cm^{-1})			$Dq \text{ cm}^{-1}$	$B \text{ cm}^{-1}$	ν_2/ν_1	β	$\beta\%$	μ_{eff} (BM)
	ν_1	ν_2	ν_3						
$\text{Co}(\text{L})(\text{OAc}) \cdot 3\text{H}_2\text{O}$	12004	25348*	22779	1334.4	807.6	2.11	0.831	16.9	4.13
$\text{Co}(\text{L})_2 \cdot 2\text{H}_2\text{O}$	11560	24431*	22258	1287.1	800.6	2.11	0.824	17.6	4.20
$\text{Ni}(\text{L})(\text{OAc}) \cdot 3\text{H}_2\text{O}$	11261	16200	26115	1126.1	568.8	1.43	0.546	45.4	3.78
$\text{Ni}(\text{L})_2 \cdot 2\text{H}_2\text{O}$	11376	16345	26238	1137.6	563.6	1.43	0.541	45.9	3.76
$\text{Cu}(\text{L})(\text{OAc}) \cdot \text{H}_2\text{O}$	20123	—	—	—	—	—	—	—	1.75
$\text{Cu}(\text{L})_2$	19998	—	—	—	—	—	—	—	1.75

* Calculated value.

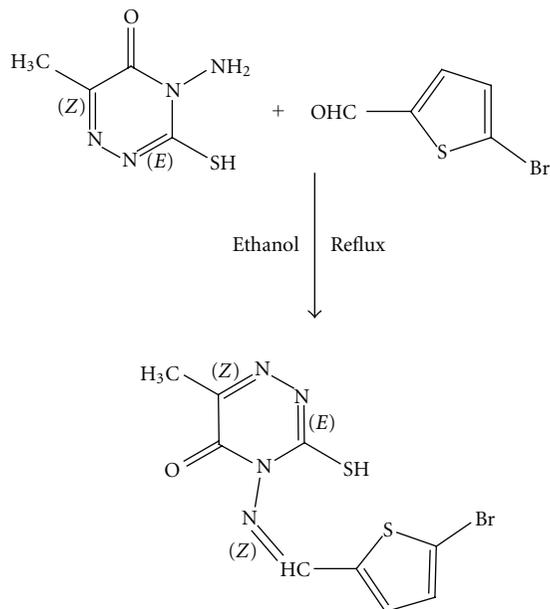


FIGURE 1: Scheme for the synthesis of Schiff base.

B, β , $\beta_{\%}$) have also been calculated (Table 3) for Ni(II) complexes by using band-fitting equation [26]. These parameters indicated significant covalent character of the metal-ligand bonds and an overlapping of ligand-metal orbitals. At room temperature Cu(II) complexes show magnetic moments 1.75 BM corresponding to one unpaired electron. The electronic spectra of Cu(II) complexes showed absorption band in the region 19998–20123 cm^{-1} attributed to ${}^2B_{1g} \rightarrow {}^2A_{1g}$ transition indicative of square planar geometry [20, 22].

3.4. Photoluminescence Emission Spectra. The photoluminescence emission spectra of the Schiff base (HL) and its 1 : 2 metal complexes are recorded in DMF at room temperature (Figure 2). HL exhibits a strong fluorescence emission at 376 nm with excitation at 265 nm. In contrast with fluorescence spectra of HL, partial fluorescence quenching phenomena are observed in its metal complexes with weak fluorescence emission at 389, 379, 381, and 374 nm for Co(II), Ni(II), Cu(II), and Zn(II) complexes, respectively with excitation at 265 nm. The fluorescence spectra results revealed that fluorescence emission intensity of Schiff bases decreased dramatically on complex formation with transition metal ions. The reason for the weak fluorescence intensity with formation of metal complexes is due to decrease in electron density on Schiff base [32, 33]. The decrease in emission maxima was in the order of HL > Zn(II) > Co(II) > Cu(II) > Ni(II).

