

FULL PAPER

Thiosemicarbazone Scaffold as a Multidentate Ligand for Transition-Metal Ions: Synthesis, Characterization, *In Vitro* Antimicrobial, Anthelmintic, DNA Cleavage, and Cytotoxic Studies

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New series of *Schiff* bases derived from *o*-substituted thiosemicarbazides and 8-formyl-7-hydroxy-4-methylcoumarin have been synthesized and their coordination tendency toward Co(II), Ni(II), and Cu(II) metal ions is studied. Analytical, spectral (IR, UV-Vis, ESR, and FAB-mass), magnetic, and thermal studies suggests octahedral geometry of the type ML₂ for all the Co(II), Ni(II), and Cu(II) complexes. The complexes are soluble in DMF/DMSO and are non-electrolytes. The *Schiff* bases and their metal complexes have been screened for antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*) and antifungal activities (*Aspergillus flavus*, *Aspergillus niger*, and *Cladosporium*) by minimum inhibitory concentration method. DNA cleavage is studied by agarose gel electrophoresis method. Metal (II) complexes show good anthelmintic activity when compared to *Schiff* bases.

Keywords: Synthesis, Anthelmintic, DNA cleavage, Cytotoxicity, Transition metal complexes.

Introduction

Multidentate *Schiff* bases have been widely used as ligands, as they can form highly stable coordination compounds. Metal complexes of S, N, and O donor ligands are gaining interest due to their diverse physicochemical properties, pronounced biological activities, and as models for metalloenzyme active sites [1][2]. *Schiff* bases and their transition-metal complexes have been studied as anticancer, antitubercular, antibacterial, antifungal, hypertensive, and hypothermic reagents [3 – 5]. Transition-metal complexes are used as catalysts for many organic reactions as well [6][7]. Thiosemicarbazides play an important role in the construction of multidentate *Schiff* bases comprising S, N, and O donor sites. Besides this, these compounds exhibit a great variety of biological activities ranging from antitumor, fungicidal, bactericidal, antibacterial, antifungal, anti-inflammatory, and antiviral activities [8 – 14]. Thiosemicarbazone drugs may act to inhibit viruses by binding to Cu ions, which are the constituents of the virus as long ago [15]. Also, thiosemicarbazones are an important class of S and N donor ligands, specifically for transition-metal ions [16 – 18].

However, coumarin and its derivatives have attracted many researchers in the organic, inorganic, and analytical research areas, and as a result numerous investigations have been done due to their diverse biological activities [19][20], interesting photophysical, photochemical, and metal-binding properties. Because of its excellent coordination tendency toward transition-metal ions, formyl coumarins are used as precursors for the synthesis of *Schiff* bases and we have reported various metal complexes of these compounds [21 – 24]. Also, it is showed that the binding of a metal ion to the coumarin moiety retains or even enhances its biological activity [20][25].

With this background in this research work, it is planned to construct some multidentate *Schiff* bases from thiosemicarbazides and formyl coumarin to study their coordination tendency toward transition-metal ions and investigate diverse biological activities. Herein, we report the synthesis and characterization of Co(II), Ni(II), and Cu(II) complexes with *Schiff* bases derived from *o*-substituted thiosemicarbazides and 8-formyl-7-hydroxy-4-methylcoumarin possessing azomethine N, S, and phenolic O donors. The *Schiff* bases and their metal complexes are screened for their antimicrobial activity and DNA cleavage of the complexes is also studied. In view of very few

reports on the anthelmintic activity studies of the metal complexes, here, we investigated the anthelmintic activity of *Schiff* bases and their metal complexes.

Results and Discussion

o-Substituted thiosemicarbazides on condensation with 8-formyl-7-hydroxy-4-methylcoumarin forms new series of *Schiff* bases (**I** – **III**) which on complexation with $\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$ / $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$ / $\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$ in EtOH form metal complexes (**1** – **9**) (Fig. 1). The elemental analysis data of *Schiff* bases and their metal complexes is presented in Tables 1 and 2, respectively. The complexes

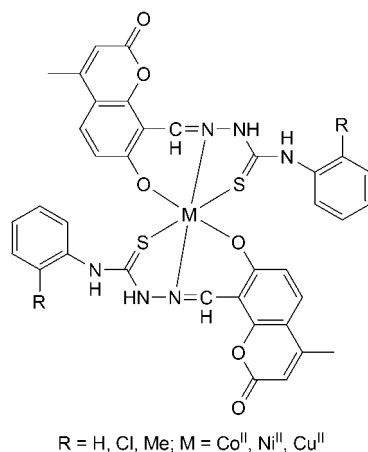


Fig. 1. Structures of metal complexes.

are colored, stable, non-hygroscopic, and insoluble in common organic solvents but soluble in DMF and DMSO. Elemental analyses showed 1:2 stoichiometry of the type ML_2 , where 'L' stands for a deprotonated ligand which exhibits thiol–thione tautomerism. The molar conductance values indicate non-electrolytic nature of the complexes in DMF.

IR Spectral Studies

The IR spectra of the *Schiff* bases exhibited two characteristic band in the region 3303 – 3190 and 3165 – 3019 cm^{-1} corresponding to two $\nu(\text{NH})$ groups. A characteristic strong band in the region 1189 – 1179 cm^{-1} is assigned to $\nu(\text{C}=\text{S})$ [26]. In addition, strong bands in the region 1728 – 1722, 1630 – 1615, and 1226 – 1218 cm^{-1} are predicted to lactonic $\nu(\text{C}=\text{O})$, $\nu(\text{C}=\text{N})$, and phenolic $\nu(\text{C}-\text{O})$ stretching frequencies, respectively.

