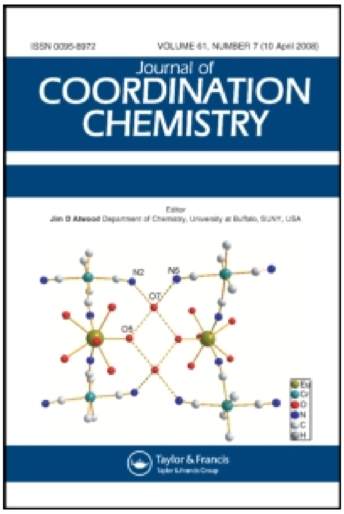

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Synthesis, spectral characterization, electrochemical and biological studies of Co(II), Ni(II) and Cu(II) complexes with thiocarbohydrazone

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Synthesis, spectral characterization, electrochemical and biological studies of Co(II), Ni(II) and Cu(II) complexes with thiocarbohydrazone

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A series of metal complexes of Co(II), Ni(II) and Cu(II) have been synthesized with the Schiff base derived from thiocarbohydrazide and 8-formyl-7-hydroxy-4-methylcoumarin. The structures of the complexes have been proposed by elemental analyses, molar conductance, spectral (IR, UV-Vis, ESR and FAB-mass), magnetic, thermal and electrochemical studies. These complexes are soluble in DMF and DMSO and molar conductance values indicate that they are non-electrolytes. Elemental analyses of the complexes confirm stoichiometry $ML \cdot 2H_2O$ [M=Co(II), Ni(II) and Cu(II)]. Spectroscopic studies indicate coordination occurs through phenolic oxygen after deprotonation and nitrogen of azomethine. The Schiff base and its complexes have also been screened for antibacterial (*Escherichia coli*, *Streptococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*) and antifungal activities (*Aspergillus niger*, *Aspergillus flavus* and *cladosporium*) by the MIC method. The brine shrimp bioassay was also carried out to study their *in vitro* cytotoxic properties.

Keywords: Synthesis; Biological activity; Electrochemical; Thiocarbohydrazone; Coumarin

1. Introduction

Metal complexes of thiocarbohydrazones and coumarins have attracted attention due to applications in analytical chemistry for the identification and estimation of both organic and inorganic compounds, antibacterial [1–3], antifungal [3, 4], antitumor [5], anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombic, antiviral, anticarcinogenic [4], anti-HIV, anticoagulant and spasmolytic activities [6]. Some thiocarbohydrazones have been used as inactivators of HSV-1 ribonucleotide reductase [7]. The hydroxycoumarins are potent metal chelators, free radical scavengers, and powerful chain-breaking antioxidants. Hydrazine groups of thiocarbohydrazone are very reactive, forming *bis* derivatives with aldehydes and ketones [8–10]. A number of metal complexes with thiocarbohydrazones have been reported from our laboratory [11–13]. Attempts have been made to obtain VO^{2+}, Co(II), Ni(II), Cu(II) and Pt(II)

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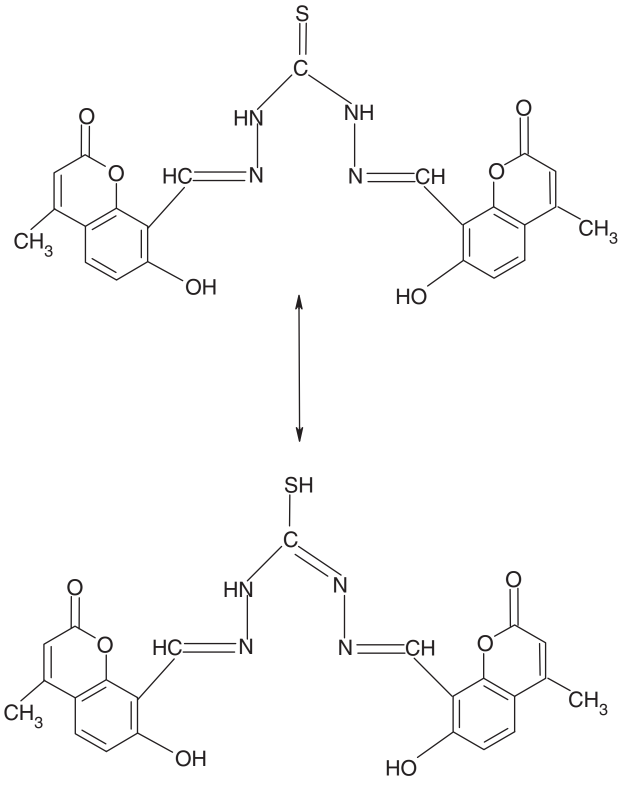


