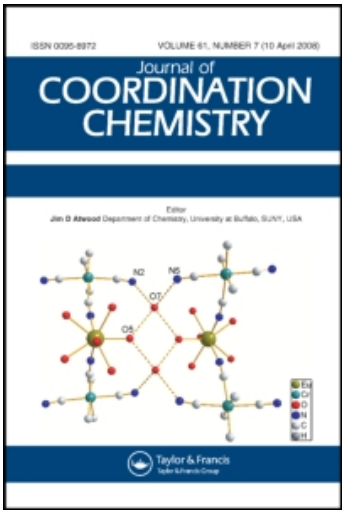

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DNA cleavage,*in vitro* antimicrobial and electrochemical studies of Co(II), Ni(II), and Cu(II) complexes with*m*-substituted thiosemicarbazide Schiff bases

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DNA cleavage, *in vitro* antimicrobial and electrochemical studies of Co(II), Ni(II), and Cu(II) complexes with *m*-substituted thiosemicarbazide Schiff bases

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Co(II), Ni(II), and Cu(II) complexes, $ML_2 \cdot 2H_2O$ have been synthesized with Schiff bases derived from *m*-substituted thiosemicarbazides and 2-methoxy benzaldehyde. The complexes are soluble in DMF/DMSO and non-electrolytes. From analytical, spectral (IR, UV-Vis, ESR, and FAB-mass), magnetic and thermal studies octahedral geometry is proposed for the complexes. The redox behavior of the complexes was investigated using cyclic voltammetry. The Schiff bases and their metal complexes have been screened for antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi*) and antifungal activities (*Aspergillus niger*, *Aspergillus flavus*, and *Cladosporium*) by *Minimum Inhibitory Concentration* method. DNA cleavage is studied by agarose gel electrophoresis method.

Keywords: Antimicrobial; DNA; Electrochemical; Spectral characterization

1. Introduction

Thiosemicarbazide-based compounds have been extensively studied [1–3]. Various Schiff bases of thiosemicarbazide (thiosemicarbazones) and their corresponding complexes have wide biological activities such as antitumor [4], antibacterial [5], and antifungal activities [6]. Thiosemicarbazones represent an important class of compounds utilized as starting materials in the synthesis of industrial products [7]. Cu and Ni complexes with Schiff base derived from N-methylthiosemicarbazone were studied spectroscopically [8]. Sharma *et al*. [9] have studied 16 ring-substituted 4-phenyl thiosemicarbazones. Transition metal complexes with tetradentate Schiff bases have been investigated as catalysts for a number of redox reactions and electrochemical reduction processes. Cyclic voltammetry is useful to investigate the mechanisms of catalysis by Schiff-base metal complexes as well as to study structure–reactivity relationships in these compounds [10–12]. Very recently, Adel *et al*. reported the

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interaction of transition/inner transition metals with Schiff bases of thiosemicarbazide ligands [13] and NS donor ligands of thiosemicarbazide [14]. From our laboratories, a number of transition metal complexes with various Schiff bases have been reported which have been physicochemically characterized and studied for their antibacterial and antifungal activity [15–18]. Interactions of transition metal complexes with DNA have been extensively 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 [19–21].

The present work synthesizes and characterizes Co(II), Ni(II), and Cu(II) complexes with Schiff bases derived from *m*-chloro/nitro substituted thiosemicarbazides and 2-methoxy benzaldehyde possessing sulfur and azomethine nitrogen donors. The electrochemistry of the Schiff bases and their complexes is investigated by cyclic voltammetry. The Schiff bases and their metal complexes are screened for their antimicrobial activity and DNA cleavage of the complexes is also studied.

2. Experimental

2.1. *Physical measurements*

Carbon, hydrogen, and nitrogen were estimated by using an Elemental Analyzer Carlo Erba EA1108 analyzer. The IR spectra of the Schiff bases and their complexes were recorded on a HITACHI-270 IR spectrophotometer from 4000 to 250 cm^{-1} in KBr disks. The electronic spectra of the complexes were measured in HPLC grade DMF on a VARIAN CARY 50-BIO UV-spectrophotometer from 200 to 1100 nm. ^{1}H-NMR spectra of ligands were recorded in D_6-DMSO 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 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 all the complexes was recorded on a CHI1110A-electrochemical analyzer (made in USA) in DMF containing 0.05 M *n*-Bu_4NClO_4 as the supporting electrolyte. The ESR spectrum was recorded on a Varian-E-4X-band EPR spectrometer with 3000 G at modulation frequency of 100 kHz under liquid nitrogen temperature using TCNE as g marker. Thermogravimetric analyses were measured from room temperature to 1000°C at a heating rate of 10°C min^{-1} on 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 and magnetic moment was measured by using a Faraday balance.