The prominent IR bands are presented in the Table 3. In comparison with the spectra of the *Schiff* bases, all the Co(II), Ni(II), and Cu(II) complexes exhibited the band of $\nu(\text{C}=\text{N})$ in the region 1600 – 1585 cm^{-1} showing the shift of band to lower wavenumbers indicating that the N-atom of the azomethine group is coordinated to the metal ion [27 – 29]. The strong band in the region 1189 – 1179 cm^{-1} of $\nu(\text{C}=\text{S})$ in *Schiff* bases, shifted to lower wavenumbers in the region 1164 – 1142 cm^{-1} in metal complexes indicates the coordination of sulfur atom. The high intensity band due to phenolic $\nu(\text{C}-\text{O})$ appeared in the region at 1226 – 1218 cm^{-1} in the *Schiff*

Table 1. Elemental analyses of *Schiff* bases **I** – **III**

<i>Schiff</i> Base	C [%]		H [%]		N [%]		S [%]		Yield [%]
	Obsd.	Calc.	Obsd.	Calc.	Obsd.	Calc.	Obsd.	Calc.	
I	61.23	61.12	4.24	4.28	11.79	11.88	9.11	9.05	75
II	55.58	55.69	3.61	3.68	10.91	10.83	8.18	8.25	71
III	62.11	62.05	4.63	4.66	11.49	11.43	8.79	8.71	78

Table 2. Elemental analyses of Co(II), Ni(II), and Cu(II) complexes along with molar conductance and magnetic moment data

Complex	Empirical formula	Color, yield [%]	M [%]		C [%]		N [%]		S [%]		Molar conductance [$\text{Ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$]	μ_{eff} [BM]
			Obsd.	Calc.	Obsd.	Calc.	Obsd.	Calc.	Obsd.	Calc.		
1	$\text{Co}(\text{C}_{18}\text{H}_{14}\text{N}_3\text{O}_3\text{S})_2$	Dark brown, 57	7.65	7.73	56.59	56.61	11.11	11.00	8.41	8.38	19.22	4.61
2	$\text{Co}(\text{C}_{18}\text{H}_{13}\text{ClN}_3\text{O}_3\text{S})_2$	Dark brown, 55	7.01	7.08	51.68	51.92	9.89	10.09	7.48	7.69	22.06	4.71
3	$\text{Co}(\text{C}_{19}\text{H}_{16}\text{N}_3\text{O}_3\text{S})_2$	Dark brown, 56	7.34	7.42	57.47	57.67	10.54	10.63	7.98	8.09	24.52	4.49
4	$\text{Ni}(\text{C}_{18}\text{H}_{14}\text{N}_3\text{O}_3\text{S})_2$	Yellowish green, 62	7.61	7.61	56.59	56.66	10.69	11.02	8.42	8.22	17.28	3.16
5	$\text{Ni}(\text{C}_{18}\text{H}_{13}\text{ClN}_3\text{O}_3\text{S})_2$	Yellowish green, 61	6.94	7.04	51.26	51.94	9.98	10.09	7.24	7.69	20.01	3.11
6	$\text{Ni}(\text{C}_{19}\text{H}_{16}\text{N}_3\text{O}_3\text{S})_2$	Yellowish green, 69	7.12	7.42	57.12	57.67	10.15	10.62	7.99	8.09	23.07	3.20
7	$\text{Cu}(\text{C}_{18}\text{H}_{14}\text{N}_3\text{O}_3\text{S})_2$	Dark green, 63	8.19	8.30	56.21	56.32	10.25	10.97	8.12	8.36	25.85	1.78
8	$\text{Cu}(\text{C}_{18}\text{H}_{13}\text{ClN}_3\text{O}_3\text{S})_2$	Dark green, 66	7.12	7.59	51.12	51.64	10.02	10.04	7.32	7.65	18.03	1.79
9	$\text{Cu}(\text{C}_{19}\text{H}_{16}\text{N}_3\text{O}_3\text{S})_2$	Dark green, 69	7.58	7.98	57.10	57.31	10.23	10.55	7.96	8.04	21.14	1.77

bases appeared as a medium to high intensity band in the 1298 – 1272 cm^{-1} regions in the complexes. These observations support the formation of M–O bonds via deprotonation. So, the –OH groups have been bonded to the metal ion *via* deprotonation. The new bands in the region of 478 – 498 cm^{-1} in all the complexes are assigned to stretching frequencies of (M–N) bonds. The bands in the region 337 – 349 cm^{-1} of far IR spectra are due to (M–S) bond formation. Thus, on the basis of IR data, it is concluded that all the metal ions are coordinated to the azomethine N, phenolic O, and S atoms providing strong evidences for the complexation of *Schiff* bases with metal (II) ions.

¹H- and ¹³C-NMR Studies of Schiff Bases

The important ¹H-NMR data of the *Schiff* bases is presented in the Table 4. In the ¹H-NMR spectrum of the *Schiff* base **I**, the –OH proton exhibited a *singlet* signal at 9.96 ppm (s, 1 H) and another *singlet* at 8.85 ppm (s, 1 H) is attributed to CH=N. A characteristic H-atom signal at 11.91 ppm (s, 1 H) is assigned to NH. In addition to

this, H-atoms appeared as *multiplet* in the region 6.95 – 7.72 ppm (m, 8 H) due to aromatic H-atoms and the *singlet* at 2.23 ppm is assigned to Me H-atom of the coumarin moiety. Similar results were evident for the other *Schiff* bases. In ¹³C-NMR spectrum of the *Schiff* bases, the signal observed around 165.0 ppm is ascribed to lactonyl carbon (C=O) of the coumarin. A characteristic signal around 155.2 ppm is assigned to (C=N). In addition to this, the characteristic signals in the region 96.2 – 134.6 ppm are assigned to aromatic C-atoms and the signals at 12.24 and 30.7 ppm are due to CH₃ and CH₂, respectively.

Electronic Spectral Studies

The Co(II) complex exhibited electron spectral absorption bands, which are the characteristic of high spin octahedral Co(II) complexes in the region 9258 – 9317 and 18,945 – 18,973 cm^{-1} corresponding to ⁴T_{1g}(F) → ⁴T_{2g}(F) (ν₁) and ⁴T_{1g}(F) → ⁴T_{1g}(P) (ν₃) transitions, respectively. The magnetic measurement for Co(II) complexes exhibited magnetic moment values of 4.49 – 4.71, which

Table 3. Important infrared frequencies (cm^{-1}) of metal complexes

Metal complex	ν(C=N) [cm^{-1}]	Phenolic ν(C–O) [cm^{-1}]	ν(C=S) [cm^{-1}]	ν(M–N) [cm^{-1}]	ν(M–S) [cm^{-1}]
1	1601	1298	1164	498	347
2	1596	1284	1158	482	345
3	1600	1291	1152	486	348
4	1586	1272	1162	482	349
5	1594	1276	1160	488	340
6	1585	1282	1158	478	337
7	1591	1286	1142	490	338
8	1599	1288	1154	492	345
9	1593	1284	1152	484	346

