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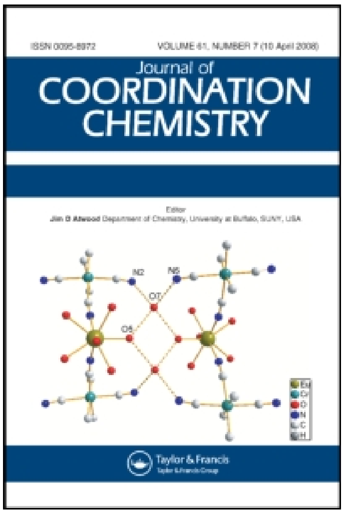

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Spectroscopic, *in vitro* antibacterial, and antifungal studies of Co(II), Ni(II), and Cu(II) complexes with 4-chloro-3-coumarinaldehyde Schiff bases

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A series of metal complexes $ML_2 \cdot 2H_2O$ [M = Co(II), Ni(II), and Cu(II)] have been synthesized with Schiff bases derived from 3-substituted-4-amino-5-mercapto-1,2,4-triazole and 4-chloro-3-coumarinaldehyde. The structures of these metal complexes have been proposed from elemental analyses, spectral (IR, UV-Vis, FAB-mass, ESR, and fluorescence), magnetic, and thermal studies. Low molar conductance values in DMF indicate that the metal complexes are non-electrolytes. Cyclic voltammetric studies suggested that the Ni(II) and Cu(II) complexes are single-electron transfer quasi-reversible. The Schiff bases and their metal complexes have been screened for *in vitro* antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella typhi*) and antifungal activities (*Candida albicans*, *Cladosporium*, and *Aspergillus niger*) by the minimum inhibitory concentration method. The Schiff base **I** and its Co(II) and Ni(II) complexes exhibit DNA cleavage activity on isolated DNA of *S. aureus* and *A. niger*.

Keywords: Antimicrobial; Coumarin; Electrochemical; Spectroscopic studies; Schiff bases; Transition metal complexes

1. Introduction

Coumarins are well-known natural products displaying a broad range of biological activities [1], anticoagulant [2, 3], antibacterial, antifungal [4], antibiotic [5], spasmolytic [6], anthelmintic [7], diuretic [8], anti-inflammatory [9], antitubercular agents [10], antihistamic [11], antidepressant [12], and antimalarial [13]. Coumarin derivatives have been studied to suggest their use as chelating agents [14, 15]. Triazole derivatives possess antibacterial, fungicidal, hypotensive, and hypothermic activities [16–18].

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Metal complexes of various Schiff bases have been extensively investigated spectroscopically and investigated for their biological properties [19–24]. Interaction of transition metal complexes with DNA has been studied for use as probes for DNA structure and potential applications in chemotherapy. Some metal complexes can induce the breakage of DNA strands. After DNA strands are cleaved, the DNA double strands break and replication ability of cancer gene is destroyed. A copper complex could cleave DNA in the presence of ascorbate or hydroquinone [25]; reductants had a critical influence on DNA cleavage. Very recently, Cu(II) complexes have been reported to be active in DNA strand scission [26–28].

Comparatively, fewer publications have been devoted to the synthesis of Schiff bases of 4-chloro-3-coumarinaldehyde [29]. Hence, in continuation with our earlier work on coumarin Schiff bases [20, 23, 24, 28], here we report the synthesis, spectroscopic, and biological studies of Co(II), Ni(II), and Cu(II) complexes of Schiff bases derived from 4-chloro-3-coumarinaldehyde and 3-substituted-4-amino-5-mercapto-1,2,4-triazole. The Schiff bases and their coordinating ability toward metal complexes have been studied by various spectroscopic (FT-IR, NMR, UV-Vis, ESR, FAB-mass, fluorescence, and CV), magnetic, and thermal studies.