Figure 1. Tautomeric structures of the Schiff base.

complexes with *bis* (diacetylmonoxime) thiocarbohydrazone Schiff base [14] and La(III) and Th(IV) complexes with the Schiff base derived from thiocarbohydrazide and thiophene-2-aldehyde [13].

There is no report on the synthesis of Co(II), Ni(II) and Cu(II) complexes with the Schiff base derived from thiocarbohydrazide and 8-formyl-7-hydraoxy-4-methyl coumarin. This ligand has donor sites with the ONNO sequence and varied coordination possibilities.

As a part of our investigation we have synthesized the Schiff base (figure 1) and the Co(II), Ni(II) and Cu(II) complexes which are characterized spectroscopically, thermally and with molar conductivities and evaluated for biological activity.

2. Experimental

2.1. *Physical measurements*

IR spectra of the Schiff base and its Co(II), Ni(II) and Cu(II) complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000–250 cm^{-1} region in KBr disks. The electronic spectra of the complexes were recorded in DMF on a VARIAN CARY 50-BIO UV-spectrophotometer in the region 200–1100 nm. The proton NMR spectra of ligand were recorded in $CDCl_3$ on a BRUKER 300 MHz spectrometer at room temperature using TMS as an internal reference. FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 KV, 10 Am) as the FAB gas. The accelerating voltage was 10 KV and the spectra were recorded at room temperature with *m*-nitrobenzyl alcohol as the matrix. The mass spectrometer was operated in the +ve ion mode. Electrochemistry of Co(II) and Cu(II) complexes were recorded on a CHI1110A-electrochemical (HCH Instruments) analyzer (made in U.S.A). Thermogravimetric analyses were measured from room temperature to 1000°C at a heating rate of 10°C min^{-1}. The data were obtained by using a PERKIN-ELMER DIAMOND TG/DTA instrument. Molar conductivity measurements were recorded on an ELICO-CM-82 T conductivity bridge with a cell having cell constant 0.51; magnetic moment was evaluated by a Faraday balance.

2.2. *Synthesis*

All chemicals were of reagent grade. 7-hydroxy-4-methyl-coumarin was obtained from Acros Chemical Company. 8-formyl-7-hydroxy-4-methyl-coumarin [15] and thiocarbohydrazide [16] were prepared as described in the literature.

2.3. *Synthesis of Schiff base*

A mixture of thiocarbohydrazide and 8-formyl-7-hydroxy-4-methylcoumarin in 1 : 2 molar proportions in an alcoholic medium containing a few drops of concentrated HCl was refluxed for 2–3 h. The product was separated, filtered, washed with alcohol and recrystallized from EtOH.

2.4. *Synthesis of Co(II), Ni(II) and Cu(II) complexes*

An alcoholic solution of Schiff base (1 mmol) was refluxed with 1 mmol of $CoCl_2 \cdot 6H_2O/NiCl_2 \cdot 6H_2O/CuCl_2 \cdot 2H_2O$ in ethanol on a steam bath for 1 h; 2 mmol of sodium acetate was added and reflux was continued for 3 h. The separated complex was filtered, washed thoroughly with water, ethanol and ether and finally dried in vacuum over fused $CaCl_2$.

