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Spectroscopic, DNA cleavage and antimicrobial studies of Co(II), Ni(II) and Cu(II) complexes of sulfur donor Schiff bases

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A series of Co(II), Ni(II) and Cu(II) complexes of the type ML_2 of Schiff bases derived from methylthiosemicarbazide and isatin/chloroisatin have been synthesized. Schiff bases exhibit thiol–thione tautomerism wherein sulfur plays an important role in the coordination. In view of analytical, spectral (IR, NMR, UV-vis, ESR, FAB-Mass) and magnetic studies, it has been concluded that all the metal complexes possess octahedral geometry. The measured molar conductance values in DMF indicate that the complexes are non-electrolytes in nature. The redox behaviors of the metal complexes are investigated by using cyclic voltammetry. The Schiff bases and their metal complexes have been screened for their antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and antifungal activities (*Aspergillus niger*, *Aspergillus flavus* and *Cladosporium*) by the minimum inhibitory concentration method. The DNA cleavage studies showed the cleavage of DNA by the agarose gel electrophoresis method.

Keywords: antimicrobial; DNA; electrochemical; spectroscopy; sulfur donor Schiff bases

1. Introduction

Metal complexes have been gaining increasing importance in recent years, particularly in the design of repository, slow-release or long-acting drugs in nutrition and in the study of metabolism (1). Metal complexes of the Schiff bases have also been widely studied due to their relevance in biological systems (2). Isatin (indole-2,3-dione) is a versatile lead molecule for designing potential bioactive agents and its derivatives were reported to possess a broad spectrum of antiviral activities (3, 4). Isatin is used to prepare a large variety of heterocyclic compounds such as indoles and also as a raw material for drug synthesis (5). Schiff bases and Mannich bases of isatin are known to possess a wide range of pharmacological properties that include antibacterial (6), anticonvulsant (7), anti-HIV (8), antifungal (9) and antiviral activities (10). Ligands consisting of sulfur donor atoms are the subject of interest in coordination chemistry. The chemistry of thiosemicarbazides has received considerable attention in view of their variable bonding modes, promising biological implications, structural diversity and ion-sensing ability (11–13). As regards

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biological implications, thiosemicarbazide metal complexes have intensively been investigated for antiviral, anticancer, antitumoral, antimicrobial, anti-amoebic and anti-inflammatory activities. The interaction of transition metal complexes with DNA has extensively been studied for their usage as probes for DNA structure and their potential application in chemotherapy. Very recently, Cu(II) complexes have been reported to be active in DNA strand scission (14–16).

In continuation of our earlier work on Schiff's base metal complexes (17–20), here we report the antimicrobial and DNA cleavage activity studies of Co(II), Ni(II) and Cu(II) metal complexes, with Schiff bases derived from methylthiosemicarbazide and isatin/chloroisatin. The structural features of the Schiff bases and their metal complexes have been studied by various spectral and analytical techniques.

2. Results and discussion

The Schiff bases (**I** and **II**) form octahedral complexes (**1–6**) 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 ethanol. All the Co(II), Ni(II) and Cu(II) complexes are colored, stable and non-hygroscopic in nature. These complexes are insoluble in common organic solvents, but soluble in DMF and DMSO. The elemental analyses showed that the Co(II), Ni(II) and Cu(II) complexes have 1:2 stoichiometry of the type ML_2 , where L stands for a deprotonated ligand. The molar conductance values are too low to account for any dissociation of the complexes in DMF, indicating the non-electrolytic nature of the complexes in DMF (Table 1).

2.1. IR spectral studies

The prominent infrared spectral data of Schiff bases and their Co(II), Ni(II) and Cu(II) complexes are presented in Table 2. The IR spectra of the Schiff bases exhibited two characteristic bands in the regions 3244–3195 and 3254–3234 cm^{-1} , respectively, due to $\nu(\text{NH})$ groups (21). A characteristic strong band at 2672–2666 cm^{-1} is ascribed to $\nu(\text{SH})$ and another characteristic strong band at 1199–1193 cm^{-1} is assigned to $\nu(\text{C}=\text{S})$ (22). These observations suggest that the Schiff bases exhibit thiol–thione tautomerism (Figure 1). In addition to this, the characteristic high-intensity bands in the regions 1617–1615 and 3202–3206 cm^{-1} and a strong band in the region 1705–1700 cm^{-1} in the IR spectra of the Schiff bases are assigned to $\nu(\text{C}=\text{N})$ (23), $\nu(\text{NH})$ of isatin moiety and $\nu(\text{C}=\text{O})$ (20, 23, 24), respectively.