2.2. *Methods*

All chemicals used were of reagent grade.

2.3. *Synthesis of m-substituted thiosemicarbazide*

Freshly distilled *m*-chloroaniline/*m*-nitroaniline (12.7 g/13.8 g) was dissolved in ammonia (20 mL) and carbon disulfide (8 mL) was added gradually with stirring in an

ice bath. Ethanol (30 mL) was added gradually with constant stirring in an ice bath until 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 that separated were filtered, washed with cold ethanol, and recrystallized from hot ethanol. The IUPAC names of the synthesized thiosemicarbazones are 4-(*m*-chlorophenyl) thiosemicarbazide and 4-(*m*-nitrophenyl) thiosemicarbazide.

2.4. *Synthesis of Schiff bases [I and II]*

The synthesis of Schiff bases (schematically presented in scheme 1) is synthesized by refluxing hot ethanolic solution (30 mL) of *m*-substituted thiosemicarbazide (0.01 M) and hot ethanolic solution (30 mL) of 2-methoxy benzaldehyde (0.01 M) for 4–5 h with addition of a few drops of hydrochloric acid. The precipitate formed during reflux was filtered, washed with cold EtOH, and recrystallized from hot EtOH.

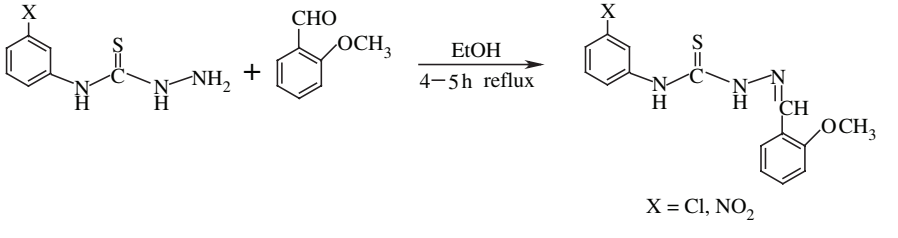
2.5. *Synthesis of Co(II), Ni(II), and Cu(II) complexes [(1)–(6)]*

An ethanol solution (45 mL) of Schiff base (2 mM) was mixed with an ethanol solution (5 mL) of 1 mM of $CoCl_2 \cdot 6H_2O/NiCl_2 \cdot 6H_2O/CuCl_2 \cdot 2H_2O$ 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 water, ethanol, ether, and finally dried in a vacuum over fused $CaCl_2$.

3. Pharmacology

3.1. *DNA cleavage experiment*

3.1.1. Preparation of culture media. DNA cleavage experiments were done according to the literature [22]. Nutrient broth [peptone, 10; yeast extract, 5; NaCl, 10; in ($g\,L^{-1}$)] was used for culturing of *Escherichia coli* and potato dextrose broth [potato, 250;



Schiff base	X	Molecular formula
I	Cl	$C_{15}H_{14}N_3OSCl$
II	NO_2	$C_{15}H_{14}N_4O_3S$

Scheme 1. Synthesis of Schiff bases.

dextrose, 20; in ($g\,L^{-1}$)] was used for the culture of *Aspergillus niger*. The 50 mL media was prepared, autoclaved for 15 min at 121°C under 15 lb pressure. The autoclaved media were inoculated with the seed culture. *E. coli* was incubated for 24 h and *A. niger* for 48 h at 37°C.

3.1.2. Isolation of DNA. The fresh bacterial culture (1.5 mL) is centrifuged to obtain the pellet which is then dissolved in 0.5 mL of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 10% SDS). To this, 0.5 mL of saturated phenol was added and incubated at 55°C for 10 min, centrifuged at 10,000 rpm for 10 min. To the supernatant, equal volume of chloroform : isoamyl alcohol (24 : 1) and 1/20th volume of 3 M sodium acetate (pH 4.8) was added. After centrifuging at 10,000 rpm for 10 min, three volumes of chilled absolute alcohol was added to the supernatant. The precipitated DNA was separated by centrifuging and the pellet was dried and dissolved in TAE buffer (10 mM tris pH 8.0, 1 mM EDTA) and stored cold.