2.5. *Analyses*

The metal contents were estimated gravimetrically by the standard method [17]. Carbon, hydrogen, nitrogen and sulfur were estimated using a C, H, N and S analyzer. The elemental analyses and molar conductance values are listed in table 1.

3. Results and discussion

The complexes are colored non-hygroscopic solids, stable in air, and soluble in DMF and DMSO. The elemental analyses show stoichiometry $ML \cdot 2H_2O$, where L stands for a doubly deprotonated ligand. The molar conductance values indicate non-electrolytes in DMF (table 1).

Table 1. Elemental analyses of the Schiff base and its Co(II), Ni(II) and Cu(II) complexes with magnetic and molar conductance data.

	M%		C%		N%		S%			
Empirical formula	Obsd	Calcd	Obsd	Calcd	Obsd	Calcd	Obsd	Calcd	Molar conductance ($Ohm^{-1} cm^{-2} M^{-1}$)	Mag. moments (μ_{eff} BM)
$C_{23}H_{18}N_4O_6S$	–	–	57.70	57.74	11.69	11.72	6.64	6.69	–	–
$Co(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	10.31	10.33	48.31	48.34	9.80	9.81	5.58	5.60	28	4.82
$Ni(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	10.25	10.28	48.35	48.37	9.78	9.80	5.60	5.61	26	3.24
$Cu(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	11.02	11.04	47.93	47.95	9.71	9.73	5.53	5.56	25	1.72

In order to establish whether the water molecules are coordinated to the metal, a weighed complex was heated for 2 h at 105°C then cooled in a desiccator and weighed again with no loss in weight, suggesting the water molecules are coordinated.

3.1. *Infrared spectra*

Infrared spectral data of the Schiff base and its Co(II), Ni(II) and Cu(II) complexes are listed in table 2 and reproduced in Supplementary data.

The Schiff base shows a broad medium band at $3120\,\mathrm{cm^{-1}}$ due to ν(NH) vibrations, suggesting that the ligand is in the thione form. A broad band of weak intensity at ca $2400\,\mathrm{cm^{-1}}$ is attributed to the ν(SH) vibration, suggesting thiol-thione tautomerism (figure 1). The high intensity band at $750\,\mathrm{cm^{-1}}$ in this Schiff base is assigned to ν(C=S). A high intensity band at $1620\,\mathrm{cm^{-1}}$ is attributed to ν(C=N) [10]. These data confirm the presence of 8-formyl-7-hydroxy-4-methylcoumarin. In the Schiff base, the intramolecular H-bonded-OH occurs at $2658\,\mathrm{cm^{-1}}$ as a broad medium band with fine structure [18]. A high intensity band at $1295\,\mathrm{cm^{-1}}$ with an additional band around $1505\,\mathrm{cm^{-1}}$ was assigned to the phenolic ν(C–O) and the absorption at $1730\,\mathrm{cm^{-1}}$ is due to ν(C=O) (lactone carbonyl).

For the Co(II), Ni(II) and Cu(II) complexes we observed the following changes: (a) A broad weak band with fine structure at $2658\,\mathrm{cm^{-1}}$, assigned to the H-bonded –OH in the Schiff base disappeared. (b) Broad bands of medium intensity (ca 3230–$3250\,\mathrm{cm^{-1}}$) are assigned to ν(NH) and a weak band at 2370–$2392\,\mathrm{cm^{-1}}$ is due to ν(SH) clearly indicating that the sulfur of the C=S group is not coordinated. (c) The medium intensity band at $1608\,\mathrm{cm^{-1}}$ due to ν(C=N) indicates that the C=N of the ligand coordinates to the metal through nitrogen. (d) A broad band at $3400\,\mathrm{cm^{-1}}$ in addition to a medium to high intensity band at $850\,\mathrm{cm^{-1}}$ are attributed to the symmetric and antisymmetric-OH stretching modes of coordinated water [19]. (e) The unaltered position of the ν(C=O) (lactone carbonyl) confirms non involvement in coordination. (f) The ν(M–O) and ν(M–N) bands have been assigned in the region 540–$550\,\mathrm{cm^{-1}}$ and 425–$450\,\mathrm{cm^{-1}}$, respectively.