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 1588–1574 cm^{-1} ; the shift to lower wave numbers indicates that the nitrogen atom of the azomethine group is coordinated to the metal ion (25). The deprotonation of the thiol group is indicated by the absence of a band around $\sim 2670 \text{ cm}^{-1}$ in all the metal complexes, which is due to $\nu(\text{S}-\text{H})$ of the Schiff bases, indicating that the metal ion is coordinated through the sulfur atom. This is further supported by the band around 772–781 cm^{-1} in the metal complexes due to $\nu(\text{C}-\text{S})$. The band corresponding to $\nu(\text{C}=\text{O})$ was shifted to lower wave frequency by about 45–40 cm^{-1} and appeared in the region 1666–1653 cm^{-1} in the metal complexes, suggesting the coordination of carbonyl oxygen to the metal ion (26). The new bands in the region 473–460 cm^{-1} in the spectra of the complexes are assigned to stretching frequencies of M–N bonds (27). The bands in the region 348–336 cm^{-1} of far-IR spectra are due to metal–sulfur bond formation (28). The unaltered position of a band due to $\nu(\text{NH})$ of isatin moiety in all the metal complexes indicates that the nitrogen atom is not involved in coordination.

Thus the IR spectral data results provide strong evidences for the complexation of Schiff bases with metal (II) ions.

Table 1. Elemental analyses of Schiff bases and their Co(II), Ni(II) and Cu(II) complexes along with molar conductance and magnetic moment data.

Comp. no.	Empirical formula	Colour/yield %	M%		C%		H%		N%		Molar conductance ($\Omega^{-1} \text{ cm}^2 \text{ mole}^{-1}$)	μ_{eff} (BM)
			Obsd.	Calcd.	Obsd.	Calcd.	Obsd.	Calcd.	Obsd.	Calcd.		
I	$\text{C}_{10}\text{H}_{10}\text{N}_4\text{OS}$	78%	–	–	51.51	51.28	4.56	4.27	23.25	23.93	–	–
II	$\text{C}_{10}\text{H}_9\text{N}_4\text{O}_3\text{SCl}$	75%	–	–	44.52	44.77	3.12	3.35	20.70	20.89	–	–
1	$\text{Co}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$	Brown/70%	11.46	11.23	45.72	45.21	3.75	3.42	21.62	21.33	27.8	4.32
2	$\text{Co}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl})_2$	Brown/66%	9.22	9.93	40.22	40.40	2.13	2.69	18.43	18.85	19.25	4.84
3	$\text{Ni}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$	Yellowish green/68%	11.66	11.06	45.29	45.80	3.27	3.43	21.17	21.37	25.88	2.92
4	$\text{Ni}(\text{C}_{10}\text{H}_9\text{N}_4\text{OSCl})_2$	Yellowish green/69%	11.66	11.06	45.43	45.80	3.17	3.43	18.24	18.88	26.64	3.32
5	$\text{Cu}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$	Dark green/65%	11.59	11.90	46.14	46.36	3.77	3.40	21.45	21.17	28.4	1.76
6	$\text{Cu}(\text{C}_{10}\text{H}_9\text{N}_4\text{OSCl})_2$	Dark green/69%	9.56	9.79	40.15	40.13	3.42	3.01	18.61	18.72	24.11	1.78

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

Compound	$\nu(\text{N-H})$	$\nu(\text{N-H})$ of isatin	$\nu(\text{C=N})$	$\nu(\text{C=O})$	$\nu(\text{C=S})$	$\nu(\text{SH})$	$\nu(\text{C-S})$	$\nu(\text{M-N})$	$\nu(\text{M-S})$
$\text{C}_{10}\text{H}_{10}\text{N}_4\text{OS}$	3244–3254	3202	1617	1705	1199	2672	–	–	–
$\text{C}_{10}\text{H}_9\text{N}_4\text{OSCl}$	3195–3234	3206	1615	1700	1193	2666	–	–	–
$\text{Co}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$	3241	3199	1586	1664	–	–	773	471	347
$\text{Co}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl})_2$	3193	3204	1575	1655	–	–	777	465	336
$\text{Ni}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$	3243	3201	1582	1666	–	–	772	460	345
$\text{Ni}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl})_2$	3192	3204	1579	1653	–	–	780	473	341
$\text{Cu}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$	3241	3198	1588	1665	–	–	776	463	348
$\text{Cu}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl})_2$	3191	3207	1574	1658	–	–	781	469	339

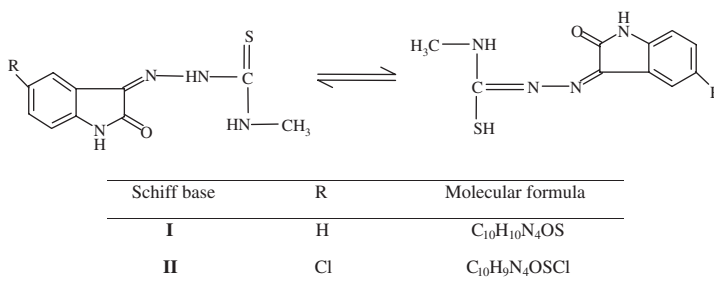


Figure 1. Structure of Schiff bases: thiol–thione tautomerism.

Table 3. NMR data of Schiff bases.

