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Synthesis, characterization, DNA cleavage and in vitro antimicrobial activities of copper(II) complexes of Schiff bases containing a 2,4-disubstituted thiazole

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Abstract Six Cu(II) complexes of Schiff base ligands of arylidene-2-(4-(4-bromo/methoxy-phenyl)thiazol-2-yl) hydrazines have been synthesized, characterized and screened for DNA cleavage and antimicrobial activities. The chemical structures of the complexes were deduced by physicochemical and spectroscopic methods. Elemental analyses indicated that the stoichiometry of the complexes is CuL_2 (L = Schiff base ligand). The DNA cleavage activities of the complexes were evaluated by agarose gel electrophoresis in the presence and absence of oxidant (H_2O_2) and free radical scavenger (DMSO). All the six complexes showed significant nuclease activity in the presence of H_2O_2, and two of the complexes showed moderate nuclease activity even in the absence of oxidant. The complexes did not show nuclease activity in the presence of free radical scavenger. The compounds were tested for activity against selected bacteria and fungi.

Introduction

Recent clinical reports have highlighted the increasing occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and other antibiotic-resistant human pathogenic microorganisms [1]. Metal complexes of some antibiotics have shown improved results against these microorganisms [2, 3]. Complexes of the quinoline group antibiotics, namely, ciprofloxacin, norfloxacin and tetracycline have been found to possess enhanced activity compared to the antibiotic alone. Thus, a Pd(II) complex of tetracycline has sixteen times more potency than the parent compound against *E.coli HB101/pBR322*, a bacterial strain resistant to tetracycline, while a Pd(II) complex of doxycycline is two times more potent than doxycycline against resistant strains [4]. Schiff bases and their transition metal complexes are well known as antibacterial, antifungal, anticancer and antiviral agents, etc. [5, 6]. The significant antimicrobial activity of Schiff bases containing a 2,4-disubstituted thiazole ring reported by us earlier [7] prompted us to synthesize their copper complexes as possible antimicrobial agents.

Transition metal complexes with Schiff base ligands have shown promising nucleolytic activity. The interaction of transition metal complexes with DNA has been studied extensively for their use as probes for DNA structure and their potential applications in chemotherapy [8]. Anticancer drugs can bind to DNA via covalent and/or non-covalent interaction(s), causing DNA damage leading to the inhibition of uncontrolled growth of cancerous cells. Bis(1,10-phenanthroline)copper(I) was the first transition metal complex shown to possess DNA cleavage activity [9], followed by derivatives of ferrous-EDTA [10, 11], various metalloporphyrins [12], *cis*-diamino dichloro platinum complexes [13] and ruthenium complexes of 4,7-diphenyl-1,10-phenanthroline [14]. Recently, some Cu(II) complexes have been reported to be active in DNA strand scission [15]. Keeping the above facts in mind, the DNA cleavage activity of the present Cu(II) complexes has been studied by gel electrophoresis.

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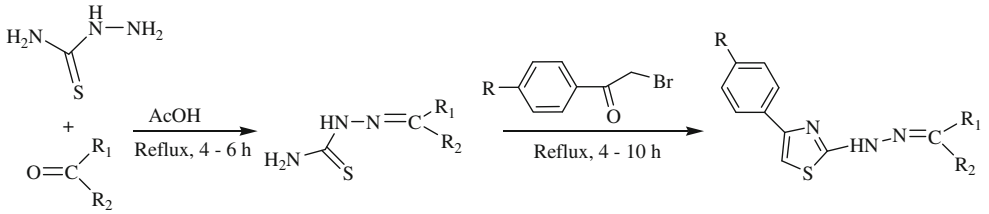
Results and discussion

The synthesis of Schiff base ligands containing a 2,4-disubstituted thiazole ring, HL^1–HL^6, is outlined in Scheme 1. The corresponding copper complexes $[Cu(L)_2]$ were obtained in three steps. In the first step, the Schiff base thiosemicarbazone was synthesized by condensing equimolar quantities of the substituted aryl aldehyde/ketone with thiosemicarbazide in methanol or ethanol in the presence of a few drops of glacial acetic acid as catalyst. In the second step, equimolar quantities of the thiosemicarbazone and substituted phenacyl bromide (4-bromo/-methoxy phenacyl bromide) were refluxed to obtain Schiff bases HL^1–HL^6, containing a 2,4-disubstituted thiazole. This reaction proceeds via the cyclization of thiosemicarbazone to the corresponding 2,4-disubstituted thiazole [7]. The thiazoles HL^1–HL^6 were then refluxed with copper(II) acetate in 2:1 M ratio, in accordance with the reported methods for similar compounds [16, 17], to obtain the desired complexes in 68–85% yields. The products were characterized by TLC, UV, FTIR, ^{1}H NMR, ^{13}C NMR, Mass spectrometry, electronic, magnetic, thermogravimetric (TG/DTA), ESR and elemental analyses. The observed molar conductances of the complexes in DMSO for 10^{-3} M solutions at room temperature indicate their non-electrolytic nature [18].

The tentative assignments of the IR bands useful for determining the ligand's mode of coordination are listed in Table 1. The FTIR spectra of the thiosemicarbazones showed bands in the range of 3,120–3,330 cm^{-1} for ν(NH) and $\nu(NH_2)$, and 1,590–1,670 cm^{-1} for the azomethine group (HC=N). The absence of a band in the range of 1,700–1,750 cm^{-1} confirms the conversion of the aldehyde/ketone group to azomethine in the product. Absorption bands in the range of 3,124–3,282 cm^{-1} for NH, and 1,604–1,663 cm^{-1} for the azomethine group were observed for the Schiff bases containing 2,4-disubstituted thiazoles. The stretching frequencies of the azomethine group ν(HC=N) in the free ligands was shifted by 14–63 cm^{-1}, being present in the range of 1,562–1,655 cm^{-1} in the complexes, which suggests participation of the azomethine nitrogen in coordination [19]. The broad peaks of the ortho phenolic OH for HL^3, HL^4, HL^5 and HL^6 were present in the region of 3,403–3,425 cm^{-1} in the free ligands, but absent from the spectra of the complexes, indicating coordination between the phenolic oxygen and copper. The phenolic ν(C–O) stretching vibration that was present in the region of 1,227–1,335 cm^{-1} for the free Schiff base