3.2. *^{1}H-NMR spectra*

The Schiff base has the characteristic resonance due to azomethine proton at 8.24 ppm. The signal located at 10.12 ppm is ascribed to the NH proton and the signal at 9.52 ppm to SH [19].

A singlet at 12.40 ppm is probably due to phenolic OH. Hydrogen bonding leads to deshielding and an increase in the frequency of the ^{1}H-NMR signal. The sharp multiplet

Table 2. Infrared frequencies (in $\mathrm{cm^{-1}}$) of the Schiff base and complexes.

Empirical formula	Coordinated water ν(OH)	ν(NH)	ν(SH)	ν(C=N)	ν(C=O)	H-bonded –OH stretching	M–N	M–O
$C_{23}H_{18}N_4O_6S$	–	3120	2400	1620	1730	2658	–	–
$Co(C_{23}H_{16}N_4O_6S)\cdot 2H_2O$	3389	3230	2386	1600	1725	–	450	540
$Ni(C_{23}H_{16}N_4O_6S)\cdot 2H_2O$	3345	3245	2370	1610	1728	–	435	545
$Cu(C_{23}H_{16}N_4O_6S)\cdot 2H_2O$	3400	3250	2392	1605	1730	–	425	550

signals of the phenyl protons are found in the region 6.4–7.6 ppm. The methyl protons of the ligand are observed as a sharp peak at 2.50 ppm.

3.3. *Electronic spectral studies*

Electronic spectra of Co(II) complexes show absorptions in the region 8000–10000 cm^{-1} and 18000–20000 cm^{-1} corresponding to ν_1 and ν_3 transitions, $^4T_{1g}$ (F) $\rightarrow$ $^4T_{2g}$ (F) (ν_1); $^4T_{1g}$ (F) $\rightarrow$ $^4T_{1g}$ (P) (ν_3) [20, 21]. In the present Co(II) complex the absorptions are at 9680 and 19845 cm^{-1}, characteristic of high spin octahedral Co(II). ν_2 is not observed because of its proximity to the strong ν_3 transition, but can be calculated [22, 23] (table 3).

The Ni(II) complex (table 3) has three bands at 10416, 16459 and 26452 cm^{-1} attributed to the $^3A_{2g} \rightarrow {}^3T_{2g}$ (ν_1); $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) (ν_2) and $^3A_{2g} \rightarrow {}^3T_{1g}$ (P) (ν_3) transitions of octahedral geometry.

Electronic spectra of the Cu(II) complex display three prominent bands, a low intensity broad band at 14524 cm^{-1} from $^2T_{2g} \leftarrow {}^2E_g$, a high intensity band at 25421 cm^{-1} due to symmetry forbidden ligand → metal charge transfer and two sharp bands at 30885 cm^{-1} and 37125 cm^{-1} due to the ligand. Electronic spectra indicate distorted octahedral geometry around Cu(II).

3.4. *Magnetic data*

The magnetic moments obtained at room temperature listed in table 1 indicate paramagnetism. The Co(II) complex has a magnetic moment of 4.82 BM, within the expected range of 4.7–5.2 BM [24, 25] for octahedral complexes. The Ni(II) complex has a magnetic moment of 3.24 BM within the range 2.5–3.5 BM for octahedral Ni(II) [25, 26]. The observed magnetic moment of the Cu(II) complex is 1.72 BM corresponding to one unpaired electron.