Schiff base	^1H NMR data (ppm)
$\text{C}_{10}\text{H}_{10}\text{N}_4\text{OS}$	11.3 (s, 1H, NH of isatin moiety), 9.2 (s, 1H, NH of thiosemicarbazone), 8.46 (s, 1H, SH), 6.99–7.61 (m, 4H, Ar-H), 3.3 (s, 3H, CH_3)
$\text{C}_{10}\text{H}_9\text{N}_4\text{OSCl}$	11.1 (s, 1H, NH of isatin moiety), 9.18 (s, 1H, NH of thiosemicarbazone), 8.42 (s, 1H, SH), 6.01–7.87 (m, 3H, Ar-H), 3.16 (s, 3H, CH_3)

2.2. ^1H NMR spectral study of Schiff bases I and II

The ^1H NMR data of both the Schiff bases are presented in Table 3 and the ^1H NMR spectrum of Schiff bases **I** and **II** are given in Supplementary material (Figures S1 and S2, respectively). In the ^1H NMR spectrum of the Schiff base **I**, the NH protons of isatin and thiosemicarbazide moieties exhibited signals at 11.3 ppm (s, 1H) and 9.2 ppm (s, 1H), respectively (29). A characteristic proton signal at 8.46 ppm (s, 1H) is assigned to the $-\text{SH}$ proton. In addition to this, the multiplet signals in the region 6.99–7.61 ppm (m, 4H) and a sharp singlet signal at 3.3 ppm (s, 3H) are due to aromatic protons and CH_3 protons, respectively (30). In the case of Schiff base **II**, the signals observed at 11.1 ppm (s, 1H) and 9.18 ppm (s, 1H) are attributed to the NH protons of isatin and thiosemicarbazide moieties, respectively. A signal at 8.42 ppm (s, 1H) is ascribed to the $-\text{SH}$ proton. The signals observed as a multiplet in the region 7.01–7.87 (m, 3H) and as a singlet at 3.16 ppm are due to aromatic and CH_3 protons, respectively.

2.3. Electronic spectral and magnetic studies

The octahedral $\text{Co}(\text{II})$ complexes exhibit electronic spectral bands in the region 8000–10,000, 14,000–16,000 and 18,000–20,000 cm^{-1} corresponding to $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{2g}(\text{F})$ (ν_1), $^4\text{T}_{1g}(\text{F})$

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

Complex No.	Transitions (cm ⁻¹)			Cald. ν_2 (cm ⁻¹)	Dq (cm ⁻¹)	B ¹ (cm ⁻¹)	% Distortion	ν_2/ν_1	LSFE	Cald.		
	ν_1	ν_2	ν_3							μ_{eff} (BM)	β	β° (%)
Co(C ₁₀ H ₉ N ₄ OS) ₂	9411	14326	19965	12885	947.80	330.28	1.666	1.586	32.49	–	0.313	68.72
Co(C ₁₀ H ₈ N ₄ OSCl) ₂	9543	15121	19952	12629	948.40	299.14	1.993	1.585	32.51	–	0.283	71.66
Ni(C ₁₀ H ₉ N ₄ OS) ₂	9808	14128	21441	13889	980.23	414.62	1.402	1.43	33.61	3.194	0.373	60.73
Ni(C ₁₀ H ₈ N ₄ OSCl) ₂	9820	14141	21830	13961	982.32	422.63	1.245	1.40	33.66	3.193	0.400	60.04

→ ⁴T_{2g} (F) (ν_2) and ⁴T_{1g} (F) → ⁴T_{2g} (P) (ν_3) transitions, respectively. In the present study, the brownish Co(II) complexes showed the absorption bands in the region 9411–9543, 14,326–15,121 and 19,665–19,952 cm⁻¹ corresponding to ν_1 , ν_2 and ν_3 transitions, respectively. These bands are the characteristic of high-spin octahedral Co(II) complexes (28). The ligand field parameters calculated are presented in Table 4. The magnetic measurement of Co(II) complexes exhibited magnetic moment values of 4.32–4.84, which agree well with the octahedral range of 4.3–5.7 BM for Co(II) complexes (31).

The greenish Ni(C₁₀H₉N₄OS)₂ complex exhibited three bands at 9808, 14,128 and 21,441 cm⁻¹ attributed to the ³A_{2g} → ³T_{2g} (ν_1), ³A_{2g} → ³T_{1g} (F) (ν_2) and ³A_{2g} → ³T_{1g} (P) (ν_3) transitions, respectively, which indicate an octahedral geometry around the Ni(II) ion (28). The ligand field parameters are given in Table 4 (32). The value of ν_2/ν_1 is found to be around 1.44. The calculated value of μ_{eff} is 3.194, which is within the range 2.8–3.5 BM, suggesting the octahedral environment. The values of the nephelauxetic parameter, β , indicate low covalent character of the metal–ligand σ bonds (33). Ni(II) complexes showed the magnetic moment values of 2.92–3.32 BM, which are within the range 2.8–3.5 BM, suggesting consistency with their octahedral environment (34). Hence, the ligand field parameters correlate with the electronic spectral and magnetic properties.

The electronic spectra of Cu(II) complexes display two prominent bands: a low-intensity broad band around 16,231 cm⁻¹ is assignable to ²E_g → ²T_{2g} transition and another high-intensity band at 26,325 cm⁻¹ is due to ligand → metal charge transfer. On the basis of electronic spectra, the distorted octahedral geometry around the Cu(II) ion is suggested (35). The Cu(II) complexes showed magnetic moment of 1.76–1.78 BM, which is slightly higher than the spin-only value (1.73 BM) expected for one unpaired electron, which offers the possibility of an octahedral geometry (36).

