



Novel Ligand 1-Benzyl-3-(4-Ethyl-Pyridin-2-Yl)-Thiourea and Cu (I) Complexes: DNA Interaction, Antibacterial and Thermal Studies

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ABSTRACT

The new ligand 1-Benzyl-3-(4-ethyl-pyridin-2-yl)-thiourea (**1**), and two copper(I) complexes [Cu(4ETU)₂Cl] (**2**), [Cu(4ETU)(B)Cl] (**3**) where 4ETU=1-Benzyl-3-(4-ethyl-pyridin-2-yl)-thiourea, B is a N,N-donor heterocyclic base, viz. 1,10-phenanthroline (phen, **3**), 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** and 1:1:1 (metal:ligand:B) for **3**. PXRD analysis provides the crystalline nature of the metal complexes. IR data coupled with electronic spectra and molar conductance values suggest that the complex **2** shows the presence of a trigonal planar geometry and the complex **3** shows the presence of a tetragonal geometry about the Cu(I) centre. Binding interactions of the complexes with calf thymus (CT) DNA have been investigated by absorption, emission, DNA thermal denaturation and viscosity studies. The phen complex displays significant binding propensity to the CT DNA giving an order: **3** (phen) > **2**. Complex **3** shows efficient oxidative cleavage of SC-DNA in the presence of H₂O₂ involving hydroxyl radical species as evidenced from the control data showing inhibition of DNA cleavage in the presence of DMSO and KI. The compounds were screened for their *in vitro* anti-bacterial activity using Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*) and Gram-negative bacteria (*Esherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*). Complexes **2** & **3** exhibits higher antibacterial activity against Gram-positive bacteria than the Gram-negative bacteria. Antibacterial activity is higher when thiourea coordinates to metal ion than the thiourea alone. The thermogram of complex **2** exhibits single step and complex **3** exhibits two step decomposition behavior.

Keywords: Antibacterial activity, Chemical nuclease activity, Copper(I) complexes, Thiourea.

INTRODUCTION

The development of precursors suitable for DNA cleavage under physiological condition is highly promising in genome research.¹ While bioinorganic metal complexes have wide variety of applications in biological, clinical, analytical foot printing, pharmacological areas, recent studies show novel research highlights in chemotherapeutics.²⁻⁵ It has been revealed that deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies due to its finger print as a genetic "code" in most living organisms. Mechanism of the interactions between DNA and small molecules or metal complexes are therefore significantly important in the research domains such as anti neoplastic medication, molecular biology, bioengineering, design of novel pharmaceutical moieties.⁶⁻¹² Sigman and co-workers have reported that the bis-(1,10-phenanthroline) copper(I) complex in presence of H₂O₂ acts as a 'chemical nuclease' that efficiently nicks DNA. It is thus the DNA cleavage through copper (I) complexes associated to heteronuclear bases an object of large discussions in the literature.^{1,13} Pt(II), Pd(II), organoruthenium(II) and iridium thiourea complexes were good DNA intercalative binders.¹⁴⁻¹⁶ Thioureas of high asymmetry and remarkable stability are being extensively studied because of their unique biological properties including antifungal, insecticidal,

fungicidal, antibacterial and herbicidal properties.¹⁷⁻¹⁹ Essentially, these 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. 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 condition, in order to design more potent antimicrobial agents.

Along these lines, a set of two different copper (I) complexes were synthesized in high yield through a reaction of CuCl with 4ETU 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 novel thiourea 4ETU (Scheme 1) containing pyridyl group together with their respective mononuclear Cu (I)-based complexes **2** and **3**. Of a particular interest, 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.

MATERIALS AND METHODS

Materials

All reagents and chemicals were obtained from Sigma Aldrich and Fluka. Solvents were further purified by standard procedures.²⁰ 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.