3.5. *ESR spectral studies*

The ESR spectrum of the Cu(II) complex is provided in Supplementary material. The $g_{\parallel}$ and $g_{\perp}$ values are 2.02 and 2.16 with g_{av} calculated to be 2.11. The Cu(II) complex shows a compressed octahedron with $g_{\parallel} < g_{\perp}$, indicating the electron is delocalized in the d_z^2 orbital [27]. The parameter G, determined as $G = (g_{\parallel} - 2)/(g_{\perp} - 2)$, is 0.125 suggesting considerable interaction in the solid state [28].

3.6. *FAB-mass spectral studies of Schiff base and its Ni(II) complex*

The FAB-mass spectrum of the Schiff base (shown in Supplementary material) has a molecular ion at *m*/*z* 479 equivalent to its molecular weight $[L+H]^+$.

The FAB-mass spectrum of Ni(II) complex of Schiff base (Supplementary material) has a molecular ion peak M^+ at *m*/*z* 571 equivalent to its molecular weight $[ML \cdot 2H_2O]^+$. The molecular ion by loss of two water molecules gave a fragment at *m*/*z* 535.

Fragmentation leads to $[ML]^+$ which undergoes demetallation to form $[L+H]^+$ at *m*/*z* 477.

Table 3. Ligand field parameters of Co(II) and Ni(II) complexes.

	Transitions (cm^{-1})											
Complex No.	ν_1	ν_2	ν_3	ν_2 Calcd (cm^{-1})	Dq (cm^{-1})	B^1 (cm^{-1})	% Distortion	ν_2/ν_1	LFSE	μ_{eff} Calcd BM	β	β°%
$Co(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	9680	–	19845	20530	1085.0	755.70	–	2.12	24.80	–	0.781	21.85
$Ni(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	10416	16459	26452	16495	1041.6	779.96	0.220	1.58	35.71	3.17	0.739	26.14

Table 4. Thermogravimetric data of Co(II), Ni(II) and Cu(II) complexes.

Empirical formula	Decomposition temperature °C	% Weight loss Obsd	% Weight loss Calcd	Inference
$Co(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	95–110	6.28	6.30	Loss of water molecule
	245–260	18.51	18.56	Loss of thiocarbohydrazide
	330–345	65.42	65.49	Loss of aldehyde moieties
$Ni(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	100–120	6.28	6.31	Loss of water molecule
	240–255	18.53	18.57	Loss of thiocarbohydrazide
	340–350	65.50	65.53	Loss of aldehyde moieties
$Cu(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	90–115	6.22	6.25	Loss of water molecule
	280–290	18.39	18.42	Loss of thiocarbohydrazide
	330–350	64.93	64.98	Loss of aldehyde moieties

3.7. *Thermogravimetric study*

Thermal decomposition of Co(II), Ni(II) and Cu(II) complexes has been studied as a function of temperature (table 4) by TGA/DTG.

In Co(II) and Ni(II) complexes the weight losses during decomposition from 95–120, 240–260 and 330–350°C correspond to loss of coordinated water molecules, thiocarbohydrazide and aldehyde moieties, respectively. The final weight corresponded to that of the metal oxide.

For the Cu(II) complex, decomposition from 90–115, 280–290°C and 330–350°C correspond to loss of coordinated water molecules, thiocarbohydrazide and aldehyde moieties, respectively; no significant decomposition with further increase in temperature. The final weight of metal oxide residue is almost equal to the calculated value.

3.8. *Kinetic study*

The Freeman and Carroll procedure [29] was used to evaluate the kinetic parameters for decomposition from a single experimental curve (figure 2) from the plot of $(\Delta \log \mathrm{d}w/\mathrm{d}t)/(\Delta \log Wr)$ *versus* $\Delta T^{-1}/\Delta \log Wr \times 10^3 K^{-1}$. The order of reaction and energies of activation are listed in table 5.