2.4. FAB-Mass spectral studies

Representative FAB-Mass spectrum of Schiff base **I** is depicted in Figure 2. The spectrum showed a molecular ion peak at m/z 234, which is equivalent to its molecular weight. In addition to this, the fragment peaks observed at m/z 219 and 145 are due to the cleavage of CH₃ and CN₂SH₂, respectively. In the case of Schiff base **II**, the molecular ion peak is observed at m/z 268, which is ascribed to C₁₀H₉N₄SOCl.

The FAB-Mass spectrum of all the complexes exhibited molecular ion peak equivalent of their molecular weight along with other fragmentation peaks. Hence, only the representative Co(C₁₀H₉N₄OS)₂, Ni(C₁₀H₉N₄OS)₂ and Cu(C₁₀H₉N₄OS)₂ complexes are discussed here. The spectrum of Co(C₁₀H₉N₄OS)₂ showed a molecular ion peak M⁺ at m/z 525, which is equivalent of its molecular weight. The fragmentation peaks observed at 436, 391, 302 and 157 correspond to C₂N₂SH₅, C₈N₂H₅O, C₂N₂SH₅ and C₈N₂H₅O, respectively. In the case of the Ni(C₁₀H₉N₄OS)₂ complex (Figure 3), the molecular ion peak M⁺ at m/z 524 corresponds to its molecular weight. The complexes exhibiting fragmentation peaks at 435, 390, 301 and 156 correspond to cleavages of C₂N₂SH₅, C₈N₂H₅O, C₂N₂SH₅ and C₈N₂H₅O, respectively. The spectrum

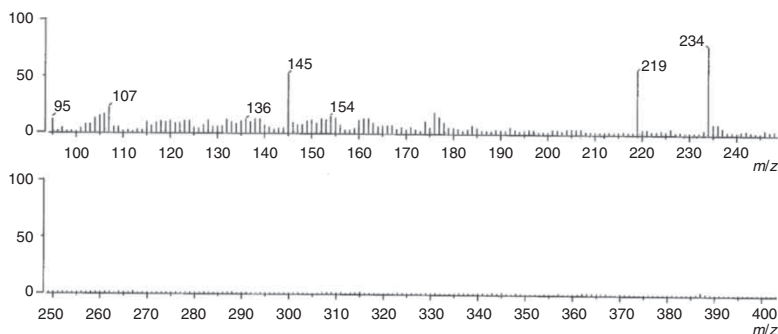
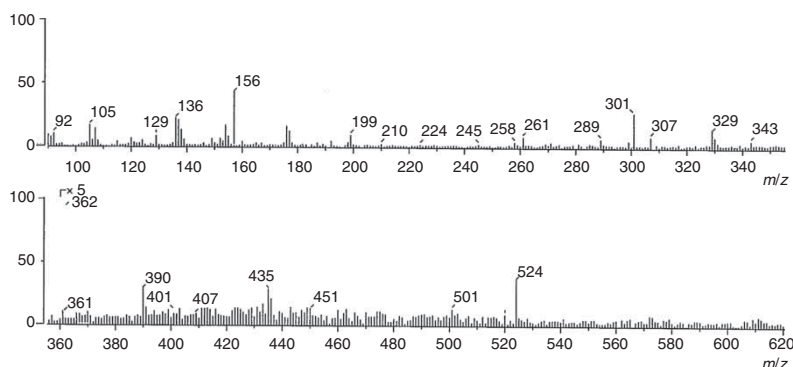


Figure 2. FAB-Mass spectrum of Schiff base I.

Figure 3. FAB-Mass spectrum of $\text{Ni}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$.

of the $\text{Cu}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$ complex exhibited a molecular ion peak M^+ , which is equivalent of its molecular weight, at m/z 529 with all other fragmentation peaks.

2.5. ESR spectral studies

The ESR spectral studies of the Cu(II) complexes provide information of the metal ion environment. The ESR spectra of the Cu(II) complexes were recorded in DMSO at liquid nitrogen temperature (LNT) and at RT. The ESR spectrum of one representative $\text{Cu}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$ complex is discussed here. The spectrum at RT shows one intense absorption band in the high field region and is isotropic due to the tumbling motion of the molecules. However, this complex at LNT shows four well-resolved peaks in the low field region. The g_{\parallel} and g_{\perp} values have been found to be 2.113 and 2.034, respectively. These values indicate that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital (37). The trend $g_{\parallel} > g_{\perp} > 2.0023$ observed for the complex indicates that the unpaired electron is localized in the $d_{x^2-y^2}$ orbital of the Cu(II) ion and is characteristic for the axial symmetry. The value of g_{av} was calculated to be 2.06. Thus, the results confirmed that the Cu(II) complexes possess distorted octahedral geometry.