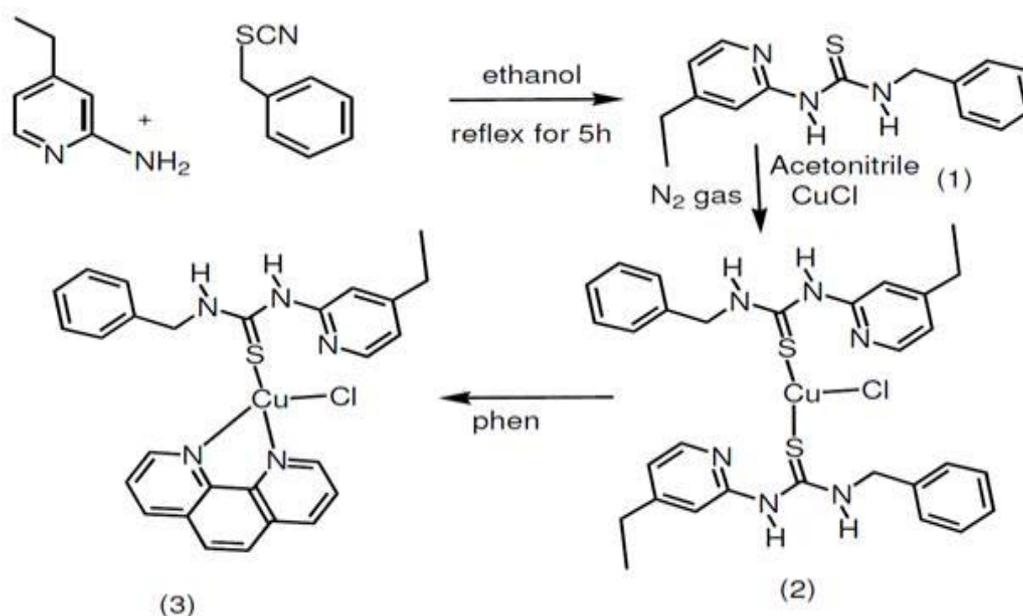
Synthesis

1-Benzyl-3-(4-ethyl-pyridin-2-yl)-thiourea (4ETU) (**1**): a solution of 2-amino-4-ethyl pyridine (0.01 mol) in ethanol (15 ml) was added to (0.01 mol) benzyl isothiocyanate in 10 ml of ethanol and refluxed with stirring for 2h. The resulting white precipitate was filtered off and washed with 5 mL cold ethanol and recrystallised from ethanol, dried (~95% Yield). m.p 142 °C, (M+H) 272.2. Anal. Calc. for C₁₅H₁₇N₃S : C, 66.39; H, 6.31; N, 15.48; S, 11.82 found: C, 66.63; H, 6.32; N, 15.54; S, 12.31%. IR, cm⁻¹ (KBr disc): 3450br, 3228s, 2925s, 2854w, 1616vs, 1534vs, 1207s, 732s, (s, strong; w, weak; br, broad; vs, very strong). ¹H NMR 12.14(s, 1H), 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, 1H), 6.91(m, 2H), 4.90(s, 2H), 2.58(q, J=7.5 Hz, 2H), 1.16(t, J=7.5 Hz, 3H)

[Cu(4ETU)₂Cl] (**2**): Copper(I) chloride (99 mg, 1.00 mmol) was dissolved in 25 mL MeCN under N₂, ligand (544 mg, 2.00 mmol) was added as a solid. The resulting suspension was stirred under nitrogen for 1 h at room temp. The white solid was collected via filtration, and washed with diethyl ether. It was dried under vacuum (~70% yield). m.p 168 °C, [M-Cl]⁺ 605.02. Anal. Calc. for C₃₀H₃₄N₆S₂CuCl : C, 56.14; H, 5.33; N, 13.09; S, 9.99 found: C, 55.99; H, 5.12; N, 12.84; S, 9.55%. IR, cm⁻¹ (KBr disc): 3446br, 3211s, 3028w, 2962m, 2925s, 2854w, 1618vs, 1574w, 1527vs, 1488m, 1344m, 1287w, 1207s, 733s, 694s, 650m (s, strong; m, medium; w, weak; br, broad; vs, very strong). ¹H NMR in DMSO d⁶ 12.10 (s, 1H), 10.63(s, 1H), 8.06-6.93(m, 16H), 4.90(s, 4H), 2.75(q, J=7.5 Hz, 4H), 1.22(t, J=7.5 Hz, 6H)

