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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

DNA cleavage and antimicrobial investigation of Co(II), Ni(II), and Cu(II) complexes with triazole Schiff bases: synthesis and spectral characterization

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Abstract The Co(II), Ni(II), and Cu(II) complexes with Schiff bases derived from 3-substituted-4-amino-5-mercapto-1,2,4-triazole and fluvastatin have been synthesized. Schiff bases exhibited thiol–thione tautomerism and coordinated to metal ion through azomethine nitrogen and thiolate sulphur atoms. Square planar geometry for all the metal complexes of the type ML_2 has been proposed in the light of analytical, spectral (IR, UV–Vis., ESR, and FAB-mass), magnetic, and thermal studies. The antimicrobial studies of Schiff bases and their metal complexes against various antibacterial (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*) and antifungal (*Aspergillus niger*, and *Pencillium Chrysogenum*) species by Minimum Inhibitory Concentration method revealed that, the metal complexes possess more healing antibacterial activity than the Schiff bases. Co(II), Ni(II), and Cu(II) complexes cleave the DNA isolated from *A. niger*.

Keywords Antibacterial · Antifungal · DNA cleavage · Spectroscopic studies · Schiff base complexes · Transition metal complexes

Introduction

Metal complexes with Schiff base ligands have played an important role since the early days of Coordination Chemistry (Holm, 1974; Yamada, 1966; Sacconi, 1966). Indeed, a great deal of study has been carried out on the synthesis and characterization of transition metal compounds with Schiff bases as ligands, mainly due to their applications in the field of organic chemistry (Katsuki, 1995; Ito and Katsuki, 1999) and in catalytic processes (Canali and Sherrington, 1999). Also, the last decade has seen an upsurge of interest in metal ion therapeutics for both diagnosis and treatment of diseases (Reichert *et al.*, 1999; Schwietert and McCue, 1999). For well over a hundred years, the synthesis and functionalization of indoles has been a major area of focus for chemists. Indoles are of great significance in view of their (i) occurrence in nature as a prominent sub-structure of a large number of alkaloids (Somei and Yamada, 2003; Hibino and Choshi, 2002) and (ii) wide-ranging biological activities (Gribble, 1995). In addition, 1,2,4-triazoles are an important class of heterocyclic compounds which are well known for their potential antimicrobial properties. The substituted 1,2,4-triazoles are associated with diverse biological activities such as, fungicidal, antimicrobial, anticonvulsant, and antiviral activities (Walser *et al.*, 1991; Ewiss *et al.*, 1986; Bhat *et al.*, 2001; Kitazaki *et al.*, 1996; Todoulou *et al.*, 1994; Xiang-Shu *et al.*, 2009). A number of metal complexes with 1,2,4-triazole ligands have been synthesized and studied for their applicability in diverse fields (Heleen *et al.*, 1991; Joost *et al.*, 1992). Such a vide spectrum of biological applications of 1,2,4-triazoles prompted us to synthesize Schiff bases, derived from triazoles and stitch them with various metal ions. Very recently, a number of metal complexes of 1,2,4-triazole Schiff bases, which

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possess very good antimicrobial properties have been reported from this laboratory (Ajaykumar *et al.,* 2009a, b; Gangadhar *et al.,* 2008; Gangadhar *et al.,* 2009a, b).

In continuation of our earlier study, we report here the synthesis, antimicrobial properties, and DNA cleavage studies of Co(II), Ni(II), and Cu(II) metal complexes with Schiff bases derived from 3-substituted-4-amino-5-mercapto-1,2,4-triazole and fluvastatin which is an indole derivative. The structural features of Schiff bases and their metal complexes have been elucidated by various spectral and analytical techniques.

Materials and methods

Materials

All the chemicals used were of reagent grade. 3-substituted-4-amino-5-mercapto-1,2,4-triazole was synthesized according to the literature (Dhaka *et al.,* 1974; Escobar-Valderrama *et al.,* 1989; Palmer and Dines, 2004; Ajaykumar *et al.,* 2009a, b). Fluvastatin was recrystallized before use.