2. Experimental

2.1. *Analysis and physical measurements*

Carbon, hydrogen, and nitrogen were estimated using Elemental Analyzer Truspec (Leco Corporation, USA). IR spectra of the Schiff bases and their Co(II), Ni(II), and Cu(II) complexes were recorded on a HITACHI-270 IR spectrophotometer (4000–250 cm^{-1}) in KBr disks. Electronic spectra of the complexes were recorded in HPLC grade DMF and DMSO on a VARIAN CARY 50-BIO UV-spectrophotometer from 200 to 1100 nm. The ^1H-NMR spectra of ligands were recorded in DMSO-d_6 on a BRUKER 300 MHz spectrometer at room temperature (RT) using TMS as an internal reference. Fluorescence spectra of Schiff bases and their complexes were recorded on a HITACHI F-7000 Fluorescence Spectrophotometer (made in Japan) using 10^{-3} mol L^{-1} in HPLC grade DMF and DMSO at RT. Electrochemistry of the metal complexes was recorded on a CHI1110A-electrochemical analyzer (made in USA) in DMF containing 0.05 M n-Bu_4NClO_4 as the supporting electrolyte. The ESR spectrum was recorded on a Varian-E-4X-band EPR spectrometer with 3000 G at modulation frequency of 100 KHz at liquid nitrogen temperature (LNT) using TCNE as "g" marker. FAB-mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 kV, 10 A) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at RT with m-nitrobenzyl alcohol as the matrix. Thermogravimetric analyses (TGA) were measured from RT to 1000°C at a heating rate of 10°C min^{-1}. The data were obtained using TA Instruments Water LLC, New Castle, Delaware, USA, Model; DCS Q 20, 2009. Molar conductivity measurements were recorded on an ELICO-CM-82 T Conductivity Bridge with a cell having cell constant 0.51. Magnetic moments of the complexes were carried out by using a Faraday balance.

2.2. *Synthesis*

All chemicals and solvents were of AR grade; metal(II) salts were used as their chlorides. 3-Substituted-4-amino-5-mercapto-1,2,4-triazoles were synthesized by reported methods [22, 30].

2.2.1. Formylation of 4-hydroxycoumarin. To a well-cooled solution of 4-hydroxycoumarin (0.02 mol) in dry DMF (0.2 mol), freshly distilled dry $POCl_3$ (0.02 mol) was added dropwise with constant stirring at 0–5°C. The reaction mixture was set aside for 30 min and then carefully poured on crushed ice with vigorous stirring. The compound 4-chloro-3-coumarinaldehyde that separated was filtered, washed successively with 5% $NaHCO_3$ and water, and then recrystallized from acetone–water giving pale yellow needles. M.p. (yield %); 115°C (80%).

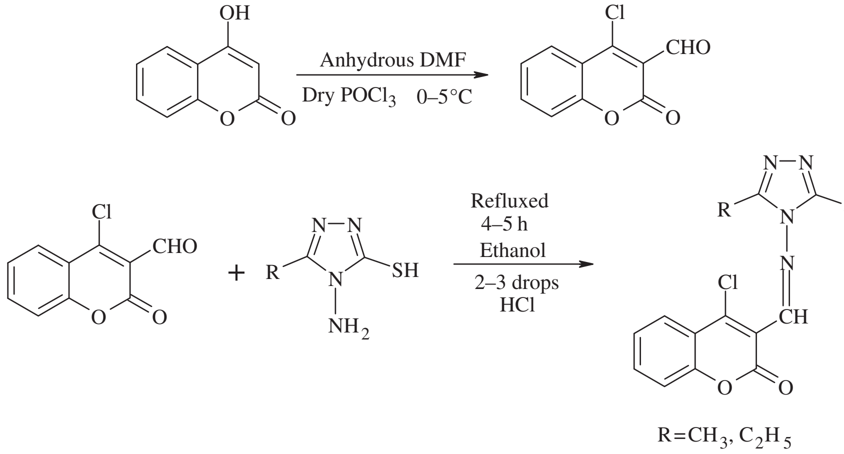
2.2.2. Synthesis of Schiff bases (I and II). The Schiff bases were synthesized by condensation of 3-substituted-4-amino-5-mercapto-1,2,4-triazole (0.01 mol) and 4-chloro-3-coumarinaldehyde (0.01 mol) dissolved in 30 mL alcoholic medium containing a few drops of concentrated HCl. The resulting mixture was refluxed for 3–4 h. The solid product which separated on evaporation of the solvent was filtered, washed with alcohol, and then recrystallized from EtOH. M.p. (Yield %); 267°C (67%); and 269°C (65%) of Schiff bases **I** and **II**, respectively.

2.2.3. Synthesis of Co(II), Ni(II), and Cu(II) complexes (1–6). An alcoholic solution (30mL) of Schiff bases (**I** and **II**; 2 mmol) was refluxed with 1 mmol of $CoCl_2 \cdot 6H_2O$/$NiCl_2 \cdot 6H_2O$/$CuCl_2 \cdot 2H_2O$ in 30 mL ethanol solution on a steam bath for 1 h. Then, 2 mmol of sodium acetate was added to the reaction mixture and reflux continued for 3 h. The separated complex was filtered, washed thoroughly with water, ethanol, and ether, and finally dried in vacuum over fused $CaCl_2$.