Scheme 1 Synthetic route to the Schiff base ligands. HL^1, R=Br, R_1=Ph, R_2=–$CH_2CH(OH)Ph$; HL^2, R=OMe, R_1=Ph, R_2=–$CH_2CH(OH)Ph$; HL^3, R=Br, R_1=H, R_2=–C_6H_3–2,4–$(OH)_2$; HL^4, R=OMe, R_1=H, R_2=–C_6H_3–2,4–$(OH)_2$; HL^5, R=Br, R_1=H, R_2=–C_6H_4OH; HL^6, R=OMe, R_1=H, R_2=–C_6H_4OH

Table 1 Infrared spectroscopic assignment (KBr, ν_{max} cm^{-1}) for HL^1–HL^6 and $[Cu(L^1)_2]$–$[Cu(L^6)_2]$

Compound	ν(O–H)	ν(N–H)	ν(C=N) azomethine	ν(C=N) thiazole	ν(C–O)	ν(Cu–N)	ν(Cu–O)
HL^1	3,340	3,171	1,663	1,521	1,241	–	–
HL^2	3,423	3,124	1,622	1,508	1,354	–	–
HL^3	3,423	3,282	1,604	1,545	1,227	–	–
HL^4	3,425	3,170	1,635	1,446	1,243	–	–
HL^5	3,403	3,153	1,604	1,511	1,240	–	–
HL^6	3,406	3,167	1,635	1,521	1,335	–	–
$[Cu(L^1)_2]$	–	3,169	1,633	1,520	1,126	680	490
$[Cu(L^2)_2]$	–	3,122	1,608	1,508	1,255	700	596
$[Cu(L^3)_2]$	–	3,280	1,655	1,546	1,245	671	470
$[Cu(L^4)_2]$	–	3,169	1,572	1,452	1,259	623	521
$[Cu(L^5)_2]$	–	3,150	1,562	1,508	1,257	684	528
$[Cu(L^6)_2]$	–	3,168	1,575	1,521	1,241	639	493

Table 2 The ^{1}H NMR spectral data[a] (δ, ppm)[b] of selected free ligands and their copper(II) complexes

Compound [empirical formula]	OH[c]	NH	HC=N (azomethine)	Aromatic protons
HL^1 [$C_{23}H_{18}BrN_3OS$]	12.1	7.7 (s, 1H)	–	6.9–7.7 (m, 14H)
HL^3 [$C_{16}H_{12}BrN_3O_2S$]	12.1 (s, 1H)	8.2 (s, 1H)	9.9 (s, 1H)	7.2–8.0 (m, 7H)
HL^5 [$C_{16}H_{12}BrN_3OS$]	9.7 (s, 1H)	8.0 (s, 1H)	7.2 (s, 1H)	7.3–7.6 (m, 8H)
[$Cu(L^1)_2$][$CuC_{46}H_{34}Br_2N_6O_2S_2$]	–	7.7 (d, 2H)	–	6.7–7.2 (m, 28H)
[$Cu(L^3)_2$][$CuC_{32}H_{22}Br_2N_6O_4S_2$]	–	8.3 (s, 2H)	11.5 (s, 2H)	6.9–7.7 (m, 14H)
[$Cu(L^5)_2$][$CuC_{32}H_{22}Br_2N_6O_2S_2$]	–	8.0 (s, 2H)	7.7 (s, 2H)	6.4–7.3 (m, 16H)

[a] Solvent DMSO-d_6/$CDCl_3$

[b] Relative to TMS

[c] At *ortho* position

Table 3 Magnetic moments and electronic spectra of HL^1–HL^6 and [$Cu(L^1)_2$]–[$Cu(L^6)_2$]

Compound [empirical formula]	Magnetic moment, μ_{eff} (B.M.)[a] at 300 K	λ_{max} (nm)	Assignments
HL^1 [$C_{23}H_{18}BrN_3OS$]	–	240, 335	π–π^*, n–π^*
HL^2 [$C_{24}H_{21}N_3O_2S$]	–	260, 340	π–π^*, n–π^*
HL^3 [$C_{16}H_{12}BrN_3O_2S$]	–	296, 320	π–π^*, n–π^*
HL^4 [$C_{17}H_{15}N_3O_3S$]	–	257, 340, 392	π–π^*, n–π^*
HL^5 [$C_{16}H_{12}BrN_3OS$]	–	235, 262, 310	π–π^*, n–π^*
HL^6 [$C_{17}H_{15}N_3O_2S$]	–	230, 265, 340	π–π^*, n–π^*
[$Cu(L^1)_2$][$CuC_{46}H_{34}Br_2N_6O_2S_2$]	1.54	260, 275, 350, 500	π–π^*, n–π^*, L–M, d–d
[$Cu(L^2)_2$] [$CuC_{48}H_{40}N_6O_4S_2$]	1.79	244, 252, 295, 457	π–π^*, n–π^*, L–M, d–d
[$Cu(L^3)_2$][$CuC_{32}H_{22}Br_2N_6O_4S_2$]	1.74	252, 392,457, 510	π–π^*, n–π^*, L–M, d–d
[$Cu(L^4)_2$] [$CuC_{34}H_{28}N_6O_6S_2$]	1.88	260, 340, 490	π–π^*, n–π^*, L–M, d–d
[$Cu(L^5)_2$][$CuC_{32}H_{22}Br_2N_6O_2S_2$]	1.70	283, 320, 410, 500	π–π^*, n–π^*, L–M, d–d
[$Cu(L^6)_2$] [$CuC_{34}H_{28}N_6O_4S_2$]	1.84	295, 457, 490	π–π^*, n–π^*, L–M, d–d

[a] *B.M.* Bohr magneton

ligands HL^3–HL^6 was shifted by 16–94 cm^{-1} in the spectra of the complexes. The band attributed to ν(NH) group remained almost unchanged in the complexes, suggesting non-coordination of the N atom of this group to the metal. In the far infrared region, bands in the region of 623–700 cm^{-1} were attributed to ν(M–N) and in the region of 470–596 cm^{-1} to ν(M–O) [20].

