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Synthesis, spectroscopic characterisation, thermal analysis, DNA interaction and antibacterial activity of copper(I) complexes with N, N′- disubstituted thiourea

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ABSTRACT

copper(I) complexes $[Cu(4MTU)_2Cl]$ (**2**), [Cu(4MTU) (B)Cl] (**3**), $[Cu(6MTU)_2Cl]$ (**5**) and [Cu(6MTU) (B)Cl] (**6**) where 4MTU = 1-Benzyl-3-(4-methyl-pyridin-2-yl)-thiourea (**1**) and 6MTU = 1-Benzyl-3-(6-methyl-pyridin-2-yl)-thiourea (**4**), B is a N,N-donor heterocyclic base, viz. 1,10-phenanthroline (phen **3**, **6**), were synthesized, characterized by various physico-chemical and spectroscopic techniques. The elemental analysis suggests that the stoichiometry to be 1:2 (metal:ligand) for **2**, **5** 1:1:1 (metal:ligand:B) for **3**, **6**. X-ray powder diffraction illustrates that the complexes have crystalline nature. IR data coupled with electronic spectra and molar conductance values suggest that the complex **2**, **5** show the presence of a trigonal planar geometry and the complex **3**, **6** show the presence of a tetrahedral geometry about the Cu(I) centre. The binding affinity towards calf thymus (CT) DNA was determined using UV-Vis, fluorescence spectroscopic titrations and viscosity studies. These studies showed that the tested phen complexes **3**, **6** bind moderately (in the order of 10^5 M^{-1}) to CT DNA. The complex **2**, **5** does not show any apparent binding to the DNA and hence poor cleavage efficiency. Complex **3**, **6** shows efficient oxidative cleavage of plasmid DNA in the presence of H_2O_2 involving hydroxyl radical species as evidenced from the control data showing inhibition of DNA cleavage in the presence of DMSO and KI. The *in vitro* antibacterial assay indicates that these complexes are good antimicrobial agents against various pathogens. Anti-bacterial activity is higher when thiourea coordinates to metal ion than the thiourea alone.

1. Introduction

In the past few years, chemical nucleases have been presented as valuable tools in genomic research, as well as promising candidates for application in chemotherapeutics [1–3]. In this context a large number of transition metal complexes such as Cu, Fe, Pt, Ru, Au, Pd etc. have been reported to mediate DNA reactions by themselves or assisted by both oxidation or reducing agents or without any assistant agents [4]. In this line lower oxidation state transition metals have been used in combination of oxygen or other forms of reactive oxygen such as hydrogen peroxide or alkylhydroperoxides to generate reactive oxygen species (ROS) that ultimately may cleave DNA by direct strand scission or base modification [5]. The reactivity of a metal complex will depend obviously on the nature of the metal complex as well as in the potential supramolecular interactions it can have with the DNA [6,7]. The bio essential element copper, and its complexes are of particular interest because they possess biologically accessible redox potentials and they are typically used, upon association with dioxygen or hydrogen peroxide, for efficient and, in some instances, selective DNA cleavage through oxidative pathways [8–10].

Sigman and co-workers have reported that the bis-(1,10-phenanthroline) copper(I) complex in presence of H_2O_2 acts as a 'chemical nuclease' that efficiently nicks DNA, and copper(I) complexes showing efficient chemical nuclease activity [11]. Pt(II), Pd(II), organoruthenium(II) and iridium thiourea complexes are good DNA intercalative binders [12–16]. Active and well-defined thiourea ligands containing thione, thiol (–N–CS–N–) groups are considered as 'privileged ligands' because of their capability to remarkably stabilize different metals in various oxidation states, and their complexes are extensively studied due to sensitivity, selectivity and synthetic flexibility towards a variety of metal ions

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[17–20]. It is thus the DNA cleavage through copper(I) complexes associated to heteronuclear bases an object of large discussions in the literature [11]. Essentially, these latter studies are set to continue toward the synthesis and characterization of novel copper(I) thiourea derivatives that are indeed anticipated to be much more capable of being antimicrobial agents [21]. However, there is a prerequisite of a thorough investigation relating to structure and to the activity of copper(I) thiourea derivatives and hence their stability under physiological conditions, in order to design more potent antimicrobial agents.

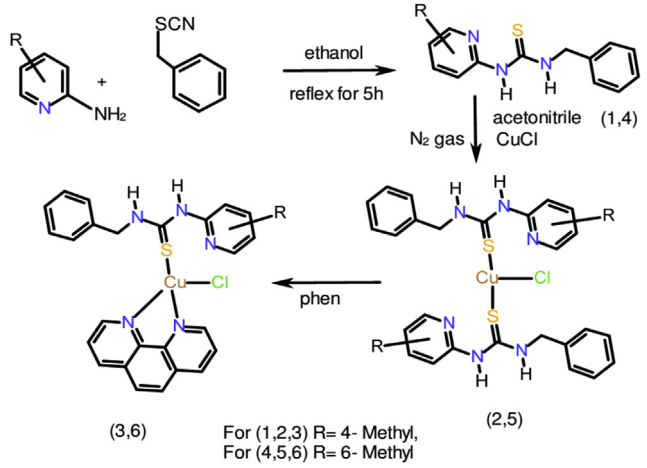
Along these lines, a set of four different copper(I) complexes were synthesized in high yield form through a reaction of CuCl with 4MTU, 6MTU and heterocyclic base phen. These Cu(I) complexes are stable and show appreciable solubility toward common polar organic solvents. This study includes the synthesis, structure elucidation and spectroscopic properties of a thiourea MTU (Scheme 1) containing pyridyl group together with their respective mononuclear Cu(I)-based complexes **2**, **3**, **5** and **6**. This study explores the possibility of Cu(I) complexes being artificial nucleases based on peculiar characteristics of metal-ligand coordination; (i) the existence of planar aromatic and/or heterocyclic ring system capable of being inserted or stacked between base pairs in the hydrophobic interior of helical double stranded DNA, (ii) the presence of nitrogen atoms that can establish hydrogen bonds with the DNA and the capacity to yield cationic copper(I) complex where the positive global charge could favor their electrostatic attraction to the anionic phosphate backbone of DNA.

2. Materials and methods

2.1. Materials

All reagents and chemicals were obtained from Sigma Aldrich and Fluka. Solvents were further purified by standard procedures [22]. Super coiled pUC19 (cesium chloride purified) DNA was purchased from Bangalore Genei (India). Agarose (molecular biology grade), ethidium bromide (EB) and calf thymus DNA (CT DNA) were obtained from Sigma (USA) and bacterial media were purchased from Himedia. The four *Salmonella typhimurium* mutant strains (histidine-dependant) TA98, TA100, TA1535 and TA1538 were procured from IMTECH, Chandigarh, India.

Tris–HCl buffer solution was prepared by using deionized, sonicated triple distilled water.



Scheme 1. Preparation of ligands and copper(I) complexes.

2.2. Syntheses

2.2.1. Synthesis of 1-Benzyl-3-(4-methyl-pyridin-2-yl)-thiourea (4MTU) (*1*), 1-Benzyl-3-(6-methyl-pyridin-2-yl)-thiourea (6MTU) (*4*)

A solution of 2-amino-4-methyl pyridine (0.01 mol) (**1**), 2-amino-6-methyl pyridine (0.01 mol) (**4**) in ethanol (15 ml) was added to (0.01 mol) benzyl isothiocyanate in 10 ml ethanol and refluxed with stirring for 2 h. The resulting white precipitate was filtered off and washed with 5 mL cold ethanol and recrystallized from ethanol, dried, Yield ~80%. The analytical data Calc. for $C_{14}H_{15}N_3S$ (**1**): C, 65.34; H, 5.87; N, 16.33; S, 12.46 found: C, 65.32; H, 5.82; N, 16.32; S, 12.77%. IR, cm^{-1} (KBr disc): 3226 m, 2981br, 2915w, 1615s, 1538vs, 1486s, 1326s, 1185s, 809s, 732s, 448vs (s, strong; m, medium; w, weak; br, broad; vs, very strong). ^{1}H NMR 10.58 (s, 1H), 8.03 (s, 1H), 7.34 (m, J = 4.5 Hz, 2H), 7.27 (m, J = 4.5 Hz, 2H), 6.99 (s, 2H), 6.87 (d, J = 7 Hz, 2H), 4.90 (d, 2H), 2.26 (s, 3H), MP 142 °C, ESI-MS in MeCN: *m/z* 257.94 $[M+H]^+$.

The analytical data Calc. for $C_{14}H_{15}N_3S$ (**4**): C, 65.34; H, 5.87; N, 16.33; S, 12.46 found: C, 65.12; H, 5.82; N, 16.22; S, 12.75%. IR, cm^{-1} (KBr disc): 3188 m, 3037br, 2912w, 1603s, 1535vs, 1452s, 1299s, 1222s, 1185s, 1152s, 785s, 716s, 449vs (s, strong; m, medium; w, weak; br, broad; vs, very strong). ^{1}H NMR 10.6 (s, 1H), 8.05 (d, J = 5.5 Hz, 1H),7.34 (d, J = 5 Hz, 2H), 7.26 (d, J = 4 Hz, 2H), 7.04 (s, 2H), 6.91 (m, 2H), 4.84 (d, J = 6.5 Hz, 2H), 2.26 (s, 3H), MP 148 °C, ESI-MS in MeCN: *m/z* 257.94 $[M+H]^+$.