*Synthesis of Schiff bases (**I–II**)*

The synthesis of Schiff bases is schematically presented in Scheme 1. The Schiff bases have been synthesized by refluxing the reaction mixture of hot ethanolic solution (30 ml) of 3-substituted-4-amino-5-mercapto-1,2,4-triazole (0.01 mol) and hot ethanolic solution (30 ml) of fluvastatin (0.01 mol) for 4-5 h with addition of 4–5 drops of conc. hydrochloric acid. The product obtained after the evaporation of the solvent was filtered, washed with cold ethanol and recrystallized from hot ethanol. Yield (M.P.): 74% (189°C) and 76% (196°C) of **SB I** and **SB II**, respectively.

Schiff base **I**: ^{1}H NMR (d_6-DMSO): 10.4 (s, 1H, SH), 10.01 (s, 1H, CH=N), 7.1–7.7 (m, 8H, Ar–H), 7.3 (s, 1H, triazole-H), 6.47–6.55 (d, 2H, –CH=CH–), 6.47–6.56 (S, 6H, isopropyl group). IR (KBr) cm^{-1}: 1616 (C=N), 2752 (SH), 1105 (C=S). FAB MS: *m/z* 405. Anal.: Obsd.(Calc.) C, 65.11 (65.18); H, 4.81 (4.93); N, 17.19 (17.28).

Schiff base **II**: ^{1}H NMR (d_6-DMSO): 10.6 (s, 1H, SH), 10.04 (s, 1H, CH=N), 7.1–7.6 (m, 8H, Ar–H), 6.47–6.56 (d, 2H, –CH=CH–), 2.38 (s, 1H, –CH_3), 6.47–6.56 (S, 6H, isopropyl group). IR (KBr) cm^{-1}: 1619 (C=N), 2753 (SH), 1102 (C=S). FAB MS: *m/z* 419. Anal.: Obsd.(Calc.) C, 65.94 (65.87); H, 5.19 (5.25); N, 16.66 (16.71).

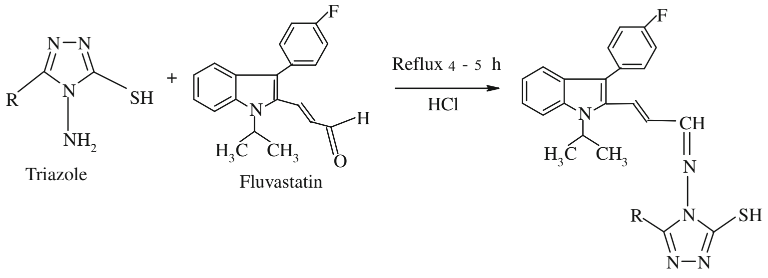
*Synthesis of Co(II), Ni(II), and Cu(II) complexes [**1–6**]*

Ethanolic solution (40 ml) of Schiff bases (2 mmol) was mixed with ethanolic solution (10 ml) of $CoCl_2{\cdot}6H_2O$/$NiCl_2{\cdot}6H_2O$/$CuCl_2{\cdot}2H_2O$ (1 mmol) and refluxed on water bath for 2 h. Then, to the reaction mixture, sodium acetate (1 mmol) was added and reflux was continued for further 2 h. The separated complex was filtered, washed thoroughly with water and ethanol, and dried in vacuum over fused $CaCl_2$. Yield of all the metal complexes lie in the range of 66–76%.

Characterization

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 4000–400 cm^{-1} region in KBr disc. The electronic spectra of the complexes were recorded in HPLC grade DMSO on a VARIAN CARY 50-BIO UV-spectrophotometer in the region of 200–1100 nm. The ^{1}H-NMR spectra of the Schiff bases 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, 10A) as the FAB gass with accelerating voltage set as 10 kV, the spectra were recorded at room temperature, and m-nitrobenzyl alcohol was used as the matrix. The mass spectrometer, was operated in the positive ion mode. The

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



R	Schiff base No.	Formula
H	**SB I**	$C_{22}H_{20}FN_5S$
CH_3	**SB II**	$C_{23}H_{22}FN_5S$

ESR spectrum was recorded under liquid nitrogen temperature (LNT) on Varian-E-4X-band EPR spectrometer, and the field set is 3000 G at modulation frequency of 100 K Hz using TCNE as ‘g’ marker. Thermogravimetric analyses data were measured from room temperature to 1000°C at a heating rate of 10°C/min. The data were obtained by using a PERKIN–ELMER DIAMOND TG/DTA instrument. Molar conductivity measurements were recorded on ELICO-CM-82 T Conductivity Bridge with a cell having cell constant 0.51, and magnetic moment measurement was carried out using Faraday balance.