3.5. Electron Paramagnetic Resonance Spectra. ESR spectra (Figure 3) of copper complexes in powder form at LNT were recorded in the X band using 9.1 GHz field modulation with g factor quoted relative to the standard marker TCNE ($g = 2.0027$). The ESR spectra of Cu(II) complexes show typical axial behavior with slightly different g_{\parallel} and g_{\perp} values. From the observed g values for Cu(L)(OAc)·H₂O at room temperature ($g_{\parallel} = 2.11$, $g_{\perp} = 2.04$, $g_{\text{av}} = 2.06$, $G = 2.84$) and for Cu(L)₂ at room temperature ($g_{\parallel} = 2.14$, $g_{\perp} = 2.06$, $g_{\text{av}} = 2.08$, $G = 2.37$), it is evident that unpaired electron is localized in the dx^2-y^2 orbital and corresponds to square planar geometry of the complexes. The $g_{\parallel} < 2.3$ value confirms the covalent character of the metal ligand bond. The axial symmetry parameter G is less than 4.0 and indicates considerable exchange interaction in the solid complex [32, 34]. No signal at half field was observed in the spectrum, ruling out the possibility of a dimeric form [35].

3.6. Thermal Studies. Thermal studies of Zn(L)₂·2H₂O and Ni(L)₂·2H₂O (Figure 4) indicate that the complexes decompose in three major steps from 50 to 800°C. The TG curves give information on the thermal stability and the product formed on heating [21, 22, 36]. The TG curve of Zn(L)₂·2H₂O consists mainly of three steps in the temperature range 50–198, 198–430, and 430–800°C. The first step seems to be consistent with the evolution of two water molecules (calcd. 4.75%, found 4.54%). The second TG step represents a mass loss (calcd. 45.9%, found 45.7%) corresponding to expulsion of organic moiety (C₁₀H₆Br₂S₂). The final steps corresponds to decomposition of triazine

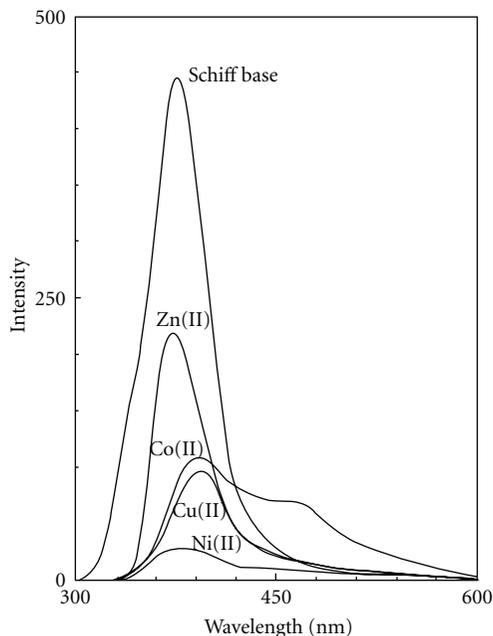


FIGURE 2: Fluorescence emission spectra of Schiff base and its metal complexes.

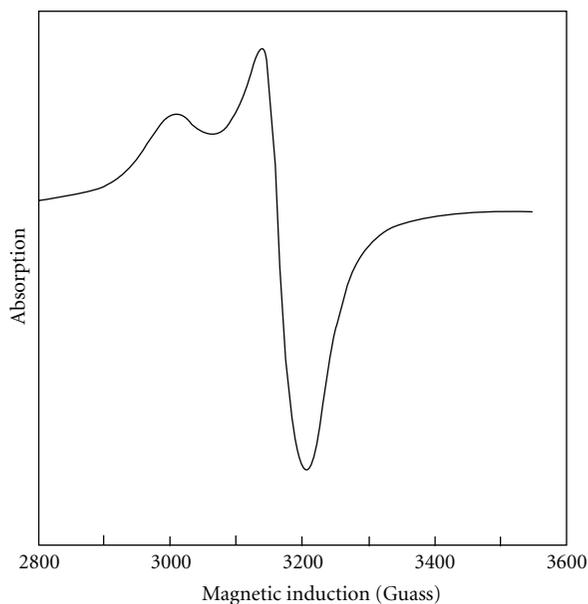


FIGURE 3: X-band ESR spectra of Cu(L)(OAc)·H₂O.

molecules at 430–800°C with mass loss of 40.8% (calcd. 40.9) of the ligand leaving zinc oxide as residue.