2.2.2. Synthesis of $[Cu(4MTU)_2Cl]$ (*2*) and $[Cu(6MTU)_2Cl]$ (*5*)

To a pre N_2 purged acetonitrile solution (15 ml) of CuCl (0.049 g, 0.5 mmol), 1-Benzyl-3-(4-methyl-pyridin-2-yl)-thiourea (4MTU) (0.257 g 1 mmol) (**2**), 1-Benzyl-3-(6-methyl-pyridin-2-yl)-thiourea (6MTU) (0.257 g 1 mmol) (**5**) was added as solid with magnetic stirring for a duration of 2 h. The resulting white precipitate was filtered off and washed with diethyl ether, dried under vacuum. Anal. Calcd. for $C_{28}H_{30}N_6S_2CuCl$ (**2**): C, 54.80; H, 4.92; N, 13.69; S,10.44 found: C, 54.26; H, 4.87; N, 13.55; S, 10.81%. IR, cm^{-1} (KBr disc): 3173br, 3000w, 2914w, 1619s, 1533vs, 1488s, 1345 m, 1207s, 936s, 815s, 695s, 444vs (s, strong; m, medium; w, weak; br, broad; vs, very strong). ^{1}H NMR in DMSO d^6 10.66 (s, 1H), 9.31(S, 2H) 8.92 (d, J = 8 Hz, 2H) 8.04(S, 1H), 7.39–6.91 (m, 12H), 4.90 (s, 4H), 2.75 (q, J = 7.5 Hz, 6H), [Yield: ~70%], MP 168 °C, ESI-MS in MeCN: *m/z* 577.12 $[M-Cl]^+$.

Anal. Calcd. for $C_{28}H_{30}N_6S_2CuCl$ (**5**): C, 54.80; H, 4.92; N, 13.69; S,10.44 found: C, 54.36; H, 4.74; N, 13.33; S, 10.63%. IR, cm^{-1} (KBr disc): 3178br, 3125w, 3026w, 2912 m, 1675s, 1522vs, 1451s, 1226s, 1065 m, 784s, 695s, 627 m (s, strong; m, medium; w, weak; br, broad; vs, very strong). ^{1}H NMR in DMSO d^6 10.71 (s, 1H), 9.41(S, 2H) 8.12 (d, J = 9 Hz, 2H) 8.08(S, 1H), 7.72–6.77 (m, 12H), 4.85 (d, J = 6.5 Hz, 4H), 2.73(q, J = 7.5 Hz, 46), [Yield: ~70%], MP 168 °C, ESI-MS in MeCN: *m/z* 577.12 $[M-Cl]^+$.

2.2.3. Synthesis of [Cu(4MTU) (phen)Cl] (*3*) and [Cu(6MTU) (phen) Cl] (*6*)

To an acetonitrile solution (15 ml) of CuCl (0.049 g, 0.5 mmol), 1-Benzyl-3-(4-methyl-pyridin-2-yl)-thiourea (4MTU) (0.1285 g, 0.5 mmol) (**3**), 1-Benzyl-3-(6-methyl-pyridin-2-yl)-thiourea (6MTU) (0.1285 g, 0.5 mmol) (**6**) was added as solid with magnetic stirring for a duration of 0.5 h. To this an acetonitrile solution of heterocyclic base [phen (0.09 g), 0.5 mmol] was added. The reaction mixture was then stirred at room temperature for 2 h with continues purging of N_2. The resulting deep red colored solid was collected by filtration and washed with diethyl ether, and finally dried in vacuum over phosphorus pentoxide, Anal. Calc. for $C_{26}H_{23}N_5SCuCl$ (**3**): C, 58.20; H, 4.32; N, 13.05; S, 5.98 found: C, 58.10; H, 4.67; N, 12.77; S, 6.06%. IR (KBr phase, cm^{-1}): 3159b,

3039w, 2904w, 1566 m, 1529vs, 1419 m, 1211s, 976s, 840s, 720s, 445 m (vs, very strong; s, strong; m, medium; w, weak; b, broad). ^{1}H NMR in DMSO d^6 10.63 (s, 1H), 9.19 (s, 1H), 8.30 (d, J = 6.5 Hz, 2H), 8.03 (d, J = 6.5 Hz, 2H), 7.45–6.86 (m, 12H), 4.89 (d, J = 7 Hz, 2H), 2.58 (t, J = 2.5 Hz, 3H), [Yield: ~70%], MP 208 °C, ESI-MS in MeCN: *m*/*z* 500.05 $[M-Cl]^+$.

Anal. Calc. for $C_{26}H_{23}N_5SCuCl$ (**6**): C, 58.20; H, 4.32; N, 13.05; S, 5.92 found: C, 58.84; H, 4.53; N, 13.20; S, 5.51%. IR (KBr phase, cm^{-1}): 3193 m, 3041b, 2905 m, 1600s, 1531vs, 1446 m, 1221s, 784s, 714s, 690s, 455s (vs, very strong; s, strong; m, medium; w, weak; b, broad). ^{1}H NMR in DMSO d^6 10.68 (s, 1H), 8.83 (s, 1H), 8.23–6.84 (m, 16H), 4.81 (d, J = 6 Hz, 2H), 2.47 (t, J = 2.5 Hz, 3H), [Yield: ~70%], MP 209 °C, ESI-MS in MeCN: *m*/*z* 500.05 $[M-Cl]^+$

The compounds showed stability in the solid state and good solubility in common organic solvents other than hydrocarbons. They were less soluble in water.

2.3. General methods

All compounds were systematically subjected to characterization tools. Elemental analyses of carbon, nitrogen, hydrogen and sulfur were performed with the Elementar varioMICRO V1.9.7 analyzer, copper and chloride with Energy dispersive spectroscopy analysis of X-rays (EDAX). Powder XRD data were recorded on Philips X'pert PRO X-ray diffractometer with graphite monochromatized Cu Kα radiation (λ = 1.541 Å).

Thermogravimetric analysis (TGA) was carried out at 10 °C min^{-1} heating rate using SDT Q600 V20.9 Build 20 Module DSC-TGA Standard under nitrogen up to 700 °C with a closed perforated aluminum pan. Conductivity measurements were made using 10^{-3} M solutions in DMF on a model Control Dynamics (India) conductivity meter and a dip-type cell, calibrated with a solution of AnalaR potassium chloride. Magnetic susceptibility data at 298 K for the polycrystalline samples of the compound were obtained using Model 300 Lewis-coil-force magnetometer of George Associates Inc. (Berkeley, USA) make. Hg $[Co(NCS)_4]$ was used as a standard.

Spectroscopic measurements include: IR spectroscopy of samples in KBr pellets over the region 400–4000 cm^{-1} in a Shimadzu spectrometer, Far IR spectroscopy of samples in polythene discs over the range 50–400 cm^{-1} in a Thermo-Nicolate 6700 spectrometer. UV and visible absorption measurements of wavelengths ranging from 200 to 800 nm using Shimadzu UV-3010 PC spectrophotometer, full scan mass spectra (MS mode) using MicroTOF LC Bruker Daltonics spectrometer equipped with an electrospray source operating in positive ion mode samples were dissolved in DMF/MeOH (50/50) solution and were injected into the apparatus by direct infusion. ^{1}H NMR (400 MHz liquid state NMR spectrometer, Bruker) was recorded in DMSO d^6 solution using tetramethylsilane (TMS) as internal standard.

2.4. Studies on DNA interaction

The UV absorbance at 260 and 280 nm of the CT-DNA solution in 5 mM Tris–HCl buffer (pH 7.2) gave a ratio of ~1: 1.9, indicating the DNA free of protein [23], where the free DNA concentration per nucleotide was $\varepsilon = 6600\ L\ mol^{-1}\ cm^{-1}$ at 260 nm [24], with an ionic strength 5×10^{-3} mol L^{-1} NaCl, and the pH 7.3.

The equilibrium binding constant (K_b) values for the interaction of the compound with CT-DNA were obtained from the absorption spectral titration data using the following equation:

$$\left(\varepsilon_a - \varepsilon_f\right)/\left(\varepsilon_b - \varepsilon_f\right) = \left(b - \left(b^2 - 2K_b^2 C_t[DNA]/s\right)^{1/2}\right)/2K_b C_t \quad (1)$$

where b is $(1 + K_b C_t + K_b\,[DNA]/2s)$, ε_a is the extinction coefficient observed for the charge transfer absorption band at a given DNA concentration, ε_f is the extinction coefficient of the complex free in solution, ε_b is the extinction coefficient of the complex when fully bound to DNA, K_b is the equilibrium binding constant, C_t is the total metal complex concentration, [DNA] represents the DNA concentration in nucleotides and s is the binding site size in base pairs. The non-linear least-squares analysis was done using Origin Lab, version 6.1 [25,26].

The apparent binding constant (K_{app}) of the complexes **2**, **5** and **3**, **6** were also determined by fluorescence spectral technique using ethidium bromide (EB) bound CT DNA solution in Tris–HCl/NaCl buffer (pH 7.2). The fluorescence intensities of EB at 600 nm (546 nm excitation) with an increasing amount of the complex concentration were recorded. Ethidium bromide was non-emissive in Tris-buffer medium due to fluorescence quenching of the free EB by the solvent molecules [27,28]. In the presence of DNA, EB showed enhanced emission intensity due to its intercalative binding to DNA. A competitive binding of the copper complexes to CT DNA could result in the displacement of EB or quenching of the bound EB by the copper (I) species decreasing its emission intensity.