2.3. *Pharmacology*

2.3.1. DNA cleavage. Preparation of culture media and DNA isolation of *Staphylococcus aureus* and *Aspergillus niger* microbial strains were done according to the literature procedure [28]. Nutrient broth (peptone, 10; Yeast extract, 5; NaCl, 10; in $g\,L^{-1}$) was used for culturing of *S. aureus* and potato dextrose broth (potato, 250; dextrose, 20; in $g\,L^{-1}$) was used for the culture of *A. niger*.

2.3.1.1. *Agarose gel electrophoresis.* Cleavage products were analyzed by agarose gel electrophoresis [31]. Test samples ($1\,mg\,mL^{-1}$) were prepared in DMF. Samples (25 µg) were added to the isolated DNA of *S. aureus* and *A. niger*, incubated for 2 h at 37°C, and then 20 μL of DNA sample (mixed with bromophenol blue dye at 1 : 1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 L) and finally loaded on agarose gel and 50 V of electricity passed through for 30 min. Removing the gel and staining with $10.0\,\mu g\,mL^{-1}$ ethidium bromide for 10–15 min gave bands observed under Vilberlourmate Gel documentation system and photographed to



Scheme 1. Synthesis of Schiff bases **I** and **II**.

determine the extent of DNA cleavage. Then, the results are compared with standard DNA marker.

2.3.2. In vitro *antibacterial and antifungal assay*

Biological activities of Schiff bases and their complexes were studied by agar and potato dextrose agar diffusion methods. The antibacterial and antifungal activities were done at 25, 50, and 100 $\mu g\,mL^{-1}$ concentrations in DMSO using four bacteria (*Escherichia coli*, *S. aureus*, *Bacillus subtilis*, and *Salmonella typhi*) and three fungi (*A. niger*, *Candida albicans*, and *Cladosporium*) by the minimum inhibitory concentration (MIC) method [32]. The bacterial strains were incubated for 24 h at 37°C and fungal strains for 48 h at 37°C. Standard antibacterial and antifungal drugs (Gentamycine and Fluconazole) were used for comparison under similar conditions.

3. Results and discussion

Synthesis of the Schiff bases is schematically presented in scheme 1. Schiff bases (**I** and **II**) with $CoCl_2 \cdot 6H_2O/NiCl_2 \cdot 6H_2O/CuCl_2 \cdot 2H_2O$ in EtOH gave octahedral complexes (**1**–**6**), where the coordination sphere of the central metal is completed by two water molecules. The Co(II), Ni(II), and Cu(II) complexes are colored, stable, non-hygroscopic, insoluble in common organic solvents and soluble in DMF and DMSO. Elemental analyses showed that Co(II), Ni(II), and Cu(II) complexes have 1 : 2 stoichiometry $ML_2 \cdot 2H_2O$, where "L" stands for a singly deprotonated ligand. The molar conductance values are too low to account for any dissociation of the complexes in DMF, indicating non-electrolytes in DMF (table 1).

Table 1. Elemental analyses of Schiff bases and their complexes along with molar conductances and magnetic moment data.

Compound no.	Empirical formula	Color/yield (%)	M (%)		C (%)		H (%)		N (%)		Molar conductance (Ω^{-1} cm^2 mole^{-1})	μ_{eff} (BM)
			Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated		
I	$C_{13}H_9N_4SClO_2$	Orange red/80	–	–	48.72	48.75	2.81	2.82	17.33	17.35	–	–
II	$C_{14}H_{11}N_4SClO_2$	Orange red/78	–	–	50.27	50.29	3.29	3.30	15.20	15.26	–	–
1	$Co(C_{26}H_{16}N_8S_2Cl_2O_4) \cdot 2H_2O$	Brown/65	8.047	8.050	42.55	42.50	2.71	2.72	15.25	15.30	26.22	4.80
2	$Co(C_{28}H_{20}N_8S_2Cl_2O_4) \cdot 2H_2O$	Brown/63	7.750	7.755	44.12	44.15	3.13	3.18	14.70	14.72	25.30	5.02
3	$Ni(C_{26}H_{16}N_8S_2Cl_2O_4) \cdot 2H_2O$	Yellowish green/69	7.921	7.924	44.60	44.65	2.72	2.75	15.33	15.35	20.06	3.20
4	$Ni(C_{28}H_{20}N_8S_2Cl_2O_4) \cdot 2H_2O$	Yellowish green/68	7.600	7.606	44.22	44.28	3.10	3.13	14.70	14.77	22.35	3.28
5	$Cu(C_{26}H_{16}N_8S_2Cl_2O_4) \cdot 2H_2O$	Dark green/65	8.531	8.534	42.30	42.37	2.70	2.68	15.20	15.22	28.15	1.77
6	$Cu(C_{28}H_{20}N_8S_2Cl_2O_4) \cdot 2H_2O$	Dark green/66	8.225	8.223	43.90	43.98	3.10	3.17	14.63	14.66	25.56	1.79

Table 2. The important infrared frequencies (in cm^{-1}) of Schiff bases and their metal complexes.

