

Effect of copper–sulphur bond on the DNA photo-cleavage activity of 2-(methylthio)ethylpyridine-2-carbaldimine copper(II) complexes

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Abstract. The binding and photo-induced DNA cleavage activity of a binary complex $[\text{CuL}_2](\text{ClO}_4)_2$ (**1**) and the *in situ* generated ternary complexes $[\text{CuLB}](\text{ClO}_4)_2$ from **1** (B: 1,10-phenanthroline, phen, **2**; dipyrido[3,2-*d*: 2',3'-*f*]quinoxaline, dpq, **3**) are studied, where L is a N_2S -donor tridentate Schiff base 2-(methylthio)ethylpyridine-2-carbaldimine. Complex **1**, structurally characterized by X-ray diffraction study, has six-coordinate meridional geometry showing CuN_4S_2 coordination. The Cu–N bond lengths are in the range of 1.968(3) to 2.158(4) Å. The Cu–S bond lengths of 2.599(2) and 2.705(2) Å are significantly long indicating weak covalent interaction between copper and sulphur atoms. The thiomethyl groups are *cis* to each other giving S–Cu–S angle of 75.82(5)°. The Cu–N(pyridyl) bond distances are longer than the Cu–N(imine) bonds. The complexes are redox active and display a quasi-reversible cyclic voltammetric response assignable to the Cu(II)/Cu(I) couple near 0.0 V vs SCE in DMF-Tris buffer (1 : 4 v/v) using 0.1 M KCl as supporting electrolyte. Electronic spectra of the complexes show a *d–d* band in the range 630 to 700 nm in DMF along with higher energy charge transfer bands. While complex **1** is a poor binder to DNA, the ternary complexes show good DNA binding propensity. The photo-nuclease activity of **1–3** is studied using UV and visible wavelengths. The DNA cleavage activity at 365 nm follows the order: **3** > **2** > **1**. The cleavage reaction involves the formation of singlet oxygen as the reactive species in a type-II process.

Keywords. DNA photo-cleavage; copper(II) complexes; Schiff base; crystal structure; phenanthroline bases.

1. Introduction

DNA cleavage activity of transition metal complexes under physiological conditions has gained considerable current interest for varied applications of such complexes in nucleic acids chemistry.^{1–15} DNA can be cleaved by hydrolytic and oxidative pathways. The hydrolytic process involves cleavage of the phosphodiester bond, leading to the formation of fragments that could be religated by enzymatic processes. The oxidative cleavage of DNA involves nucleobase oxidation and/or degradation of sugar by abstraction of deoxyribose hydrogen atom(s). Such cleavage can occur by chemical or photochemical means. The photo-induced oxidative DNA cleavage has advantages in therapeutic applications as only a source of light is used in this process instead of any external additives like reducing or oxidizing agents that are necessary for observing “chemical nuclease” acti-

ty. The photo-cleavage of DNA with visible light irradiation is of importance in photodynamic therapy (PDT) of cancer.^{16–20} Photofrin[®] which is a currently used PDT drug for lung and esophageal cancers is a mixture of hematoporphyrin and its derivatives. This drug is active on laser irradiation at 630 nm to generate singlet oxygen as the reactive species. Among non-porphyrinic species, DNA-binding and photo-cleavage activities of polypyridyl 4*d*- and 5*d*-metal complexes are studied in detail.^{21–26} In contrast, the chemistry of copper-based photo-nucleases has remained relatively unexplored.²⁷ Copper being a bio-essential element, its complexes find more applications in nucleic acid chemistry as compared to the heavier transition elements.

We have recently shown that non-porphyrinic binary and ternary copper(II) complexes are efficient photo-cleavers of DNA on UV or visible light irradiation.^{28–36} The ligands or metal salts alone are found to be cleavage-inactive. However, when a photosensitizing ligand and a DNA binder are covalently bonded to a copper(II) centre, the complex becomes

Dedicated to the memory of the late Professor Bhaskar G Maiya

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cleavage-active. Mechanistic studies have revealed that metal-assisted light-induced DNA cleavage involves photo-excitation of the charge transfer and $d-d$ bands.²⁹ We have also observed that sulphur-containing ligands act as photosensitizers and the photosensitizing effect is greater when the sulphur is bound to the metal centre and the concerned moiety lacks p -conjugation. The choice of sulphur-containing Schiff base ligand is based on the fact that thio or thione moieties are known to show efficient inter-system crossing to the triplet state on photo-irradiation.^{37–39} Excited states with longer lifetimes can cause oxidative damage of DNA directly by a type-I process or can activate molecular oxygen from its stable triplet state ($^3\text{O}_2$) to the cytotoxic singlet state ($^1\text{O}_2$) in a type-II process.⁴