[Cu(4ETU)(phen)Cl] (**3**): Copper(I) chloride (99 mg, 1.00 mmol) was dissolved in 25 mL MeCN under N₂, ligand (272 mg, 1.00 mmol) was added as a solid. The resulting suspension was stirred under nitrogen for 1 h at room temp. To this was added acetonitrile solution of heterocyclic base [phen (180 mg, 1.00 mmol)]. The reaction mixture was then stirred at room temperature for 2 h with continues purging of N₂. The resulting deep red colored solid that was collected by filtration and washed with Et₂O, and finally dried in vacuum over P₄O₁₀ (~70% yield). m.p 197 °C, [M-Cl]⁺ 514.21. Anal. Calc. for C₂₇H₂₅N₅SCuCl : C, 58.90; H, 4.58; N, 12.72; S, 5.82 found: C, 58.17; H, 4.38; N, 12.65; S, 5.95%. IR (KBr phase, cm⁻¹): 3446b, 3211s, 2962w, 2925m, 2854w, 1618vs, 1527s, 1488m, 1344m, 1207s, 732s, 694m (vs, very strong; s, strong; m, medium; w, weak). ¹H NMR in DMSO d⁶ 12.13(s, 1H), 10.6(s, 1H), 8.33-6.91(m, 16H), 4.89 (s, 2H), 2.58(q, J=7.5 Hz, 2H), 1.16(t, J=7.5 Hz, 3H)

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



Scheme 1: Schematic representation of formation of ligand and Cu(I) complexes

General methods

All compounds were systematically subjected to characterization tools. Elemental analyses of carbon, nitrogen, hydrogen and sulphur were performed with the Elemental varioMICRO V1.9.7 analyzer, copper and chloride performed 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 K α radiation ($\lambda=1.541 \text{ \AA}$). Thermo gravimetric analysis (TGA) was carried out at $10^\circ\text{C min}^{-1}$ heating rate using SDT Q600 V20.9 Build 20 Module DSC-TGA Standard under nitrogen up to 600°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. $\text{Hg}[\text{Co}(\text{NCS})_4]$ was used as a standard. Electrochemical measurements were made at 25°C on an EG&G PAR model 253 VersaStat potentiostat/galvanostat with electrochemical analysis software 270 using a three electrode setup consisting of a glassy carbon working, platinum wire auxiliary and a saturated calomel reference electrode (SCE) in DMF containing 0.1M KCl. The electrochemical data were uncorrected for junction potentials.

IR spectra in the range $400\text{-}4000 \text{ cm}^{-1}$ were recorded using Shimadzu spectrometer in KBr pellets, far IR spectra in the range $50\text{-}400 \text{ cm}^{-1}$ were recorded using Thermo Nicolet 6700 spectrometer in polythene discs. UV and visible absorption spectra of samples were recorded using Shimadzu UV-3010 PC spectrophotometer, full scan mass spectra (MS mode) of samples were recorded using MicroTOF LC Bruker Daltonics spectrometer. ^1H NMR spectra were recorded using Bruker spectrometer (500 MHz) in DMSO d^6 solution using tetramethylsilane (TMS) as internal standard. For stability of complexes, UV spectra were recorded as a function of time $t = 0, 1, 2, 3, 4$ and 5 h . All spectroscopic measurements were performed at 25°C .

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.²¹ The concentration of CT-DNA was measured from the band intensity at 260 nm with a known ϵ value ($6600 \text{ M}^{-1} \text{ cm}^{-1}$).²² Absorption titration measurements were done by varying the concentration of CT-DNA keeping the metal complex concentration constant in 5 mM Tris-HCl/5 mM NaCl buffer (pH 7.2). Samples were kept for equilibrium before recording each spectrum. The intrinsic binding constant (K_b) for the interaction of the complexes with CT-DNA were determined from a plot of $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ vs.