DNA cleavage experiment

Preparation of culture media

Potato dextrose broth [potato, 250; dextrose, 20; in (g/l)] was used for the culture of *A. niger*. The 50-ml media was prepared, autoclaved for 15 min at 121°C under 15-lb pressure. The autoclaved media of *A. niger* was inoculated with the seed culture and incubated for 48 h. at 37°C.

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 mixture, 0.5 ml of saturated phenol was added, incubated at 55°C for 10 min, and then 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. Then, the solution was centrifuged at 10,000 rpm for 10 min, and to the supernatant, three volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation, and the pellet was dried and dissolved in TAE buffer (10 mM tris pH 8.0, 1 mM EDTA), and stored in cold condition.

Agarose gel electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method. Test samples (1 mg/ml) were prepared in DMF. The samples (25 μg) were added to the isolated DNA of *A. niger*. The samples were incubated for 2 h at 37°C, and then, 20 μl of DNA sample (mixed with bromophenol blue dye @ 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 l) and finally loaded on agarose gel. Then, a constant potential of 50 V for around 30 min was applied. The gel was removed and stained with 10.0 μg/ml ethidium bromide for 10–15 min. The bands were observed under Vilberlourmate Gel documentation system and photographed to determine the extent of DNA cleavage. Then, the results are compared with standard DNA marker.

In vitro antibacterial and antifungal studies

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 methods. The antibacterial and antifungal activities were done at 100, 200, and 500 μg/ml concentrations in DMF solvent using four bacteria (*E. coli*, *S. aureus*, *P. aeruginosa*, and *B. subtilis*) and two fungi (*A. niger* and *P. chrysogenum*) strains by the minimum inhibitory concentration (MIC) method (Sadana *et al.,* 2003). These bacterial strains were incubated for 24 h at 37°C, and fungi strains were incubated for 48 h at 37°C. Standard antibacterial (*Streptomycin*) and antifungal drug (*Nyastatin*) were used for comparison under similar conditions. Activity was determined by measuring the diameter of the zone of inhibition (mm).

Results and discussion

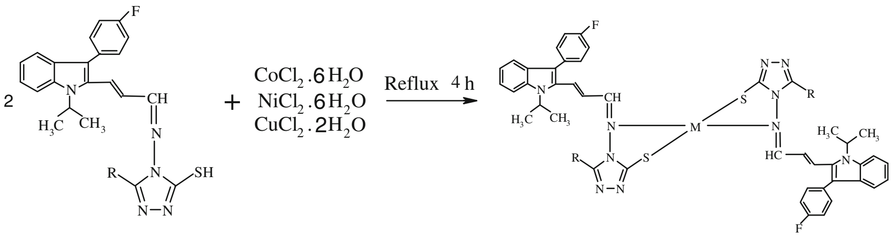
The Schiff bases (**I** and **II**) form square–planar complexes (**1–6**) with $CoCl_2 \cdot 6H_2O/NiCl_2 \cdot 6H_2O/CuCl_2 \cdot 2H_2O$ in ethanol (Scheme 2). All the Co(II), Ni(II), and Cu(II) complexes are stable and non-hygroscopic in nature. The complexes are insoluble in common organic solvents 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 singly deprotonated ligand, which exhibits thiol–thione tautomerism (Fig. 1). 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). Several attempts were made to develop the single crystal of the complexes. However, those attempts failed due to insolubility of the complexes in common organic solvents.

IR spectral studies

The IR spectral data results provide strong evidences for the complexation of Schiff bases with metal (II) ions (Table 2).