Compound no.	ν(OH)	ν(NH)	ν(SH)	Lactonyl ν(C=O)	ν(C=N)	ν(M–N)	ν(M–S)
I	–	3145	2762	1720	1615	–	–
II	–	3179	2782	1716	1628	–	–
1	3410	3142	–	1717	1608	478	378
2	3427	3177	–	1715	1609	473	345
3	3410	3144	–	1722	1607	475	368
4	3419	3174	–	1716	1609	469	341
5	3433	3140	–	1718	1605	471	360
6	3434	3175	–	1719	1608	470	358

3.1. *IR spectra*

IR spectra of the Schiff bases were compared with the spectra of the metal complexes. IR spectra (table 2) of the Schiff bases show characteristic bands due to ν(NH) and ν(SH) at 3145–3179 and 2762–2782 cm^{-1}, respectively. Another band at 1090–1092 cm^{-1} is assigned to ν(C=S). These observations suggest that the Schiff bases exhibit thiol–thione tautomerism (figure 1). Strong bands at 1716–1720, 1615–1628, and 1387–1394 cm^{-1} in the Schiff bases are assigned to ν(C=O), ν(C=N), and phenolic ν(C–O), respectively.

In comparison with spectra of the Schiff bases, all metal complexes (table 2) exhibit downward shift of about 10–20 cm^{-1} of ν(C=N), indicating the participation of azomethine nitrogen in coordination to the metal ion. Deprotonation of the thiol is indicated by the absence of a band in the metal complexes at 2762–2782 cm^{-1}, due to ν(SH) of Schiff bases, indicating that the Schiff bases are coordinated to the metal through the thiolate sulfur. This is further supported by the lower frequency band at 752–762 cm^{-1} in the metal complexes due to ν(C–S). The bands observed at 1716–1720 cm^{-1} in the free ligand have been assigned to ν(C=O). Practically no effect on these frequencies after complexation indicates non-involvement of these groups in coordination. The new bands in the region of 469–478 and 341–378 cm^{-1} in the spectra of the metal complexes are assigned to stretching frequencies of ν(M–N) and ν(M–S) bond formation, respectively. The presence of coordinated water in the complexes is indicated by a broad band in the region 3410–3434 cm^{-1} and two weak bands at 760–800 and 710–720 cm^{-1} due to ν(–OH) rocking and wagging modes of vibrations, respectively [33].

Thus, IR spectral data provide strong evidence for coordination of Schiff bases to the metal(II) ions through azomethine nitrogen and thiolate sulfur.

3.2. *^{1}H- and ^{13}C-NMR spectral studies of Schiff bases*

In the ^{1}H-NMR spectrum (table 3) of **II**, a singlet at 13.84 ppm (s, 1H) is ascribed to –NH and a sharp signal at 10.93 ppm (s, 1H) is attributed to –SH. These observations suggest that the Schiff bases exist in thiol–thione tautomerism. A singlet at 8.68 ppm (s, 1H) is assigned to –CH=N group. The multiplet observed at 6.9–7.7 ppm (m, 4H) is due to aromatic protons. The quartet and triplet at 2.58 and 1.25 ppm are due to $–CH_2$ and $–CH_3$, respectively.

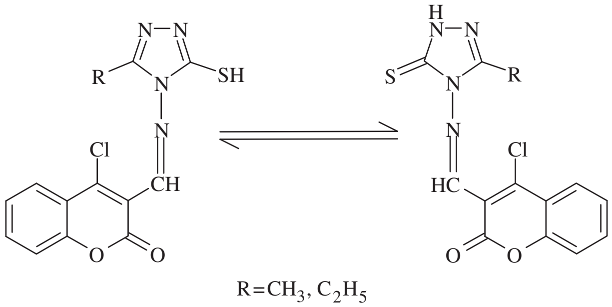


Figure 1. Structure of Schiff bases: thiol–thione tautomerism.

Table 3. The important ^{1}H- and ^{13}C-NMR data of Schiff bases.