2.6. Electrochemistry

All the complexes were studied for their electrochemical behavior. Both the Cu(II) complexes exhibited similar electrochemical properties. A cyclic voltammogram of $\text{Cu}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl})_2$

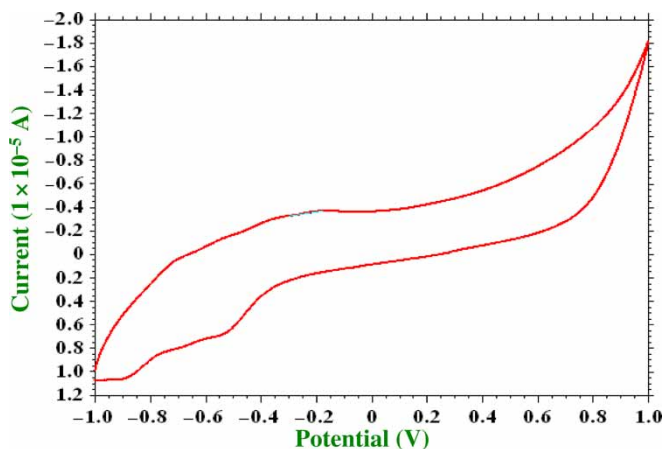


Figure 4. Cyclic voltammogram of $\text{Cu}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl})_2$.

(Figure 4) displays a reduction peak at $E_{pc} = -0.5\text{ V}$ with a corresponding oxidation peak at $E_{pa} = -0.25\text{ V}$. The peak separation of this couple (ΔE_p) is 0.25 at 0.1 V and increases with increasing scan rate. The most significant feature of the Cu(II) complex is the Cu(II)/Cu(I) couple. The difference between forward and backward peak potentials can provide a rough evaluation of the degree of reversibility of one electron transfer reaction. The analyses of cyclic voltametric responses with the scan rate varying from 50 to 300 mV s^{-1} give the evidence for quasi-reversible one electron transfer process. The ratio of cathodic to anodic peak height was less than 1. However, the peak current increases with increasing square root of the scan rates. This establishes the electrode process as diffusion-controlled (38). The separation in peak potentials increases at higher scan rates. These characteristic features are consistent with the quasi-reversibility of Cu(II)/Cu(I) couple.

The cyclic voltammogram of the $\text{Co}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl}_2)_2$ complex is depicted in Figure 5. It exhibits the first reduction peak at $E_{pc} = -0.65\text{ V}$ with an associated oxidation peak at $E_{pa} = -0.13\text{ V}$ and second reduction peak at $E_{pc} = 0.24\text{ V}$ with an associated oxidation peak at $E_{pa} =$

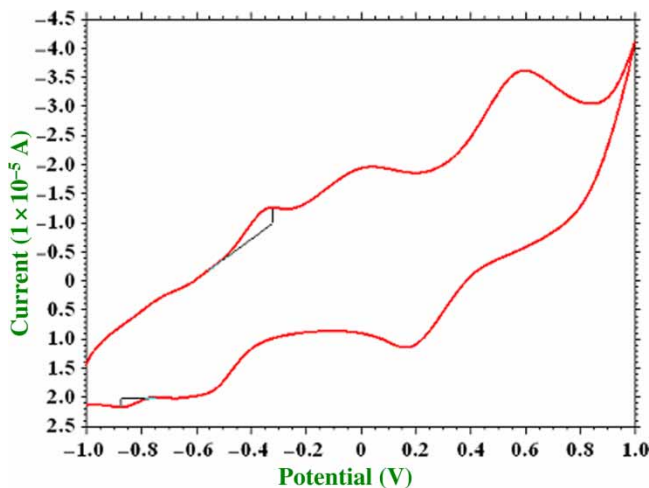


Figure 5. Cyclic voltammogram of $\text{Co}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl}_2)_2$.

0.59 V corresponding to Co(II)/Co(I) and Co(I)/Co(0), respectively. The peak separation of this (ΔE_p) is 0.52 and 0.35 V at a scan rate of 0.1 V s^{-1} for the first and second redox couples, respectively. This Co(II) complex also has a quasi-reversible character and the peak current rises with increasing square root of the scan rates. The difference between forward and backward peak potentials can provide a rough evaluation of the degree of the reversibility.

2.7. Pharmacological results

2.7.1. In vitro antibacterial and antifungal activity

The antibacterial studies inferred that the Schiff bases (**I** and **II**) were highly active against *Pseudomonas aeruginosa* and *Escherichia coli* and moderately active against *Salmonella typhi* and *Staphylococcus aureus*. All the Co(II), Ni(II) and Cu(II) metal complexes showed potential antibacterial activity against all the bacterial strains (Table 5) than the respective Schiff bases. In the case of antifungal activity, the Schiff bases and their Co(II), Ni(II) and Cu(II) complexes

Table 5. Antimicrobial results of Schiff bases and their metal complexes.