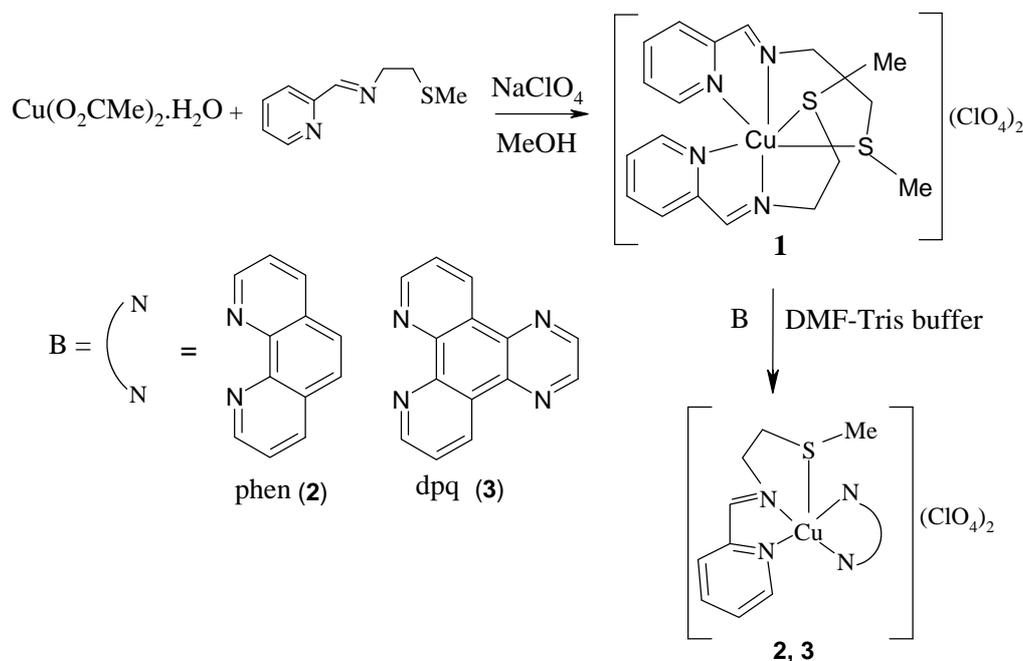
Design of the metal complex as a photo-cleaver of DNA necessitates the presence of a photosensitizer and a DNA binder. Here, we report the synthesis and crystal structure of a binary complex $[\text{CuL}_2](\text{ClO}_4)$ (**1**), with a photosensitizable sulphur-containing tridentate Schiff-base ligand 2-(methylthio)ethylpyridine-2-carbaldehyde (L) (scheme 1). To incorporate a DNA binder, we have prepared two ternary complexes of formulation $[\text{CuLB}](\text{ClO}_4)_2$, where B is a heterocyclic base, viz. 1,10-phenanthroline (phen, **2**) and dipyrido[3,2-d : 2',3'-f]quinoxaline (dpq, **3**). Copper complexes of phenanthroline bases like phen

and dpq are known to bind DNA efficiently.^{40–42} The dpq ligand in **3** is a photosensitizer as DNA intercalator quinoxalines are known to cleave DNA at 365 nm by the photo-excited $^3(n-p^*)$ and/or $^3(p-p^*)$ states in the absence of any external additives.⁴³ The significant result of the present study is the light-induced DNA cleavage activity of the complexes at 365 nm. The Cu–S bond length in **1** is found to be an important factor in promoting metal-assisted light-induced DNA cleavage activity.

2. Experimental

2.1 Materials

All reagents and chemicals were purchased from commercial sources and used without further purification. Solvents used for electrochemical and spectroscopic studies were purified and dried by standard procedures before use. The supercoiled (SC) pUC19 DNA (caesium chloride purified) was purchased from Bangalore Genie (India). The calf thymus (CT) DNA, agarose (molecular biology grade) and ethidium bromide (EB) were obtained from Sigma. Tris (hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer was prepared using deionized and sonicated triple-distilled water. The ligand L was prepared by



Scheme 1. The reaction pathways for the synthesis of the binary complex $[\text{CuL}_2](\text{ClO}_4)$ (**1**) and *in situ* generation of the ternary complexes $[\text{CuLB}](\text{ClO}_4)_2$ (B: phen, **2**; dpq, **3**).