Schiff base	^{1}H-NMR (DMSO-d_6) (ppm)	^{13}C-NMR (DMSO-d_6) (ppm)
I	12.56 (s, 1H, NH), 10.92 (s, 1H, SH), 8.67 (s, 1H, CH=N), 6.9–7.9 (m, 4H, Ar-H), 1.3 (t, 3H, CH_3)	91.8, 116.6, 117.2, 124.0, 124.8, 133.5 (coumarin), 154.35 (C=N), 166.5 (C=O), 185.3, 179.2 (triazole), 11.30 (CH_3)
II	13.84 (s, 1H, NH), 10.93 (s, 1H, SH), 8.68 (s, 1H, CH=N), 6.9–7.7 (m, 4H, Ar-H), 2.58 (q, 2H, CH_2), 1.25 (t, 3H, CH_3)	95.2, 116.7, 117.8, 125.3, 125.4, 133.6 (coumarin), 154.6 (C=N), 166.9 (C=O), 187, 183 (triazole), 11.33 (CH_3), 31.7 (CH_2)

In ^{13}C-NMR spectrum (table 3) of the Schiff base **II** the signal observed at 166.9 ppm is ascribed to lactonyl carbon (C=O) of the coumarin. A characteristic signal at 154.6 ppm is assigned to (C=N). In addition to this, the characteristic signals in the region 95.2–133.6 ppm are assigned to aromatic carbons and the signals at 11.24 and 31.7 ppm are due to CH_3 and CH_2, respectively.

3.3. *Electronic spectral studies*

The cobalt(II) complexes exhibited two distinct bands at 9920–9938 and 18,860–20,660 cm^{-1} corresponding to $^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}(F)$ (ν_1) and $^{4}T_{1g}(F) \rightarrow {}^{4}T_{1g}(P)$ (ν_3), which suggests an octahedral geometry around cobalt(II) [34]. The ν_2 band that involves a two-electron transition is not observed because of its proximity to the strong ν_3 transition. Electronic spectra of nickel(II) complexes showed d–d bands in the region 10,450–10,790, 16,333–16,889, and 26,824–27,620 cm^{-1}, assigned to $^{3}A_{2g}(F) \rightarrow {}^{3}T_{2g}(F)$ (ν_1), $^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(F)$ (ν_2), and $^{3}A_{2g}(F) \rightarrow {}^{3}T_{1g}(P)$ (ν_3) transitions, respectively. Table 4 shows the ligand field parameters ($Dq, B', \beta, \beta^{\circ}\%, \nu_2/\nu_1$, and LFSE) [35]. Racah parameter B' is 806.918–826.650 cm^{-1}, less than the free ion value of 1040 cm^{-1} indicating covalent complex. The ratio ν_2/ν_1 was 1.563–1.565, well within the range 1.40–1.61 indicative of octahedral geometry [36]. The value of $\beta^{\circ}\%$, 23.587, further supports octahedral geometry around Ni(II) [37].

Table 4. Ligand field parameters of Ni(II) complexes.

Complex no.	Transitions (cm^{-1}) ν_1	ν_2	ν_3	ν_2 Calculated (cm^{-1})	Dq (cm^{-1})	B^1 (cm^{-1})	Distortion (%)	ν_2/ν_1	LSFE	μ_{eff} Calculated (BM)	β	β° (%)
3	10,450	16,333	26,824	16,629.76	1045	806.918	1.785	1.563	35.829	3.171	0.764	23.587
4	10,790	16,869	27,620	17,149.75	1079	826.650	1.520	1.565	36.994	3.160	0.783	21.719

Electronic spectra of Cu(II) complexes showed absorptions in the region 14,555–14,760 cm^{-1} attributed to $^2T_{2g} \leftarrow {}^2E_g$ transition, indicative of distorted octahedral geometry [38].

3.4. *Magnetic studies*

Table 1 lists magnetic moments obtained at RT. The magnetic measurement for Co(II) complexes exhibited magnetic moment values of 4.80–5.02 BM, which agree with octahedral range 4.3–5.2 BM. Ni(II) complexes showed the magnetic moment values of 3.20–3.28 BM within the range 2.8–3.5 BM [39], consistent with their octahedral environment. Cu(II) complexes showed magnetic moments of 1.77–1.79 BM, slightly higher than the spin-only value 1.73 BM expected for one unpaired electron, offering the possibility of octahedral geometry [40].