3.9. *Electrochemistry*

Electrochemical properties of the complexes were studied on a CHI1110A-Electrochemical analyzer in DMF containing 0.05 M n-Bu_4NClO_4 as supporting electrolyte. A cyclic voltammogram of Co(II) displays a reduction peak at $E_{pc} = -0.375\,V$ with a corresponding oxidation peak at $E_{pa} = -0.075\,V$. The peak separation of this couple (ΔE_p) is 0.3 V at scan rate 0.1 V and increases with scan rate. The most significant feature of the Co(II) complex is the Co(II)/Co(I) couple which is a quasi-reversible one electron oxidation (Supplementary data). 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 rate, establishing a diffusion controlled electrode process [30]. The separation in peak potentials increases at higher scan rates consistent with the quasi-reversibility of the Co(II)/Co(I) couple.

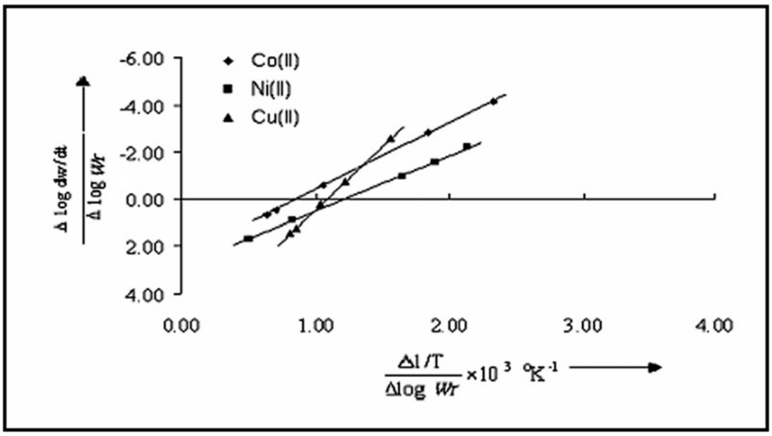


Figure 2. Kinetics of thermal decomposition study of Co(II), Ni(II) and Cu(II) complexes.

Table 5. Kinetics of thermal decomposition of Co(II), Ni(II) and Cu(II) complexes.

Complex No.	Empirical formula	$\Delta \log dw/dt/\Delta \log Wr$	$\Delta T^{-1}/\Delta \log Wr \times 10^3$ ($^\circ K^{-1}$)	Order of reaction	Energy of activation E_a (Kcal mol^{-1})
1	$Co(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	−4.1933	2.331	0.87	13.29
		−2.8400	1.839		
		−0.5500	1.059		
		0.4821	0.702		
		0.6902	0.632		
2	$Ni(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	−2.201	2.1453	1.2	10.83
		−1.560	1.8924		
		−0.950	1.654		
		0.895	0.827		
		1.724	0.498		
3	$Cu(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	−2.5672	1.5726	1.1	24.58
		−0.7500	1.2210		
		0.2568	1.0430		
		1.2700	0.8510		
		1.4700	0.8140		

The Cu(II) complex exhibits a reduction peak at $E_{pc} = 0.075\,V$ with a direct re-oxidation peak at $E_{pa} = 0.672\,V$ corresponding to the Cu(II)/Cu(I) couple (Supplementary data). The peak separation (ΔE_p) is 0.597 V. This Cu(II) complex is also quasi-reversible as the separation in peak potential is higher than 59 mV and the peak currents rise with increasing $\nu^{1/2}$.

4. Biological activities

4.1. In vitro *antibacterial and antifungal assay*

The Schiff base and its corresponding Co(II), Ni(II) and Cu(II) complexes were screened for their biological activity by using four bacteria, *E. coli*, *S. aureus*, *S. pyogenes* and *P. aeruginosa* and three fungi namely *A. niger*, *A. flavus* and *cladosporium* by the MIC method [31]. The bacteria were subcultured in agar medium. The stock solution (1 mg mL^{-1}) was prepared by dissolving 10 mg of the test compound in 10 mL of DMF and was used to prepare concentrations of 100, 50 and 25 µg mL^{-1}.

The plates were incubated immediately at 37°C for 20 h for bacteria and 48 h for fungi. The activity was determined by measuring the diameter of the zone showing complete inhibition (mm). Growth inhibition was compared with standard drugs. In order to clarify any effect of DMF on the biological screening, separate studies were carried out with DMF and showed no activity against any bacterial strains.