Schiff-base condensation using pyridine-2-carboxaldehyde and 2-mercaptoethylamine followed by methylation with methyl iodide in presence of sodium methoxide. Dipyrido[3,2-*d*: 2',3'-*f*]quinoxaline (dpq) was prepared by a literature procedure.⁴⁴

2.2 Physical measurements

Elemental analysis was performed using a Thermo Finnigan Flash EA 1112 CHNSO analyser. Infrared and electronic spectra were recorded on Perkin–Elmer Spectrum One and Perkin–Elmer Lambda 35 spectrophotometers respectively. Molar conductivity measurements were made using a Control Dynamics (India) conductivity-meter. Electrochemical measurements were carried out at 25°C on an EG&G PAR model 253 Versa Stat potentiostat/galvanostat with electrochemical analysis software 270 using a three-electrode set up comprising a glassy carbon working electrode, platinum wire auxiliary and saturated calomel reference electrode (SCE). KCl (0.1 M) was used as a supporting electrolyte in DMF–Tris buffer medium (1 : 4 *v/v*). Room temperature magnetic susceptibility data were obtained with a model 300 Lewis coil-force magnetometer of George Associates Inc. (Berkeley, USA) make. Susceptibility data were corrected for diamagnetic contribution.⁴⁵

2.3 Synthesis of [CuL₂](ClO₄)₂ (I)

The complex was prepared by reacting 0.5 g (2.8 mmol) quantity of the Schiff base (L) with 0.2 g (1.0 mmol) copper(II) acetate-hydrate, in 25 ml methanol under stirring for 1 h at 25°C. On completion of the reaction, a methanolic solution of sodium perchlorate (0.3 g, 2.5 mmol) was added. The product was obtained as a dark green solid on slow concentration of the solution. The solid was isolated, washed with diethyl ether and finally dried in vacuum over P₄O₁₀ (yield: 78%).

Analysis: Calc. for C₁₈H₂₄N₄CuO₈S₂Cl₂: C, 34.72; H, 3.97; N, 9.03%. Found: C, 34.86; H, 4.23; N, 8.85%. IR (KBr phase, cm⁻¹): 3484*br*, 3033*m*, 1646*m*, 1600*s*, 1569*w*, 1475*w*, 1473*m*, 1445*m*, 1375*w*, 1302*m*, 1248*w*, 1143*s*, 1089*vs*, 1021*w*, 776*s*, 636*s*, 626*s*, 506*m*, 418*m* (*br*, broad; *w*, weak; *m*, medium; *s*, strong; *vs*, very strong). $\mu_{\text{eff}} = 1.9 \mu_{\text{B}}$ at 300 K. $\Lambda_{\text{M}} = 156 \Omega^{-1} \text{cm}^2 \text{M}^{-1}$ at 25°C in DMF (complex = 10⁻³ M). The complex was found to be soluble in polar organic solvents and less soluble in water.

Caution! Perchlorate salts of metal complexes containing organic ligands are potentially explosive

and small quantities should be taken at a time and handled with care.

2.4 X-ray crystallographic procedures

Single crystals of complex **1** were obtained by a diffusion technique in which an acetonitrile solution of the complex was layered with diethyl ether. The unit cell parameters and the intensity data of a crystal mounted on a glass fiber using epoxy cement were obtained using a Bruker SMART APEX CCD diffractometer, equipped with a fine focus 1.75 kW sealed tube Mo-K_α X-ray source, with increasing ω (width 0.3 deg frame⁻¹) at a scan speed of 15 s frame⁻¹. The SMART software was used for data acquisition and the SAINT software for data abstraction. The data were corrected for absorption.⁴⁶ Structure solution and refinement were done using SHELX system of programs.⁴⁷ Hydrogen atoms were placed at their calculated positions and refined using a riding model. The non-hydrogen atoms were refined anisotropically. Crystal data and structure refinement parameters are given in table 1. Perspective view of the complex was obtained using ORTEP.⁴⁸ Detailed crystallographic data in the CIF format have been deposited with the Cambridge Crystallographic Data Center, CCDC No. 256343. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: <http://www.ccdc/cam.ac.uk>).