Viscometric titrations were performed with a SchottGerate AVS 310 Automated Viscometer. The viscometer was thermostated at 37 °C in a constant temperature bath. The concentration of DNA was 200 μM in NP and the flow times were measured with an automated timer, and each sample was measured three times, and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ vs. [complex]/[DNA], where η is the viscosity of DNA in the presence of complex and η_0 that of DNA alone. Viscosity values were calculated from the observed flowing time of DNA-containing solutions (t) corrected for that of buffer alone (t_0), $\eta = (t - t_0)$.

2.5. DNA cleavage

The gel was prepared warming up 0.8% weight by volume of agarose (from Sigma Aldrich) in 1× Tris acetate buffer (1× TAE, which was obtained by dilution of 100 ml of 10× TAE, supplied by SIGMA, in 1 L of water). The same 1× TEA buffer was used as working buffer.

The extent of cleavage of supercoiled (SC) DNA in the presence of the complex and oxidizing agent H_2O_2 was determined by agarose gel electrophoresis. The gel electrophoresis was carried out by using gel trays of 210–150 mm with a 16-toothed comb to produce the sample wells. A Electrophoresis Power Supply Bangalore- Genei (India) system was used as a constant voltage supply set to 60 V and 15 mA. In a typical reaction, supercoiled pUC19 DNA (0.2 μg), taken in 50 mM Tris-HCl buffer (pH 7.2) containing 50 mM NaCl, was treated with the complex. The extent of cleavage was measured from the intensities of the bands using UVITEC Gel Documentation System. Due corrections were made for the low level of nicked circular (NC) form present in the original supercoiled (SC) DNA sample and for the low affinity of EB binding to SC compared to NC and linear forms of DNA [29]. The error range observed in determining %NC form from the gel electrophoresis experiments was ±3–5%.

For mechanistic investigations, inhibition reactions were done on adding the reagents prior to the addition of the complex. The solutions were incubated for 1 h in a dark chamber at 37 °C followed by addition to the loading buffer containing 25%

bromophenol blue, 0.25% xylene cyanol, and 30% glycerol (2 μL), and the solution was finally loaded on 0.8% agarose gel containing 1.0 μg/ml ethidium bromide (EB).

2.6. Antibacterial activity

The antibacterial activity was tested against seven clinical isolates like *Bacillus subtilis*, *Bacillus mycodies, Staphylococcus aureus, Yersinia enterocolitica, Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*. The test organisms were maintained on nutrient agar slants. *In-vitro* antibacterial activity was determined by agar well-diffusion method as described by Mukherjee et al. [30]. The overnight bacterial culture was centrifuged at 8000 rpm for 10 min. The bacterial cells were suspended in saline to make a suspension of 10^5 CFU/mL and used for the assay. Plating was carried out by transferring the bacterial suspension to a sterile Petri plate, mixed with molten nutrient agar medium, and allowing the mixture to solidify. About 75 μL of the sample (2 mg/mL) was placed in the wells. Plates were incubated at 37 °C and activity was determined by measuring the diameter of the inhibition zones. The bactericidal assays were performed in triplicate.

3. Results and discussion

A set of four different copper (I) complexes were synthesized in high yield form through a reaction of CuCl with 4MTU, 6MTU and heterocyclic base phen by the continuous purging of N_2. The elemental analysis closely corresponded to spectroscopic measurements. The purity of compounds is reflected in elemental analysis (complete data is given in synthesis part) and in their appropriate spectral patterns. The elemental analysis suggests that the stoichiometry to be 1:2 (metal:ligand) for **2**, **5** and 1:1:1 (metal:ligand:B) for **3**, **6**. Presence of copper and chloride is confirmed through the EDAX analysis of complexes.

3.1. Infra-red spectroscopy

IR data of ligand and the complexes were recorded as KBr pallets in the region 4000–400 cm^{-1}, the spectra of the complexes are similar to that of uncoordinated N-heterocycles except for minor shifts in the position of the peaks. selected IR spectroscopic vibration bands of **1**–**6** listed in Table 1 (Fig. 1), where the ν(C═S) occurs between 740 and 680 cm^{-1} for the free ligand, is shifted toward lower frequency upon complexation, as observed for the other thione compounds [31] and ν(NH) around 3200 cm^{-1} is slighly shifted to higher wave numbers in Cu(I)-complex indicates the existence of the thione form of the ligand in the solid state of the complex. ν(CuN) band ~470 cm^{-1} in compound **3**, **6** indicates the coordination via nitrogen of phen to copper centre [32]. The IR spectral data of the complexes suggest that the ligands bind to copper (I) via sulfur of thiourea. Further it is supported by far IR spectra of the complexes which display peaks due to ν(CuS) in the range 180–250 cm^{-1}. The ν(CuS) at 250 cm^{-1} indicates for Cu(I) in three coordination mode [33] of the complex **2**, **5** and the ν(CuS) at 220 cm^{-1} indicates for Cu(I) in four coordination mode of the complex **3**, **6**. Both the complexes displays peak due to ν(CuCl), band ~175 cm^{-1} indicating coordination of Cl to Cu(I) centre [34].

3.2. Electronic absorption spectra

The electronic absorption spectra of compounds **2**, **3**, **5** and **6** (Fig. 2, Table 1) show two intense broad bands with maxima in the range of ~270 and ~325 nm; the high energy band can be attributed to ligand $\pi \rightarrow \pi^*$ transitions on the pyridine and benzene aromatic rings, where as the lower energy band can be considered as a thione originating CT transition at the C═S bond [35]. But they do not register any band characteristic to Cu (II) ions in the visible region. This confirms the observation that the compounds contain copper in the +1 oxidation state [36,37]. None of the thione bands is found to express any significant shift upon coordination to copper. So that the complexes adopt the trigonal planar geometry for **2**, **5** and tetragonal geometry around the copper(I) centre for **3**, **6**.

The time dependent stability of complexes **2**, **5** and **3**, **6** measured over 5 h at pH 7.4, 25 °C showed no significant changes in UV–Vis spectral line shapes, but more appropriately loss in signal intensity of complex **2**, **5** in the later part of the time scale [38]. The rate of changes in the spectra for the complex **2**, **5** and **3**, **6** followed an order, **3**, **6** > **2**, **5**. A result of this clearly indicates the complexes are stable in solution state to carry out the DNA binding and cleavage studies of the compounds.

3.3. ^1H NMR spectra

The ^1H NMR spectra of the thiourea ligand and its diamagnetic Cu(I) complexes were recorded in DMSO d^6 solution using tetramethylsilane (TMS) as internal standard. The chemical shifts of the different types of protons of the ligand and its diamagnetic Cu(I) complexes data are given in synthesis, ^1H NMR chemical shifts of ligand and its diamagnetic Cu(I) complexes were compared with that of parent thiourea, (Fig. 3) in which ^1H NMR signals of former shift to lower fields upon binding to metal ions as expected to observe at N–H and N′-H sites respectively at 10.63 and 9.41 ppm. The NMR results are in good agreement with the FT-IR spectra suggesting that the nitrogen is not involved in coordination with Cu(I) in the complex **2**, **5** and leaving the coordination occur through C═S. In complex **3**, **6** also N–H proton resonance is not shifted indicating that the coordination through C═S of thiourea and nitrogen of phen to Cu(I) center.

3.4. ESI MS spectra

ESI mass spectra (Fig. 4) of complexes **2**, **5** exhibits base peak at

Table 1
Physical and analytical data of the compounds.

Sl. No	Compound	ν[a] (NH)	ν(C═S)	ν(CuS)	ν(CuN)	ν(CuCl)	Λ[b]	Electronic spectral data λ_{max} (nm)
1	1-Benzyl-3-(4-methyl-pyridin-2-yl)-thiourea	3226	732	–	–	–	–	315 (4800)
2	$[Cu(4MTU)_2Cl]$	3173	695	248	–	178	23	255 (24500) 315 (17500)
3	[Cu(4MTU) (phen)Cl]	3159	720	220	445	175	32	275 (26800) 330 (4500)
4	1-Benzyl-3-(6-methyl-pyridin-2-yl)-thiourea	3188	722	–	–	–	–	315 (25200)
5	$[Cu(6MTU)_2Cl]$	3178	695	254	–	176	27	239 (19120) 315 (25200)
6	[Cu(6MTU) (phen)Cl]	3193	714	221	455	175	40	270 (21600) 294 (23520)