Compound	Concentration ($\mu\text{g mL}^{-1}$)	% Inhibition against bacteria				MIC		
		<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>A. flavus</i>	<i>Cladosporium</i>	<i>A. niger</i>
C ₁₀ H ₁₀ N ₄ OS	10	44	40	57	48	51	55	45
	30	66	56	62	65	54	63	61
	50	73	58	65	68	66	66	64
	100	78	69	76	70	73	76	69
C ₁₀ H ₉ N ₄ OSCl	10	52	44	51	45	58	56	57
	30	59	64	55	60	59	66	66
	50	61	69	63	68	69	74	78
	100	75	73	78	70	77	78	85
Co(C ₁₀ H ₉ N ₄ OS) ₂	10	67	60	68	57	78	75	71
	30	76	73	78	68	84	85	84
	50	83	82	80	79	91	89	91
	100	94	89	85	90	97	94	95
Co(C ₁₀ H ₈ N ₄ OSCl) ₂	10	65	57	75	68	76	74	80
	30	77	64	85	79	80	79	85
	50	84	81	84	79	87	88	90
	100	93	91	96	93	98	95	96
Ni(C ₁₀ H ₉ N ₄ OS) ₂	10	63	56	65	56	90	80	90
	30	78	63	75	68	91	93	93
	50	79	75	79	78	95	94	97
	100	94	86	94	86	100	99	100
Ni(C ₁₀ H ₉ N ₄ OSCl) ₂	10	65	57	60	55	88	82	86
	30	73	58	70	69	95	86	88
	50	79	64	88	73	97	92	90
	100	88	79	89	85	99	95	95
Cu(C ₁₀ H ₉ N ₄ OS) ₂	10	61	71	71	51	84	74	77
	30	74	78	76	65	90	79	87
	50	79	80	84	78	95	91	96
	100	90	82	90	80	98	93	98
Cu(C ₁₀ H ₈ N ₄ OSCl) ₂	10	65	63	73	65	85	76	76
	30	73	69	75	66	87	81	79
	50	88	84	81	79	89	95	84
	100	98	97	94	85	98	96	94
<i>Gentamycin</i>	100	100	100	100	100	–	–	–
<i>Flucanazole</i>	100	–	–	–	–	100	100	100

Table 6. MIC results ($\mu\text{g mL}^{-1}$).

Compound	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>A. flavus</i>	<i>Cladosporium</i>	<i>A. niger</i>
$\text{C}_{10}\text{H}_{10}\text{N}_4\text{OS}$	25	10	25	25	10	>100
$\text{C}_{10}\text{H}_9\text{N}_4\text{OSCl}$	25	10	25	25	25	>100
$\text{Co}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$	10	10	10	10	10	10
$\text{Co}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl})_2$	10	10	25	10	10	10
$\text{Ni}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$	10	10	10	10	10	10
$\text{Ni}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl})_2$	10	10	10	10	10	25
$\text{Cu}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$	25	>100	–	25	>100	>100
$\text{Cu}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl})_2$	25	>100	–	>100	>100	>100

were found to be highly active. All the metal complexes possess higher antifungal activity than the Schiff bases. This higher antimicrobial activity of the metal complexes, compared with that of Schiff bases, is perhaps due to the change in structure due to coordination, and chelating tends to make metal complexes act as more powerful and potent bacteriostatic agents, thus inhibiting the growth of the microorganisms (39, 40). Moreover, coordination reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups within the chelate ring system formed during the coordination. This process, in turn, increases the lipophilic nature of the central metal atom, which favors its permeation more efficiently through the lipid layer of the microorganism, thus destroying them more aggressively. The minimum inhibitory concentration (MIC) of some selected compounds, which showed significant activity against selected bacterial and fungi species, was determined. The results indicated that these compounds are the most active in inhibiting the growth of the tested organisms at a concentration of $10 \mu\text{g mL}^{-1}$ (Table 6).

2.7.2. DNA cleavage activity

The representative Schiff base $\text{C}_{10}\text{H}_{10}\text{N}_4\text{OS}$ and its $\text{Co}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$, $\text{Ni}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$ and $\text{Cu}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$ complexes are studied for their DNA cleavage activity by the agarose gel electrophoresis method against DNA of *E. coli*.

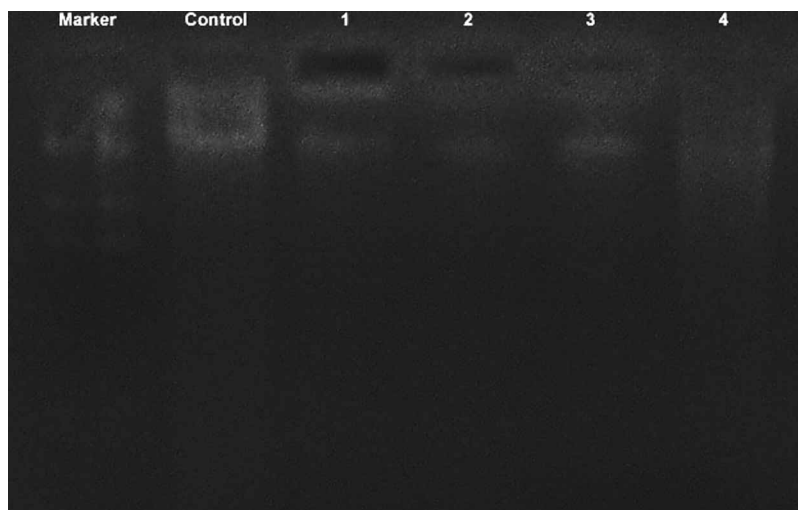


Figure 6. DNA cleavage on genomic DNA of *E. coli*. M, standard molecular weight marker; Control, control DNA of *E. coli*; lane 1, *E. coli* DNA treated with $\text{C}_{10}\text{H}_{10}\text{N}_4\text{OS}$; lane 2, *E. coli* DNA treated with $[\text{Co}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2]$; lane 3, *E. coli* DNA treated with $[\text{Ni}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2]$; lane 4, *E. coli* DNA treated with $[\text{Cu}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2]$.