4.2. *Minimum inhibitory concentration (MIC)*

Some compounds showing promising antibacterial/antifungal activity were selected for minimum inhibitory concentration studies. The minimum inhibitory concentration was determined by assaying at 100, 50 and 25 µg mL^{-1} concentrations along with standards at the same concentrations.

The microbial results are summarized in tables 6 and 7 (figures 3 and 4). The ligand shows moderate activity towards *S. aureus* and *S. pyogenes* and low activity towards *E. coli* and *P. aeruginosa*. The Cu(II) complex shows high activity towards *E. coli*, *S. pyogenes* and *P. aeruginosa*; Co(II) and Ni(II) complexes are weakly active.

For antifungal activity, the ligand shows high activity against *A. flavus*, *A. niger* and less towards *cladosporium*. The Co(II) complex shows high activity against *A. niger* and *A. flavus* and less towards *cladosporium*. The Ni(II) complex shows high activity (almost equal to standard) in lower concentration towards *A. flavus*, as also observed for Cu(II) complex towards *A. flavus* and *A. niger* with only moderate activity towards *cladosporium*.

Table 6. Antibacterial results of Schiff base and its Co(II), Ni(II) and Cu(II) complexes.

		Percentage of zone inhibition			
Compound	Conc (µg mL^{-1})	*E. coli*	*S. aureus*	*S. pyogenes*	*P. aeruginosa*
$C_{23}H_{18}N_4O_6S$	100	24	52	45	18
	50	21	59	32	15
	25	23	58	30	10
$Co(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	100	15	45	49	53
	50	12	35	25	58
	25	16	28	23	55
$Ni(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	100	41	50	48	57
	50	25	40	50	48
	25	21	32	45	42
$Cu(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	100	72	58	68	69
	50	67	46	60	58
	25	51	33	53	55
Gentamycin	100	100	100	100	100
	50	100	100	100	100
	25	100	100	100	100

Table 7. Antifungal results of Schiff base and its Co(II), Ni(II) and Cu(II) complexes.

Compound	Conc ($\mu g\,mL^{-1}$)	Percentage of inhibition		
		A. flavus	*Cladosporium*	*A. niger*
$C_{23}H_{18}N_4O_6S$	100	70	59	78
	50	67	53	72
	25	54	50	68
$Co(C_{23}H_{16}N_4O_6S)\cdot 2H_2O$	100	68	57	50
	50	62	51	44
	25	60	50	41
$Ni(C_{23}H_{16}N_4O_6S)\cdot 2H_2O$	100	85	58	47
	50	89	42	–
	25	87	38	–
$Cu(C_{23}H_{16}N_4O_6S)\cdot 2H_2O$	100	87	68	82
	50	89	57	87
	25	80	51	85
Flucanazole	100	100	100	100
	50	100	100	100
	25	100	100	100

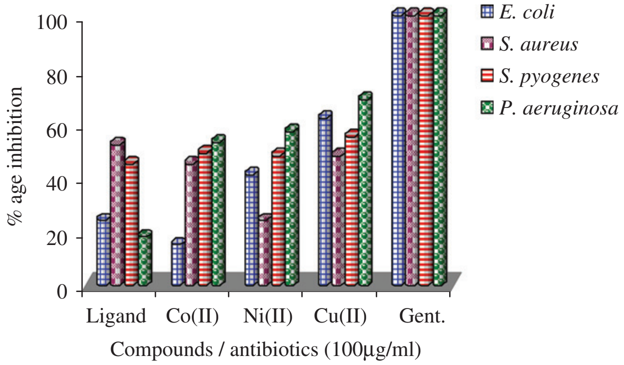


Figure 3. Antibacterial study of the Schiff base and its Co(II), Ni(II) and Cu(II) complexes.