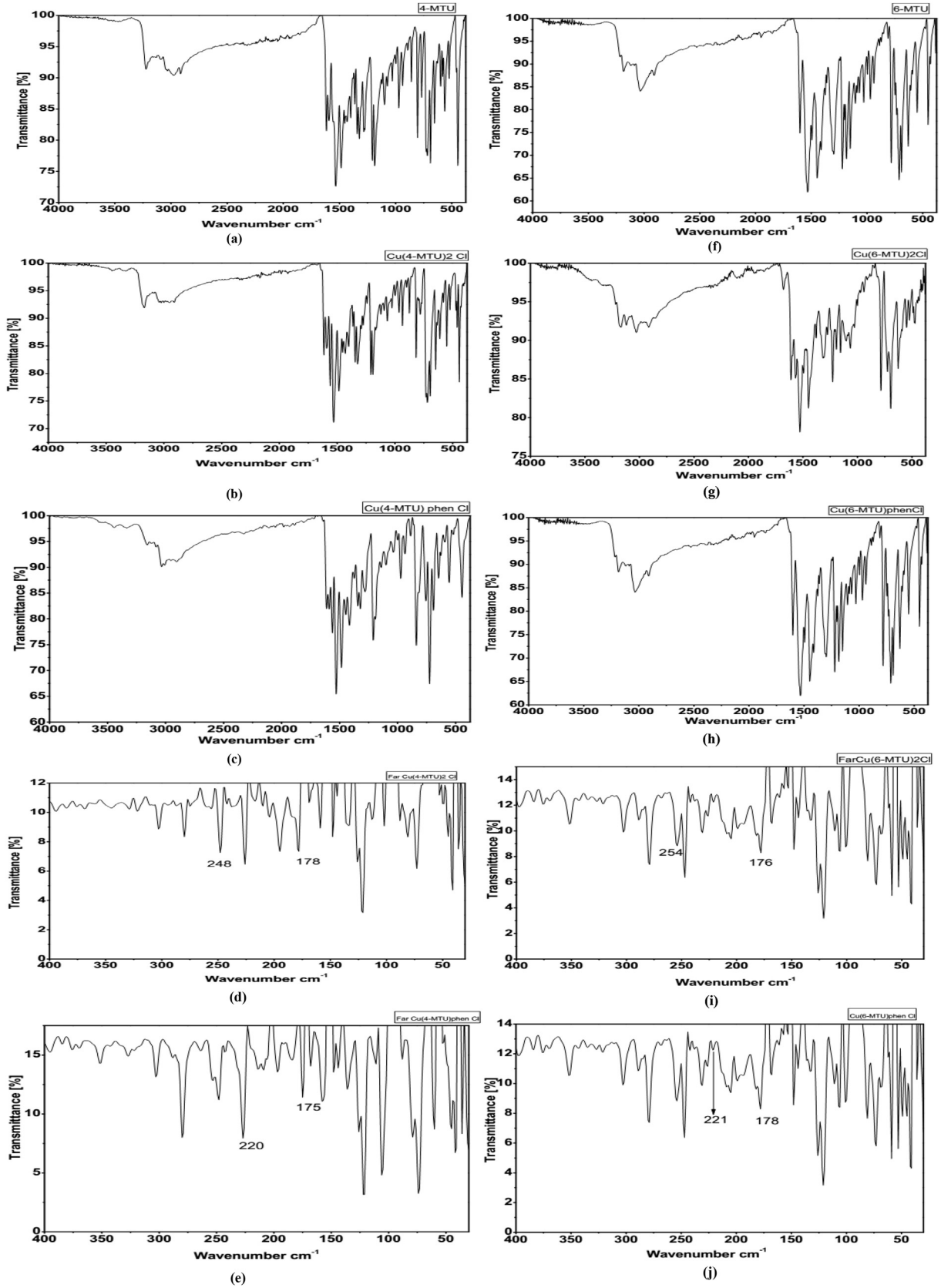


Fig. 1. IR spectra of compounds in KBr phase (**a)** 4MTU, (**b**) $Cu(4MTU)_2$**Cl**, **(c)** Cu(4MTU)phen Cl. Far IR spectra of compounds in pressed Polythene disks **(d)** $Cu(4MTU)_2$**Cl** & **(e)** Cu(4MTU)phen Cl. IR spectra of compounds in KBr phase (**f**) 6MTU, **(g)** $Cu(6MTU)_2$**Cl**, **(h)** Cu(6MTU)phen Cl. Far IR spectra of compounds in pressed Polythene disks **(i)** $Cu(6MTU)_2$**Cl** & **(j)** Cu(6MTU)phen Cl.

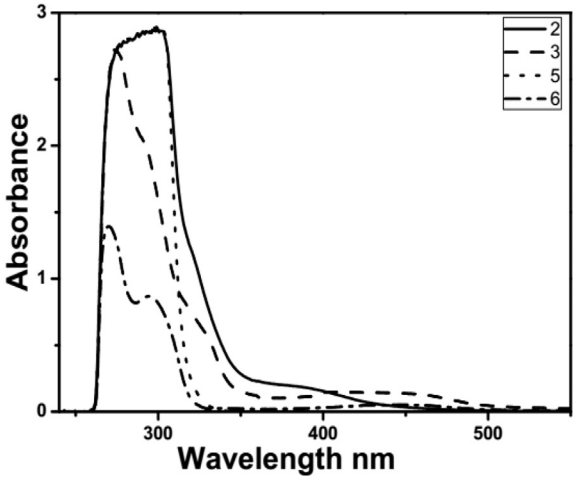


Fig. 2. UV–Vis absorption spectra of complexes $Cu(4MTU)_2$**Cl** (**-**), Cu(4MTU)phen Cl(**- -**), $Cu(6MTU)_2$**Cl(…)** and Cu(6MTU)phen Cl**(-.-)**. in DMF at 25 °C.

m/*z* 577.12 indicating the presence of complex cation $[Cu(MTU)_2]^+$. The ESI mass spectra of complexes **2**, **5** also exhibits peak at *m*/*z* 320.12 which can be assigned to $[Cu(MTU)]^+$. ESI mass spectra of complexes **3**, **6** exhibits base peak at *m*/*z* 500.05 indicating the presence of complex cation $[Cu(MTU)(phen)]^+$. The ESI mass spectra of complexes **3**, **6** also exhibits peak at *m*/*z* 283.91 which can be assigned to $[Cu(phen)+K]^+$.

Molar conductance values of 10^{-3} M solutions of complexes fall in the range of 23–40 $Scm^2\ mol^{-1}$. These values are lower than that expected for a 1:1 electrolyte in DMF, indicating that the compounds act as non-electrolytes, i.e., the anions are coordinated to the metal ions [39](Table 1).

3.5. TG and DTA

The thermal behavior of 4MTU and 6MTU Copper(I) compounds recorded from ambient temperature up to 600 °C in nitrogen flow do not show any loss in weight up to 190 °C, reveals that crystal water molecules and coordinated water molecules are not present in the compounds [40]. The TG curves in the 190–400 °C range suggest that the loss in weight for complexes **2**, **5** corresponds to elimination of 2 molecules of MTU ligand with a DTGmax 258 °C. The TG curves in the 190–285 °C range suggest that the loss in weight for complex **3**, **6** corresponds to elimination of one molecule of MTU ligand with a DTGmax 208 °C and 285–560 °C range suggest that the loss in weight one molecule of phen with a DTGmax 396 °C. In both cases the remaining residues are metal oxides. These results are in accord with the composition of the compounds. The thermograms for complexes **2**, **5** & **3**, **6** are given in Fig 5. The stages of decomposition, temperature ranges, the temperature of the greatest rate of decomposition (DTAmax), the evolved products, as well as the found and calculated mass loss percentages of complexes **2**, **5** and **3**, **6** are given in Table 2.

3.5.1. Powder X-ray diffraction

To obtain further evidence about the structure of the metal complexes X-ray diffraction was performed. The powder XRD patterns indicate crystalline nature for the complexes [41]. It can be easily seen that the pattern of the thiourea differs from its metal complexes, which may be attributed to the formation of a well-defined distorted crystalline structure.

3.6. Interactions of Cu(I)-complexes with DNA

DNA Binding Properties of the compound **2**, **3**, **5** and **6** to the calf thymus (CT) DNA, the electronic absorption spectral studies have been performed. The panoply of absorption signals of the compound **3**, **6** as a function of increasing concentration of CT DNA is shown in Fig. 6. It is observed from graph that the minor bathochromic shift of 1–3 nm along with significant hypochromicity. The bis ligand compounds does show weak binding to the DNA due to less extended planarity compared to phen, which is consistent with its trend in hypochromism (Table 3). The relatively higher binding propensity of the phen compounds is possibly due to the presence of extended planar aromatic ring in phen backbone structure. Previous studies on bis-phen copper complex have explored that this complex binds to DNA either by partial intercalation or binding of one phen ligand to the minor groove while the other phen making favorable contacts within the groove [42,43]. The binding nature of the phen complex toward DNA is appeared to be as similar as that has been observed in the case of bis-phen species.

The emission spectral method is used to study the relative binding of the compound to CT-DNA. The emission intensity of ethidium bromide (EB) is used as a spectral probe as EB shows no apparent emission intensity in buffer solution because of solvent quenching and shows an enhancement of the emission intensity when intercalatively bound to DNA [44]. The binding of the compound to DNA decreases the emission intensity of EB. The relative binding propensity of the compound to DNA is measured from the extent of reduction of the emission intensity Fig. 7, Table 3. The reduction of the emission intensity of EB on increasing the complex concentration could be caused due to displacement of the DNA bound EB by the copper(I) thiourea compound.