DNA-binding studies are important for the rational design and construction of new and more efficient drugs targeted to DNA (41). The electrophoresis analysis clearly revealed that the Schiff base **I** and their metal complexes have acted on DNA as there was a difference in molecular weight between the control and the treated DNA samples. The difference was observed in the bands of lanes 1–4 compared with the control DNA of *E. coli* (Figure 6). This shows that the control DNA alone does not show any apparent cleavage, whereas the Schiff base and its complexes do show. However, the nature of reactive intermediates involved in the DNA cleavage by the complexes has not been clear. The results indicated the important role of coordination of sulfur to the metal in these isolated DNA cleavage reactions. As the compound was observed to cleave the DNA, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome.

3. Experimental

3.1. Analysis and physical measurements

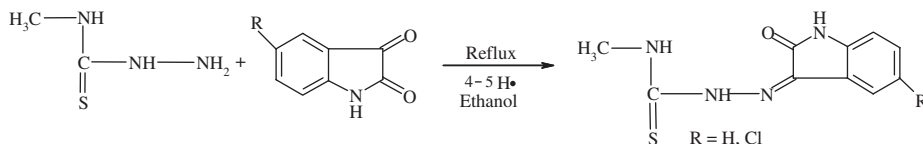
Carbon, hydrogen and nitrogen were estimated by using Elemental Analyzer Carlo Erba EA1108 analyzer. The IR spectra of the Schiff bases and their Co(II), Ni(II) and Cu(II) complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000–250 cm^{-1} region in a KBr disc. The electronic spectra of the complexes were recorded in HPLC-grade DMF and DMSO solvents on a VARIAN CARY 50-BIO UV spectrophotometer in the region 200–1100 nm. The ^1H NMR spectra of ligands were recorded in D_6 -DMSO on a BRUKER 300 MHz spectrometer at RT 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 A m) as the FAB gas. The accelerating voltage was 10 kV, the spectra were recorded at RT and m-nitrobenzyl alcohol was used as the matrix. The mass spectrometer was operated in the positive ion mode. The electrochemistry of all the complexes was recorded on a CHI1110A-electrochemical analyzer (made in the USA) in DMF containing 0.05 M *n*-Bu₄NCIO₄ as the supporting electrolyte. The ESR spectrum was recorded on a Varian-E-4X-band EPR spectrometer and the field is set at 3000 G at modulation frequency of 100 kHz under LNT using TCNE as a 'g' marker. Molar conductivity measurements were recorded on an ELICO-CM-82 T Conductivity Bridge with a cell having a cell constant of 0.51 and the magnetic moment was carried out by using Faraday balance.

3.2. Methods

All the chemicals used were of reagent grade.

3.2.1. Synthesis of 3-methylthiosemicarbazone

Freshly distilled methyl amine (3.4 mL) was dissolved in ammonia (20 mL), and carbon disulfide (8 mL) was added to it gradually with stirring in an ice bath. Ethanol (30 mL) was added to it gradually with constant stirring in an ice bath until the carbon disulfide was completely dissolved. The reaction mixture was then allowed to stand for 2–3 h. An aqueous sodium chloroacetate (0.1 m) 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 ethanol and recrystallized from hot ethanol. Yield (m.p.): 81% (130°C).



Scheme 1. Synthesis of Schiff bases.

3.2.2. Synthesis of Schiff bases **I** and **II**

The synthesis of Schiff bases is presented in Scheme 1. The Schiff bases have been synthesized by refluxing the reaction mixture of hot ethanolic solution (30 mL) of 3-methylthiosemicarbazone (0.01 mol) and hot ethanolic solution (30 mL) of isatin/chloroisatin (0.01 mol) for 4–5 h with addition of four to five drops of hydrochloric acid. The precipitate formed during reflux was filtered, washed with cold EtOH and recrystallized from hot EtOH.

Yield (m.p.): 78% (234 °C) and 75% (252 °C) Schiff bases **I** and **II**, respectively.

3.2.3. Synthesis of Co(II), Ni(II) and Cu(II) complexes (**I–6**)

An alcoholic solution (45 mL) of Schiff bases (2 mmol) was mixed with an alcoholic solution (5 mL) of 1 mmol of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ / $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ / $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and refluxed on a water bath for 2 h. Then, to the reaction mixture, 2 mmol of sodium acetate was added, and reflux was continued for 3 h. The separated complex was filtered, washed thoroughly with water, ethanol, ether and finally dried in a vacuum over fused CaCl_2 . The yield of the metal complexes lies in the range 65–70%.