2.5 DNA binding experiments

The experiments were carried out in Tris–HCl buffer (50 mM, pH 7.2) using a solution of calf thymus (CT) DNA which gave a ratio of UV absorbance at 260 and 280 nm in the range 1.8 to 1.9 : 1 indicating that the DNA is sufficiently free from protein.⁴⁹ The CT DNA concentration was determined by absorption spectroscopy using the ϵ value of 6600 M⁻¹ cm⁻¹ at 260 nm.⁵⁰ Absorption titration experiments were carried out with a complex concentration of 50 μM , varying the CT DNA concentration from 80 to 720 μM . The intrinsic binding constant K_b was determined from the [DNA]/($\epsilon_a - \epsilon_f$) vs. [DNA] plot using the relation: $[\text{DNA}]/(\epsilon_a - \epsilon_f) = [\text{DNA}]/(\epsilon_b - \epsilon_f) + 1/K_b(\epsilon_b - \epsilon_f)$, where [DNA] is the concentration of DNA. The absorption coefficients ϵ_a , ϵ_f and ϵ_b correspond to apparent absorption coefficient of the

complex, extinction coefficient of the free copper complex and extinction coefficient of the copper(II) complex in the completely DNA bound form respectively.

Relative binding of the binary and ternary complexes was also studied by the fluorescence spectral method using ethidium bromide (EB) bound CT DNA solution.⁵¹ The fluorescence intensities at 600 nm (546 nm excitation) of EB with increase of the complex concentration were recorded at 25°C. EB was non-emissive in the buffer medium due to the fluorescence-quenching effect of the solvent molecules. In presence of DNA, EB showed enhanced emission due to intercalative binding to DNA. Competitive binding of the copper complex to CT DNA resulted in the displacement of bound EB or quenching of its emission thus reducing the emission intensity. The apparent binding constant (K_{app}) was calculated from the relation: $K_{EB}[EB] = K_{app}[\text{complex}]$, where

$K_{EB} = 1.0 \times 10^7 \text{ M}^{-1}$ and the concentration of EB is 1.3 mM.⁵¹

2.6 DNA cleavage experiments

The extent of cleavage of supercoiled (SC) pUC19 DNA (0.8 mL, 0.5 mg) to its nicked circular (NC) form was determined by agarose gel electrophoresis in Tris-HCl buffer (50 mM, pH 7.2) containing NaCl (50 mM). In the cleavage reactions, the 33 mM complexes in 18 mL buffer were photo-irradiated using monochromatic UV or visible light. The samples were then incubated for 1 h at 37°C followed by addition to the loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol (3 mL) and finally loaded on 0.8% agarose gel containing 1.0 mg/mL ethidium bromide. Electrophoresis was carried out at 40 V for 2 h in Tris-acetate EDTA (TAE) buffer. Bands were visualized by UV light and photographed to determine the extent of DNA cleavage from the intensities of the bands using UVITEC Gel Documentation System. Due corrections were made for the trace of NC DNA present in the SC DNA sample and for the low affinity of EB binding to SC DNA in comparison to the NC form.⁴⁴ The wavelengths used for the photo-induced DNA cleavage experiments were 365 nm (12 W) and low powered CW He-Ne laser of 632.8 nm (Scientifica-Cook make, UK, 3 mW) in the absence of any external additives. Control experiments were done for mechanistic investigations using different reagents that were added to DNA prior to the addition of the complex.

Table 1. Selected crystallographic data and structure refinement for **1**.

Empirical formula	C ₁₈ H ₂₄ Cl ₂ CuN ₄ O ₈ S ₂
Formula weight (g mol ⁻¹)	622.97
Temperature (K)	293(2)
<i>I</i> (Å) (Mo-K _α)	0.71073
Crystal system	Orthorhombic
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁ (no. 19)
<i>a</i> (Å)	9.963(6)
<i>b</i> (Å)	14.014(9)
<i>c</i> (Å)	18.866(12)
<i>α</i> = <i>β</i> = <i>γ</i> (°)	90.00
<i>V</i> (Å ³)	2634(3)
<i>Z</i>	4
<i>r</i> _{calc} (g cm ⁻³)	1.571
<i>m</i> (Mo-K _α) (m ⁻¹)	12.39
Crystal size (mm)	0.40 × 0.19 × 0.12
<i>F</i> (000)	1276
Theta range for data collection	1.81 to 26.06°
Index ranges	-12 ≤ <i>h</i> ≤ 11, -17 ≤ <i>k</i> ≤ 17, -22 ≤ <i>l</i> ≤ 22
Refinement method	Full-matrix least-squares on <i>F</i> ²
Data/restraints/parameters	5131/0/316
Goodness-of-fit on <i>F</i> ²	1.0301
<i>R</i> 1 ^a [<i>I</i> > 2 <i>s</i> (<i>I</i>)]	0.0458
<i>R</i> 1 [all data]	0.0504
<i>wR</i> 2 ^b [<i>I</i> > 2 <i>s</i> (<i>I</i>)]	0.1295
<i>wR</i> 2 [all data]	0.1348