To investigate further the binding modes of the compound viscosity measurements on solutions of CT-DNA incubated with the compound were carried out. Because the viscosity of a DNA solution is sensitive to the addition of organic drugs and metal compound bound by intercalation, we examined the effect on the specific relative viscosity of DNA upon addition of compound. The relative specific viscosity (η/η_0), (η and η_0 are the specific viscosities of DNA in the presence and absence of a complex, respectively) of DNA reflects the increase in contour length associated with the separation of DNA base pairs caused by intercalation. The relative viscosity of DNA and contour length follows the equation: $(\eta/\eta_0) = (L/L_0)^{1/3}$, where L and L_0 are the contour length of DNA in the presence and absence of complex respectively [45]. A classical intercalator such as EtBr could cause a significant increase in the viscosity of DNA solutions, in contrast, a partial and/or non-classical intercalation of the ligand could bend or kink DNA resulting in a decrease in its effective length with a concomitant increase in its viscosity [46]. The plots of relative viscosities with R = [Com]/[DNA] are shown in Fig. 8. The change in relative viscosity for the compound **3** and **6** are more than that for the compound **2** and **5** suggesting greater DNA binding propensity of the phen complexes in comparison to the bis ligand complexes, but this change is less compared to that of potential classical intercalators, e.g. ethidium bromide. This is consistent with the observed trend shown by other optical methods and suggests primarily an electrostatic and/or groove binding nature of the complex.

3.7. DNA cleavage activity

DNA cleavage activity of the compounds **1**–**6** in the presence of hydrogen peroxide has been studied using plasmid supercoiled (SC) pUC19 DNA (33.3 μM, 0.2 μg) in 50 mM Tris-HCl buffer/50 mM NaCl (*p*H 7.2). The extent of DNA cleavage, observed by gel

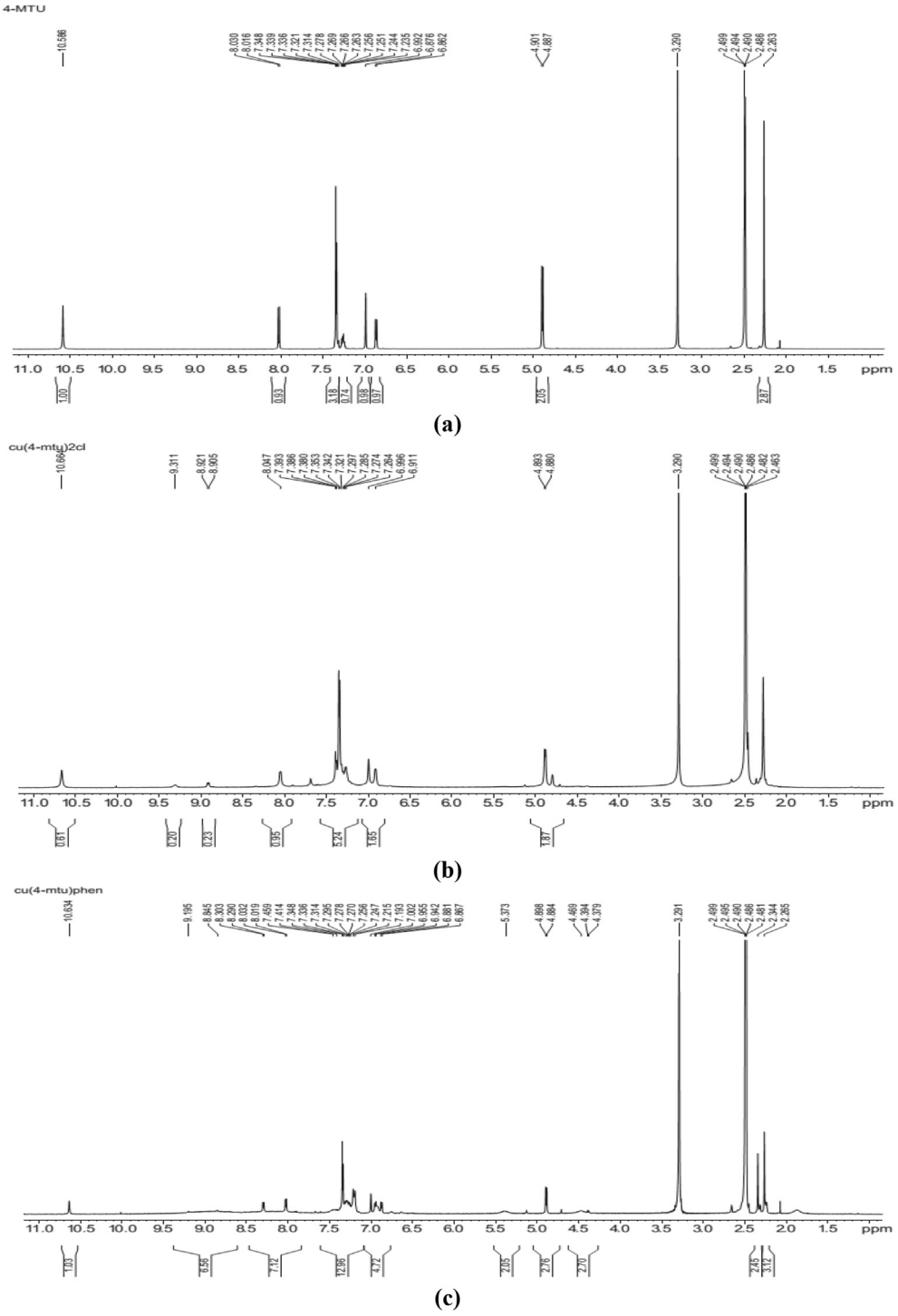


Fig. 3. H^1 NMR spectra of compounds in DMSO d^6 solution using tetramethylsilane (TMS) as internal standard (**a**) 4MTU, (**b**) $Cu(4MTU)_2$**Cl**, (**c**) Cu(4MTU)phen Cl, (**d**) 6MTU, (**e**) $Cu(4MTU)_2$**Cl** & (**f**) Cu(4MTU)phen Cl.

electrophoresis, gives the order: **6** > **3** > **2** > **5**. A 30 μM concentration of **3** and **6** completely cleaves SC DNA into its nicked circular (NC) form on reaction with 200 μM H_2O_2 Fig. 9(**a**), (**b**), Tables 4a and 4b. The DNA cleavage activity order for **1**–**6** follows the relative binding propensities of the compounds to DNA. Control experiments using only H_2O_2 or the compounds alone do not show any apparent cleavage of SC DNA under similar reaction conditions. The reaction of SC DNA with CuCl in the presence of H_2O_2 does not show any cleavage activity. Addition of hydroxyl radical scavengers like DMSO or KI significantly inhibits the cleavage. The inhibitory effect is large with the DMSO as this reagent could reduce the effect of the oxidizing agent by reacting with the externally added hydrogen peroxide. The DNA cleavage reaction of the compound in the presence of H_2O_2 probably proceeds through the hydroxyl radical pathway and/or "copper-oxo" intermediate as the reactive species. The pathways involved in the DNA cleavage reactions are believed

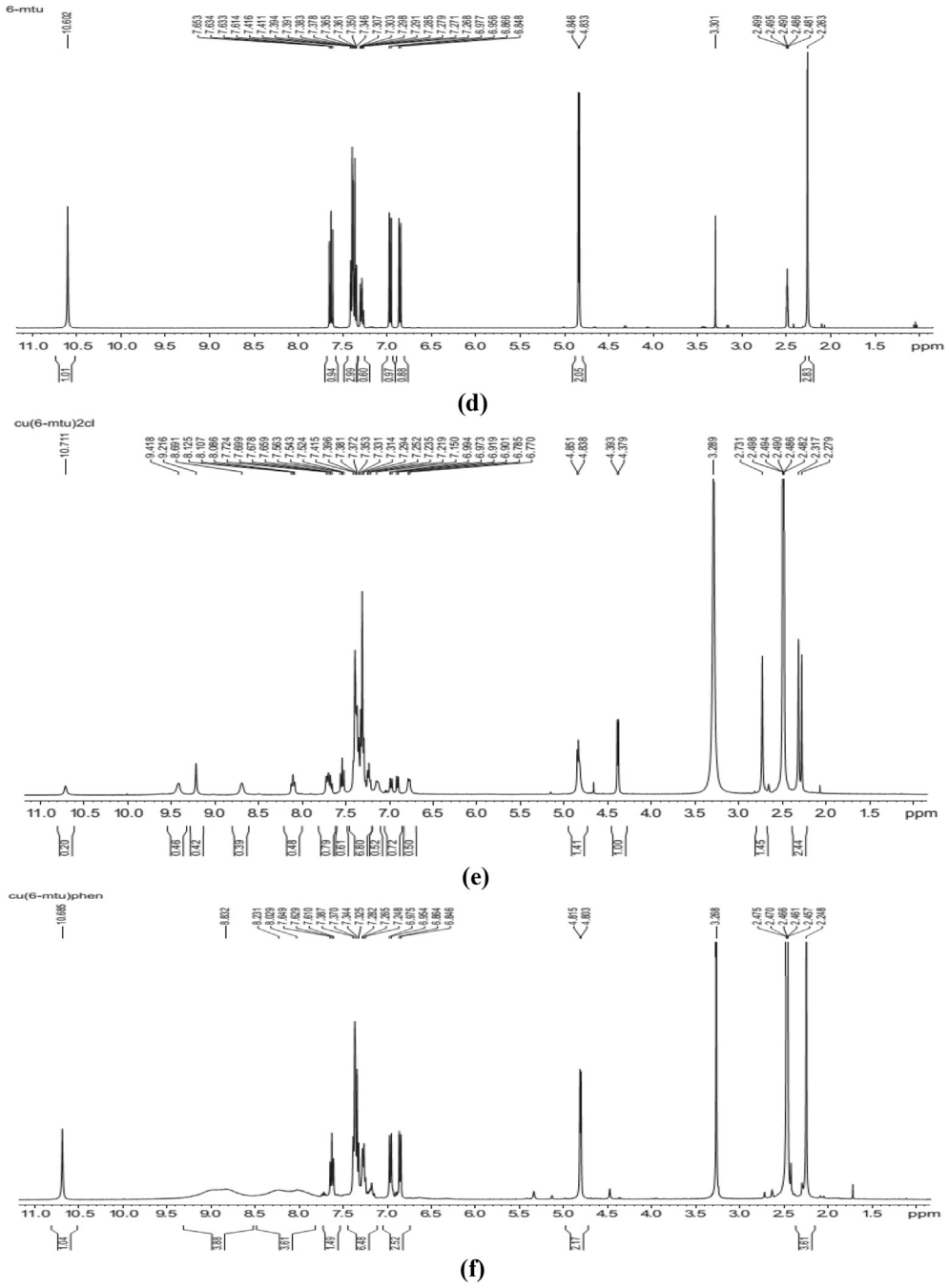


Fig. 3. (*continued*).