TGA studies of Ni(L)₂·2H₂O indicated first weight loss (4.3%) in the range of 50–235°C, attributed to the loss of two water molecules (calcd. 4.8%). The second weight loss of 49.9% (calcd. 50.0%) from 235–300°C corresponds to loss of organic moiety (C₁₀H₆Br₂N₂S₂). In the last step of decomposition triazine moiety comes out in the range 300–800°C with mass loss of 37.1% (Calcd. 37.5%). The final residual weight is 9.1% (calcd. 9.8%) corresponding to NiO.

TABLE 4: Antibacterial activity of synthesized compounds through agar well diffusion method.

Compound	Diameter of growth of inhibition zone (mm) ^a			
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
HL	16.3	18.3	—	—
Co(L)(OAc)·3H ₂ O	18.3	18.3	—	—
Co(L) ₂ ·2H ₂ O	16.3	19.6	—	—
Ni(L)(OAc)·3H ₂ O	17.3	18.0	—	—
Ni(L) ₂ ·2H ₂ O	17.3	19.6	—	—
Cu(L)(OAc)·H ₂ O	16.3	18.3	—	—
Cu(L) ₂	20.6	22.6	—	—
Zn(L)(OAc)·3H ₂ O	19.3	21.3	—	—
Zn(L) ₂ ·2H ₂ O	17.3	19.6	—	—
Ciprofloxacin	26.6	24.0	25.0	22.0

—No activity.

^aValues, including diameter of the well (8 mm), are means of three replicates.

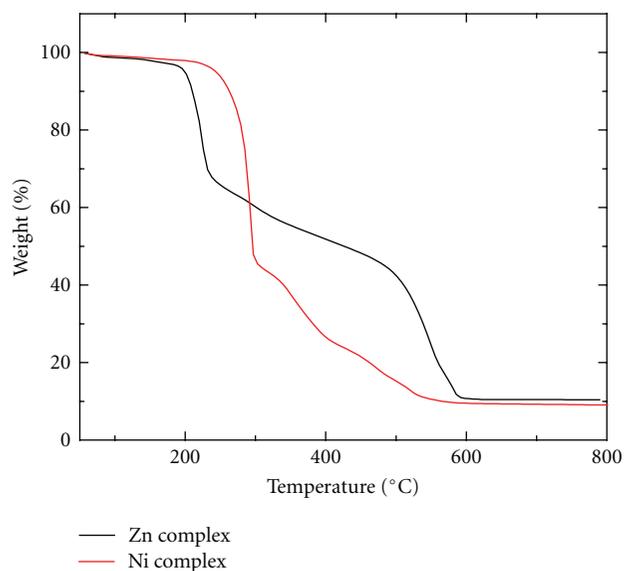


FIGURE 4: Thermogravimetric curves of Zn(L)₂·2H₂O and Ni(L)₂·2H₂O.

3.7. Antimicrobial Discussion. The ligand (HL), metal complexes, standard drugs, and DMSO solvent were screened separately for their antibacterial activity against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria and antifungal activity against *Aspergillus niger* and *Aspergillus flavus*. The microbial results are summarized in Tables 4, 5, and 6. The antimicrobial studies suggested that the Schiff bases are biologically active and their metal complexes showed significantly enhanced antibacterial and antifungal activity against microbial strains in comparison to the free ligands. Positive controls (standard drug) produced significantly sized inhibition zones against the tested bacteria and fungi; however, negative control (DMSO) produced no observable inhibitory effect against any of the test organisms.

Tested chemical compounds showed zone of inhibition ranging between 16 mm and 22 mm against the Gram-positive bacteria. On the basis of zone of inhibition produced

TABLE 5: Minimum inhibitory concentration (MIC) (μg/mL) of compounds.