$[\text{DNA}]$ using the absorption spectral titration data using the equation:

$$\frac{(\epsilon_a - \epsilon_f)}{(\epsilon_b - \epsilon_f)} = \frac{\left(b - \left(b^2 - 2K_b C_t \frac{[\text{DNA}]}{s} \right)^{1/2} \right)}{2K_b C_t}$$

where b is $(1 + K_b C_t + K_b [\text{DNA}]/2s)$, 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, $[\text{DNA}]$ 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 6.1.²³

The apparent binding constant (K_{app}) of the complexes were 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.²⁴ 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.

DNA-melting experiments were carried out by monitoring the absorbance of CT-DNA ($200 \mu\text{M}$ NP) at 260 nm with varying temperature in the absence and presence of the complexes in 2:1 ratio of DNA and the complex with a ramp rate of $0.5^\circ\text{C}/\text{min}$ in phosphate buffer (pH 6.85) using a Peltier system attached to the UV-Vis spectrophotometer.

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 $200 \mu\text{M}$ 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. $[\text{complex}]/[\text{DNA}]$, 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), $\eta = (t - t_0)$.

DNA cleavage

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

cleavage of super coiled (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 25 mA. In a typical reaction, super coiled pUC19 DNA (0.2 μ g), 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 super coiled (SC) DNA sample and for the low affinity of EB binding to SC compared to NC and linear forms of DNA.²⁵ The error range observed in determining %NC form from the gel electrophoresis experiments was ± 3 to 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 μ L), and the solution was finally loaded on 0.8% agarose gel containing 1.0 μ g/ml ethidium bromide (EB).

Antibacterial activity

The antibacterial activity was tested against six clinical isolates like *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *K. pneumoniae*. The test organisms were maintained on nutrient agar slants. In-vitro antibacterial activity was determined by the agar well-diffusion method as described by Mukherjee et al.²⁶ The overnight bacterial culture was centrifuged at 8000 rpm for 10 min. The bacterial cells were suspended in saline to make a suspension of 105 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 μ L 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 assay was carried out in triplicate.

RESULTS AND DISCUSSION

A set of two different novel copper (I) complexes were synthesized in high yield through a reaction of CuCl with 4ETU and heterocyclic base phen by the continuous purging of N_2 . The elemental analysis closely corresponded to spectroscopy measurements. Particularly, the purity of compounds is reflected in elemental analysis and in their appropriate spectral patterns. The elemental analysis suggests that the stoichiometry to be 1:2 (metal:ligand) for **2** and 1:1:1(metal:ligand:B) for **3** (B= 1,10 phenanthroline). Presence of copper and chloride is confirmed through the EDAX analysis of complexes.

IR spectra

The IR spectra of the metal complexes clearly indicate the bonding mode of ligand to the metal ion. The prominent IR spectral data is given in Table 1.

In the IR spectra of ligand, a strong intensity band is observed in the range of 740-720 cm^{-1} . This band is shifted towards lower side about 10-20 cm^{-1} which is in the range of 720-680 cm^{-1} in the case of copper(I) complexes. This supports the fact that, ligand coordinate to the metal ion through the sulphur of C=S group in both the complexes.²⁷ Both the complexes exhibited a broad medium intensity band in the 3220-3175 cm^{-1} region, which is ascribed to the $\nu(NH)$ vibration. In the spectrum of ligand this band was observed in the region 3230-3220 cm^{-1} , this supports the fact that, there is no considerable shift in the $\nu(NH)$ vibrations in case of complexes compared to the ligand, indicates non-involvement of NH group in the coordination. The presence of weak band at 470 cm^{-1} corresponding to the $\nu(CuN)$ vibration further confirms the coordination via nitrogen of phen to copper centre in complex **3**.²⁸ Further it is supported by far IR spectra of the complexes display peaks due to $\nu(CuS)$ in the range 180–250 cm^{-1} . The $\nu(CuS)$ at 250 cm^{-1} indicates for Cu(I) in three coordination mode²⁹ of the complex **2** and the $\nu(CuS)$ at 220 cm^{-1} indicates for Cu(I) in four coordination mode of the complex **3**. Both the complexes displays peak due to $\nu(CuCl)$, band ~ 175 cm^{-1} indicates the coordination of Cl to the Cu(I) centre.³⁰

Electronic absorption spectra

The electronic absorption spectra of compounds **1-3** (Table 1) show two intense broad band's 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, whereas the lower energy band can be considered as a thione originating CT transition at the C=S bond.³¹ But they do not register any bands characteristic to Cu (II) ions in the visible region. This confirms the observation that the compounds contain copper in the +1 oxidation state.³² The coordination sphere of the d^{10} Cu (I) in particular is highly labile and easily distorted, with regular and irregular linear, trigonal, and tetrahedral coordination geometries being well-documented.³³ 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** and tetragonal geometry around the copper (I) centre for **3**.