Table 4. Important ¹H-NMR ((D₆)DMSO) data of *Schiff* bases

<i>Schiff</i> Base	δ(H) [ppm]
I	9.96 (s, 1 H, OH), 8.85 (s, 1 H, CH=N), 11.91 (s, 1 H, NH), 6.95 – 7.72 (m, 8 H, Ar–H), 2.23 (s, 3 H, Me)
II	10.04 (s, 1 H, OH), 8.89 (s, 1 H, CH=N), 11.85 (s, 1 H, NH), 6.93 – 7.69 (m, 7 H, Ar–H), 2.38 (s, 3 H, Me)
III	9.89 (s, 1 H, OH), 8.79 (s, 1 H, CH=N), 11.94 (s, 1 H, NH), 6.93 – 7.69 (m, 7 H, Ar–H), 2.42 (s, 3 H, Me), 2.10 (s, 3 H, Me)

Table 5. Ligand field parameters of the Co(II) and Ni(II) complexes

Complex	Transitions [cm^{-1}]			ν ₂ calc. [cm^{-1}]	Dq [cm^{-1}]	B ¹ [cm^{-1}]	Distortion [%]	ν ₂ /ν ₁	LFSE	μ _{eff} calc. [BM]	β	β° [%]
	ν ₁	ν ₂	ν ₃									
Co(C ₁₈ H ₁₄ N ₃ O ₃ S) ₂	9258	–	18,945	15,127	942	231	–	1.597	32.29	4.49	0.219	78.07
Co(C ₁₈ H ₁₃ ClN ₃ O ₃ S) ₂	9317	–	18,973	15,312	947	227	–	1.623	32.47	4.47	0.215	78.48
Ni(C ₁₈ H ₁₄ N ₃ O ₃ S) ₂	9808	14,128	21,441	13,589	980	373	3.961	1.440	33.62	3.194	0.354	64.60
Ni(C ₁₈ H ₁₃ ClN ₃ O ₃ S) ₂	9825	14,371	21,842	13,969	982	422	2.873	1.463	33.68	3.193	0.400	59.99

agreed with octahedral range [30], which further support the electronic spectral results. The ligand field parameters are presented in the Table 5.

In case of Ni(II) complexes, three bands which are 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) were observed in the region 9808 – 9825, 14,128 – 14,371, and 21,441 – 21,842 cm^{-1} transitions, respectively. This suggests an octahedral geometry around Ni(II) ion [31]. Ni(II) complexes showed the magnetic moment values of 3.11 – 3.20 are well within the range of 2.8 – 3.5 BM, suggesting the consistency with their octahedral environment. Ligand field parameters are presented in the Table 5 and it can be observed that the ligand field parameters correlate the electronic spectral and magnetic susceptibility results.

The electronic spectra of Cu(II) complexes displayed a low-intensity broad band around 16,534 cm^{-1} is assignable to ${}^2E_g \rightarrow {}^2T_{2g}$ transition and another high intensity band at 25,543 cm^{-1} due to ligand \rightarrow metal charge transfer. On the basis of electronic spectra, distorted octahedral geometry around Cu(II) ion is suggested. The Cu(II) complexes showed magnetic moment 1.77 – 1.79 BM, slightly higher than the spin-only value 1.73 BM expected for one unpaired electron, which offers possibility of an octahedral geometry [32]. On the basis of electronic spectra, distorted octahedral geometry around Cu(II) ion is suggested [33][34].

ESR Spectrum of Cu(II) (7) Complex

The ESR spectrum of Cu(II) complex(9) at liquid N_2 temperature in DMSO using TCNE as g marker (2.00277) showed g_{\parallel} and g_{\perp} value to be 2.0864 and 2.0372, respectively. The g_{av} was calculated to be 2.0536. Here, $g_{\parallel} > g_{\perp}$ suggest that the electron is localized in the $d_{x^2-y^2}$ orbital of the Cu(II) ion and this is the

characteristic for the axial symmetry [35]. All these results confirmed that the Cu(II) complexes possess distorted octahedral geometry and $g_{\parallel} < 2.3$, so that the complex exhibits appreciable covalent nature.

Mass Spectral Studies of Schiff Bases and Their Complexes

The FAB-mass spectrum of a representative Schiff base I has been depicted in Fig. 2. The spectrum showed a molecular ion peak at m/z 353 which is equivalent to its molecular weight. The LC/MS spectrum of Schiff base III has been studied using C-18 column. The spectrum of Schiff base III shows its molecular ion peak at m/z 366 (M^+) corresponds to its molecular weight which clearly confirms the formation Schiff base. The FAB-mass spectra of Co(II) (1), Ni(II) (4), and Cu(II) (7) complexes showed a molecular ion peak at m/z 763, 762, and 767, respectively, those are equivalent of their molecular weights along with other fragment ions. The FAB-mass spectrum of Cu(II) (7) complex is reproduced in Fig. 3.

Electrochemistry

A cyclic voltammogram of Co(II) complex displays a reduction peak at $E_{pc} = -0.983$ V with a corresponding oxidation peak at $E_{pa} = -0.743$ V. The peak separation of this couple (ΔE_p) is 0.24 V at 100 mV/s and increases with increase in scan rate. The most significant feature of the Co(II) complex is the Co(II)/Cu(I) quasi-reversible couple (Fig. 4). The difference between forward and backward peak potentials can provide a rough evaluation of the degree of the reversibility of one electron-transfer reaction. The analysis of cyclic voltametric responses with the varying scan rates 50 – 200 mV/s gave the evidence