The ^{1}H NMR spectra were recorded in DMSO-d_6/$CDCl_3$, and spectral data of the free ligands and their copper(II) complexes are presented in Table 2. The aromatic protons were present as multiplets in the range of 6.9–8.0 ppm for the free Schiff base ligands, which were slightly shifted downfield for the complexes. This was assigned to a decrease in the local electron density in the complexes [21]. The azomethine proton of all the six Schiff base ligands was observed in the range of 7.2–9.9 ppm, while peaks at 7.7–8.2 ppm were observed for the NH group. A singlet in the range of 9.7–12.1 ppm was assigned to the ortho phenolic group proton. The Ph-OCH_3 protons of Schiff bases HL^2, HL^4 and HL^6 were present in the range of 3.6–3.9 ppm. The ^{1}H NMR spectra of the copper complexes exhibited peaks which were either shifted downfield or absent compared to the free ligands. Thus, the phenolic –OH peak was absent in the spectra of the complexes. The peak at 7.7–8.2 ppm due to NH remained at almost the same position, indicating the non-coordination of NH to the metal.

The ^{13}C NMR spectra were consistent with the ^{1}H NMR spectra. Changes in the chemical shifts for the azomethine carbon and carbon attached to the phenolic –OH were observed in the complexes. The aryl carbon and azomethine carbon of all the Schiff base ligands exhibited peaks in the region of 111–135 and 132–143 ppm, respectively. Peaks in the region of 100–108, 147–157 and 160–171 ppm were assigned to C_5, C_4 and C_2 of the thiazole ring, respectively. A peak at 55–56 ppm was observed for the methoxy carbon of the ligands HL^2, HL^4 and HL^6. In the complexes, the usual azomethine resonances were shifted ca. 5–14 ppm downfield, suggesting involvement of the nitrogen lone pair in coordination. The aryl carbons showed peaks in the

111–135 ppm region. The phenolic aryl carbon was present at 158–160 ppm, hence shifted ca. 3–7 ppm downfield, consistent with coordination of the phenolic oxygen.

The electronic spectra of the compounds were recorded in DMF and are presented in Table 3. The spectra of the free Schiff base ligands showed two main absorption bands in the region of 230–392 nm, assigned to π–π^* transitions of the aromatic ring and phenolic chromophore. This band was shifted in the spectra of the complexes. A band in the region of 310–392 nm was assigned to the n–π^* transition of the azomethine chromophore. The equivalent transitions of the complexes were shifted in comparison with the free ligands, due to coordination of the imine nitrogen atom to the metal [22]. A transition in the range of 410–457 nm for the complexes was assigned to ligand to metal charge transfer (LMCT), while a band at 490–510 nm was attributed to metal d–d transitions, which is compatible with the complexes having a planar or distorted tetrahedral structure [23].

The magnetic moments of the complexes were determined at room temperature and are presented in Table 3. All of these copper(II) complexes were paramagnetic, with magnetic moments in the range of 1.54–1.88 B.M., considerably lower than the value expected for the spin-only contribution in a d^9 system. This suggests a probable antiferromagnetic interaction between Cu(II) atoms and consequently a planar or distorted tetrahedral arrangement of the four donor atoms around the metal [24]. Thus, the electronic spectra and magnetic moments suggest a planar or distorted tetrahedral geometry for the complexes [25].

The ESR spectra of the complexes were recorded as polycrystalline samples at room temperature (RT), and the data are presented in Table 4. The spectra exhibited anisotropic signals with $g|| = 2.111 - 2.213, g\perp = 2.017 - 2.059$ and $g_{iso/av} = 2.065 - 2.110$ calculated from the formula $1/3[g|| + 2g^{\perp}]$, characteristic of axial symmetry [26]. Since the g|| and $g\perp$ values are close to 2 and $g|| > g\perp$, a planar geometry is suggested around Cu(II), corresponding to elongation along the four fold symmetry *Z*-axis [27]. The trend $g|| > g\perp > g_e$ (2.0023) shows that the unpaired electron is localized in the d_{x2-y2} orbital of the Cu(II) atom in these complexes [28]. In addition, exchange coupling interaction between two Cu(II) centres is explained by the Hathaway expression $G = (g|| - 2.0023)/(g\perp - 2.0023)$. The deviation of '*g*' values from the free electron value (2.0023) may be due to angular momentum contribution in the complexes.

According to Hathaway, if $G < 4.0$, a considerable exchange coupling is present in the solid complex [27]. In the present case, the *G* values of the complexes $[Cu(L^3)_2]$, $[Cu(L^5)_2]$ and $[Cu(L^6)_2]$ are in the range of 1.982–3.593, indicating considerable exchange interaction. If the $G > 4.0$, the exchange interaction is negligible which is the case for complexes $[Cu(L^1)_2]$, $[Cu(L^2)_2]$ and $[Cu(L^4)_2]$. Kivelson and Neiman showed that for an ionic environment g|| is normally 2.3 or larger, but for a covalent environment g|| is less than 2.3. In the present study, the g|| values for the complexes are in the range of 2.111–2.213; consequently, the environment is essentially covalent.

The mass spectra of the free ligands showed the expected peaks. For example, HL^1 showed the molecular ion peak at *m/z* 464 (M^+, 80), which matches with its molecular formula $C_{23}H_{18}BrN_3OS$. A peak at *m/z* 416 (M + 1, 100%) was observed for HL^2 which is in conformity with the molecular formula $C_{24}H_{21}N_3O_2S$. A peak at *m/z* 326 (M + 1, 100%) for HL^6 fits with the molecular formula $C_{17}H_{15}N_3O_2S$. Similarly, the complex $[Cu(L^1)_2]$ showed a molecular ion peak M^+ at *m/z* 990 which is equivalent to its molecular weight. Molecular ion peaks M^+ at *m/z* 892 for $[Cu(L^2)_2]$ and at *m/z* 841 for $[Cu(L^3)_2]$ were observed and are equivalent to their molecular weights. Complexes $[Cu(L^4)_2]$, $[Cu(L^5)_2]$ and $[Cu(L^6)_2]$ showed molecular ion peaks M^+ at *m/z* 744, 810, and 712, respectively. In addition, the spectra also showed fragment ion peaks including demetallated products of the copper complexes. For example, peaks at *m/z* 648 and 340 were assigned to removal of two methoxy and two hydroxyl groups [M-$C_2H_8O_4$] and demetallation, respectively, in the case of $[Cu(L^4)_2]$.