Compound	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
HL	128	64
Co(L)(OAc)·3H ₂ O	128	64
Co(L) ₂ ·2H ₂ O	128	64
Ni(L)(OAc)·3H ₂ O	128	64
Ni(L) ₂ ·2H ₂ O	128	64
Cu(L)(OAc)·H ₂ O	128	64
Cu(L) ₂	64	32
Zn(L)(OAc)·3H ₂ O	64	32
Zn(L) ₂ ·2H ₂ O	128	64
Ciprofloxacin	6.25	6.25

TABLE 6: Antifungal activity of Schiff base and metal complexes.

Compound	Mycelial growth inhibition (%)	
	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>
HL	45.2	46.6
Co(L)(OAc)·3H ₂ O	47.7	51.1
Co(L) ₂ ·2H ₂ O	48.8	52.2
Ni(L)(OAc)·3H ₂ O	45.5	48.8
Ni(L) ₂ ·2H ₂ O	43.3	46.6
Cu(L)(OAc)·H ₂ O	45.5	48.8
Cu(L) ₂	58.8	63.3
Zn(L)(OAc)·3H ₂ O	56.6	61.1
Zn(L) ₂ ·2H ₂ O	51.1	56.6
Fluconazole	81.1	77.7

against the test bacterium, Cu(L)₂ was found to be most effective against *B. subtilis* and *S. aureus*, with zone of inhibition of 20.6 mm and 22.6 mm, respectively. However other tested compounds showed moderate antibacterial activity (Table 4). Tested chemical compounds did not exhibit any activity against Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. In the whole series,