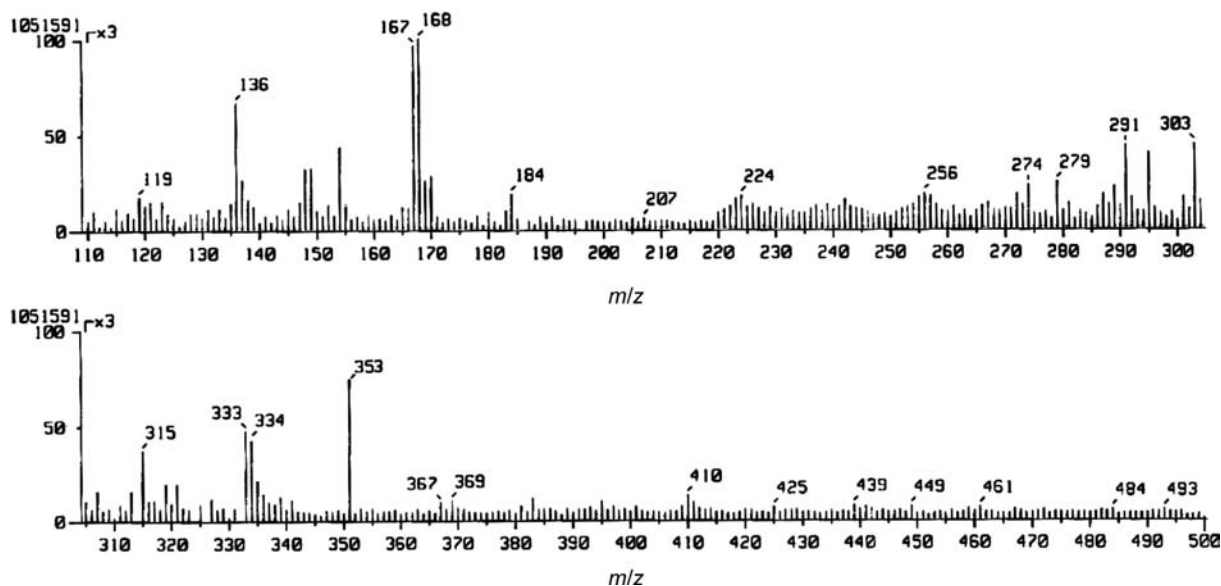


Fig. 2. FAB-MS spectrum of Schiff base I.

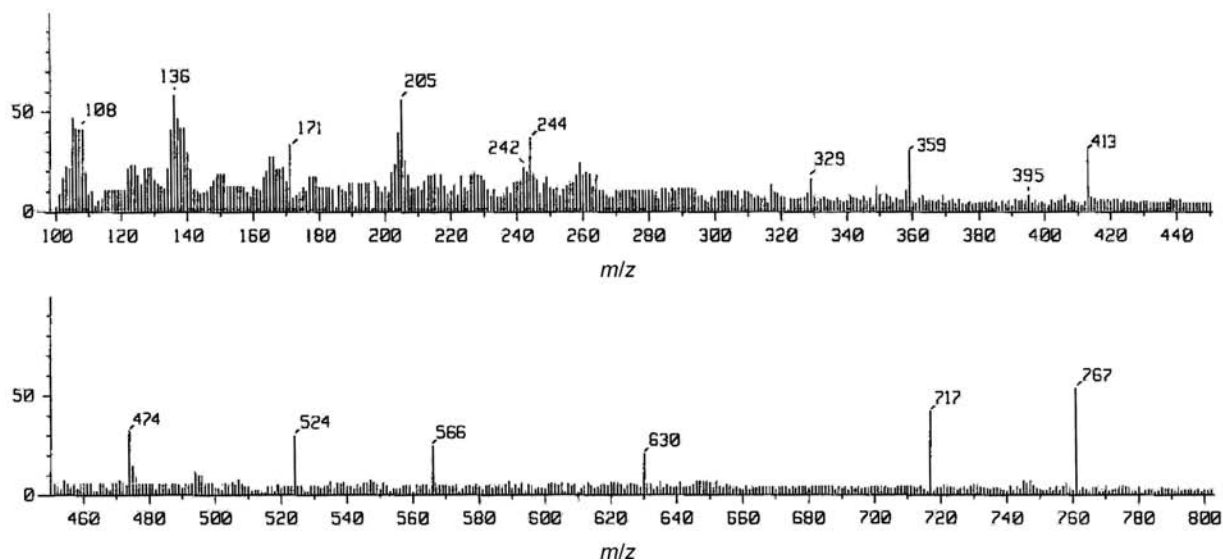


Fig. 3. FAB-MS spectrum of Cu(II) complex (7).

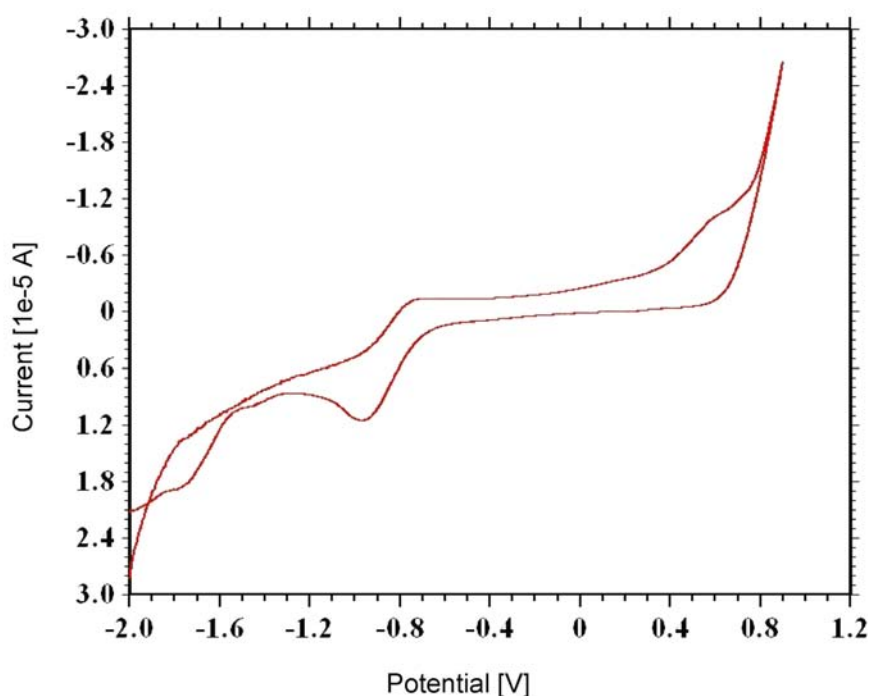


Fig. 4. Cyclic voltammogram of Co(II) complex (2).

for quasi-reversible one electron oxidation state. The ratio of cathodic to anodic peak height was < 1 .