The time dependent stability of **2** and **3** 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 compound **2** in the later part of the time scale³⁴ was observed. The rate of changes in the spectra for the compound **2** and **3** followed an order, **3** > **2**.

Electrochemical Data: The oxidation behavior of the homoleptic and heteroleptic copper(I) complexes was studied by cyclic voltammetry. Both the compounds

undergo a reversible one-electron oxidation process, which can be attributed to the $\text{CuI} \rightarrow \text{CuII}$ couple.³⁵ Relevant measurements are provided in the Table 1. The redox potentials range from 0.15-0.4 V for the homoleptic complex $[\text{Cu}(\text{4ETU})_2\text{Cl}]$ and 0.18-0.6 V for the heteroleptic complex $[\text{Cu}(\text{4ETU})(\text{phen})\text{Cl}]$.

¹H NMR spectra

The proton NMR spectra of the ligand and its diamagnetic Cu(I) complexes were recorded in DMSO d_6 solution using tetramethylsilane (TMS) as internal standard. ¹H NMR chemical shifts of ligand and its diamagnetic Cu(I) complexes were compared with that of parent thiourea, 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 12.14 and 10.6ppm. 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** and leaving the coordination occur through C=S. In complex **3** also N-H proton resonance is not shifted indicating that the coordination through C=S of thiourea and nitrogen of phen to Cu(I) centre.

Molar conductance values of 10^{-3} M solutions are lower than that expected for 1:1 electrolyte in DMF, indicating that the compounds act as non-electrolytes, i.e., the anions are coordinated to the metal ions.³⁶ (Table 1)

Table 1: Physicochemical data of the compounds

Compound	ν^a (NH)	$\nu(\text{C}=\text{S})$	$\nu(\text{CuS})$	$\nu(\text{CuN})$	$\nu(\text{CuCl})$	CV ^b	Λ^c	Electronic spectral data λ_{max} (nm)
1-Benzyl-3-(4-ethyl-pyridin-2-yl)-thiourea	3228	732	---	---	---	---	---	270(1500) 320(4800)
$[\text{Cu}(\text{4ETU})_2\text{Cl}]$	3211	695	250	---	175	0.15-0.4 V	29	255(24500) 315(17500)
$[\text{Cu}(\text{4ETU})(\text{phen})\text{Cl}]$	3176	720	220	470	175	0.18-0.6	34	275(26800) 330(4500)

Note: ^a In KBr phase; ^b Cyclic voltammetry (CV) in DMF-0.1 M TBAP, $E_{1/2} = 0.5(E_{\text{pa}} + E_{\text{pc}})$ for the Cu(I)/Cu(II) couple, $\Delta E_p = (E_{\text{pa}} - E_{\text{pc}})$ at 10 mV s^{-1} , where E_{pa} is anodic and E_{pc} is cathodic peak potential, respectively; ^c Λ_m , molar conductance in DMF medium ($\Omega \text{ cm}^2 \text{ mol}^{-1}$) 25°C.