3.1.3. Agarose gel electrophoresis. Cleavage products were analyzed by agarose gel electrophoresis [22]. Test samples ($1\,mg\,mL^{-1}$) were prepared in DMF and added (25 μg) to the isolated DNA of *E. coli* and *A. niger*. The samples were incubated for 2 h at 37 °C and then 20 μL of DNA sample (mixed with bromophenol blue dye in 1 : 1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g tris base, pH 8.0, and 0.5 M EDTA/1 L), and finally loaded on agarose gel with 50 V for 30 min. Removing the gel and staining with $10.0\,\mu g\,mL^{-1}$ ethidium bromide for 10–15 min gave bands under Vilberlourmate Gel documentation system and photographed to determine the extent of DNA cleavage as compared with standard DNA marker.

3.1.4. *In vitro* antibacterial and antifungal assay. The biological activities of the Schiff bases and their complexes have been studied for antibacterial and antifungal activities by agar and potato dextrose agar diffusion methods, respectively. The antibacterial and antifungal activities were done at 10, 30, 50, and $100\,\mu g\,mL^{-1}$ concentrations in DMF solvent using four bacteria (*E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi*) and three fungi (*A. niger*, *A. flavus*, and *cladosporium*) by the MIC method [23]. 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 (Flucanozole) was used for comparison under similar conditions.

4. Results and discussion

The Schiff bases (I and II) form complexes (**1**–**6**) with $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, and $CuCl_2 \cdot 2H_2O$ in ethanol. The 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 $ML_2 \cdot 2H_2O$, where L stands for a deprotonated ligand which exhibits thiol–thione tautomerism. The structure of I is presented in figure 1. The molar conductance values indicate non-electrolytic nature of the complexes in DMF (table 1).

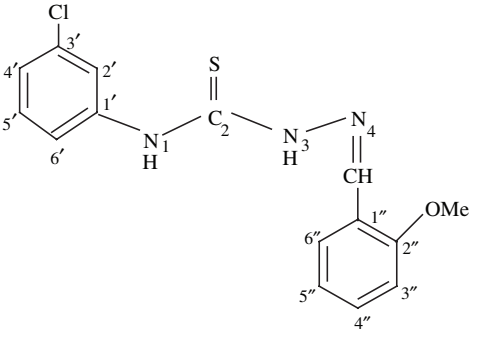


Figure 1. Structure of Schiff base I.

To establish whether water present in the complexes is coordinated, the weighed complex was heated for 2 h at 105°C, cooled in a desiccator, and weighed again with no loss in weight [24], suggesting that water present in the complexes is coordinated.

4.1. *IR spectral studies*

The prominent infrared spectral data of the Schiff bases and their complexes are presented in "Supplementary material".

The IR spectra of the Schiff bases exhibit two characteristic bands due to ν(NH) at 3270–3152 cm^{-1} corresponding to two ν(NH) groups. A strong band at 2672–2666 cm^{-1} is ascribed to ν(SH) and another band at 1199–1187 cm^{-1} is assigned to ν(C=S) [25]. Both ν(SH) and ν(C=S) bands are of equal intensity; these observations suggest that the Schiff bases exhibit thiol–thione tautomerism with 1 : 1 ratio. High intensity bands at 1605 and 1596 cm^{-1} in the IR spectra of I and II are assigned to ν(C=N) [26].

In comparison with the spectra of the Schiff bases, all Co(II), Ni(II), and Cu(II) complexes exhibit ν(C=N) at 1559–1597 cm^{-1} shifted to lower wave numbers, indicating that azomethine is coordinated to metal [26–28]. Deprotonation of the thiol is indicated by the absence of a band at ~2672 cm^{-1} in all complexes, which is due to ν(S–H), indicating that the metal is coordinated through sulfur. This is further supported by the band at 786–769 cm^{-1} in the complexes due to ν(C–S). New bands at 473–466 cm^{-1} are assigned to stretching of M–N bonds [29]. The band at 341–338 cm^{-1} of far IR-spectra is due M–S [30]. The presence of coordinated water in the complexes is confirmed by a broad band at 3436–3394 cm^{-1} and two weak bands at 750–800 and 700–720 cm^{-1} due to $\nu(OH_2)$ rocking and wagging modes, respectively [31].

The IR spectral data provide strong evidence for complexation of Schiff bases with metal(II).