4. Pharmacology

4.1. DNA cleavage experiment

4.1.1. Preparation of culture media

DNA cleavage experiments were carried out according to the literature (42, 43). Nutrient broth (peptone, 10 g l^{-1} ; yeast extract, 5 g l^{-1} ; NaCl, 10 g l^{-1}) was used for the culturing of *E. coli*. The 50 mL medium was prepared and autoclaved for 15 min at 121 °C under 15-lb pressure. The autoclaved medium was inoculated with the seed culture. The *E. coli* was incubated for 24 h.

4.1.2. Isolation of DNA

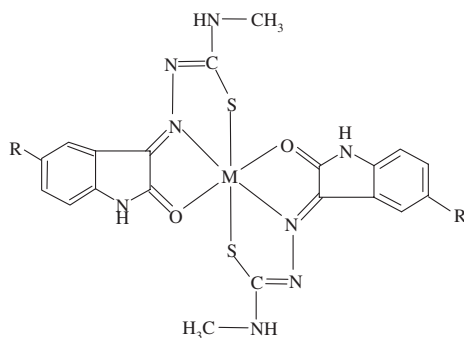
The fresh bacterial culture (1.5 mL) was centrifuged to obtain the pellet, which was then dissolved in 0.5 mL of lysis buffer (100 mM Tris pH 8.0, 50 mM EDTA, 10% sodium dodecyl sulphate (SDS)). To this, 0.5 mL of saturated phenol was added and incubated at 55 °C for 10 min. It was then centrifuged at 10,000 rpm for 10 min, and to the supernatant, an equal volume of chloroform:isoamyl alcohol (24:1) and 1/20th volume of 3 M sodium acetate (pH 4.8) were added. Then, this solution was centrifuged at 10,000 rpm for 10 min and to the supernatant, 3 vol of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in a TAE buffer (10 mM Tris pH 8.0, 1 mM EDTA) and stored in cold conditions.

4.1.3. Agarose gel electrophoresis

Cleavage products were analyzed by the agarose gel electrophoresis method (42, 43). Test samples (1 mg mL^{-1}) were prepared in DMF. The samples ($25 \mu\text{g}$) were added to the isolated DNA of *E. coli*. The samples were incubated for 2 h at 37°C . Then $20 \mu\text{L}$ of DNA sample (mixed with bromophenol blue dye at a 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with a standard DNA marker containing TAE buffer ($4.84 \text{ g Tris base, pH 8.0, 0.5 M EDTA per 1 L}$) and finally loaded on agarose gel and a constant electricity of 50 V was passed for around 30 min. The gel was removed and stained with $10.0 \mu\text{g mL}^{-1}$ ethidium bromide for 10–15 min and the bands observed under Vilberlourmate Gel documentation system and photographed to determine the extent of DNA cleavage. Then, the results were compared with that of a standard DNA marker.

4.2. In vitro antibacterial and antifungal assay

The biological activities of synthesized Schiff bases and their Co(II), Ni(II) and Cu(II) complexes have been studied for their antibacterial and antifungal activities by agar and potato dextrose agar diffusion method, respectively. The antibacterial and antifungal activities were done at 10, 30, 50 and $100 \mu\text{g mL}^{-1}$ concentrations in DMF solvent by using four bacteria (*E. coli*, *S. aureus*, *P. aeruginosa* and *S. typhi*) and three fungi (*Aspergillus niger*, *A. flavus* and *Cladosporium*) by the MIC method (44). These bacterial strains were incubated for 24 h at 37°C and fungal strains were incubated for 48 h at 37°C . Standard antibacterial (Gentamycin) and antifungal drugs (Fluconazole) were used for comparison under similar conditions.



R = H, Cl

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

Complex no.	R	M	Molecular formula
1	H	Co(II)	$\text{Co}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$
2	Cl	Co(II)	$\text{Co}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl})_2$
3	H	Ni(II)	$\text{Ni}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$
4	Cl	Ni(II)	$\text{Ni}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl})_2$
5	H	Cu(II)	$\text{Cu}(\text{C}_{10}\text{H}_9\text{N}_4\text{OS})_2$
6	Cl	Cu(II)	$\text{Cu}(\text{C}_{10}\text{H}_8\text{N}_4\text{OSCl})_2$

Figure 7. Structure of metal complexes.

5. Conclusion

The newly synthesized Schiff bases act as tridentate ligands and exhibit thiol–thione tautomerism. The metal ion is coordinated through the azomethine nitrogen, lactonyl oxygen and sulfur atom via deprotonation. On the basis of analytical, spectral and magnetic studies, the structure given in Figure 7 has been proposed for the metal complexes.

The Cu(II) and Co(II) complexes exhibited the one electron transfer quasi-reversible redox couple. The Schiff bases and their metal complexes were found to be highly active against some of the bacterial and fungal species. The activity is significantly increased on coordination. The DNA cleavage studies revealed that the complexes show non-specific cleavage of DNA.

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