The IR spectra of the Schiff bases exhibited a characteristic high intensity band in the region of 1619–1616 cm^{-1}, assigned to v(HC=N). In comparison with the spectra of the Schiff bases, all the Co(II), Ni(II) and Cu(II) complexes exhibited the band of v(HC=N) in the region of

Scheme 2 Synthesis of metal complexes



R	M	Formula	Complex No.
H	Co(II)	$Co(C_{22}H_{19}FN_5S)_2$	**1**
CH_3	Co(II)	$Co(C_{23}H_{21}FN_5S)_2$	**2**
H	Ni(II)	$Ni(C_{22}H_{19}FN_5S)_2$	**3**
CH_3	Ni(II)	$Ni(C_{23}H_{21}FN_5S)_2$	**4**
H	Cu(II)	$Cu(C_{22}H_{19}FN_5S)_2$	**5**
CH_3	Cu(II)	$Cu(C_{23}H_{21}FN_5S)_2$	**6**

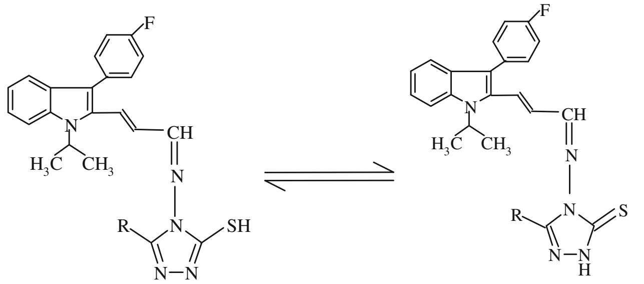


Fig. 1 Structure of Schiff bases I and II: Thiol–thione tautomerism

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

Compound number	Empirical formula	M%		C%		H%		N%		Molar conductance $Ohm^{-1}\ cm^2\ mol^{-1}$	μ_{eff} (BM)
		Obsd.	Calcd.	Obsd.	Calcd.	Obsd.	Calcd.	Obsd.	Calcd.		
SB-I	$C_{22}H_{20}FN_5S$	–	–	65.11	65.18	4.81	4.93	17.19	17.28	–	–
SB-II	$C_{23}H_{22}FN_5S$	–	–	65.94	65.87	5.19	5.25	16.66	16.71	–	–
1	$Co(C_{22}H_{19}FN_5S)_2$	6.791	6.805	60.96	60.89	4.36	4.38	16.14	16.15	8.2	3.57
2	$Co(C_{23}H_{21}FN_5S)_2$	6.610	6.592	61.58	61.67	4.72	4.69	15.72	15.64	6.9	3.58
3	$Ni(C_{22}H_{19}FN_5S)_2$	6.692	6.697	60.88	60.97	4.31	4.39	16.08	16.16	10.1	D
4	$Ni(C_{23}H_{21}FN_5S)_2$	6.442	6.487	61.72	61.74	4.59	4.69	15.71	15.66	9.5	D
5	$Cu(C_{22}H_{19}FN_5S)_2$	7.197	7.233	60.58	60.61	4.28	4.36	16.13	16.07	11.6	1.82
6	$Cu(C_{23}H_{21}FN_5S)_2$	7.102	7.007	61.57	61.40	4.65	4.67	15.52	15.57	11.2	1.83

1608–1607 cm^{-1} showing the shift of band to lower wave numbers indicating that the azomethine nitrogen atom is coordinated to the metal ion (Azza Abu-Hussen and Adel Emara, 2004). A characteristic strong band in the region of 2753–2752 cm^{-1} and another band around 1100 cm^{-1} in the spectra of the Schiff bases are ascribed to ν(SH) of triazole and to ν(C=S) (Sen *et al.,* 1998), respectively. These observations suggest that, the Schiff bases exhibit thiol–thione tautomerism. The deprotonation of the thiol group is indicated by the absence of a band around 2753 cm^{-1} in all the metal complexes indicating that the metal is coordinated through sulphur atom. This is further supported by the band around 747–742 cm^{-1} in the metal complexes due to ν(C–S). The new bands in the region of 470–462 and 419–406 cm^{-1} in the IR-spectra of the complexes are assigned to stretching frequencies of (M–N) and metal-sulphur bond formation, respectively.