Thermal decomposition of the complexes was studied by TG/DTA. The complexes did not show any weight loss up to 200 °C, which reveals that both crystalline and coordinated water were absent from the complexes. The TG curves in the range of 210–330 °C suggested that the loss in weight

Table 4 ESR spectral data of the Cu(II) complexes as polycrystalline sample

Compound [empirical formula]	Temperature	g||	g⊥	$g^a_{iso/av}$	G^b
$[Cu(L^1)_2][CuC_{46}H_{34}Br_2N_6O_2S_2]$	RT	2.193	2.033	2.086	5.848
$[Cu(L^2)_2\ [CuC_{48}H_{40}N_6O_4S_2]$	RT	2.162	2.017	2.065	9.529
$[Cu(L^3)_2][CuC_{32}H_{22}Br_2N_6O_4S_2]$	RT	2.212	2.059	2.110	3.593
$[Cu(L^4)_2]\ [CuC_{34}H_{28}N_6O_6S_2]$	RT	2.213	2.046	2.101	4.630
$[Cu(L^5)_2][CuC_{32}H_{22}Br_2N_6O_2S_2]$	RT	2.204	2.056	2.105	3.642
$[Cu(L^6)_2]\ [CuC_{34}H_{28}N_6O_4S_2]$	RT	2.111	2.056	2.074	1.982

[a] $g_{iso/av} = 1/3[g|| + 2g\perp]$

[b] $G = (g|| - 2.0023)/(g\perp - 2.0023)$

corresponds to decomposition of the ligands. In all cases, the remaining residues were metal oxide (CuO).

Antibacterial and antifungal activities

Antibacterial activities of the complexes were evaluated against various Gram-negative and Gram-positive pathogenic bacterial strains, namely, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Vibrio cholerae*. Antifungal activities were evaluated against *Candida albicans, Cryptococcus neoformans*, *Aspergillus flavus* and *Chrysosporium tropicum*. The antibacterial and antifungal activities were evaluated by the agar disc diffusion method, and minimum inhibitory concentrations (MIC) were determined by serial dilution as per the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [29]. The solvent, DMSO, did not show any inhibition against the tested organisms.

The results of antibacterial screening of the free Schiff bases and their copper(II) complexes are presented in Table 5. The free ligands showed moderate or no antibacterial activity. The complex $[Cu(L^1)_2]$ showed significant antibacterial activity against both *S. aureus* and *V. cholerae*. $[Cu(L^2)_2]$ showed good antibacterial activity against *V. cholerae*, while $[Cu(L^5)_2]$ showed good antibacterial activity against *K. pneumoniae*. Thus, the complexes showed enhanced antibacterial activity compared to their free ligands. Ciprofloxacin (100 μg/ml) was used as standard.

The results of antifungal screening of all the Schiff base ligands and their complexes are presented in Table 5. The free ligands showed moderate or no antifungal activity. The complex $[Cu(L^1)_2]$ showed significant antifungal activity against all the four fungal strains tested. In contrast, no significant change in the antifungal activity of $[Cu(L^2)_2]$ was observed compared with its free ligand. Except for $[Cu(L^2)_2]$, the results showed enhanced antifungal activity of the copper(II) complexes compared to their free ligands. Fluconazole (100 μg/ml) was used as standard.

Nuclease activity

The DNA cleavage activities of the copper(II) complexes were evaluated by agarose gel electrophoresis. To investigate the probable mechanism of nuclease activity, experiments were also performed in the presence and absence of oxidant (H_2O_2) and free radical scavenger (DMSO). Cleavage in plasmid DNA was detected by UV illumination in the presence of ethidium bromide (EB), which is the most widely used intercalative agent and fluorescence probe for DNA structure [30]. When the supercoiled (SC) form of DNA is nicked, it gives rise to an open circular (OC) relaxed form and further cleavage gives

Table 5 Antibacterial and antifungal activities of the ligands HL^1–HL^6 and the complexes $[Cu(L^1)_2]$–$[Cu(L^6)_2]$

Compound	Microbial species									
	Bacteria						Fungi			
	E.coli	*S.aureus*	*S.typhi*	*P. aeruginosa*	*K. pneumoniae*	*V. cholerae*	*C. albicans*	*C. neoformans*	*A. flavus*	*C. tropicum*
HL^1	–	20 (12.5)	–	–	–	20 (12.5)	22 (6.25)	21 (6.25)	20 (6.25)	19 (6.25)
HL^2	–	–	–	–	–	19 (25)	–	–	16 (25)	–
HL^3	–	–	–	–	–	–	–	–	–	–
HL^4	–	–	–	<10 (50)	–	–	–	–	–	–
HL^5	–	–	–	–	<10 (50)	–	–	–	–	–
HL^6	–	–	–	–	–	–	14 (25)	15 (25)	–	16 (25)
$[Cu(L^1)_2]$	–	22 (6.25)	–	–	–	21 (6.25)	24 (6.25)	23 (6.25)	22 (6.25)	20 (6.25)
$[Cu(L^2)_2]$	–	–	–	–	–	21 (12.5)	–	–	17 (25)	–
$[Cu(L^3)_2]$	–	–	–	–	–	–	–	–	–	–
$[Cu(L^4)_2]$	–	–	–	<10 (50)	–	–	–	–	–	–
$[Cu(L^5)_2]$	–	–	–	–	14 (25)	–	–	–	–	–
$[Cu(L^6)_2]$	–	–	–	–	–	–	16 (12.5)	16 (25)	–	18 (12.5)
Ciprofloxacin	29 (6.25)	28 (6.25)	22 (6.25)	27 (3.12)	26 (6.25)	22 (6.25)	–	–	–	–
Fluconazole	–	–	–	–	–	–	25 (6.25)	25 (6.25)	23 (6.25)	24 (6.25)
DMSO	–	–	–	–	–	–	–	–	–	–

Diameter of inhibition zone was measured in mm. MIC values are given in brackets. MIC (μg/ml) = Minimum Inhibitory Concentration, i.e. the lowest concentration of drug which completely inhibits bacterial or fungal growth. – indicates no activity. Ciprofloxacin and fluconazole were used as standard for antibacterial and antifungal activity, respectively

a linear form. In gel electrophoresis, SC shows the fastest migration, followed by linear form and lastly the OC form. The present complexes at micromolar concentrations, after 2 h incubation with DNA, showed the nucleolytic activities presented in Fig. 1a, b and c. To investigate the mechanism of nucleolytic activity, experiments were also performed in the presence of H_2O_2 as oxidant and DMSO as a free radical scavenger. The complexes exhibited significant nucleolytic activity in the presence of H_2O_2.