4.2. *^{1}H NMR spectral study of Schiff bases I and II*

In the ^{1}H-NMR spectra of the Schiff bases, the NH proton exhibited two singlets at 9.98(s, 1H)/9.24 ppm (s, 1H) and 9.92(s, 1H)/9.28 ppm (s, 1H) [32]. The characteristic signals at 8.46 ppm and 8.42 ppm (s, 1H) are assigned to –CH=N. Sharp singlets at 3.87 ppm (s, 1H) and 3.91 ppm (s, 1H) are attributed to SH. The multiplets in the region

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

Compound No.	Empirical formula	Color/yield (%)	M (%)		C (%)		H (%)		N (%)		Molar conductance ($ohm^{-1}cm^2M^{-1}$)	μ_{eff} (BM)
			Obsd	Calcd	Obsd	Calcd	Obsd	Calcd	Obsd	Calcd		
I	$C_{15}H_{14}N_3OSCl$	70	–	–	56.11	56.42	4.53	4.388	13.02	13.16	–	–
II	$C_{15}H_{14}N_4O_3S$	72	–	–	54.32	54.54	4.12	4.242	12.612	12.72	–	–
1	$Co(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$	Brown/68	8.02	8.07	49.18	49.24	4.12	4.10	11.50	11.49	22.8	4.51
2	$Co(C_{15}H_{13}N_4O_3S)_2 \cdot 2H_2O$	Brown/65	7.74	7.835	47.71	47.80	3.958	3.98	14.80	14.87	19.22	4.74
3	$Ni(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$	Yellowish green/67	7.89	7.945	49.22	49.31	4.11	4.109	11.45	11.50	21.25	3.12
4	$Ni(C_{15}H_{13}N_4O_3S)_2 \cdot 2H_2O$	Yellowish green/68	7.71	7.71	47.81	47.87	3.889	3.989	14.82	14.89	20.67	3.31
5	$Cu(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$	Dark green/65	8.41	8.571	48.85	48.97	4.01	4.08	11.20	11.42	19.4	1.77
6	$Cu(C_{15}H_{13}N_4O_3S)_2 \cdot 2H_2O$	Dark green/64	8.30	8.322	47.50	47.55	3.922	3.963	14.72	14.79	23.3	1.79

6.95–7.81 and 7.01–7.87 ppm (m, 8H) (m, 8H) are due to aromatic protons and singlets at 3.47 ppm (s, 3H) and 3.46 ppm are due to OCH_3. NMR results support the IR inferences and confirm the thiol–thione tautomerism of the Schiff bases.

4.3. *Electronic spectral and magnetic studies*

The Co(II) complexes exhibited bands in the region 8000–10,000 cm^{-1} and 18,000–20,000 cm^{-1} corresponding to ν_1 and ν_3 transitions attributed to $^4T_{1g}$ (F) $\rightarrow$ $^4T_{2g}$ (F) (ν_1) and $^4T_{1g}$ (F) $\rightarrow$ $^4T_{1g}$ (P) (ν_3). The brownish Co(II) complexes showed absorption bands at 9256–9312 and 18,965–18,952 cm^{-1} corresponding to ν_1 and ν_3, characteristic of high spin octahedral Co(II) complexes [31]. However, ν_2 is not observed because of its proximity to the strong ν_3 transition. The ligand field parameters calculated are presented in "Supplementary material". Magnetic measurements for the Co(II) complexes have magnetic moment values of 4.51–4.74 which agree with octahedral range [33] supporting the electronic spectral results.

The greenish $Ni(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$ exhibited three bands at 10,363, 15,878, and 26,334 cm^{-1} attributed to $^3A_{2g} \rightarrow {}^3T_{2g}$ (ν_1), $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) (ν_2), and $^3A_{2g} \rightarrow {}^3T_{1g}$ (P) (ν_3), respectively, which indicate octahedral Ni(II) [34]. The ligand field parameters (Supplementary material) show ν_2/ν_1 to be 1.527 and the μ_{eff} value to be 3.174, suggesting octahedral Ni(II). Hence, the ligand field parameters [35] correlate to the electronic spectral and magnetic properties.

The electronic spectra of Cu(II) complexes display two prominent bands. A low intensity broad band at 16,582 cm^{-1} is assignable to $^2E_g \rightarrow {}^2T_{2g}$ transition and a high intensity band at 25,642 cm^{-1} is due to ligand $\rightarrow$ metal charge transfer. The electronic spectra suggest distorted octahedral geometry around Cu(II) [36]. The Cu(II) complexes magnetic moment (1.77–1.79 BM) is consistent with octahedral geometry [37].