to be analogous to those proposed by Sigman and co-workers for the chemical nuclease activity of bis(phen)copper species [11,43].

3.8. Antibacterial activity

Gram-positive and Gram-negative bacteria can be differentiated in the physical appearance of their cell envelopes. The compounds were screened for their *in-vitro* antibacterial activity against both Gram-positive and Gram-negative bacteria. *In-vitro* antibacterial activity was determined by the agar well-diffusion method. The percentage zone of inhibition data are represented in Table 5. The data reviles that a series of novel compounds **2**, **3**, **5** and **6** were found to be highly active. Notably, they exhibited much more antibacterial activity than the standard drug ciprofloxacin, while compounds **1**, **4** inactive against both Gram-positive and Gram-negative bacteria. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlapping of the ligand orbital and the partial sharing of the positive charge of the metal ion with the donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes, interferes with enzyme activity and may lead to cell apoptosis.

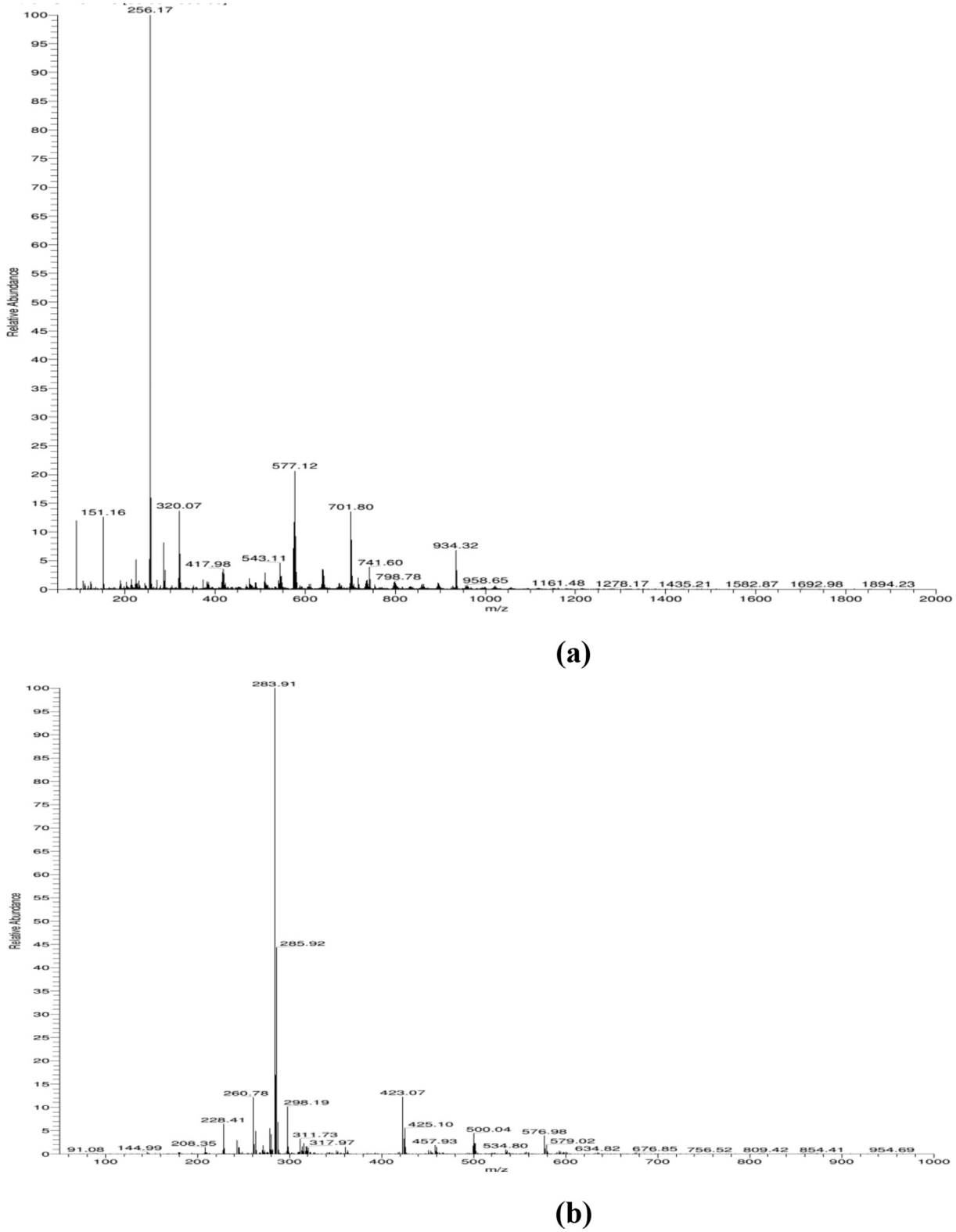
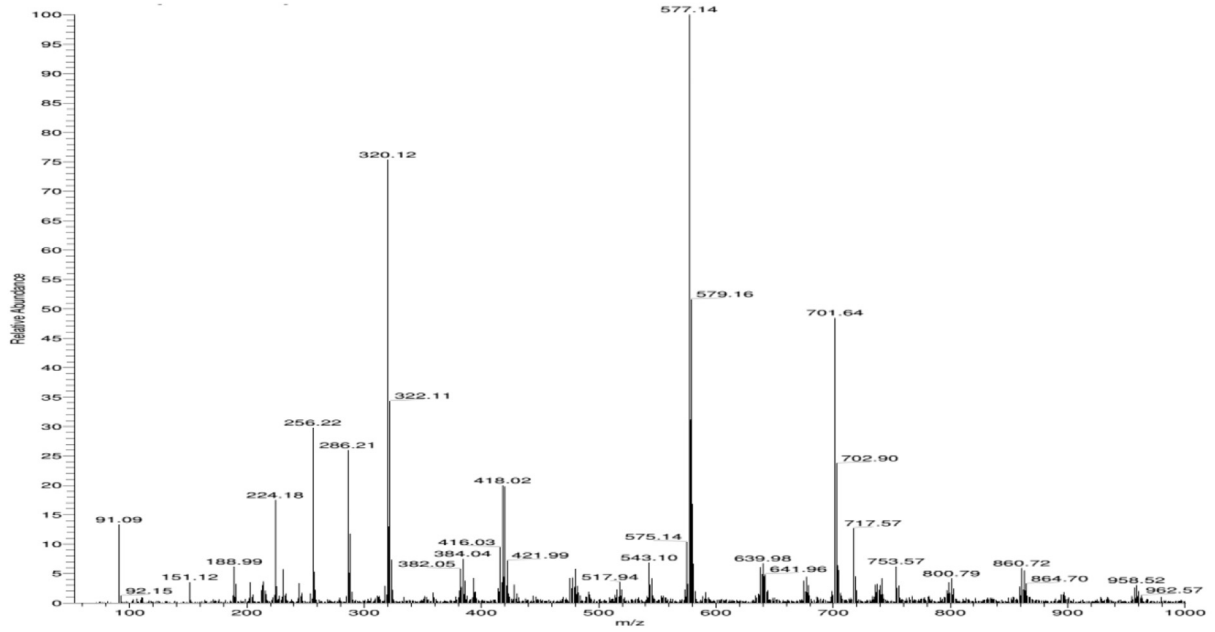