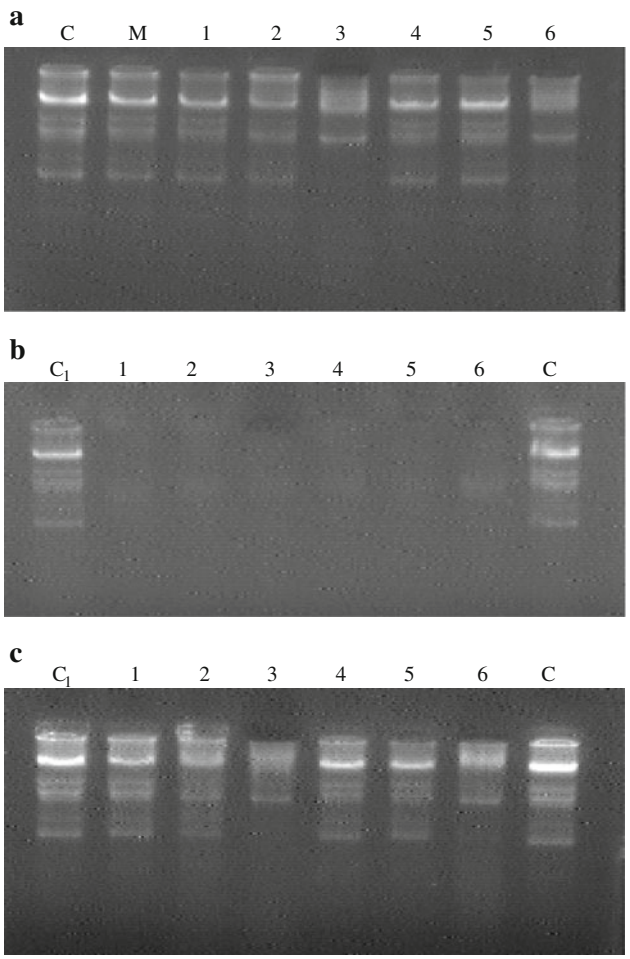


Fig. 1 **a** Gel electrophoresis photograph showing the effects of Cu(II) complexes on plasmid DNA on 1% agarose gel. *Lane C*: Control (Untreated DNA), *Lane M*: Copper salt, *Lane 1*: DNA + Complex I, *Lane 2*: DNA + Complex II, *Lane 3*: DNA + Complex III, *Lane 4*: DNA + Complex IV, *Lane 5*: DNA + Complex V, *Lane 6*: DNA + Complex VI. Complexes I–VI represent $[Cu(L^1)_2]$–$[Cu(L^6)_2]$, respectively. **b** Gel electrophoresis photograph showing the effects of Cu(II) complexes on plasmid DNA in the presence of H_2O_2 on 1% agarose gel. *Lane C1*: Untreated DNA + H_2O_2, *Lane 1*: DNA + Complex I + H_2O_2, *Lane 2*: DNA + Complex II + H_2O_2, *Lane 3*: DNA + Complex III + H_2O_2, *Lane 4*: DNA + Complex IV + H_2O_2, *Lane 5*: DNA + Complex V + H_2O_2, *Lane 6*: DNA + Complex VI + H_2O_2, *Lane C*: Control (untreated DNA). Complexes I–VI represent $[Cu(L^1)_2]$–$[Cu(L^6)_2]$, respectively. **c** Gel electrophoresis photograph showing the effects of Cu(II) complexes on plasmid DNA in the presence of DMSO on 1% agarose gel. *Lane C1*: Untreated DNA + DMSO, *Lane 1*: DNA + Complex I + DMSO, *Lane 2*: DNA + Complex II + DMSO, *Lane 3*: DNA + Complex III + DMSO, *Lane 4*: DNA + Complex IV + DMSO, *Lane 5*: DNA + Complex V + DMSO, *Lane 6*: DNA + Complex VI + DMSO, *Lane C*: Control (Untreated DNA). Complexes I–VI represent $[Cu(L^1)_2]$–$[Cu(L^6)_2]$, respectively

Many copper(II) complexes are known to cleave DNA more efficiently in the presence of oxidants such as hydrogen peroxide and ascorbic acid. Possible reaction mechanisms of DNA cleavage by the copper(II) complexes in the presence of H_2O_2 have been proposed by several research groups [31, 32]. The more pronounced nuclease activity of these complexes in the presence of oxidant (Fig. 1b), compared to that without oxidant (Fig. 1a), may be due to the increased production of hydroxyl radicals. The complexes $Cu(L^3)_2$ and $Cu(L^6)_2$ showed moderate nuclease activity (Fig. 1a, lanes 3 and 6) while $Cu(L^1)_2$, $Cu(L^2)_2$, $Cu(L^4)_2$ and $Cu(L^5)_2$ did not show any nuclease activity in the absence of oxidant (Fig. 1a, lanes 1, 2, 4 and 5). The nuclease activity of the complexes did not change in the presence of free radical scavenger (Fig. 1c, lanes 1-6). The purity of the isolated plasmid DNA was checked by UV–Vis spectroscopy. The ratio of UV absorbance at 260 and 280 nm was 1.68, indicating that the DNA was sufficiently free from RNA and/or protein.