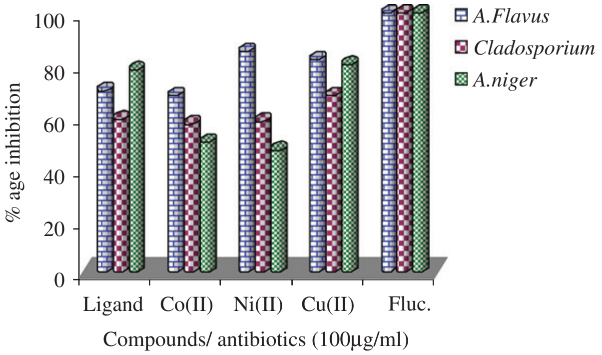


Figure 4. Antifungal study of the Schiff base and its Co(II), Ni(II) and Cu(II) complexes.

4.3. In vitro *cytotoxicity*

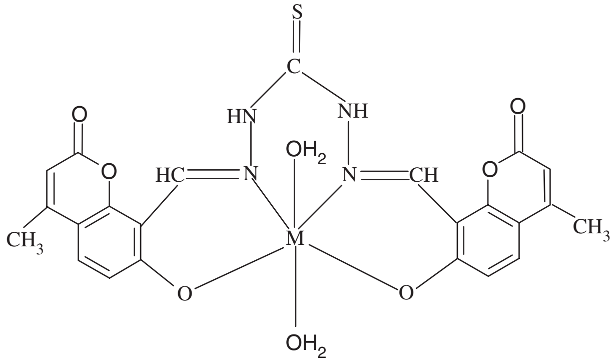
The Schiff base and complexes were screened for their cytotoxicity (brine shrimp bioassay) using protocol of Meyer *et al.* [32]. Brine shrimp (Artemia salina leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm) filled with artificial seawater, which was prepared with a commercial salt mixture and doubly-distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the minor compartment was open to ordinary light.

After two days, nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 mL of DMF. From this stock solutions 100, 50 and 10 $\mu g\,mL^{-1}$ were transferred to 9 vials (three for each dilution were used for each test sample and LD_{50} is the mean of three values) and one vial was kept as control having 2 mL of DMF only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 mL of seawater and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with seawater to 5 mL per vial. After 24 h the number of survivors was counted. Data were analyzed by a Finney computer program to determine the LD_{50} values [33].

The data, table 8, show only the Cu(II) complex displayed cytotoxic activity as $LD_{50} = 8.974 \times 10^{-4}\,M\,mL^{-1}$ against Artemia salina, while all other compounds were almost inactive for this assay.

Table 8. Brine shrimp bioassay data of the ligand and its Co(II), Ni(II) and Cu(II) complexes.

Compound	LD_{50} ($M\,mL^{-1}$)
$C_{23}H_{18}N_4O_6S$	5.392×10^{-3}
$Co(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	2.439×10^{-3}
$Ni(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	1.692×10^{-3}
$Cu(C_{23}H_{16}N_4O_6S) \cdot 2H_2O$	8.974×10^{-4}



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

Figure 5. Octahedral structure of Co(II), Ni(II) and Cu(II) complexes.

5. Conclusion

The synthesized Schiff base is a tetradentate ligand through azomethine nitrogen and phenolic oxygen atoms to the metal ions. The bonding of the ligand to metal was confirmed by the analytical, IR, electronic, magnetic, ESR, FAB mass, thermal and electrochemical studies.

Among the Co(II), Ni(II) and Cu(II) complexes Cu(II) shows high activity towards *E. coli*, *S. pyogenes* and *P. aeruginosa* and Ni(II) complex shows high active (almost equal to standard) in lower concentration towards *A. Flavus*, also observed for Cu(II) complex towards *A. flavus* and *A. niger*; remaining Co(II) and Ni(II) complex activities are slight.

The structures shown in figure 5 are proposed for $ML \cdot 2H_2O$ (M=Co(II), Ni(II) and Cu(II)).

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