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

The FAB-mass spectrum of I, depicted in "Supplementary material", shows a molecular ion peak at m/z 319 equivalent to its molecular weight. Fragment peaks observed at m/z 288, 199, and 126 are due to the cleavage of OCH_3, C_7H_5, and CN_2SH, respectively. For II, the molecular ion peak is observed at m/z 330, ascribed to $C_{15}H_{14}N_4O_3S$.

The FAB-mass spectra of $Co(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$, $Ni(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$ and $Cu(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$ showed molecular ion peaks M^+ at m/z 731, 730, and 735 equivalent to their molecular weight. All $[MHL \cdot 2H_2O]^+$ undergo demetallation to form $[L + H]^+$ at m/z 319. $Co(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$ complex showed a fragment at m/z 695 ascribed to the loss of two waters. The complexes of II showed molecular ion peaks at m/z 753, 752, and 757 equivalent of their molecular weight and undergo demetallation to form $[L + H]^+$ at m/z 330.

4.5. *ESR spectrum of 5*

The ESR spectrum of **5** (Supplementary material) at RT shows one intense absorption in the high field region, isotropic due to tumbling of the molecules. However, at LNT

Table 2. Thermogravimetric data of **1**, **3**, and **5**.

Compound No.	Decomposition temperature (°C)	Weight loss (%)		Metal oxide (%)		Inference
		Obsd	Calcd	Obsd	Calcd	
1	145–174	4.8	4.9	10.1	10.3	Loss due to coordinated water molecules
	230–270	32.8	32.8			Loss due to 2-methoxy benzaldehyde
	315–365	54.4	54.4			Loss due to thiosemicarbazide
3	140–185	4.9	4.9	10.0	10.1	Loss due to coordinated water molecules
	215–255	32.7	32.9			Loss due to 2-methoxy benzaldehyde
	300–355	54.4	54.5			Loss due to thiosemicarbazide
5	140–165	4.9	4.9	10.7	10.7	Loss due to coordinated water molecules
	210–260	32.5	32.7			Loss due to 2-methoxy benzaldehyde
	310–355	54.1	54.1			Loss due to thiosemicarbazide

four well-resolved peaks in low field region are observed. The $g_{||}$ and $g_{\perp}$ values have been found to be 2.1009 and 2.015, respectively. The trend $g_{||} > g_{\perp} > 2.0023$ indicates that the unpaired electron is localized in the $d_{x^2-y^2}$ orbital of Cu(II) and is characteristic for axial symmetry [38]. Thus, ESR spectral data confirm that **5** possesses distorted octahedral geometry.

4.6. *Thermal studies of metal complexes*

The thermal behavior of Co(II), Ni(II), and Cu(II) complexes are almost the same; only $Co(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$, $Ni(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$, and $Cu(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$ will be discussed.

The thermal decomposition of **1**, **3**, and **5** took place in three steps as indicated by DTG peaks around 140–185, 210–270, and 300–365°C corresponding to the loss of coordinated water, 2-methoxy benzaldehyde and *m*-substituted thiosemicarbazide, respectively. TGA studies confirm coordination of water to the metal ions. Both waters depart in a narrow (~45 K) one step process. Finally, the metal complexes decompose gradually with the formation of metal oxide above 365°C. The TG/DTG spectrum of **3** is presented in "Supplementary material". The proposed chemical change with temperature and the percentage of metal oxide obtained are given in table 2.

4.7. *Electrochemistry*

All complexes were studied for their electrochemical behavior. Both Cu(II) complexes exhibit similar electrochemical properties. A cyclic voltammogram of $Cu(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$ (Supplementary material) displays a reduction peak at $E_{pc} = -1.41$ V with a corresponding oxidation peak at $E_{pa} = -0.79$ V. The peak separation of this couple (ΔE_p) is 0.62 V at 0.1 V s^{-1} and increases with the increase in 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 provides a rough evaluation of the degree of the reversibility of one electron transfer reaction. The analysis of cyclic voltammetric responses with scan rate varying from 50 to

300 mV s^{-1} gives the evidence for quasi-reversible one electron transfer. The ratio of cathodic to anodic peak height was less than one. However, the peak current increases with the increase of the square root of the scan rates, establishing the electrode process as diffusion controlled [39]. The separation in peak potentials increases at higher scan rates, consistent with quasi-reversibility of the Cu(II)/Cu(I) couple.

The cyclic voltammogram of $Ni(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$ (Supplementary material) exhibits a reduction peak at $E_{pc} = -1.352$ V with oxidation peak at $E_{pa} = -0.682$ V corresponding to the Ni(II)/Ni(I) couple. The peak separation of this couple (ΔE_p) is 0.67 V. This Ni(II) complex also has a quasi-reversible character as the separation in peak potential that is higher than 59 mV and the peak currents rise 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.