Conclusion

Six copper(II) complexes of Schiff base ligands of arylidene-2-(4-(4-bromo/methoxy-phenyl) thiazol-2-yl) hydrazines have been synthesized, and their chemical structures were deduced by physicochemical and spectroscopic methods. The complexes were formulated as $[CuL_2]$, where the ligand L acts as bidentate chelate with O and N as donating atoms. Gel electrophoresis experiments showed pronounced DNA cleavage activity of the complexes in the presence of an oxidant. Antimicrobial screening showed enhanced antibacterial and antifungal activities of the complexes compared to the free ligands. Hence, the present work contributes to the development of novel copper complexes as nucleolytic and antimicrobial agents.

Experimental

Chemicals and solvents were obtained from Merck, Genei (Bangalore), S.D. Fine, Himedia (Mumbai) and Aldrich and were of analytical reagent grade or purified by standard methods prior to use. Melting points were determined in open-glass capillaries on a Stuart-SMP10 melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FTIR-8400 s spectrophotometer using KBr

pellets in the range of 4,000–400 cm^{-1}. Electronic absorption spectra were recorded on a Shimadzu UV-1700 spectrophotometer using DMSO as solvent in the range of 200–800 nm. Magnetic moment measurements of powder samples were carried out on a Vibrating Sample Magnetometer (Lakeshore 7410) at room temperature. Molar conductances of the complexes were measured in DMSO (10^{-3}M) using a digital conductivity meter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL AL300 FTNMR spectrometer operating at 300/75 MHz and TMS (tetramethylsilane) was used as internal standard. ^{1}H NMR and ^{13}C NMR chemical shifts are reported in ppm downfield from TMS. Mass spectra were recorded on a VG-AUTOSPEC spectrometer. ESR spectra were recorded on a JEOL JES-FA200 ESR spectrometer at room temperature. TG and DTA measurements were recorded on a Labsys TG-DTA16 instrument at a heating rate of 10 °C min^{-1}. In all cases, the 50–800 °C temperature range was studied under air. Elemental analyses were done on a Heraeus CHN rapid analyser. Copper contents were determined by standard methods.

General procedure for the synthesis of the thiosemicarbazone Schiff bases

The thiosemicarbazones were prepared by our earlier reported method [7]. To a solution of thiosemicarbazide (1.82 g, 20 mmol) in aqueous ethanol (40 ml), a solution of aryl aldehyde/ketone (20 mmol) in ethanol or methanol (40 ml) was added slowly at 60–70 °C with continuous stirring. The mixture was refluxed, and the progress of reaction was monitored by TLC. After the completion of the reaction (4–6 h), the mixture was cooled, the precipitate filtered off and washed with ice-cold water then dried in air. Finally, the product was recrystallized from aqueous ethanol (1:3). The compounds were soluble in MeOH, EtOH, DMSO and DMF.

Characterization data of the compounds are given below

1-(2-hydroxy-1,2-diphenylethylidene)thiosemicarbazide: M.P. 169 °C; Yield: 72%; IR (KBr, ν_{max} cm^{-1}): 3,320 (–OH), 3,240 (-NH_2), 3,132 (–NH), 1,658 (C=N azomethine), 1,034 (C=S); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.0–7.5 (m, 10H, aromatic H), 8.4 (s, 1H, NH), 9.5 (s, 1H, –OH); ^{13}C NMR (DMSO-d_6) δ (ppm): 114.4–127.8 (Aryl-CH), 131.4–133.9 (Aryl-C); % Elemental analysis found (Calc.) for $C_{15}H_{14}N_3OS$: C, 63.0 (63.1); H, 5.4 (5.3); N, 14.7 (14.7).

1-(2,4-dihydroxybenzylidene)thiosemicarbazide: M.P. 208 °C; Yield: 80%; IR (KBr, ν_{max} cm^{-1}): 3,541 (-OH, br.), 3,220 (-NH_2), 3,140 (-NH), 1,620 (HC=N azomethine), 1,087 (C=S); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.1–7.4 (m, 3H, aromatic H), 8.2 (s, 1H, –N=CH), 9.0 (s, 1H, NH), 11.4 (s, 1H, –OH); ^{13}C NMR (DMSO-d_6) δ (ppm): 124.8–128.2 (Aryl-CH), 132.0–134.8 (Aryl-C), 140.0 (HC=N); % Elemental analysis found (Calc.) for $C_8H_9N_3O_2S$: C, 45.3 (45.4); H, 4.3 (4.2); N, 19.7 (19.8).

1-(2-hydroxybenzylidene)thiosemicarbazide: M.P. 227 °C; Yield: 82%; IR (KBr, ν_{max} cm^{-1}): 3,403 (-OH, br.), 3,230 (-NH_2), 3,174 (-NH), 1,570 (HC=N azomethine), 1,052 (C=S); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.2–7.5 (m, 4H, aromatic H), 7.6 (s, 1H, –N=CH), 8.6 (s, 1H, NH), 11.2 (s, 1H, –OH); ^{13}C NMR (DMSO-d_6) δ (ppm): 121.5–126.9 (Aryl-CH), 132.0–133.6 (Aryl-C), 136.5 (HC=N); % Elemental analysis found (Calc.) for $C_8H_9N_3OS$: C, 49.1 (49.2); H, 4.6 (4.6); N, 21.4 (21.5).

Synthesis of HL^1–HL^6

The compounds HL^1–HL^6 were prepared by the reported method [7] with slight modification. To a solution of the appropriate thiosemicarbazone in ethanol or methanol, an equimolar quantity of substituted phenacyl bromide (4-bromo/methoxy phenacyl bromide) in methanol was added and the mixture was stirred for 10 min. The mixture was refluxed on a water bath for 4–10 h with monitoring of progress of the reaction by TLC. The product was recrystallized from ethanol or chloroform. Characterization data of the compounds are given in Tables 1, 2 and 3.

Synthesis of $[Cu(L^1)_2]$–$[Cu(L^6)_2]$

To a hot (60–70 °C) solution of HL (10 mmol) in ethanol or methanol (30 ml), a solution of $Cu(AcO)_2$ (5 mmol) in absolute ethanol (30 ml) was added dropwise with stirring. The reaction mixture started to change colour immediately upon addition of $Cu(AcO)_2$. Stirring was then continued for 1–2 h at 80 °C. The resulting precipitate was filtered off, washed with ethanol, dried *in vacuo* over P_4O_{10} and stored in a desiccator over $CaCl_2$. Analytical and physicochemical data for the complexes are given in Table 6, and their proposed molecular structures are presented in Fig. 2.