^a*R*1 = $\sum ||F_o| - |F_c|| / \sum |F_o|$, ^b*wR*2 = $\{ \sum [w(F_o^2 - F_c^2)^2 / \sum [w(F_o^2)^2]]^{1/2} \}$, where $w = 1 / [s^2(F_o^2) + (AP)^2 + BP]$ where $P = [\max(F_o^2, 0) + 2F_c^2] / 3$, A and B values are 0.1084 and 0.0000 respectively

3. Results and discussion

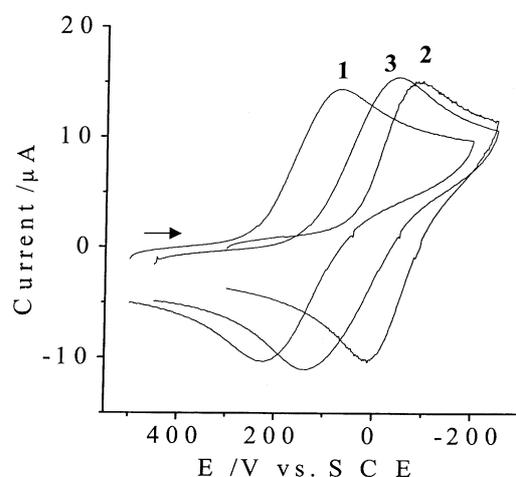
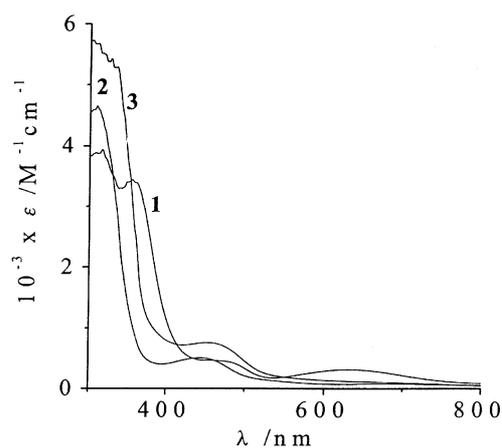
3.1 Synthesis and general aspects

The binary complex [CuL₂](ClO₄)₂ (**1**), prepared in high yield from the reaction of copper(II) acetate with the Schiff base and subsequently isolated as a perchlorate salt, is characterized from analytical and physicochemical data (table 2, scheme 1). The molecular structure of this complex is obtained from X-ray diffraction study. Two ternary complexes of formulation [CuLB](ClO₄)₂ (B: phen, **2**; dpq, **3**) having phenanthroline bases are prepared using **1** as a precursor under *in situ* reaction conditions for DNA binding and cleavage studies. Complexes **2** and **3** having phen and dpq ligands are expected to show efficient DNA binding and cleavage properties, as

Table 2. Physicochemical data on $[\text{CuL}_2](\text{ClO}_4)_2$ (**1**) and $[\text{CuLB}](\text{ClO}_4)_2$ (B = phen, **2**; dpq, **3**).