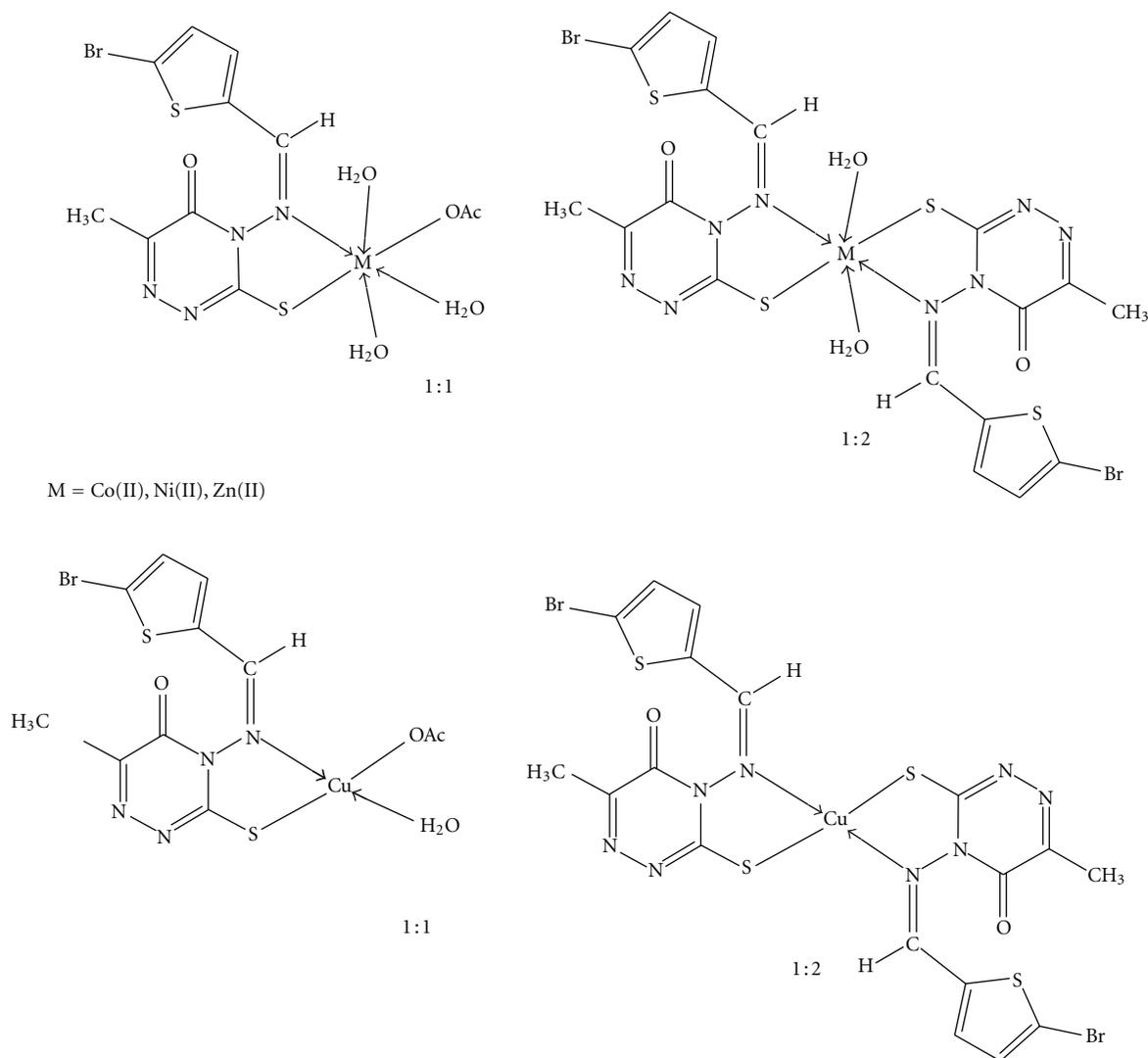


FIGURE 5: Proposed structures of metal complexes.

the MIC of chemical compounds ranged between 32 and 128 $\mu\text{g}/\text{mL}$ against Gram-positive bacteria. Two compounds $\text{Cu}(\text{L})_2$ and $\text{Zn}(\text{L})(\text{OAc}) \cdot 3\text{H}_2\text{O}$ were found to be best as they exhibit the lowest MIC of 32 $\mu\text{g}/\text{mL}$ against *B. subtilis* and 64 $\mu\text{g}/\text{mL}$ against *S. aureus* (Table 5). The results of our study are in accordance with the reports of earlier workers [13, 19, 21, 24], which also showed that the antibacterial activity of ligands is greatly enhanced when it is coordinated to metal ions. The Schiff base is found to show antifungal activity, and its metal complexes show enhanced activity as compared to the uncoordinated compound. Of the nine chemical compounds screened for their antifungal activity, three compounds $\text{Cu}(\text{L})_2$, $\text{Zn}(\text{L})(\text{OAc}) \cdot 3\text{H}_2\text{O}$, and $\text{Zn}(\text{L})_2 \cdot 2\text{H}_2\text{O}$ showed more than 50% inhibition of mycelial growth against *Aspergillus flavus* whereas three compounds namely $\text{Cu}(\text{L})_2$, $\text{Zn}(\text{L})(\text{OAc}) \cdot 3\text{H}_2\text{O}$, and $\text{Zn}(\text{L})_2 \cdot 2\text{H}_2\text{O}$, showed more than 55% inhibition of mycelial growth against *A. niger* (Table 6). The overtone's concept [37] and Tweedy's chelation theory [38] can be used to explain the enhancement in antimicrobial activity of the metal complexes.

4. Conclusions

The present work describes the synthesis of cobalt, nickel, copper, and zinc complexes of bidentate Schiff base derived from the condensation reaction of 5-bromothiophene-2-carboxaldehyde with 4-amino-3-mercapto-6-methyl-5-oxo-[1,2,4]triazine. The physical (magnetic measurements, molar conductance, TG), spectral (UV-Vis, IR, ^1H NMR, fluorescence, ESR), and analytical data for these complexes clearly suggest that the Schiff base is coordinated to metal ions through its azomethine nitrogen and sulphur. The presence of coordinated water in metal complexes was confirmed by IR and TG studies. The Schiff base exhibits a strong fluorescence emission contrast to this partial fluorescence quenching phenomena is observed in its metal complexes. The proposed structures of metal complexes are presented in Figure 5. The antimicrobial studies suggested that the Schiff bases were found to be biologically active and their metal complexes show significantly enhanced antibacterial and antifungal activity against microbial strains in comparison to

the free ligands, thus, exhibiting their broad spectrum nature and can become a new antimicrobial agent, after further biological studies.