3.5. *ESR spectrum of $Cu(C_{26}H_{16}N_8S_2Cl_2O_4) \cdot 2H_2O$*

ESR spectral studies of Cu(II) complexes provide information of the metal ion environment. The ESR spectrum of $Cu(C_{26}H_{16}N_8S_2Cl_2O_4) \cdot 2H_2O$ recorded in DMSO at LNT and at RT show $g_{||}$ and $g_{\perp}$ values of 2.0754 and 2.0551, respectively. These values indicate that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital [41]. The trend $g_{||} > g_{\perp} > 2.0023$ indicates that the unpaired electron is localized in the $d_{x^2-y^2}$ orbital of Cu(II), characteristic of axial symmetry. Thus, the results suggest that $Cu(C_{26}H_{16}N_8S_2Cl_2O_4) \cdot 2H_2O$ possesses distorted octahedral geometry.

3.6. *FAB-mass spectral studies of Schiff bases and their complexes*

The FAB-mass spectrum of **I** (figure S1 in Supplementary material) exhibited a molecular ion peak at m/z 320 which is equivalent to its molecular weight. The fragmentation peaks at m/z 305, 272, and 166 are ascribed to the cleavage of CH_3, SH, and triazole, respectively. For **II**, the molecular ion peak at m/z 334 is ascribed to $C_{14}H_{12}N_4SClO_2$.

The FAB-mass spectrum of $Co(C_{26}H_{16}N_8S_2Cl_2O_4) \cdot 2H_2O$ (figure S2 in Supplementary material) is representative of $Ni(C_{26}H_{16}N_8S_2Cl_2O_4) \cdot 2H_2O$ and $Cu(C_{26}H_{16}N_8S_2Cl_2O_4) \cdot 2H_2O$ showing molecular ion peaks M^+ at m/z 733, 732, and 737 equivalent to $[ML_2 \cdot 2H_2O]^+$. The molecular ion by loss of two water molecules gave fragment ion peaks at *m*/*z* 697, 696, and 701 for $[ML_2]^+$. All the metal complexes undergo demetallation to form $[L+H]^+$ observed at *m*/*z* 320.

3.7. *Thermal analyses*

The thermal behavior of Co(II), Ni(II), and Cu(II) complexes are all almost the same. Representative thermogravimetric (TG)/derivative thermogravimetric (DTG) curves of $Co(C_{26}H_{16}N_8S_2Cl_2O_4)\cdot 2H_2O$, $Ni(C_{26}H_{16}N_8S_2Cl_2O_4)\cdot 2H_2O$, and $Cu(C_{26}H_{16}N_8S_2Cl_2O_4)\cdot 2H_2O$ show the following characteristic features.

The thermal decomposition of $Co(C_{26}H_{16}N_8S_2Cl_2O_4)\cdot 2H_2O$, $Ni(C_{26}H_{16}N_8S_2Cl_2O_4)\cdot 2H_2O$, and $Cu(C_{26}H_{16}N_8S_2Cl_2O_4)\cdot 2H_2O$ took place in three steps, as indicated by DTG peaks around 185–200°C, 215–255°C, and 300–355°C corresponding to mass loss of coordinated water molecule, triazole, and coumarinaldehyde, respectively. Hence, the TGA studies confirm the coordination of water to the metal ions. Metal oxide formed above 560°C. The thermogram of $Cu(C_{26}H_{16}N_8S_2Cl_2O_4)\cdot 2H_2O$ is shown in figure S3 in Supplementary material. The nature of the proposed chemical change with the temperature range and the percentage of metal oxide obtained are given in table 5.

3.8. *Electrochemistry*

Electrochemical properties of the complexes were studied on a CHI1110A-Electrochemical analyzer in DMF containing $0.05\,\mathrm{mol\,L^{-1}}$ *n*-Bu_4NClO_4 as the supporting electrolyte. A cyclic voltammogram of $Cu(C_{26}H_{16}N_8S_2Cl_2O_4)\cdot 2H_2O$ (figure S4 in Supplementary material) displays a reduction peak at $E_{pc}=0.2917\,\mathrm{V}$ with a corresponding oxidation peak at $E_{pa}=0.4579\,\mathrm{V}$. The peak separation of this couple (ΔE_p) is 0.1662 V at 0.1 V and increases with scan rate. The most significant feature of the Cu(II) complex is the Cu(II)/Cu(I) couple. The difference between forward and back peak potentials can provide a rough evaluation of the degree of reversibility of one-electron transfer reaction. Analyses of cyclic voltammetric responses with scan rate varying from 50 to $250\,\mathrm{mV\,s^{-1}}$ gives evidence for quasi-reversible one-electron oxidation. The ratio of cathodic to anodic peak height was less than one; however, the peak current increases with increase of the square root of the scan rates, establishing the electrode process as diffusion controlled [42]. The separation in peak potentials increases at higher scan rates. These characteristic features are consistent with quasi-reversibility of the Cu(II)/Cu(I) couple.