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

In the ^{1}H-NMR spectrum of the Schiff base and **II**, the thiol group exhibited proton signal at 10.6 ppm (s, 1H, SH). A

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

Compound number	ν(HC=N)	ν(SH)	ν(C=S)	C–S	M–N	M–S
$C_{22}H_{20}FN_5S$	1616	2752	1105	–	–	–
$C_{23}H_{22}FN_5S$	1619	2753	1102	–	–	–
$Co(C_{22}H_{19}FN_5S)_2$	1608	–	–	744	466	406
$Co(C_{23}H_{21}FN_5S)_2$	1608	–	–	743	466	408
$Ni(C_{22}H_{19}FN_5S)_2$	1607	–	–	747	470	416
$Ni(C_{23}H_{21}FN_5S)_2$	1608	–	–	744	468	411
$Cu(C_{22}H_{19}FN_5S)_2$	1608	–	–	742	462	419
$Cu(C_{23}H_{21}FN_5S)_2$	1608	–	–	743	466	416

characteristic proton signal at 10.04 ppm (s, 1H, –CH=N) is assigned to aldimine proton. A sharp signal at 2.38 ppm (s, 3H, CH_3) and signals in the region 7.1–7.6 ppm (m, 8H, Ar–H) are due to methyl protons of triazole moiety and aromatic protons, respectively. In addition to this, the isopropyl attached to indole ring exhibited signals in the region 1.65–1.78 ppm and the resonance observed at 6.47–6.56 ppm (m, 2H) are ascribed to –CH=CH– group, respectively. In the case of Schiff base **I**, all the signals due to various differently positioned protons were observed in accordance with Schiff base **II**) The resonance due to proton of triazole ring is observed in the aromatic region and a signal due to CH_3 is absent in the proton NMR spectrum of Schiff base **I**. Thus, the NMR results support the formation of Schiff bases in line with IR inferences.

Electronic spectral and magnetic studies

The electronic spectra of Co(II) complexes exhibited absorption bands in the region of 16365–16380 cm^{-1} corresponding to $^1A_{1g} \rightarrow {}^1B_{1g}$ which indicate the square planar geometry for the Co(II) complexes. The magnetic moment values of the Co(II) complexes (**1** and **2**) are 3.57 and 3.58 BM, respectively, and it supports the square planar geometry for the present Co(II) complexes (Duta and Syamal, 1992). The Ni(II) complexes exhibited two bands in the region 18800–18810 and 21635–31640 cm^{-1} attributed to the $^1A_{1g} \rightarrow {}^1B_{1g}$ and $^1A_{1g} \rightarrow {}^1B_{2g}$ transitions, respectively, which indicate square planar geometry around Ni(II) ion. This has been supported by the diamagnetic nature of the complexes **3** and **4**. The Electronic spectra of Cu(II) complexes display a broad band around 17475–17505 cm^{-1} assignable to $^2B_{1g} \rightarrow {}^2A_{1g}$ transitions (Kumar *et al.,* 1999). In addition to this, two weak shoulders appearing in the region 20765–20875 and 11350–11555 cm^{-1} may be ascribed to $^2B_{1g} \rightarrow {}^2E_g$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ transitions, respectively. These assignments suggests the square planar geometry for the Cu(II) complexes under investigation. The magnetic moments of 1.82 and 1.83 BM for the Cu(II) complexes (**5** and **6**), respectively further supports the square planar geometry for these complexes.