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References

- [1] J. H. K. K. Hirschberg, A. Ramzi, R. P. Sijbesma, and E. W. Meijer, "Ureidotriazine-based supramolecular copolymers," *Macromolecules*, vol. 36, no. 5, pp. 1429–1432, 2003.
- [2] M. Yanase, M. Matsuoka, Y. Tatsumi et al., "Thermodynamic study on supramolecular complex formation of fullerene with calix[5]arenes in organic solvents," *Tetrahedron Letters*, vol. 41, no. 4, pp. 493–497, 2000.
- [3] M. Pattarawarapan, S. Reyes, Z. Xia, M. C. Zaccaro, H. U. Saragovi, and K. Burgess, "Selective formation of homo- and heterobivalent peptidomimetics," *Journal of Medicinal Chemistry*, vol. 46, no. 17, pp. 3565–3567, 2003.
- [4] J. R. Galán-Mascarós, J. M. Clemente, and K. R. Dunbar, "Synthesis, structure and magnetic properties of the one-dimensional chain compound $\{K[Fe(1,3,5\text{-triazine-2,4,6-tricarboxylate})(H_2O)_2] \cdot 2H_2O\}_\infty$," *Journal of the Chemical Society, Dalton Transactions*, no. 13, pp. 2710–2713, 2002.
- [5] H. O. Esser, G. Dupllis, C. Vogel, G. J. Marco, P. C. Kearney, and D. D. Kaufman, *Herbicides Chemistry, Degradation and Mode of Action*, Marcel Dekker, New York, 2nd edition, 1976.
- [6] C. Courme, N. Gresh, M. Vidal et al., "Synthesis of aryl phosphates based on pyrimidine and triazine scaffolds," *European Journal of Medicinal Chemistry*, vol. 45, no. 1, pp. 244–255, 2010.
- [7] P. De Hoog, P. Gamez, W. L. Driessen, and J. Reedijk, "New polydentate and polynucleating *N*-donor ligands from amines and 2,4,6-trichloro-1,3,5-triazine," *Tetrahedron Letters*, vol. 43, no. 38, pp. 6783–6786, 2002.
- [8] H. S. Patel and V. C. Patel, "Polyimides containing *s*-triazine ring," *European Polymer Journal*, vol. 37, no. 11, pp. 2263–2271, 2001.
- [9] N. R. El-Brollosy, "Synthesis and reactions of some new 1,2,4-triazine derivatives of biological interest," *Phosphorus, Sulfur and Silicon and Related Elements*, vol. 163, pp. 77–89, 2000.
- [10] S. Bräse, S. Dahmen, F. Lauterwasser, N. E. Leadbeater, and E. L. Sharp, "Polymer-bound 1-aryl-3-alkyltriazines as modular ligands for catalysis. Part 2: screening immobilized metal complexes for catalytic activity," *Bioorganic and Medicinal Chemistry Letters*, vol. 12, no. 14, pp. 1849–1851, 2002.
- [11] B. Erb, J. P. Kucma, S. Mourey, and F. Struber, "Polymer-supported triazines as smart reagents for the alkylation of carboxylic acids," *Chemistry-A European Journal*, vol. 9, no. 11, pp. 2582–2588, 2003.
- [12] B. A. Goodman, C. G. Palivan, H. Palivan, and S. Tomas, "Local structure is critical for superoxide dismutase activity in copper complexes: relationship between EPR parameters, structure and activity in some sterically hindered copper(II) bis(hydrazono-triazine) complexes," *Applied Magnetic Resonance*, vol. 25, no. 1, pp. 13–28, 2003.
- [13] K. Singh, Y. Kumar, P. Puri, C. Sharma, and K. R. Aneja, "Synthesis, spectroscopic, thermal and antimicrobial studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes with Schiff base derived from 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine," *Medicinal Chemistry Research*, vol. 21, no. 8, pp. 1708–1716, 2012.