The $Ni(C_{26}H_{16}N_8S_2Cl_2O_4)\cdot 2H_2O$ complex exhibits a reduction peak at $E_{pc}=0.4541\,\mathrm{V}$ with a direct re-oxidation peak at $E_{pa}=0.7490\,\mathrm{V}$ corresponding to the Ni(II)/Ni(I) couple. The peak separation of this couple (ΔE_p) is 0.2251 V at 0.1 V and increases with scan rate, consistent with quasi-reversibility of the Ni(II)/Ni(I) couple.

3.9. *Fluorescence studies*

Emission spectra of the Schiff base **I** and its metal complexes were investigated in DMF and DMSO. Schiff base **I** is characterized by an emission band at 535 nm in DMF and 527 in DMSO. Upon addition of 2–3 drops of aqueous alkali (2% NaOH) to **I**, we

Table 5. TG data of Co(II), Ni(II), and Cu(II) complexes of Schiff base **I**.

Complex no.	Decomposition temperature (°C)	Weight loss (%)		Metal oxide (%)		Inference
		Observed	Calculated	Observed	Calculated	
1	190	4.911	4.916	10.23	10.25	Loss of coordinated water molecules
	220	34.65	34.69			Loss of triazole moieties
	355	52.38	52.40			Loss of coumarin moieties
3	197	4.918	4.920	10.10	10.17	Loss of coordinated water molecules
	225	34.69	34.70			Loss of triazole moieties
	370	52.45	52.47			Loss of coumarin moieties
5	185	4.888	4.890	10.71	10.73	Loss of coordinated water molecules
	217	34.46	34.47			Loss of triazole moieties
	307	52.10	52.13			Loss of coumarin moieties

observed the λ_{max} values at 509 nm in DMF and 512 in DMSO from formation of phenoxide anion. The Co(II), Ni(II), and Cu(II) complexes of **I** are characterized by emission bands at 468, 442, and 472 nm, respectively in DMF (figure S5 in Supplementary material). There was a decrease in intensity of fluorescence of Co(II), Ni(II), and Cu(II) complexes in both DMF and DMSO solutions. Previous studies reported that transition metal ions decrease the fluorescence quite effectively [24, 28]. Schiff base **I** and its complexes are fluorescent in nature.

3.10. *Pharmacology results*

A number of authors [20–24, 43–47] have investigated the biological properties of transition metal complexes of triazoles as well as coumarin derivatives.

The Schiff bases and their metal complexes were evaluated for antimicrobial activity (table 6). Schiff bases (**I** and **II**) were potentially active against *S. aureus* and *A. niger* and moderately active against *S. typhi*. Both Schiff bases and their Co(II), Ni(II), and Cu(II) complexes showed high antibacterial activity against all the bacterial strains. All the metal complexes possess higher antifungal activity than the Schiff bases. Khanmohammadi and Abnosi [47] studied the antimicrobial activities of Co(II), Ni(II), and Cu(II) chelates of 4-amino-3-(4-pyridine)-5-mercapto-1,2,4-triazole and reported that the activity of metal complexes were higher than the Schiff bases [47]. Bagihalli *et al*. [20] reported the *in vitro* antibacterial and antifungal activities of Co(II), Ni(II), and Cu(II) complexes with 1,2,4-triazole Schiff bases [33] to be higher than the Schiff bases due to the change in structure due to coordination. Incorporation of methyl and ethyl group exhibit differences in activity (**5** is more active than **6** toward *S. typhi*).

The MIC of some selected compounds, which showed significant activity against selected bacterial and fungal strains, indicated that these compounds were most active in inhibiting the growth of the tested organisms at 10 mg mL^{-1} (table 7).

Table 6. Antimicrobial results of Schiff bases and their metal complexes.