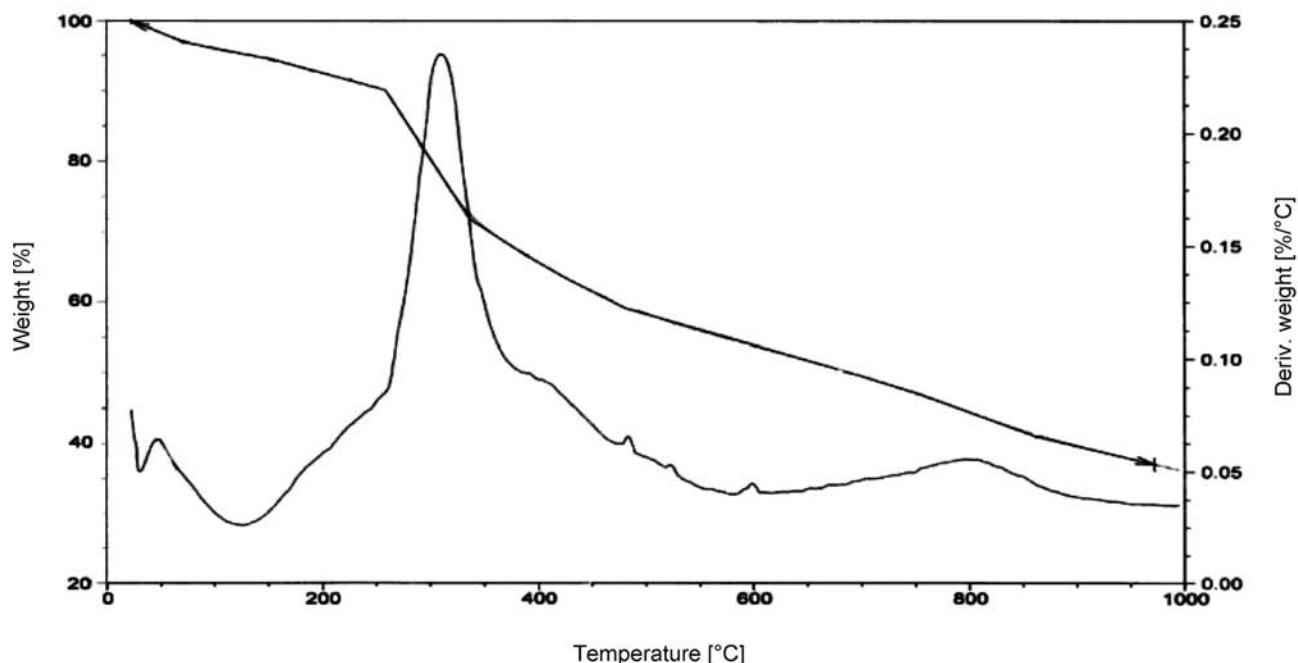
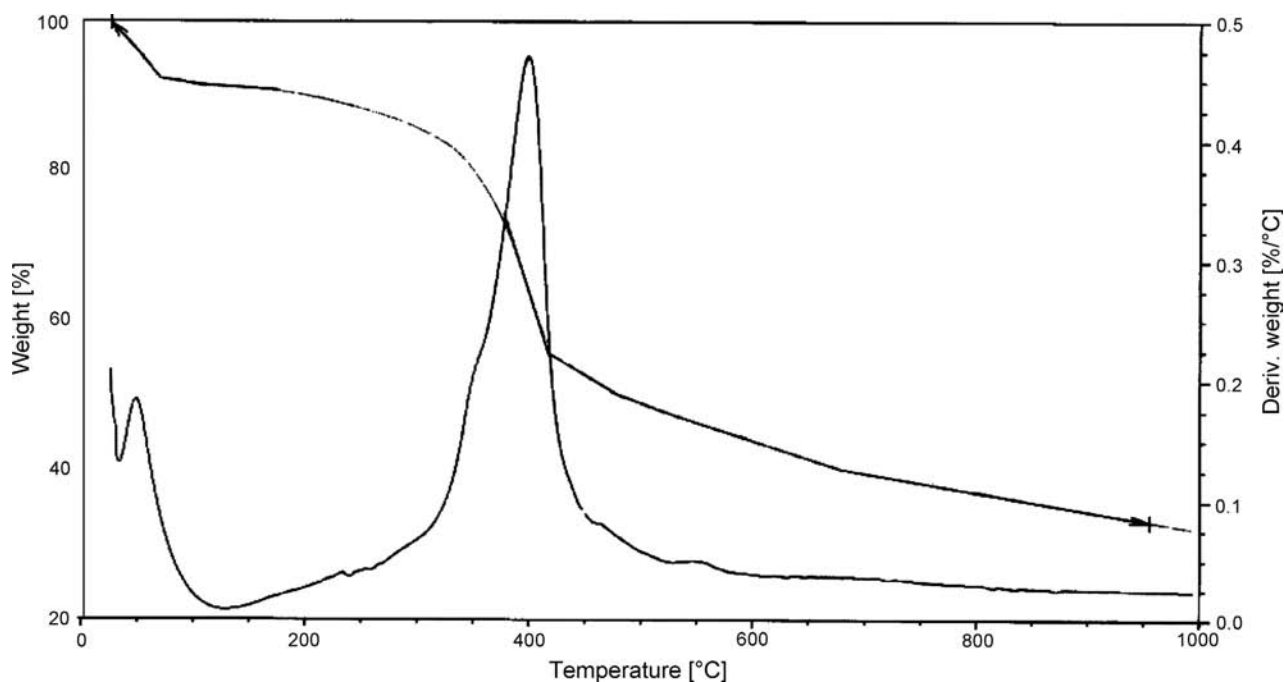
Thermal Analyses

The thermal degradation pattern of Co(II), Ni(II), and Cu(II) complexes have been studied as a function of temperature. The thermograms of representative Co($C_{18}H_{14}N_3O_3S$)₂ and Cu($C_{18}H_{14}N_3O_3S$)₂ complexes have been presented in Figs. 5 and 6, respectively. The thermal

decomposition of Co(II) (1) and Cu(II) (7) complexes took place in single step due to the loss of organic moiety in the region 315 – 325 °C with mass loss of 44.27% (calc. 44.22%) and 400 – 430 °C with the mass loss of 44.23% (calc. 44.22%), respectively.