5. Pharmacological results

5.1. In vitro *antibacterial and antifungal activity*

Antibacterial studies indicated that I and II were potentially active against *P. auregenosa* and *S. typhi* and moderately active against *S. aureus*. All the Co(II), Ni(II), and Cu(II) metal complexes showed higher antibacterial activity against all the bacterial strains (table 3) than the respective Schiff bases. For antifungal activity, the Schiff bases and their Co(II), Ni(II), and Cu(II) complexes were highly active. All the metal complexes possess higher antifungal activity than the Schiff bases. The metal complexes are powerful and potent bactereostatic agents, inhibiting the growth of the microorganisms [40–44].

The minimum inhibitory concentration (MIC) of some selected compounds, which showed significant activity against selected bacterial and fungi species, were determined. The results indicated that these compounds were most active in inhibiting the growth of the tested organisms at 10 μg mL^{-1} (table 4).

5.2. *DNA cleavage activity*

$C_{15}H_{14}N_3OSCl$, $Co(C_{15}H_{13}N_3OSCl)_2 \cdot 2H_2O$, and $Cu(C_{15}H_{13}N_4O_3S)_2 \cdot 2H_2O$ were studied for their DNA cleavage activity by agarose gel electrophoresis against DNA of *A. niger* and *E. coli*.

DNA-binding studies are important for construction of new and more efficient drugs targeted to DNA [45]. Most DNA interaction studies are carried out on calf thymus DNA [46, 47]. Here we studied DNA cleavage on DNA isolated from *A. niger* and *E. coli*. The electrophoresis clearly revealed that the Schiff base and both complexes act on DNA as there was molecular weight difference between the control and the treated DNA samples. The difference was observed in the bands of lanes 1–3 compared to the control DNA of *A. niger* (Supplementary material) and control DNA of *E. coli*. The control DNA alone does not show any apparent cleavage, whereas Schiff base and complexes do. However, the nature of reactive intermediates involved in DNA cleavage by the complexes is not clear. The results indicated the important role of metal in these isolated DNA cleavage reactions. As the compound was observed to cleave

Table 3. Antimicrobial results of Schiff bases and their metal complexes.

Compound No.	Concentration ($\mu g\,mL^{-1}$)	% Inhibition against bacteria				% Inhibition against fungi		
		E. coli	*S. aureus*	*P. aeruginosa*	*S. typhi*	*A. flavus*	*Cladosporium*	*A. niger*
I	10	13	22	13	14	51	75	63
	30	19	36	60	23	57	82	69
	50	35	49	67	74	68	86	81
	100	49	59	72	80	78	92	88
II	10	19	25	19	22	49	66	59
	30	28	39	55	29	57	79	69
	50	41	56	64	66	67	86	78
	100	61	67	71	82	75	93	89
1	10	70	54	79	77	78	76	81
	30	76	66	83	83	84	83	88
	50	83	78	83	89	86	90	91
	100	94	90	91	92	97	93	94
2	10	64	56	68	75	77	75	79
	30	76	58	81	79	80	79	84
	50	83	70	83	87	87	87	89
	100	92	81	93	92	95	94	95
3	10	46	56	63	64	65	90	72
	30	57	66	72	71	70	94	75
	50	65	75	77	76	78	94	88
	100	78	83	80	83	89	97	91
4	10	44	54	57	59	57	81	66
	30	53	61	70	68	69	88	77
	50	64	72	77	71	73	88	80
	100	76	82	82	81	87	90	84
5	10	49	36	54	50	65	72	69
	30	52	75	62	61	73	78	81
	50	62	83	72	68	78	85	91
	100	73	93	74	70	97	95	94
6	10	84	83	83	82	76	90	88
	30	90	90	88	85	78	92	91
	50	95	95	93	89	86	93	94
	100	98	98	96	95	95	96	97
Gentamycin	100	100	100	100	100	–	–	–
Flucanozole	100	–	–	–	–	100	100	100

Table 4. Minimum inhibitory concentration ($\mu g\,mL^{-1}$).