- [14] A. Dornow, H. Mengel, and P. Marx, "Über 1, 2, 4-triazine, I darstellung einiger neuer s-triazolo[3.2-c]-s-triazine," *Chemische Berichte*, vol. 97, pp. 2173–2178, 1964.
- [15] A. I. Vogel, *Book of Quantitative Chemical Analysis*, Addison-Wesley/Longman, London, UK, 5th edition, 1999.
- [16] K. R. Aneja, C. Sharma, and R. Joshi, "Fungal infection of the ear: a common problem in the north eastern part of Haryana," *International Journal of Pediatric Otorhinolaryngology*, vol. 74, no. 6, pp. 604–607, 2010.
- [17] K. R. Aneja, C. Sharma, and R. Joshi, "In vitro efficacy of amaltas (*Cassia fistula L.*) against the pathogens causing otitis externa," *Jundishapur Journal of Microbiology*, vol. 4, no. 3, pp. 175–183, 2011.
- [18] N. Gopalakrishna Bhat and B. Narayana, "Complexes of Ag(I), Tl(I), Zn(II), Hg(II), Cd(II), Pd(II), Co(II) and Ni(II) with 4-[(4-Dimethylamino-benzylidene)-amino]-5-ethyl-2,4-dihydro-[1,2,4] triazole-3-thione (HDEDT) and 4-[(Benzylidene)amino]-6-(*t*-butyl)-4H-[1,2,4]- triazole-3-thione-5-one (HBBTT)," *Synthesis and Reactivity in Inorganic, Metal-Organic and Nano-Metal Chemistry*, vol. 35, no. 3, pp. 253–262, 2005.
- [19] K. Singh, D. P. Singh, M. Singh Barwa, P. Tyagi, and Y. Mirza, "Some bivalent metal complexes of Schiff bases containing N and S donor atoms," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 21, no. 6, pp. 749–755, 2006.
- [20] K. Singh, Y. Kumar, and M. S. Barwa, "Synthesis, characterization and thermal studies of Co(II), Ni(II), Cu(II) and Zn(II) Complexes of some Schiff bases derived from 4-amino-3-mercapto-6- methyl-5-oxo-1,2,4 triazine," *South African Journal of Chemistry*, vol. 63, pp. 169–174, 2010.
- [21] G. B. Bagihalli, P. G. Avaji, S. A. Patil, and P. S. Badami, "Synthesis, spectral characterization, in vitro antibacterial, antifungal and cytotoxic activities of Co(II), Ni(II) and Cu(II) complexes with 1,2,4-triazole Schiff bases," *European Journal of Medicinal Chemistry*, vol. 43, no. 12, pp. 2639–2649, 2008.
- [22] K. Singh, M. S. Barwa, and P. Tyagi, "Synthesis, characterization and biological studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes with bidentate Schiff bases derived by heterocyclic ketone," *European Journal of Medicinal Chemistry*, vol. 41, no. 1, pp. 147–153, 2006.
- [23] A. J. Atkins, D. Black, R. L. Finn et al., "Synthesis and structure of mononuclear and binuclear zinc(II) compartmental macrocyclic complexes," *Dalton Transactions*, no. 9, pp. 1730–1737, 2003.
- [24] A. K. Singh, O. P. Pandey, and S. K. Sengupta, "Synthesis, spectral characterization and biological activity of zinc(II) complexes with 3-substituted phenyl-4-amino-5-hydrazino-1, 2, 4-triazole Schiff bases," *Spectrochimica Acta—Part A*, vol. 85, no. 1, pp. 1–6, 2012.
- [25] M. A. Phaniband, S. D. Dhumwad, and S. R. Pattan, "Synthesis, characterization, antimicrobial, and DNA cleavage studies of metal complexes of coumarin Schiff bases," *Medicinal Chemistry Research*, vol. 25, pp. 493–502, 2010.
- [26] A. B. P. Lever, *Inorganic Spectroscopy*, Elsevier, Amsterdam, The Netherlands, 1968.
- [27] V. P. Singh and P. Gupta, "Synthesis and spectral studies of metal(II) complexes with succinic acid dihydrazones and their