Nuclease activity

DNA cleavage experiments were performed according to the method described in the literature [33]. Luria–Bertani (LB) broth was used for the culture of *S. typhi*. A 50 ml

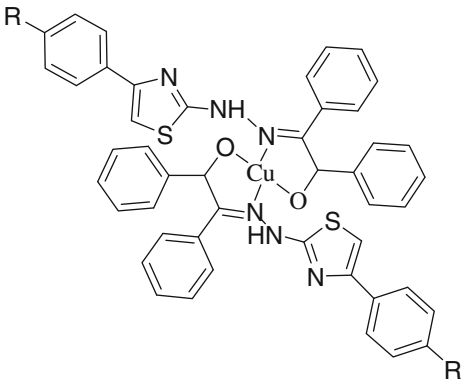
Table 6 Analytical and physicochemical data of the Cu(II) complexes

Cu(II) complex	Empirical formula	Colour	M.W.[a]	M.P. (°C)[b]	Yield (%)	Elemental analysis, found (Calc.)[c] [%]			
						C	H	N	Cu
$[Cu(L^1)_2]$	$[CuC_{46}H_{34}Br_2N_6O_2S_2]$	Green	990.26	>280	80	55.7 (55.8)	3.4 (3.4)	8.4 (8.4)	6.4 (6.4)
$[Cu(L^2)_2]$	$[CuC_{48}H_{40}N_6O_4S_2]$	Green	892.52	>280	68	64.6 (64.5)	4.5 (4.5)	9.4 (9.4)	7.1 (7.1)
$[Cu(L^3)_2]$	$[CuC_{32}H_{22}Br_2N_6O_4S_2]$	Dark brown	842.04	>280	85	45.6 (45.6)	2.6 (2.6)	9.9 (9.9)	7.5 (7.5)
$[Cu(L^4)_2]$	$[CuC_{34}H_{28}N_6O_6S_2]$	Dark brown	744.30	>280	83	54.9 (54.9)	3.8 (3.8)	11.2 (11.3)	8.5 (8.5)
$[Cu(L^5)_2]$	$[CuC_{32}H_{22}Br_2N_6O_2S_2]$	Brown	810.00	>280	70	47.3 (47.4)	2.7 (2.7)	10.3 (10.3)	7.8 (7.8)
$[Cu(L^6)_2]$	$[CuC_{34}H_{28}N_6O_4S_2]$	Brown	712.30	>280	72	57.3 (57.4)	3.9 (3.9)	11.7 (11.8)	8.8 (8.9)

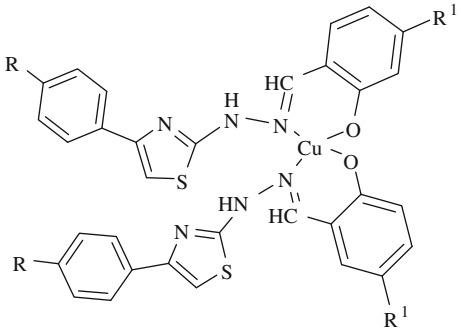
[a] Molecular weight of the compound

[b] Melting point of the compound at their decomposition

[c] Elemental analyses for C, H and N were within ±0.4% of the theoretical value



$[Cu(L^1)_2]$ and $[Cu(L^2)_2]$



$[Cu(L^3)_2]$, $[Cu(L^4)_2]$, $[Cu(L^5)_2]$ and $[Cu(L^6)_2]$

Fig. 2 Proposed molecular structures of the Cu(II) complexes. For $HL^1/HL^3/HL^5$; R=Br and R^1=OH/H; For $HL^2/HL^4/HL^6$: R=OMe and R^1=OH/H

batch of broth was autoclaved for 15 min at 121 °C under 15 lb pressure. The medium was then cooled to 45 °C, inoculated with the seed culture and incubated at 37 °C for 24 h. Isolation of plasmid DNA from pure cultures of *S. typhi* was carried out by conventional methods [34]. Briefly, the fresh bacterial culture (12 ml) was centrifuged (3,000 rpm, 10 min) to obtain a pellet, which was dissolved in 4 ml of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 10% SDS), and 0.5 ml of saturated phenol was added followed by incubation at 55 °C for 10 min. The resultant mixture was centrifuged at 10,000 rpm for 10 min and to the supernatant, an equal volume of chloroform and isoamyl alcohol (24:1) mixture and 1/10th volume of 3 M sodium acetate (pH 4.8) were added and the mixture was further centrifuged under the same conditions. After that, 3 volumes of chilled absolute alcohol were added and the so obtained DNA was separated by centrifugation. Finally, the pellet was dried and dissolved in TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA) and stored cool.

Samples of the different Cu(II) complexes were prepared in DMF (μM), and 4 μl was added separately to the isolated and purified plasmid DNA of *S. typhi*. The DNA-sample mixtures were mixed thoroughly and incubated at 37 °C for 2 h. The DNA cleavage activities of the complexes were determined by agarose gel electrophoresis [33]. Solid agarose gel was prepared by dissolving 500 mg of agarose in 50 ml of 1 × TBE buffer (45 mM Tris, 45 mM H_3BO_3, 1 mM EDTA) by boiling and poured into a gel cassette fitted with a comb. When the gel attained ca. 45 °C, 3 μl of Ethidium bromide solution (10 μg/ml) was added. The gel was allowed to solidify, and then the comb was removed carefully to leave the wells intact. The gel was placed in an electrophoresis chamber flooded with 1 × TBE buffer. Subsequently, 10 μl of DNA sample after incubation for 2 h at 37 °C was added to the loading buffer (5 μl) containing 0.25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol. The solution was loaded carefully into the wells, and the electrophoresis was carried out for 90 min at a constant 60 V in the buffer. Finally, the gel was removed carefully and the bands were observed under a UV transilluminator. The illuminated gel was photographed using an Alpha Innotech Corporation Instrument. The efficiency of the DNA cleavage was measured by determining the ability of the complex to form open circular and nicked circular

DNA from its supercoiled form by estimating the intensities of the bands after gel electrophoresis.