Compound No.	*E. coli*	*P. aeruginosa*	*S. typhi*	*A. flavus*	*Cladosporium*	*A. niger*
I	25	10	25	25	10	>100
II	25	10	25	25	25	>100
1	10	10	10	10	10	10
2	10	10	25	10	10	10
3	10	10	10	10	10	10
4	10	10	10	10	10	25
5	25	>100	–	25	>100	>100
6	25	>100	–	>100	>100	>100

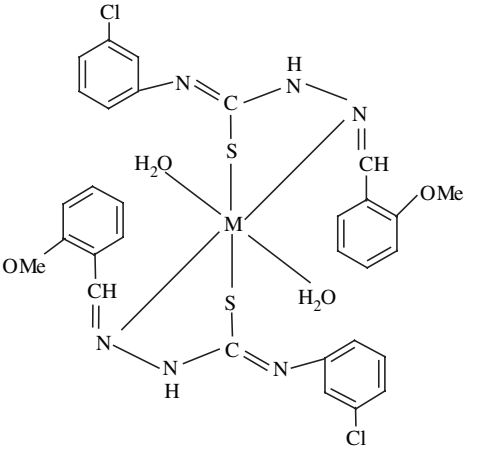


Figure 2. Structure of Co(II) **1**, Ni(II) **3**, and Cu(II) **5** complexes.

DNA, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome.

6. Conclusions

The synthesized Schiff bases are bidentate ligands and exhibit thiol–thione tautomerism. The metal ion is coordinated through the azomethine nitrogen and sulfur in thiol form *via* deprotonation. The structures of Co(II), Ni(II), and Cu(II) complexes are presented in figure 2. The bonding of ligand to metal ion is confirmed by analytical, spectral, magnetic, and thermal studies. The electrochemistry of the Cu(II) and Ni(II) metal complexes in DMF exhibit quasi-reversible one electron transfer. The Schiff bases and their metal complexes were highly active against some of the antibacterial and antifungal species. The activity is significantly increased on coordination. The DNA cleavage studies reveal that the complexes showed non-specific cleavage of DNA isolated from *A. niger* and *E. coli*.

References

[1] S.R. Bhomik. *J. Coord. Chem.*, **58**, 795 (2005).
[2] S.K. Chattopadhyay, S. Seth, T.C.W. Mak. *J. Coord. Chem.*, **55**, 259 (2002).
[3] J. Valdes-Martinez, R.A. Toscana, J. Ramirez-Ortiz. *Polyhedron*, **14**, 579 (1995).
[4] D. Kovala-Demertzi, M.A. Demertzis, J.R. Miller. *J. Inorg. Biochem.*, **86**, 555 (2001).
[5] N.C. Kasuga, K. Onodera, S. Nakano. *J. Inorg. Biochem.*, **100**, 1176 (2006).
[6] N.C. Kasuga, K. Sekino, C. Koumo. *J. Inorg. Biochem.*, **84**, 55 (2001).
[7] S. Allah, A.M. Abid. *Phosphorus, Sulfur Silicon Relat. Elem.*, **75**, 170 (2001).
[8] E. Mustafa, K. Sancak, I. Degirmencioglu, K. Serbest. *J. Mol. Struct.*, **46**, 882 (2008).
[9] V.K. Sharma, S. Srivastava, A. Srivastava. *Bioinorg. Chem. Appl.*, **2007**, 1 (2007).