Antibacterial and Antifungal Studies

The microbial results were systematized in Tables 6 and 7, respectively. In bacteriological studies, the results

Fig. 5. TG/DTA curve of Co(II) complex (**1**).Fig. 6. TG/DTA curve of Cu(II) complex (**7**).

were compared with the standard drug gentamycine. It was observed that some of the *Schiff* bases were found potentially active against all bacterial strains. *Schiff* base **I** showed high activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi*. The metal(II) complexes (**1** – **9**) of these *Schiff* bases (**I** – **III**) were also screened against the same bacterial strains. It was evident that overall potency of the uncoordinated

compounds was enhanced on coordination with metal ions. In antifungal activity, the results were compared with the standard drug fluconazole. All the *Schiff* bases showed high activity against fungal species. *Schiff* bases **I** and **II** showed very high activity. An interesting feature is that the *Schiff* base **III** showed high activity against *Cladosporium*. Co(II), Ni(II), and Cu(II) complexes (**1** – **9**) of these *Schiff* bases showed much enhanced

Table 6. Antibacterial and antifungal results of Schiff Bases (L I – L III)

Schiff Base	Concentration [μg/ml]	Inhibition against bacteria [%]				Inhibition against fungi [%]		
		<i>E. coli</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>A. flavus</i>	<i>Cladosporium</i>	<i>A. niger</i>
I	100	45	46	48	67	85	74	75
	50	40	40	49	66	84	73	74
	30	50	44	50	66	83	72	73
II	100	59	59	43	60	88	71	78
	50	59	59	42	60	87	69	76
	30	56	58	43	60	86	70	77
III	100	61	57	57	69	87	88	84
	50	58	57	56	70	86	86	71
	30	59	56	57	71	88	87	74
DMF	100	6	6	6	6	6	6	6
	50	6	6	6	6	6	6	6
	30	6	6	6	6	6	6	6
Gentamycine	100	99	100	99	100	–	–	–
Fluconazole	100	–	–	–	–	100	99	100

Table 7. Antibacterial and antifungal results of Co(II), Ni(II), and Cu(II) complexes **1** – **9** and standard

Compound	Concentration [μg/ml]	Inhibition against bacteria [%]				Inhibition against fungi [%]		
		<i>E. coli</i>	<i>S. aureus</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>Cladosporium</i>
1	100	57	59	65	73	85	77	80
	50	52	53	64	72	84	80	79
	30	50	51	62	69	85	80	78
2	100	69	62	50	64	86	71	79
	50	63	61	51	63	88	73	81
	30	66	61	50	62	87	74	81
3	100	70	62	68	73	90	59	79
	50	69	60	69	74	89	61	78
	30	69	61	70	74	87	61	80
4	100	84	68	50	69	74	85	98
	50	85	68	50	67	75	84	99
	30	85	69	53	68	78	85	97
5	100	81	56	69	80	86	78	80
	50	81	60	68	80	87	79	80
	30	82	61	66	81	87	80	81
6	100	69	61	59	65	89	80	84
	50	69	64	56	65	88	81	82
	30	68	59	59	66	88	81	81
7	100	71	64	69	74	89	58	84
	50	71	64	70	75	90	57	81
	30	70	64	70	73	91	57	82
8	100	81	73	61	82	89	84	90
	50	81	71	64	84	89	81	88
	30	81	72	61	82	89	81	89
9	100	53	60	65	72	88	81	82
	50	53	59	65	69	88	82	82
	30	54	62	65	70	88	83	79
DMF	100	7	7	6	5	7	7	5
	50	6	6	7	6	7	6	6
	30	7	6	6	4	6	7	6
Gentamycine	100	99	100	100	99	–	–	–
Fluconazole	100	–	–	–	–	100	99	99

activity as compared to the uncoordinated compounds. It can be explained on the basis that the chelation tends to make Schiff bases act as more powerful and potent bacteriostatic agents, thus inhibiting the growth of bacteria

and fungi more than the parent Schiff bases. The factors, such as solubility, conductivity, dipole moment, and cell permeability mechanism (influenced by the presence of metal ions), may be possible reasons for the increase in activity.

DNA Cleavage Studies

Three Cu(II) complexes (**7** – **9**) were studied for their DNA cleavage activity by agarose gel electrophoresis method (Fig. 7). The difference was observed in the bands (Lane A – C) compared to the control DNA of *Escherichia coli*. The gel after the electrophoresis clearly revealed that all compounds have the cleavage activity. Complexes **7** – **9** have acted on DNA, as there was a molecular weight difference between the control and the treated DNA samples. The gel of samples A – C clearly shows the diminished intensity of DNA band in all the

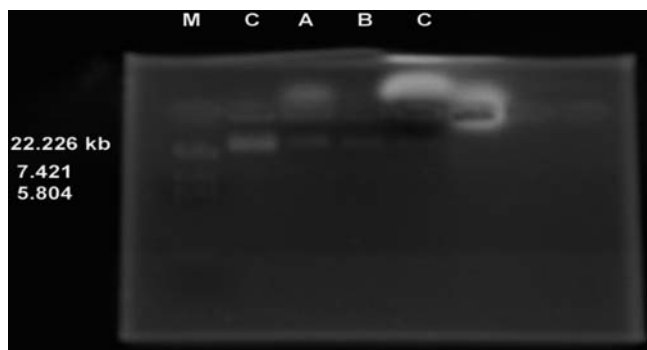


Fig. 7. M: Standard molecular weight marker; C: *E. coli*-control DNA of *E. coli*; Lanes A – C: *E. coli* DNA treated with Cu(II) complexes **7** – **9**, respectively.

treated samples indicating the activity of all samples. But, sample C has cleaved DNA completely as the band is missing. This suggests the role of metal ion on complexation with *Schiff* bases in DNA cleavage as control DNA alone does not show any apparent cleavage. This can be ascribed to inhibition of the growth of the pathogenic organism by cleaving the genome.

Anthelmintic Activity Studies

The anthelmintic activity of the *Schiff* bases and their corresponding metal complexes were tested on Indian earth worms (*Pheretima posthuma*) and the results are presented in the Table 8. The results showed that the *Schiff* base metal complexes exhibited higher anthelmintic activity compared to their corresponding *Schiff* bases. Among the Co(II), Ni(II), and Cu(II) complexes, the complexes of Co(II) and Cu(II) showed more activity and the remaining Ni(II) complexes are less active.