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

The FAB-mass spectrum of representative Schiff base-**II**, and its metal complexes are discussed here. The spectrum of Schiff base-**II** showed a molecular ion peak M^+ at *m/z* 419, equivalent to its molecular weight. In addition to this, the fragmentation peaks observed at *m/z* 305, 248, and 183 are due to the cleavage of $C_3H_4N_3S$, C_3H_7N, and C_4H_3N, respectively. The results confirm the formation of Schiff base-**II** supporting the IR and NMR results. The FAB-mass spectra of Co(II) (**2**), Ni(II) (**4**)) and Cu(II) (**6**) complexes of Schiff base-**II** showed a molecular ion peak M^+ at *m/z* 895, 894, and 899, respectively, equivalent their molecular weight which supports the formation of respective metal complexes of the type ML_2. All the fragments of the species $[M2HL_2]^+$, which underwent demetallation to form $[L + H]^+$ gave a fragment ion peak at *m/z* 418.

ESR studies of $Cu(C_{23}H_{21}FN_5S)_2$ complex

ESR spectrum of Cu(II) complex has been studied under liquid nitrogen temperature using TCNE as a 'g' marker. The four coordinate Cu(II) complexes at LNT show well-resolved four copper hyperfine lines, characteristic of monomeric Cu(II) complexes and superhyperfine lines due to azomethine nitrogen. The 'g' tensor values of Cu(II) complex can be used to derive the ground state. In square planar complexes, the unpaired electron lies in the $d_{x^2-y^2}$ orbital giving $^2B_{1g}$ as the ground state with $g_{\|} > g_{\perp}$. For the present Cu(II) complex, $g_{\|}$ and $g_{\perp}$ values have been found to be 2.5958 and 2.3777, respectively, and G value is 1.5774. Hence, it is clear from the results that, the $g_{\|} > g_{\perp} > 2$ and $G < 4$ which is consistent with $d_{x^2-y^2}$ ground state (Ray and Kauffman, 1990; Anthonisamy and Murugesan 1998) suggesting square planar geometry for the Cu(II) complex. Thus, the ESR spectral results provide further evidence to the magnetic and electronic spectral results.

Thermal decomposition study of metal complexes

The thermal behavior of Co(II), Ni(II), and Cu(II) complexes has been studied as a function of temperature. The thermal behavior of all the complexes is almost same. Hence, only the representative Co(II) (**2**), Ni(II) (**4**), and Cu(II) (**6**) complexes have been discussed here. The thermal analyses (TGA and DTA) of $Co(C_{23}H_{21}FN_5S)_2$ complex showed that the two fluvastatin and triazole moieties of Schiff base were lost in the regions of 215–325 centered at 272°C and 400–475 centered at 451°C corresponding to the mass losses of 64.92% (calc. 65.02%) and 28.26% (calc. 28.37%), respectively (Fig. 2). Finally, the formation of metal oxide took place at above 500°C. The $Ni(C_{23}H_{21}FN_5S)_2$ complex loses its fluvastatin and triazole moieties in the regions of 205–320 centered at 274°C and 355–405 centered at 379°C corresponding to mass losses of 64.98% (calc. 65.10%) and 28.34% (calc. 28.41%), respectively. The formation of metal oxide took place at 425°C. In the case of $Cu(C_{23}H_{21}FN_5S)_2$ complex, the loss of fluvastatin and triazole moieties were found to be in the regions of 205–285 centered at 251°C and 300–370 centered at 327°C corresponding to the mass losses of 64.59% (calc. 64.73%) and 28.12% (calc. 28.25%), respectively. Finally, the metal oxide formed at 400°C. Thus, the TGA and DTA analyses of this study support the ML_2 ratio of the metal complexes in the absence of coordination of water molecule.

Biological results

DNA cleavage studies

The representative Co(II) **2**, Ni(II) **4**, and Cu(II) **6** complexes were studied for their DNA cleavage activity by agarose gel electrophoresis method.

Figure 3 shows the results of oxidative DNA cleavage experiments carried out with the complexes of Co(II), Ni(II), and Cu(II) by agarose gel electrophoresis method. Control experiments clearly revealed that the untreated DNA does not show any cleavage (Fig. 3; Lane-C) whereas, all the metal complexes have exhibited cleavage activity on DNA. The difference in the migration was observed in the Lanes 1, 2, and 3 of Co(II), Ni(II), and Cu(II) complexes, respectively, compared to the control DNA of *A. niger* (Lane-C). This shows that the control DNA alone does not show any apparent cleavage whereas Co(II), Ni(II), and Cu(II) complexes have shown the same. 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 metal ions in the isolated DNA cleavage reaction. From these results, we infer that the Co(II), Ni(II), and Cu(II) complexes act as a potent nuclease agents. 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.