[10] H. Temel, B. Ziyadanogullari, I. Aydin, F. Aydin. *J. Coord. Chem.*, **58**, 1177 (2005).
[11] T. Santos Silva, R. Leticia, T. Roberta, L.Z. Sonia, R.W. Louro, H. Beraldo. *J. Coord. Chem.*, **62**, 958 (2009).
[12] A.D. Kulkarni, S.A. Patil, P.S. Badami. *Int. J. Electrochem. Sci.*, **4**, 729 (2009).
[13] A.A.E. Adel, H.S. Seleem, A.M. Madyan. *J. Coord. Chem.*, **62**, 2582 (2009).
[14] N. Raman, R. Jeyamurugan. *J. Coord. Chem.*, **62**, 2387 (2009).
[15] N.B. Reddy, P.G. Avaji, P.S. Badami, S.A. Patil. *J. Coord. Chem.*, **61**, 1827 (2008).
[16] P.G. Avaji, B.N. Reddy, P.S. Badami, S.A. Patil. *Transition Met. Chem.*, **31**, 842 (2006).
[17] G.B. Bagihalli, S.A. Patil. *J. Coord. Chem.*, **62**, 1700 (2009).
[18] G.B. Bagihalli, P.G. Avaji, S.A. Patil, P.S. Badami. *Eur. J. Med. Chem.*, **43**, 2639 (2008).
[19] J. Liu, T. Zhang, T. Lu, L. Qu, H. Zhou, Q. Zhang, L. Ji. *J. Inorg. Biochem.*, **92**, 269 (2002).
[20] V.G. Vaidyanathen, B.U. Nair. *J. Inorg. Biochem.*, **93**, 271 (2003).
[21] P.R. Reddy, K.S. Rao, B. Satyanarayana. *Tetrahedron Lett.*, **47**, 7311 (2006).
[22] T.A. Brown. *Essential Molecular Biology – A Practical Approach*, Vol. 1, 2nd Edn, Oxford University Press, New York (1990).
[23] A.K. Sadana, Y. Miraza, K.R. Aneja, O. Prakash. *Eur. J. Med. Chem.*, **38**, 533 (2003).
[24] P.G. Avaji, P.S. Badami, S.A. Patil. *J. Coord. Chem.*, **61**, 1884 (2008).
[25] G. Singh, P.A. Singh, K. Singh, D.P. Singh, R.N. Handa, S.N. Dubey. *Proc. Natl Acad. Sci. Ind.*, **72**, 87 (2002).
[26] G.A. Bain, D.X. West, J. Krejci, J.V. Martinez, S.H. Ortega, R.A. Toscanob. *Polyhedron*, **16**, 855 (1997).
[27] M. Joseph, A. Sreekanth, V. Suni, M.R.P. Kurup. *Spectrochim. Acta, Part A*, **64**, 637 (2006).
[28] K.P. Deepa, K.K. Aravindakshan. *Synth. React. Inorg. Met.-Org. Chem.*, **30**, 1601 (2000).
[29] S. Chandra, L.K. Gupta. *Spectrochim. Acta, Part A*, **61**, 269 (2005).
[30] K. Nakamoto. *Infrared Spectra of Inorganic and Coordination Compounds*, Wiley-Interscience, New York (1970).
[31] K. Singh, M.S. Barwa, P. Tyagi. *Eur. J. Med. Chem.*, **41**, 147 (2006).
[32] A.A.E. Adel, M.I.A. Omima. *Transition Met. Chem.*, **32**, 889 (2007).
[33] P.P. Dholakiya, M.N. Patel. *Synth. React. Inorg. Met.-Org. Nano-Met. Chem.*, **32**, 819 (2002).
[34] S. Chandra, A. Kumar. *J. Indian Chem. Soc.*, **84**, 325 (2007).
[35] N. Saha, N. Mukherjee. *Polyhedron*, **31**, 135 (1984).
[36] H. Liu, H. Wang, F. Gao, D. Niu, Z. Lu. *J. Coord. Chem.*, **60**, 2671 (2007).
[37] D.P. Singh, R. Kumar, V. Malik, P. Tyagi. *Transition Met. Chem.*, **32**, 1051 (2007).
[38] R.L. Dutta, A. Syamal. Electron spin resonance responses. In *Elements of Magnetochemistry*, 2nd Edn, p. 206, East-West Press, New Delhi (1992).
[39] A.J. Bard, L.R. Faulkner. *Electrochemical Methods*, 2nd Edn, John Wiley, New York (2001).
[40] Z.H. Chohan, C.T. Supuran, A. Scozzafava. *J. Enzyme Inhib. Med. Chem.*, **19**, 79 (2004).
[41] Z.H. Chohan, M. Praveen. *Appl. Organomet. Chem.*, **15**, 617 (2001).
[42] Z.H. Chohan, H. Pervez, S. Kausar, C.T. Supuran. *Synth. React. Inorg. Met.-Org. Chem.*, **3**, 529 (2002).
[43] Z.H. Chohan, H. Pervez, A. Rauf, C.T. Supuran. *Met.-Based Drugs*, **84**, 2 (2002).
[44] Z.H. Chohan, A. Scozzafava, C.T. Supuran. *J. Enzyme Inhib. Med. Chem.*, **18**, 259 (2003).
[45] M.J. Waring. In *Drug Action at the Molecular Level*, G.C.K. Roberts (Ed.), Vol. 7, p. 67, Maemillar, London (1977).
[46] M.A. Neelakantan, F. Rusairaj, J. Dharmaja, S. Johnsonraja, T. Jeyakumar, M. Sankarayana Pillai. *Spectrochim. Acta, Part A*, **71**, 1599 (2008).
[47] N. Raman, S. Thalamuthu, J. Dhaveethuraja, M.A. Neelakandan, S. Banerjee. *J. Chil. Chem. Soc.*, **53**, 1450 (2008).