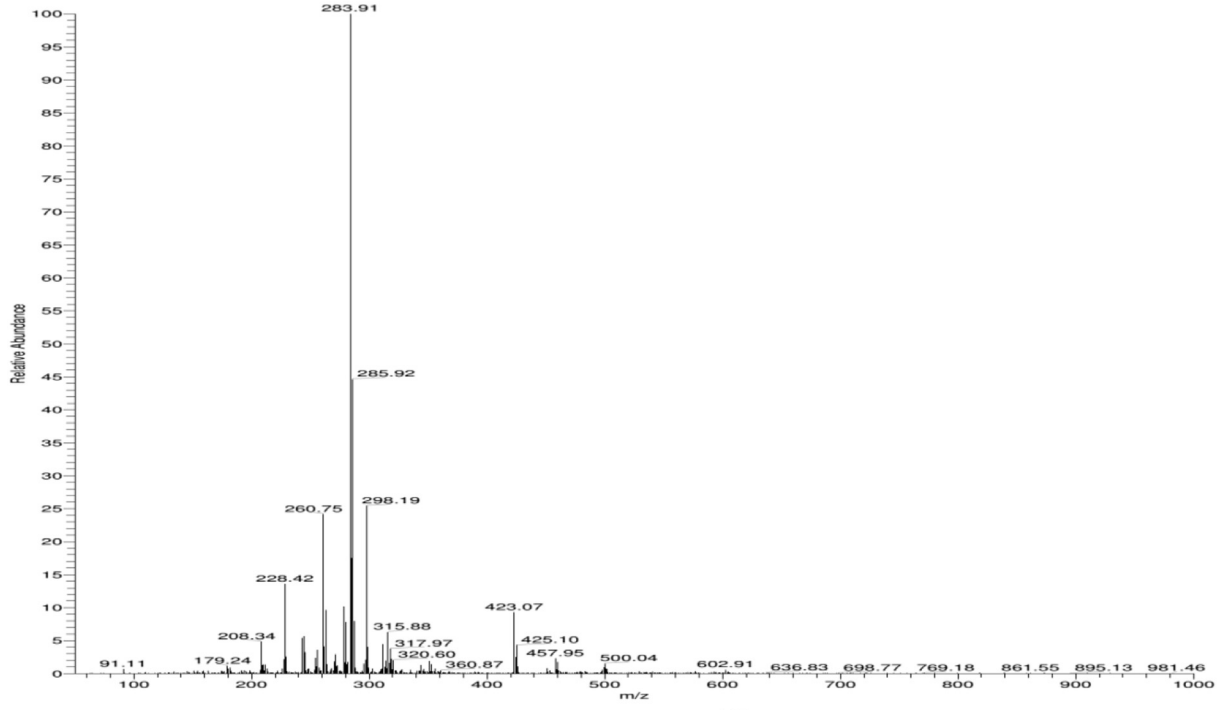
Fig. 4. ESI MS spectra of compounds in MeCN solution (**a**) Cu(4MTU)$_2$**Cl**, (**b**) Cu(4MTU)phen Cl, **(c)** Cu(4MTU)$_2$**Cl** & **(d)** Cu(4MTU)phen Cl.

4. Conclusion

A series of novel Cu(I) complexes derived from 1-Benzyl-3-(4-methyl-pyridin-2-yl)-thiourea and 1-Benzyl-3-(6-methyl-pyridin-2-yl)-thiourea has been synthesized and all the four complexes structurally characterized. The spectral data are in consistent with the proposed structure of ligands and its Cu(I) complexes. Amongst the metal complexes explored, the phen complexes displays



(c)



(d)

Fig. 4. (*continued*).

significant binding propensity with CT DNA providing an order: **6**, **3** >> **2**, **5**. The complexes **2**, **5** does not show any measurable binding interaction to the DNA and hence its poor cleavage efficiency. Complexes **3**, **6** show efficient oxidative cleavage of SC-DNA in the presence of hydrogen peroxide involving hydroxyl radical species. Pathways involving hydroxyl radicals in the DNA cleavage reactions are proposed from control studies, which show inhibition of the cleavage in the presence of the hydroxyl radical scavengers DMSO and KI. The DNA cleavage activity of transition metal complexes with bio-essential constituents like copper and active thiourea showing DNA cleavage have the potential for applications in antibacterial agents. All complexes show a profound antibacterial activity against both Gram-negative and Gram-positive bacteria than ligands.

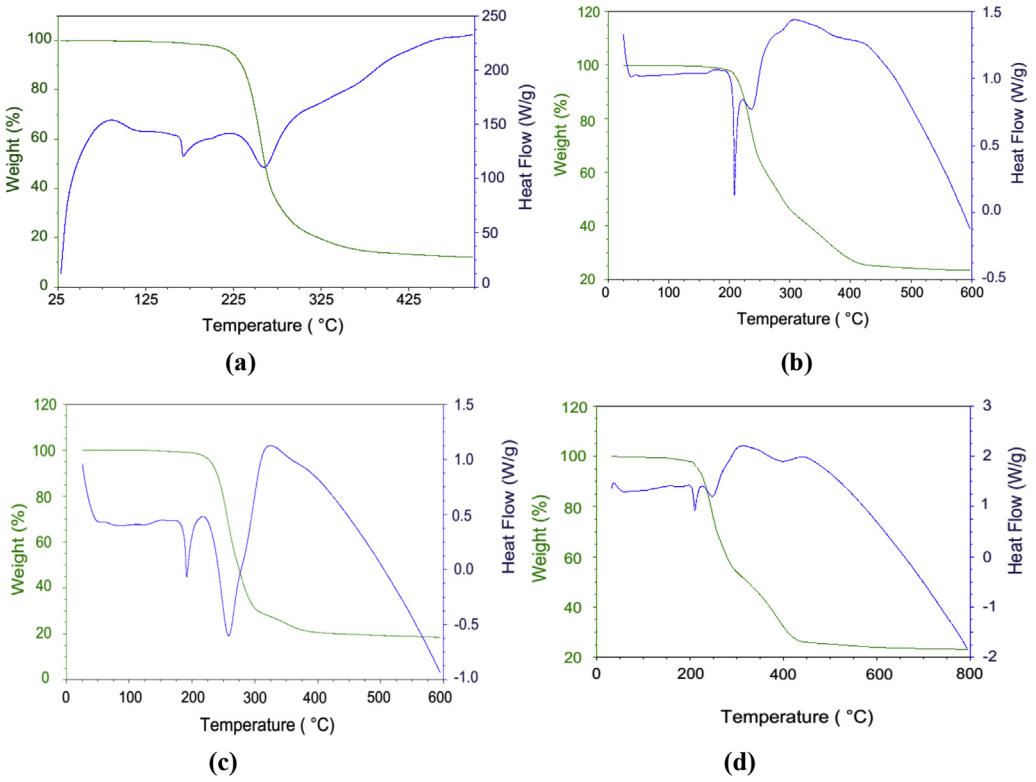


Fig. 5. TG and DTA curves of complexes **(a)** $Cu(4MTU)_2Cl$, **(b)** Cu(4MTU)phenCl, **(c)** $Cu(6MTU)_2Cl$, **(d)**Cu(6MTU)phenCl.

Table 2
Thermo analytical results for the investigated compounds.

Complex	Stage	TG results temperature range (°C)	DTA results temperature peak (°C)	Weight loss (%)		Evolved moiety
				Found	Calculated	
2	Single	190–400	258 (Endo)	83.92	83.52	MTU (2 mol)
	Residue	>450		12.07	12.97	CuO
3	I	190–285	208 (Endo)	47.68	47.90	MTU
	II	285–560	307 (Exo)	28.35	33.00	Phen
	Residue	>567		23.20	22.0	CuO + Cl
5	Single	190–400	258 (Endo)	79.69	83.52	MTU (2 mol)
	Residue	>450		18.60	18.59	CuO + Cl
6	I	190–285	209 (Endo)	47.17	47.90	MTU
	II	285–560	392 (Endo)	28.9	33.00	Phen
	Residue	>567		23.13	21.32	CuO + Cl

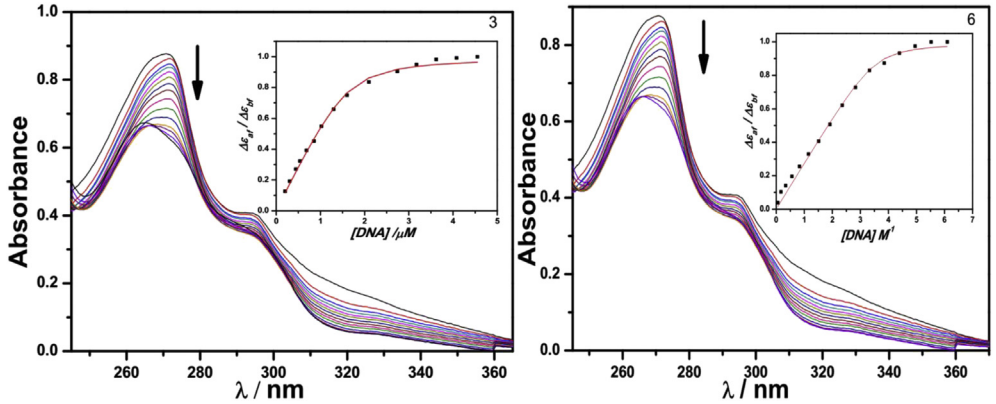


Fig. 6. Absorption spectral traces on addition of CT DNA to the solution **3** and **6** (shown by arrow). The inset shows the best least-squares fit of $\Delta\varepsilon_{af}/\Delta\varepsilon_{bf}$ vs. [DNA] of compound **3** and **6**.

Table 3
DNA binding data of the Cu(I) complexes.