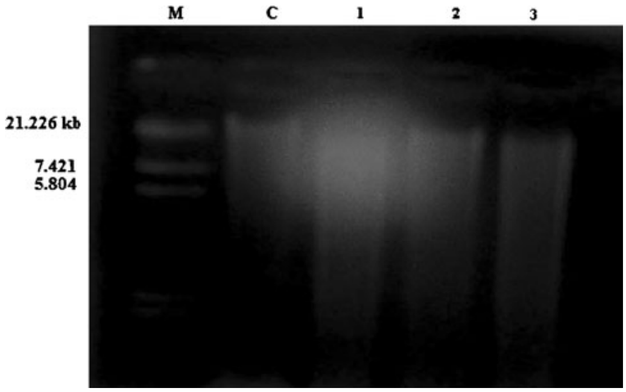
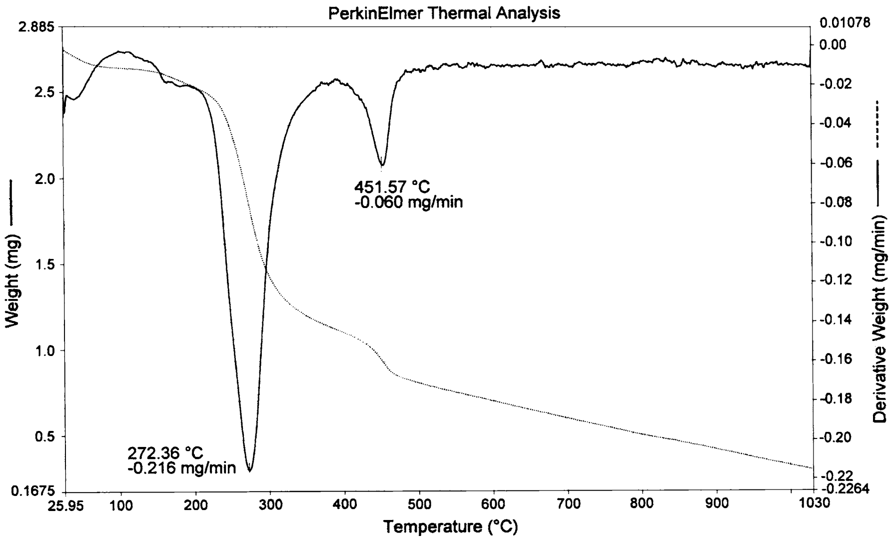


Fig. 3 DNA cleavage studies of Co(II) (**2**), Ni(II) (**4**), and Cu(II) (**6**) complexes. M: Standard Molecular weight Marker; C—Control DNA of *A. niger*; *Lane 1*: *A. niger* DNA treated with Co(II); *Lane 2*: *A. niger* DNA treated with Ni(II); *Lane 3*: *A. niger* DNA treated with Cu(II)

In vitro antibacterial and antifungal activity

The microbial results are systematized in Table 3. The antibacterial and antifungal studies suggested that the

Fig. 2 TGA/DTA spectra of Co(II) (**2**) complex



Schiff bases were found to be biologically active, and some of their metal complexes showed significantly enhanced antibacterial and antifungal activities. It is, however, known (Chohan *et al.*, 2004; Chohan and Praveen, 2001) that the chelating tends to make the Schiff bases act as more powerful and potent bactereostatic agents, thus inhibiting the growth of bacteria and fungi more than the parent Schiff bases. It is assumed that factors, such as solubility, conductivity, dipole moment, and cell permeability mechanism (influenced by the presence of metal ions) may contribute to the increase in the activities of the metal complexes relative to Schiff bases.