Antimicrobial activities

The microorganisms were obtained from the culture collection of the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Antimicrobial activities were evaluated according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) using the agar disc diffusion method [29]. Briefly, a 24/48-h-old culture of selected bacterium or fungus was mixed with sterile physiological saline (0.85%), and the turbidity was adjusted to the standard MacFarland scale inoculum of 0.5 [$\sim 10^6$ colony forming units (CFU) per millilitre]. Petri plates containing 20 mL of Mueller–Hinton Agar (MHA, Hi-Media) were used for all the bacteria tested. Fungi were cultured in Sabouraud's dextrose agar (SDA)/potato dextrose agar (PDA) (Hi-Media) and purified by single spore isolation technique. The inoculum was spread on the surface of the solidified media, and Whatman no. 1 filter paper discs (6 mm in diameter) impregnated with the test compound (20 μg/disc) were placed on the plates. Ciprofloxacin (5 μg/disc, Hi-Media) was used as positive control for bacteria and fluconazole (10 μg/disc, Hi-Media), was used as positive control for fungi. A paper disc impregnated with DMSO was used as negative control. Plates inoculated with the bacteria were incubated for 24 h at 37 °C, and the fungal cultures were incubated for 72 h at 25 °C. Inhibition zone diameters were measured in millimetres. All the tests were performed in triplicate, and the average was taken as the final value.

Minimum inhibitory concentrations (MIC), the lowest concentration which completely inhibits visible growth (turbidity in liquid media), were determined according to the guidelines of NCCLS document M27-A [29]. Stock solutions (100 μg/ml) of the test compounds, ciprofloxacin and fluconazole were prepared in DMSO. From this, serial dilutions (50, 25… 3.12 μg/ml) were prepared to determine the MIC. All the determinations were done in triplicate, and the average was taken as the final value. The standard antibiotic ciprofloxacin (100 μg/ml) for bacteria and fluconazole (100 μg/ml) for fungi were used as positive controls, and 100 μl of DMSO was used as a negative control.

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References

1. Viksveen P (2003) Homeopathy 92:99–107
2. Shaikh AR, Giridhar R, Yadav MR (2007) Int J Pharm 332(1–2):24–30
3. Uivarosi V, Neagoe S, Aldea V, Niţulescu A (2001) Roum Arch Microbiol Immunol 60(3):267–277
4. Guerra W, de Andrade AE, de Souza Monteiro AR, Bucciarelli-Rodriguez M, Chartone-Souza E, Nascimento AM, Fontes AP, Moyec L, Pereira-Maia EC (2005) J Inorg Biochem 99:2348–2354
5. Wang M, Wang LF, Li YZ, Li QX, Xu ZD, Qu DM (2001) Trans Met Chem 26:307–310
6. Radhakrishanan PK, Indrasen P, Nair CG (1994) Polyhedron 3:67–70
7. Bharti SK, Nath G, Tilak R, Singh SK (2010) Eur J Med Chem 45:651–660
8. Liu Y, Wang N, Mei W, Chen F, Li-Xin H, Jian L, Wang R (2007) Trans Met Chem 32:332–337
9. Travers AA (1993) In: DNA-Protein Interactions. Chapman and Hall, London
10. Sigman DS (1986) Acc Chem Res 19:180–186
11. Schultz PG, Taylor JS, Dervan PB (1982) J Am Chem Soc 104:6861–6863
12. Ward B, Skorobogaty A, Dabrowiak JC (1986) Biochemistry 25:6875–6883
13. Groves JT, Farrell TP (1989) J Am Chem Soc 111:4998–5000
14. Barnard CF, Clear MJ, Hydes PC (1987) Chem. Britain 22:1001–1004
15. Reddy PR, Rao KS, Satyanarayana B (2006) Tetrahydron Lett 47:7311–7315
16. Cukurovali A, Yilmaz I (2006) Trans Met Chem 31:207–213
17. Yilmaz I, Cukurovali A (2003) Trans Met Chem 28:399–404
18. Geary WJ (1971) Coord Chem Rev 7:81–122
19. Sengupta SK, Pandey OP, Srivastava BK (1998) Trans Met Chem 23:349–353
20. Bellamy LJ (1958) The infrared spectra of complex molecules, 2nd edn. Chapman & Hall, Methuen
21. Rai A, Sengupta SK, Pandey OP (2005) Spectrochim Acta A 61:2761–2765
22. Koksal M, Tumer M, Serin S (1997) Synth React Inorg Met Org Chem 27(9):775–786
23. Lever ABP (1968) In: Inorganic electronic spectroscopy. Elsevier, Amsterdam, p 337
24. Lopez-Garzon R, Moreno-Carrettero MN, Salas-Peregrin MA, Salas-Peregrin JM (1993) Polyhedron 12:507–512
25. Greenwood NN, Earnshaw A (2001) Chemistry of the elements, 2nd edit, vol 28. Butterworth Heinemann, p 1192
26. Fouda MF, Abd-Elzaher MM, Shadofa MM, El Saied FA, Ayad MI, El Tabl AS (2008) Trans Met Chem 33:219–228
27. Chandra S, Kumar U (2005) Spectrochim Acta A 61:219–224
28. Gudasi KB, Patil SA, Vadavi RS, Shenoy RV, Nethaji M (2006) Trans Met Chem 31:586–592
29. NCCLS (National Committee for Clinical Laboratory Standards) (1997) Performance standards for antimicrobial disc susceptibility test, 6th edn. Approved Standard, M2-A6. NCCLS, Wayne
30. Wilson WD, Ratmeyer L, Zhao M, Strekowski L, Boykin D (1993) Biochemistry 32:4098–4104
31. Sigman DS, Mazumder A, Perrin DM (1993) Chem Rev 93:2295
32. Kobayashi T, Kunita M, Nishino S, Matsushima H, Tokii T, Masuda H, Einaga H, Nishida Y (2000) Polyhedron 19:2639–2648
33. Brown TA (1990) Mol Biol 1:51–52
34. Sambrook J, Russell DW (2006) Preparation of plasmid DNA by alkaline lysis with SDS: minipreparation, molecular cloning, a laboratory manual, 3 edn, vol 1. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 1.32–1.34