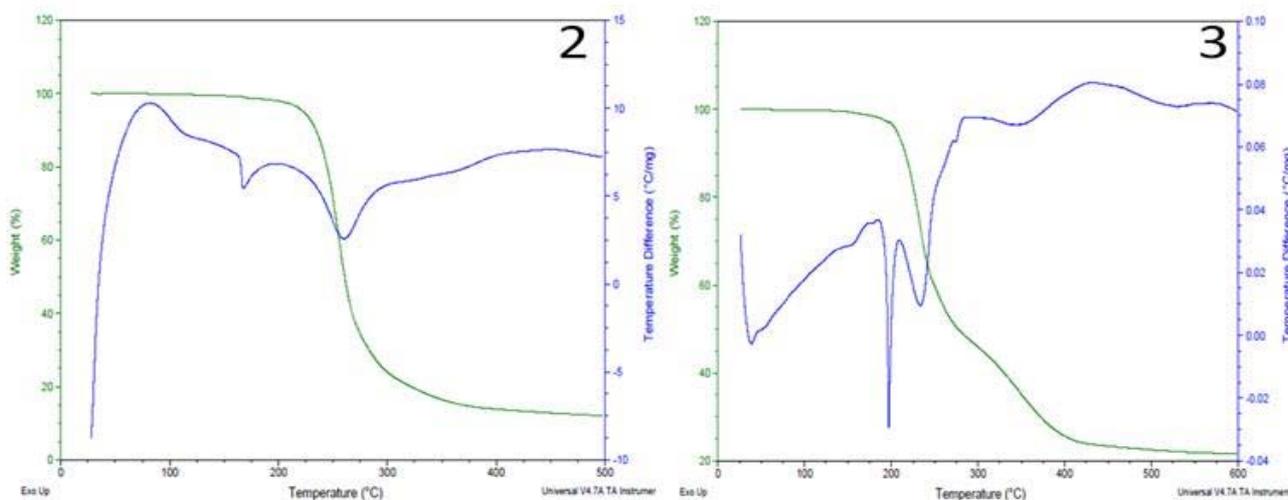


Figure 1: TG and DTA curves of complex **2** & **3**. See analytical characterization section for more details

Table 2: Thermo analytical results for the investigated compounds

Sample	Stage	TG temperature range (°C)	DTA peak (°C)	Weight loss (%)		Evolved moiety
				Found	Calculated	
$[\text{Cu}(\text{4ETU})_2\text{Cl}]$	Single	190-400	260(Endo)	84.60	84.68	ETU(2moles) CuO
	Residue	>450		12.05	12.42	
$[\text{Cu}(\text{4ETU})(\text{phen})\text{Cl}]$	I	190-285	197(Endo)	49.77	49.27	ETU
	II	285-560	234(Endo)	27.9	32.22	Phen
	Residue	>567		21.63	21.0	CuO