In Vitro Cytotoxicity Studies

Cytotoxic bioassay. All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay). It is evident from the data recorded in Table 9 that only five compounds (**2**), (**5**), and (**8**) displayed potent cytotoxic activity as LD_{50} 7.839×10^{-4} , 5.842×10^{-4} , and

Table 8. Anthelmintic activity of the *Schiff* bases and their metal complexes

Compound	Concentration [$\mu\text{g/ml}$]	Time for paralysis [min]	Time for death [min]
Albendazole	10	4.23 ± 0.51	6.13 ± 0.24
DMSO	10	–	–
$\text{C}_{18}\text{H}_{15}\text{N}_3\text{O}_3\text{S}$	5	2.82 ± 0.005	3.40 ± 0.5
	10	3.80 ± 0.003	1.56 ± 0.3
$\text{C}_{18}\text{H}_{14}\text{ClN}_3\text{O}_3\text{S}$	5	1.11 ± 0.005	3.50 ± 0.03
	10	0.12 ± 0.006	1.75 ± 0.1
$\text{C}_{19}\text{H}_{17}\text{N}_3\text{O}_3\text{S}$	5	0.16 ± 0.008	1.16 ± 0.06
	10	0.10 ± 0.001	1.05 ± 0.2
$\text{Co}(\text{C}_{18}\text{H}_{14}\text{N}_3\text{O}_3\text{S})_2$	5	1.01 ± 0.009	1.15 ± 0.07
	10	1.60 ± 0.002	2.5 ± 0.2
$\text{Co}(\text{C}_{18}\text{H}_{13}\text{ClN}_3\text{O}_3\text{S})_2$	5	0.18 ± 0.009	1.05 ± 0.09
	10	0.10 ± 0.002	1.6 ± 0.08
$\text{Co}(\text{C}_{19}\text{H}_{16}\text{N}_3\text{O}_3\text{S})_2$	5	1.10 ± 0.007	2.1 ± 0.2
	10	0.20 ± 0.002	1.15 ± 0.07
$\text{Ni}(\text{C}_{18}\text{H}_{14}\text{N}_3\text{O}_3\text{S})_2$	5	1.13 ± 0.007	1.5 ± 0.2
	10	0.81 ± 0.002	1.5 ± 0.07
$\text{Ni}(\text{C}_{18}\text{H}_{13}\text{ClN}_3\text{O}_3\text{S})_2$	5	0.66 ± 0.010	1.15 ± 0.07
	10	0.50 ± 0.019	2.5 ± 0.3
$\text{Ni}(\text{C}_{19}\text{H}_{16}\text{N}_3\text{O}_3\text{S})_2$	5	2.12 ± 0.006	3.40 ± 0.5
	10	0.70 ± 0.003	1.55 ± 0.1
$\text{Cu}(\text{C}_{18}\text{H}_{14}\text{N}_3\text{O}_3\text{S})_2$	5	0.11 ± 0.006	1.44 ± 0.03
	10	0.15 ± 0.003	1.75 ± 0.1
$\text{Cu}(\text{C}_{18}\text{H}_{13}\text{ClN}_3\text{O}_3\text{S})_2$	5	1.75 ± 0.008	2.75 ± 0.06
	10	0.10 ± 0.002	1.05 ± 0.2
$\text{Cu}(\text{C}_{19}\text{H}_{16}\text{N}_3\text{O}_3\text{S})_2$	5	2.17 ± 0.009	1.15 ± 0.07
	10	1.11 ± 0.003	1.60 ± 0.30

Table 9. Brine shrimp bioassay data of the ligands **I** – **III** and their metal(II) complexes **1** – **9**

Compound	Empirical formula	LD_{50} [mol/ml]
I	$C_{18}H_{15}N_3O_3S$	4.517×10^{-3}
II	$C_{18}H_{14}ClN_3O_3S$	5.842×10^{-3}
III	$C_{19}H_{17}N_3O_3S$	4.459×10^{-3}
1	$Co(C_{18}H_{14}N_3O_3S)_2$	6.945×10^{-3}
2	$Co(C_{18}H_{13}ClN_3O_3S)_2$	7.839×10^{-4}
3	$Co(C_{19}H_{16}N_3O_3S)_2$	5.135×10^{-3}
4	$Ni(C_{18}H_{14}N_3O_3S)_2$	2.517×10^{-3}
5	$Ni(C_{18}H_{13}ClN_3O_3S)_2$	5.842×10^{-4}
6	$Ni(C_{19}H_{16}N_3O_3S)_2$	4.459×10^{-3}
7	$Cu(C_{18}H_{14}N_3O_3S)_2$	6.945×10^{-3}
8	$Cu(C_{18}H_{13}ClN_3O_3S)_2$	8.839×10^{-4}
9	$Cu(C_{19}H_{16}N_3O_3S)_2$	5.135×10^{-3}

8.839×10^{-4} m/ml, respectively, against *Artemia salina*, while all other compounds were almost inactive for this assay.

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Experimental Part

All chemicals used were of reagent grade. 7-Hydroxy-4-methylcoumarin was obtained from *Acros Chemical Company*. 8-Formyl-7-hydroxy-4-methylcoumarin [24] was prepared as described in the literature.

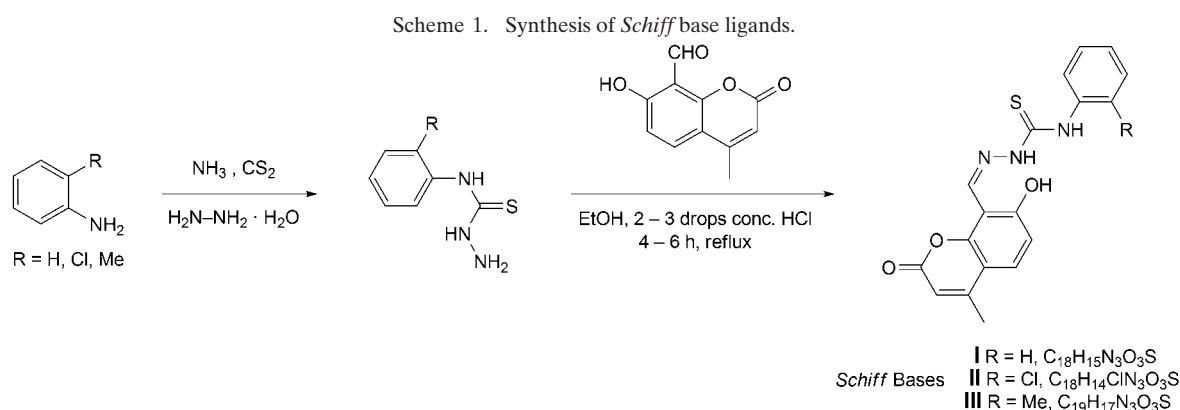
Molar conductivity measurements were done on a *ELICO-CM-82 T Conductivity Bridge* with a cell having cell constant 0.51. Elemental analyses were done by a *Elemental Analyzer Carlo Erba EA1108*. IR Spectra were recorded on a *HITACHI-270* IR spectrophotometer in KBr disks from 4000 to 250 cm^{-1} . ^1H - and ^{13}C -NMR spectra of *Schiff* bases were recorded at r.t. in $(\text{D}_6)\text{DMSO}$ on a *Bruker 300 MHz* spectrometer using TMS as an