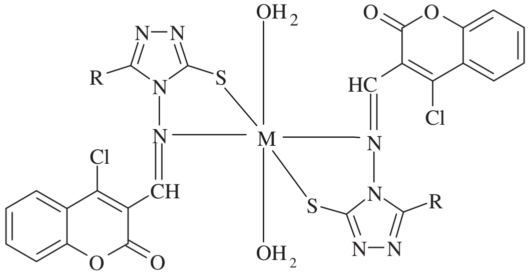
		Inhibition against bacteria (%)				Inhibition against fungi (%)		
Compound	Concentration ($\mu g\,mL^{-1}$)	*E. coli*	*S. aureus*	*B. subtilis*	*S. typhi*	*C. albicans*	*Cladosporium*	*A. niger*
I	25	43	46	42	25	48	49	50
	50	51	66	54	29	51	50	52
	100	62	71	65	38	65	62	66
II	25	42	48	43	27	46	47	44
	50	54	56	52	31	52	56	58
	100	63	66	61	39	68	61	68
1	25	53	63	53	30	52	60	70
	50	60	71	62	35	66	68	72
	100	79	88	73	42	71	73	82
2	25	51	64	59	33	55	66	72
	50	63	72	69	40	75	71	72
	100	76	89	77	42	80	79	81
3	25	56	63	59	33	59	58	66
	50	64	73	69	37	69	65	70
	100	75	80	76	44	78	75	85
4	25	53	66	59	36	54	61	68
	50	64	75	62	41	68	68	71
	100	77	88	70	49	76	71	81
5	25	58	66	50	31	16	35	30
	50	59	68	53	38	35	38	41
	100	70	70	68	40	37	53	54
6	25	56	61	53	32	14	33	45
	50	58	65	56	33	45	42	49
	100	69	66	71	41	56	51	55
Gentamycine	100	100	100	100	100	–	–	–
Fluconazole	100	–	–	–	–	100	100	100

Table 7. MIC ($\mu g\,mL^{-1}$) of selected compounds against selected bacteria.

Compound	*E. coli*	*S. aureus*	*B. subtilis*	*S. typhi*	*C. albicans*	*Cladosporium*	*A. niger*
I	25	10	25	25	10	25	10
II	25	10	25	25	25	10	10
1	10	10	10	10	10	10	10
2	10	25	10	10	10	10	10
3	10	10	10	10	10	10	10
4	10	25	10	10	10	25	25
5	25	>100	25	25	>100	>100	>100
6	25	>100	25	>100	>100	>100	>100
Gentamycine	10	10	10	10	–	–	–
Fluconazole	–	–	–	–	10	10	10

3.11. *DNA cleavage studies*

Schiff base **I**, Co(II) (**1**), and Ni(II) (**3**) were studied for their DNA cleavage activity by agarose gel electrophoresis. Lanes L2, C1, and C2 of figure S6 in "Supplementary material" represent the cleavage activity of **I**, **1**, and **3**, respectively, on isolated DNA



R=CH_3, C_2H_5

M=Co(II), Ni(II), Cu(II)

R	M	Formula	Complex no.
CH_3	Co(II)	$Co(C_{26}H_{16}N_8S_2Cl_2O_4) \cdot 2H_2O$	**1**
C_2H_5	Co(II)	$Co(C_{28}H_{20}N_8S_2Cl_2O_4) \cdot 2H_2O$	**2**
CH_3	Ni(II)	$Ni(C_{26}H_{16}N_8S_2Cl_2O_4) \cdot 2H_2O$	**3**
C_2H_5	Ni(II)	$Ni(C_{28}H_{20}N_8S_2Cl_2O_4) \cdot 2H_2O$	**4**
CH_3	Cu(II)	$Cu(C_{26}H_{16}N_8S_2Cl_2O_4) \cdot 2H_2O$	**5**
C_2H_5	Cu(II)	$Cu(C_{28}H_{20}N_8S_2Cl_2O_4) \cdot 2H_2O$	**6**

Figure 2. Structure of metal complexes.

of *S. aureus*. The difference in migration was observed compared to control DNA of *S. aureus* (Lane Ct1). **1** and **3** show cleavage activity on isolated DNA of *A. niger* (Lanes C3 and C4, respectively, of figure S6 in Supplementary material). Control DNA alone does not show any apparent cleavage whereas **I**, **1**, and **3** show a cleavage. However, the nature of reactive intermediates involved in DNA cleavage by the complexes is not clear. The results indicate the important role of metal ion in these isolated DNA cleavage reactions. We infer that **I**, **1**, and **3** are potent nuclease agents inhibiting growth of the pathogenic organism by cleaving the genome.

4. Conclusion

The synthesized Schiff bases exhibit thiol–thione tautomerism and act as singly deprotonated ligands. The metal ion is coordinated through the azomethine nitrogen and sulfur *via* deprotonation. Octahedral structures of Co(II), Ni(II), and Cu(II) complexes are shown in figure 2. The bonding of ligand to metal is confirmed by analytical, spectral, magnetic, and thermal studies. Electrochemical properties of the Cu(II) and Ni(II) complexes exhibit one-electron quasi-reversible electron transfer. The metal complexes have higher antimicrobial activities than the ligands. Schiff base **I**, **1**, and **3** cleave DNA as compared to control *S. aureus* and *A. niger* DNA.

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