- biological activity," *Pharmaceutical Chemistry Journal*, vol. 42, no. 4, pp. 196–202, 2008.
- [28] N. Raman, K. Pothiraj, and T. Baskaran, "DNA interaction, antimicrobial, electrochemical and spectroscopic studies of metal(II) complexes with tridentate heterocyclic Schiff base derived from 2'-methylacetoacetanilide," *Journal of Molecular Structure*, vol. 1000, no. 1–3, pp. 135–144, 2011.
- [29] S. A. Patil, S. N. Unki, A. D. Kulkarni, V. H. Naik, and P. S. Badami, "Co(II), Ni(II) and Cu(II) complexes with coumarin-8-yl Schiff-bases: spectroscopic, in vitro antimicrobial, DNA cleavage and fluorescence studies," *Spectrochimica Acta—Part A*, vol. 79, no. 5, pp. 1128–1136, 2011.
- [30] G. G. Mohamed, M. M. Omar, and A. A. Ibrahim, "Biological activity studies on metal complexes of novel tridentate Schiff base ligand. Spectroscopic and thermal characterization," *European Journal of Medicinal Chemistry*, vol. 44, no. 12, pp. 4801–4812, 2009.
- [31] T. Rosu, M. Negoiu, S. Pasculescu, E. Pahontu, D. Poirier, and A. Gulea, "Metal-based biologically active agents: synthesis, characterization, antibacterial and antileukemia activity evaluation of Cu(II), V(IV) and Ni(II) complexes with antipyrine-derived compounds," *European Journal of Medicinal Chemistry*, vol. 45, no. 2, pp. 774–781, 2010.
- [32] B. A. Yamgar, V. A. Sawant, S. K. Sawant, and S. S. Chavan, "Copper(II) complexes of thiazolylazo dye with triphenylphosphine and N_3^- or NCS^- as coligands: synthesis, spectral characterization, electrochemistry and luminescence properties," *Journal of Coordination Chemistry*, vol. 62, no. 14, pp. 2367–2374, 2009.
- [33] Q. H. Wang, W. Weng, J. M. Liu, L. Z. Cai, and G. C. Guo, "Synthesis, crystal structure, and fluorescence properties of several schiff-base compounds," *Journal of Coordination Chemistry*, vol. 59, no. 5, pp. 485–492, 2006.
- [34] P. B. Maravalli, K. B. Gudasi, and T. R. Goudar, "Thermal studies of cobalt(II), nickel(II) and copper(II) 3-substituted-4-salicylideneamino-5-mercapto-1,2,4-triazole complexes," *Transition Metal Chemistry*, vol. 25, no. 4, pp. 411–414, 2000.
- [35] K. Kumar Narang, J. Prasad Pandey, and V. Prasad Singh, "Synthesis, characterization and physicochemical studies of some Copper(II) tetrathiocyanato dithallate(I) complexes with hydrazides and hydrazones," *Polyhedron*, vol. 13, no. 4, pp. 529–538, 1994.
- [36] A. H. Osman, "Synthesis and characterization of cobalt(II) and nickel(II) complexes of some schiff bases derived from 3-hydrazino-6-methyl[1,2,4] triazin-5(4H)one," *Transition Metal Chemistry*, vol. 31, no. 1, pp. 35–41, 2006.
- [37] Y. Anjaneyulu and R. P. Rao, "Preparation, characterization and antimicrobial activity studies on some ternary complexes of Cu(II) with acetyl acetone and various salicylic acids," *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry*, vol. 16, no. 3, pp. 257–272, 1986.
- [38] N. Dharmaraj, P. Viswanathamurthi, and K. Natarajan, "Ruthenium(II) complexes containing bidentate Schiff bases and their antifungal activity," *Transition Metal Chemistry*, vol. 26, no. 1–2, pp. 105–109, 2001.