internal standard. UV/VIS studies of the complexes were carried out in HPLC grade DMF on a *VARIAN CARY 50-BIO* UV-spectrophotometer. Magnetic moment was measured using a *Faraday* balance. A *JEOL SX 102/DA-6000* mass spectrometer/data system was used to record FAB-MS in positive-ion mode at r.t. Argon/Xenon (6 kV, 10 Am) is used as the FAB gas at 10 kV accelerating voltage with *m*-nitrobenzyl alcohol as the matrix. ESR studies of Cu(II) complexes were carried out on a *Varian-E-4X-band EPR* spectrometer using TCNE as g marker. Electrochemistry of all the complexes was recorded on a *CHI110A*-electrochemical analyzer (made in USA) in DMF containing 0.05M Bu_4NClO_4 as the supporting electrolyte. The three-electrode system consisted of a glassy carbon electrode (3 mm diameter) as a working electrode, a Ag/AgCl (3M KCl) as reference electrode and a platinum wire as auxiliary electrode was used. Thermogravimetric analyses were measured on a *Perkin-Elmer Diamond* TG/DTA instrument from r.t. to 1000° at a heating rate of $10^\circ/\text{min}$.

Synthesis of *o*-Substituted Thiosemicarbazides

Freshly distilled *o*-substituted anilines (3.42 ml) were dissolved in NH_3 (20 ml) and CS_2 (8 ml) was gradually added to it with constant stirring in an ice bath. Then, EtOH (30 ml) was added to the reaction mixture with constant stirring in an ice bath until CS_2 was completely dissolved. The reaction mixture was then allowed to stand for 2 – 3 h. An aqueous sodium chloroacetate (0.1M) solution was added followed by hydrazine hydrate (10 ml). The reaction mixture was stirred for 2 – 3 h and allowed to stand overnight. The crystals separated were filtered, washed with cold EtOH, and recrystallized from hot EtOH.

Synthesis of Schiff Bases I – III. The synthesis of *Schiff* bases is presented in *Scheme 1*. To a hot EtOH solution (20 ml) of *o*-substituted thiosemicarbazide (0.01M) was added hot EtOH solution (15 ml) of 8-formyl-7-hydroxy-4-methylcoumarin (0.01M) followed by refluxing for 4 – 5 h with addition of a catalytic amount of HCl. The



precipitate formed during reflux was filtered, washed with cold EtOH, and recrystallized from hot EtOH.

Synthesis of Co(II), Ni(II), and Cu(II) Complexes (1 – 9). An EtOH solution (10 ml) of 1 mM $\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$ / $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$ / $\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$ was mixed with an EtOH solution (35 ml) of Schiff base (2 mM) and refluxed in a water bath for 2 h. Then, to the reaction mixture, 2 mM (in 15 ml solution) of sodium acetate was added and the reflux was continued for 3 h. The separated complex was filtered, washed thoroughly with H_2O , EtOH, ether, and finally dried in a vacuum over fused CaCl_2 .

Biological Studies

In Vitro Antibacterial and Antifungal Assay. The antibacterial and antifungal activities of the Schiff bases and their metal complexes have been studied by agar and potato dextrose agar diffusion methods, respectively. The antibacterial and antifungal activities were done at 30, 50, and 100 mg/ml concentrations in DMF solvent using four bacteria (*E. coli*, *S. aureus*, *P. aeruginosa*, and *S. typhi*) and three fungi (*A. niger*, *A. flavus*, and *Cladosporium*) by the MIC method [36]. These bacterial strains were incubated for 24 h at 37 °C and fungal strains for 48 h at 37 °C. Standard antibacterial (gentamycin) and antifungal drug (flucanazole) was used for comparison under similar conditions.

DNA Cleavage Experiment. DNA cleavage experiments were done according to the method reported in literature [21][37]. Isolation of DNA and preparation of culture media were followed as mentioned in the standard reported method, and the cleavage products were analyzed by agarose gel electrophoresis method. To determine the extent of DNA cleavage, the results were compared with standard DNA marker.

Anthelmintic Activity Studies. Anthelmintic activity assay was performed on an adult Indian earthworms, *P. posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings using the method given in the literature [38][39]. Earthworms (*P. posthuma*) of 3 – 4 cm long and 0.1 – 0.2 cm in width were collected from moist soil and washed with normal saline to remove all fecal matter were used for the experimental protocol. The compounds were subjected against earthworms at 10 mg/ml using albendazole as a standard drug. The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50 °C) which stimulated the movement, if the worm was alive.

In Vitro Cytotoxicity. The synthesized Schiff bases and its Co(II), Ni(II), and Cu(II) complexes were screened for their cytotoxicity (brine shrimp bioassay) using protocol of Meyaer et al. [40]. 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 double 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 2 days, nauplii were collected by a pipette from the lighter 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 µg/ml were transferred to nine vials (three for each dilutions 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 2 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 [41].

Conclusions

The newly synthesized Schiff bases act as tridentate ligands. The metal ion is coordinated through the azomethine N, S, and phenolic O atoms via deprotonation. The bonding of ligand to metal ion is confirmed by the analytical, spectral, magnetic, and thermal studies. The Schiff bases and their metal complexes were found to be highly active against some of the bacterial and fungal species. The DNA cleavage studies revealed that the complexes shown nonspecific cleavage of DNA. The Schiff bases and their metal complexes also showed good anthelmintic activity compared to standard drug. The activity is significantly increased on coordination. Some of the metal complexes showed good cytotoxic activity.

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