Sl. No	Complex	K_a/M^{-1}	K_b/M^{-1} [S]
2	$[Cu(4MTU)_2Cl]$	5.46×10^5	–
3	[Cu(4MTU) (phen)Cl]	8.53×10^5	$8.6 (\pm 0.7) \times 10^5$ [0.27 (±0.01)]
5	$[Cu(6MTU)_2Cl]$	3.72×10^5	–
6	[Cu(6MTU) (phen)Cl]	2.0×10^6	$1.6 (\pm 0.6) \times 10^6$ [0.59 (±0.02)]

Note: K_a = Equilibrium binding constant from absorption spectroscopy.
K_b = Apparent binding constant from EB displacement assay by emission spectroscopy.

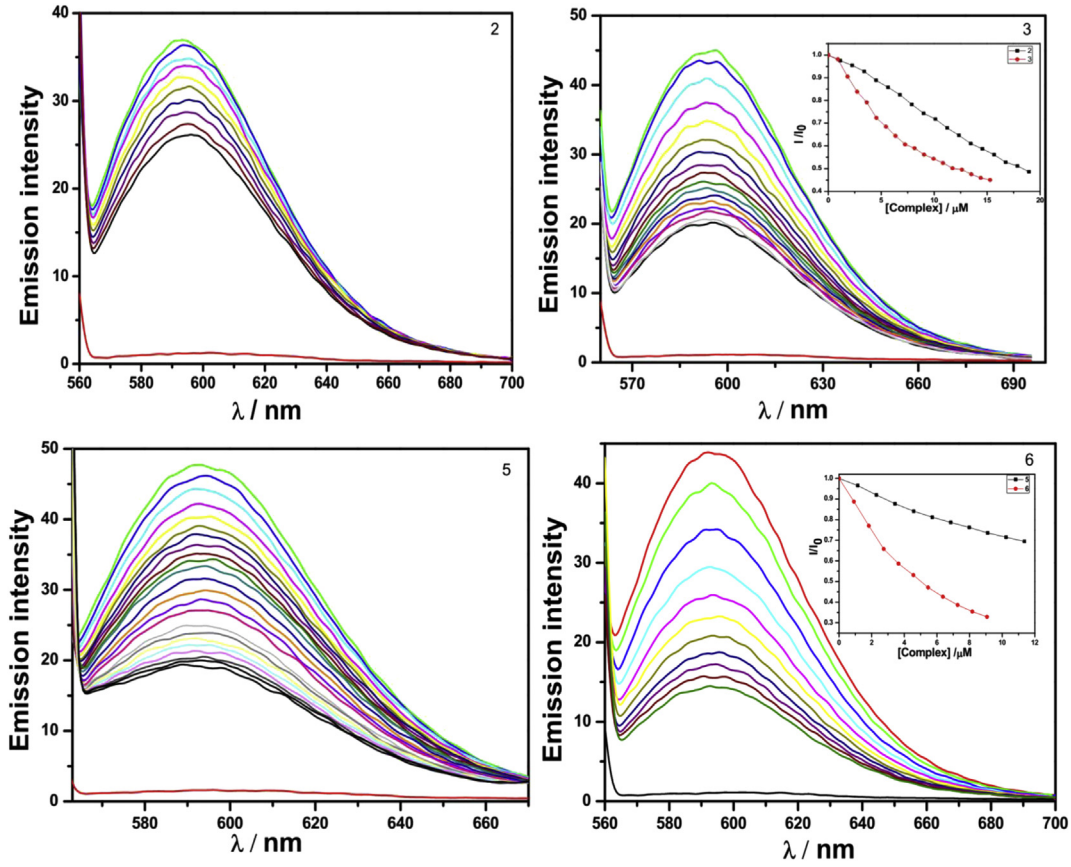


Fig. 7. Emission spectral changes on addition of complexes **2**, **3** and **5**, **6** to the CT-DNA bound to ethidium bromide. The inset shows the effect of addition of increasing concentration of the complexes **2** (■) and **3** (●), **5** (■) and **6** (●) to an ethidium bromide bound CT-DNA solution in a 5 mM Tris-HCl buffer (pH, 7.2) at 25 °C.

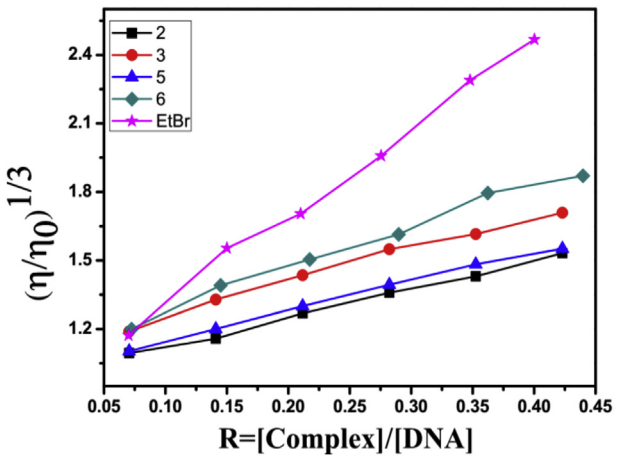


Fig. 8. Change in relative specific viscosity of CT-DNA (200 μM) with addition of complexes 2(■), 3(●), (▲), (♦) and ethidium bromide (★) in 5 mM Tris–HCl buffer medium at 37 ± 0.1 °C.

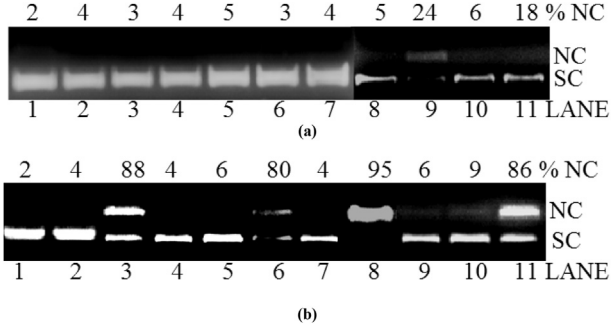


Fig. 9. Gel electrophoresis diagram showing the oxidative cleavage of SC pUC19 DNA (0.2 μg, 30 μM b.p.) by the compounds **1**–**6** in the presence of H_2O_2 (176 μM) in 50 mM Tris-HCl/NaCl buffer (pH, 7.2) containing 10% DMF. **(a)** lane-1, DNA control; lane-2, DNA + H_2O_2; lane-3, DNA + CuCl; lane-4, DNA +1; lane-5, DNA +1 + H_2O_2; lane-6, DNA + 4; lane-7, DNA +4 + H_2O_2; lane-8, DNA +2; lane-9, DNA +2 + H_2O_2; lane-10, DNA +5; lane-11 + H_2O_2; and **(b)** lane-1, DNA control; lane-2, DNA + 3; lane-3, DNA + 3 + H_2O_2; lane-4, DNA + 3 + H_2O_2 + DMSO; lane-5, DNA + 3 + H_2O_2+ KI; lane-6, DNA +3 + H_2O_2 + NaN_3; lane-7, DNA +6; lane-8, DNA +6 + H_2O_2; lane-9, DNA + 6 + H_2O_2 + DMSO; lane-10, DNA + 6 + H_2O_2+ KI; lane-11, DNA +3 + H_2O_2 + NaN_3.

Table 4a
Selected cleavage data of SC DNA pUC19 by ligands and complexes (**2**, **5**).

Sl. No.	Condition	SC%	NC%
1	DNA control	98	2
2	DNA + H_2O_2(2 μM)	96	4
3	DNA + CuCl(50 μM)	97	3
4	DNA + 1 (100 μM)	96	4
5	DNA + 1 (100 μM) + H_2O_2	95	5
6	DNA + 4 (100 μM)	97	3
7	DNA + 4 (100 μM) + H_2O_2	96	4
8	DNA + 2 (100 μM)	95	5
9	DNA + 2 (100 μM) + H_2O_2	76	24
10	DNA + 5 (100 μM)	94	6
11	DNA + 5 (100 μM) + H_2O_2	82	18

Table 4b
Selected cleavage data of SC DNA pUC19 by complexes (**3**, **6**) with controls compounds.

Sl. No.	Condition	SC%	NC%
1	DNA control	98	2
2	DNA +3 (30 μM)	96	4
3	DNA + 3 (30 μM) + H_2O_2	12	88
4	DNA + 3 (30 μM) + H_2O_2+ DMSO	96	4
5	DNA + 3 (30 μM) + H_2O_2+ KI	94	6
6	DNA + 3 (30 μM) + H_2O_2+ NaN_3	20	80
7	DNA + 6 (30 μM)	96	4
8	DNA + 6 (30 μM) + H_2O_2	5	95
9	DNA + 6 (30 μM) + H_2O_2+ DMSO	94	6
10	DNA + 6 (30 μM) + H_2O_2+ KI	91	9
11	DNA + 6 (30 μM) + H_2O_2+ NaN_3	14	86

Table 5
Antibacterial activity of the compounds **1**–**6** of concentration 2 mg/mL: zone of inhibition values (in %).

Compounds	Gram-positive				Gram-negative		
	S. aureus (MTCC 3160)	*Bacillus mycoides* (MTCC 645)	*B. subtilis* (MTCC 441)	*Yersinia enterocolitica* (MTCC 4848)	*P. aeruginosa* (MTCC 741)	*K. pneumoniae* (MTCC 109)	*Proteus mirabilis* (MTCC 743)
1	–	–	–	–	–	–	–
2	17	17	16	12	22	17	18
3	24	23	26	28	30	26	27
4	–	–	–	–	–	–	–
5	16	16	15	14	18	16	16
6	30	32	32	30	34	31	27

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