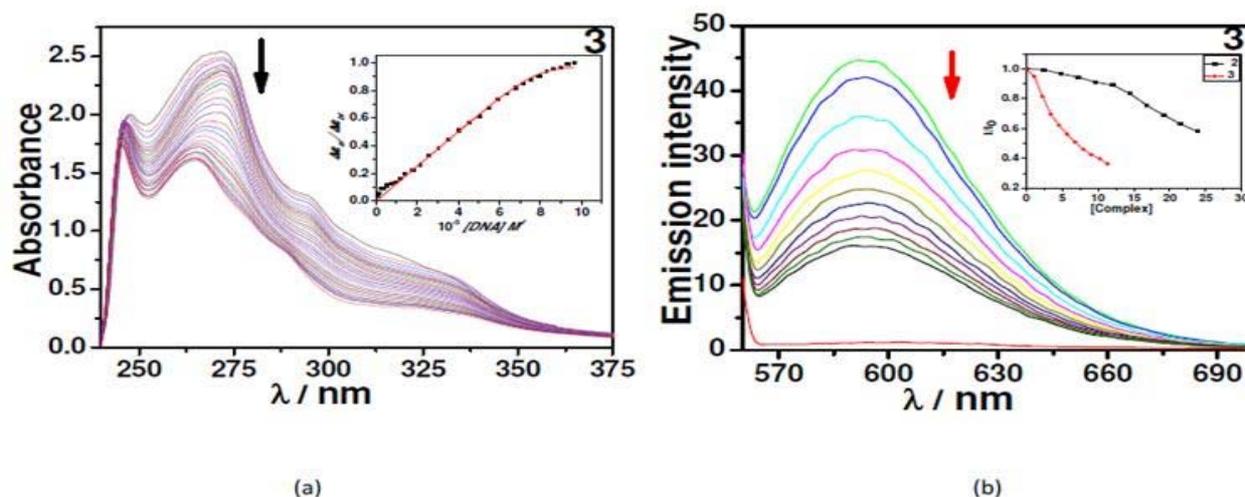


Figure 2: (a) Absorption spectral traces on addition of CT DNA to the solution **3** (shown by arrow). The inset shows the best least-squares fit of $\Delta\epsilon_{af} / \Delta\epsilon_{bf}$ vs. [DNA] of compound **3**. (b) Emission spectral changes on addition of complex **3** to the CT-DNA bound to ethidium bromide (shown by arrow). The inset shows the effect of addition of increasing concentration of the complexes **2** (●) and **3** (■) to an ethidium bromide bound CT-DNA solution in a 5 mM Tris-HCl buffer (pH, 7.2) at 25°C.

TG and DTA

The thermal behavior of 4ETU Copper(I) compound recorded from ambient temperature up to 600°C in nitrogen flow, do not show any loss in weight upto 190°C, reveals that crystal water molecules and coordinated water molecules are not present in the compound.³⁷ The TG curves in the 190–400°C range suggest that the loss in weight for complex **2** corresponds to elimination of 2 molecules of 4ETU ligand with a DTGmax 260°C. The TG curves in the 190–285°C range suggest that the loss in weight for complex **3** corresponds to elimination of 1 molecule of 4ETU ligand with a DTGmax 197°C and 285–560°C range suggest that the loss in weight for 1 molecule of phen with a DTGmax 234°C. In both cases the remaining residues are metal oxides. These results are in accord with the composition of the compound. The TG/DTA diagram for complex **2** & **3** are given in Figure 1. 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 complex **2** and **3** are given in Table 2.

Powder X-ray diffraction

To obtain further evidence about the structure of the metal complexes X-ray diffraction was performed. The diffractograms obtained for the thiourea copper complexes indicate crystalline nature for the complexes.³⁸ 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.

DNA binding properties

The binding of the complexes **2** and **3** with the calf thymus (CT) DNA has been studied by electronic absorption spectral technique. The panoply of absorption signals of the compound **3** as a function of increasing concentration of CT DNA is shown in Figure 2(a). It is clear

from graph that the minor bathochromic shift of 1-3 nm along with significant hypochromicity. K_b and s values for the phen complex, **3** are $1.29(\pm 0.8) \times 10^6$ and $0.39(\pm 0.07)$, respectively. The bis ligand compound does show weak binding to the DNA due to less extended planarity compared to phen, which is consistent with its trend in hypochromism. The relatively higher binding propensity of the phen compound 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 favourable contacts within the groove.^{39,40} 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.⁴¹ The binding of the complexes to DNA decreases the emission intensity of EB. The relative binding propensity of the complexes to DNA is measured from the extent of reduction of the emission intensity Figure 2(b). The apparent binding constant (K_{app}) values for **2** and **3** are 3.61×10^5 and $1.46 \times 10^6 \text{ M}^{-1}$, respectively. The comparatively high DNA binding propensity of the **3** in comparison to complex **2** could be due to the presence of planar aromatic ring facilitating non-covalent interactions with the DNA molecule. 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. The denaturation of DNA from double strand to single strand results in absorption hyperchromism around 260 nm. The binding of metal compound to the double stranded DNA usually stabilizes

the duplex structure to some extent depending on the strength of interaction with nucleic acid.⁴² The binding should lead to an increase in the melting temperature (T_m) of DNA as compared to DNA itself. The binding of the complex **3** results in a moderate increase $\sim 2(\pm 0.1)$ °C in the melting temperature (ΔT_m) of CT-DNA suggesting primarily electrostatic and/or groove binding nature of the compound.

Viscosity measurements of complexes were carried out with solutions of CT-DNA incubated with the complexes. The viscosity of a DNA solution is sensitive to the addition of organic drugs and metal complex 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.⁴³ 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.⁴⁴ The change in relative viscosity for the compound **3** is more than that for the compound **2** suggesting greater DNA binding propensity of the phen complex in comparison to the bis ligand complex, 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.