In the case of *antibacterial* studies, it is observed that both the Schiff bases were found to be active against *S. aureus* and *B. subtitlis*. All the Co(II), Ni(II), and Cu(II) metal complexes exhibited much enhanced activity against *S. aureus*, *B. subtitlis*, and *P. aerugenosa*. The activities of metal complexes are in the order of Co(II) > Ni(II) > Cu(II) and that against bacterial species are in the order of *S. aureus* > *P. auregenosa* > *B. subtitles*.

From the *antifungal* studies, it is clear that the Co(II), Ni(II), and Cu(II) complexes are found to possess higher antifungal activities than the respective Schiff bases. The activities of metal complexes are in the order of Co(II) > Ni(II) > Cu(II), and that against the fungi species are in the order of *A. niger* > *P. crysogenum*.

The minimum inhibitory concentration (MIC) of some selected compounds, which showed significant activity against selected bacterial and fungi species, was determined, and the MIC values of these compounds indicated that these compounds are the most active in inhibiting the growth of the tested organisms at a 10 µg/ml concentration (Table 4). It has been reported in past that the metal complexes with Schiff bases possess a high antimicrobial activity (Ajaykumar *et al.*, 2009a, b; Gangadhar *et al.*, 2008; Rehman *et al.*, 2005; Gangadhar *et al.*, 2009a, b).

Table 3 Antimicrobial results of metal complexes

Compound	Conc. (µg ml^{-1})	Growth inhibition against bacteria in mm				Growth inhibition against fungi in mm	
		S. aureus	*B. subtilis*	*P. auregenosa*	*E. coli*	*A. Niger*	*P. Crysogenum*
$C_{22}H_{20}FN_5S$	100	8	8	7	7	10	8
	200	10	10	10	8	10	8
	500	13	12	12	12	13	12
$C_{23}H_{22}FN_5S$	100	12	8	8	8	12	8
	200	12	10	9	10	12	8
	500	14	14	12	12	14	12
$Co(C_{22}H_{19}FN_5S)_2$	100	14	13	10	8	14	14
	200	14	14	10	11	14	14
	500	17	16	14	13	18	17
$Co(C_{23}H_{21}FN_5S)_2$	100	14	10	14	10	14	14
	200	16	11	14	10	16	15
	500	18	12	16	12	20	18
$Ni(C_{22}H_{19}FN_5S)_2$	100	12	10	7	7	13	12
	200	12	12	12	7	13	12
	500	15	14	16	13	15	15
$Ni(C_{23}H_{21}FN_5S)_2$	100	10	10	10	7	14	13
	200	14	11	13	7	16	13
	500	18	14	16	12	20	16
$Cu(C_{22}H_{19}FN_5S)_2$	100	8	7	8	6	11	11
	200	12	12	13	10	11	11
	500	16	15	16	13	15	14
$Cu(C_{23}H_{21}FN_5S)_2$	100	6	7	7	6	14	13
	200	12	10	8	9	14	13
	500	16	14	15	13	17	15
Streptomycin	500	28	29	35	25	–	–
Nyastatin	500	–	–	–	–	26	25

Less than 12 mm—inactive; 12–16 mm—moderately active; above 16 mm—highly active

Table 4 Results of minimum inhibitory concentration (μg/ml)

Compound	*S. aureus*	*B. subtilis*	*P. auregenosa*	*E. coli*	*A. Niger*	*P. Crysogenum*
$Co(C_{22}H_{19}FN_5S)_2$	10	15	15	20	10	15
$Co(C_{23}H_{21}FN_5S)_2$	10	20	10	25	10	10
$Ni(C_{22}H_{19}FN5S)_2$	15	15	10	20	15	15
$Ni(C_{23}H_{21}FN_5S)_2$	10	15	10	20	10	10
$Cu(C_{22}H_{19}FN_5S)_2$	15	20	15	25	15	15
$Cu(C_{23}H_{21}FN_5S)_2$	15	20	15	25	10	10

Conclusion

On the basis of spectroscopic results, the square planar geometry is proposed for all the newly synthesized complexes. All these complexes cleaved the DNA of *A. niger*. The results show that the metal complexes possess higher antibacterial and antifungal activities than the Schiff bases. Thus, the biological studies revealed the important role of metal ions in the biological systems in line with other studies.

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