DNA cleavage activity

DNA cleavage activity of the compounds **1-3** in the presence of hydrogen peroxide has been studied using plasmid supercoiled (SC) pUC19 DNA (33.3 μ M, 0.2 μ g) in 50 mM Tris-HCl buffer/50 mM NaCl (pH 7.2). The extent of DNA cleavage, observed by gel electrophoresis, gives the order: **3** > **2** > **1**. A 30 μ M concentration of **3** completely cleaves SC DNA into its nicked circular (NC) form on reaction with 200 μ M H_2O_2 (Figure 3, Table 3). The DNA cleavage activity order for **1-3** 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 to be analogous to those proposed by Sigman and co-workers for the chemical nuclease activity of bis(phen)copper species.^{1,40}

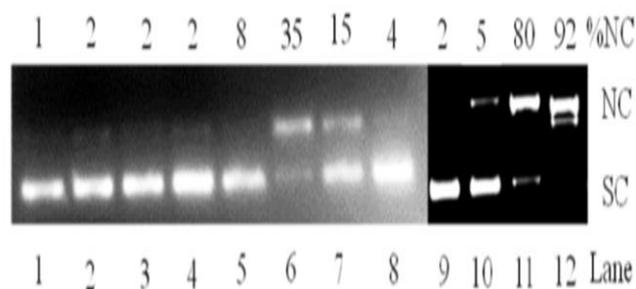


Figure 3: Gel electrophoresis diagram showing the oxidative cleavage of SC pUC19 DNA (33.3 μ M) by the compounds **1-3** in the presence of H_2O_2 (176 μ M) in 50 mM Tris-HCl/NaCl buffer (pH, 7.2) containing 10% DMF. Reaction details are given in table 3 in which the entry number corresponds to the lane number of this figure.

Table 3: Selected cleavage data of SC DNA pUC19 by complexes

Condition	SC%	NC%
DNA control	99	1
DNA + H_2O_2 (2 μ M)	98	2
DNA + CuCl(50 μ M)	98	2
DNA + Ligand (100 μ M)	96	4
DNA + Ligand(100 μ M) + H_2O_2	94	6
DNA + 2(100 μ M) + H_2O_2	65	35
DNA + 2(100 μ M)	85	15
DNA + 3(30 μ M)	96	4
DNA + 3(30 μ M) + H_2O_2 + DMSO	98	2
DNA + 3(30 μ M) + H_2O_2 + KI	95	5
DNA + 3(30 μ M) + H_2O_2 + NaN ₃	20	80
DNA + 3(30 μ M) + H_2O_2	8	92

Antibacterial activity

For the bacterial organisms, both Gram-positive and Gram-negative bacteria were used. 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 activities. In-vitro antibacterial activity was determined by the agar well-diffusion method. The percentage zone of inhibition data are represented in Table 4. The data reveals that the compounds **2** and **3** from this novel series were found to be highly active. Notably, they exhibited much more antibacterial activity than the standard drug ciprofloxacin, while compound **1** has comparable potency against Gram-positive bacteria and is inactive against 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.

Table 4: Antibacterial activity of the compounds 1-3: zone of inhibition (in %)

Compounds	Gram-positive			Gram-negative		
	<i>S. aureus</i> (MTCC 3160)	<i>M. luteus</i> (MTCC 106)	<i>B. subtilis</i> (MTCC 441)	<i>E. coli</i> (MTCC 443)	<i>P. aeruginosa</i> (MTCC 741)	<i>K. pneumoniae</i> (MTCC 109)
1	-	07	08	-	-	-
2	12	15	15	12	14	14
3	25	26	27	22	24	22

CONCLUSION

A study of two different Copper (I) complexes associated to heterocyclic based ligands has been explored. Amongst the metal complexes explored, the phen complex displays significant binding propensity with CT DNA providing an order: **3** >> **2**. The complex **2** does not show any measurable binding interaction to the DNA and hence its poor cleavage efficiency. Complex **3** shows 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. Complexes show a profound antibacterial activity against Gram-negative bacteria *Bacillus subtilis*.

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