Complex	I_{max} (nm) [ϵ ($\text{M}^{-1} \text{cm}^{-1}$)] ^a		Cu(II)/Cu(I) couple ^b			
	CT band	<i>d</i> – <i>d</i> band	$E_{1/2}$ (V) [ΔE_p (mV)]	i_{pa}/i_{pc}	K_b^c (M^{-1})	K_{app}^d (M^{-1})
1	356 (1720), 469 (230)	631 (160)	0.15 (150)	0.9	3.1×10^2	7.0×10^3
2	257 (4660), 443 (500)	679 (75)	–0.05 (75)	0.7	1.1×10^3	1.6×10^4
3	256 (5930), 301 (5880), 454 (790)	696 (90)	0.046 (180)	0.6	5.6×10^3	5.4×10^5

^aIn DMF. ^bIn DMF–Tris buffer (1 : 4 v/v) using 0.1 M KCl as supporting electrolyte. $E_{1/2} = 0.5(E_{pa} + E_{pc})$, $\Delta E_p = E_{pa} - E_{pc}$, where E_{pa} and E_{pc} are anodic and cathodic peak potentials, respectively. Scan rate: 50 mVs^{-1} . i_{pc} and i_{pa} are cathodic and anodic peak currents respectively. ^cIntrinsic CT DNA binding constant by absorption spectroscopic method. ^dApparent CT DNA binding constant from ethidium bromide displacement assay

**Figure 1.** Cyclic voltammograms of **1–3** in DMF–Tris buffer (1 : 4, pH 7.2)/KCl (0.1 M) at a scan speed of 50 mVs^{-1} .**Figure 2.** Electronic spectra of **1–3** in DMF.

the presence of such a planar heterocyclic base in transition metal complexes is known to enhance their DNA cleavage activity.^{1,2}

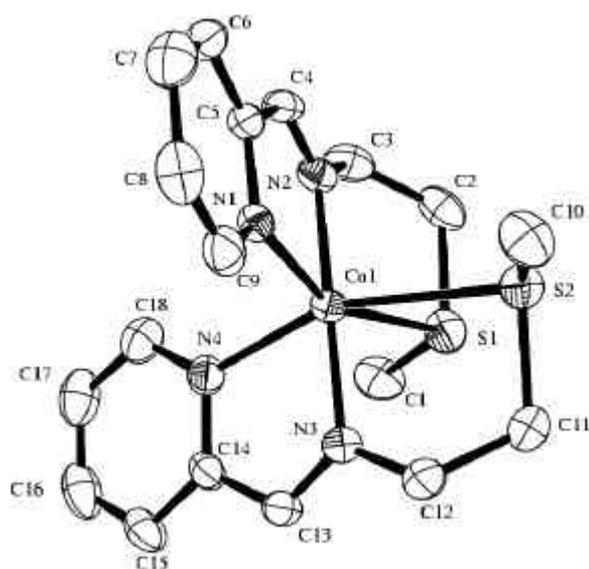
Complexes **1–3** are redox active and display a quasi-reversible cyclic voltammetric response near 0.0 V vs SCE in DMF–Tris buffer (1 : 4 v/v)/0.1 M KCl (figure 1). The redox process is assignable to the Cu(II)/Cu(I) couple. The $E_{1/2}$ value varies in the order: **1** > **3** > **2**. Complex **1** with two sulphur-containing Schiff-bases stabilises the copper(I) state more than the ternary structures. Again, the dpq complex **3** with extended aromatic ring stabilises the reduced state more than its phen analogue. We have not observed any formation of binary species $[\text{CuB}_2]^{2+}$ during the generation of the ternary complexes in cyclic voltammetric studies when **1** (1.0 mmol in 5 ml DMF–Tris buffer) is reacted with the heterocyclic base (B) in a 1 : 1 molar ratio. The electronic spectral data of the complexes in DMF show the presence of a *d*–*d* band in the range 630 to 700 nm (figure 2). Complexes **2** and **3** display significantly lower energy *d*–*d* bands than **1**, possibly due to the structural differences between the binary and ternary complexes (scheme 1). The *d*–*d* band being in the PDT window of 600–800 nm makes these complexes potential systems to explore the DNA cleavage activity on red-light irradiation. The complexes also exhibit charge transfer bands near 450 nm which could be assigned to the sulphur to copper(II) charge transfer LMCT band.

3.2 Crystal structure

Complex $[\text{CuL}_2](\text{ClO}_4)_2$ (**1**) has been structurally characterized by single crystal X-ray crystallography. An ORTEP view of the cationic complex is shown in figure 3 and selected bond distances and angles are given in table 3. The complex crystallizes in the orthorhombic space group $P2_12_12_1$ with four molecules in the unit cell. The crystallographic asymmetric unit consists of the mononuclear copper(II) complex

Table 3. Selected bond lengths (Å) and angles (°) for **1**.

Cu(1)–N(1)	2.143(4)	N(2)–Cu(1)–S(2)	98.88(11)
Cu(1)–N(2)	1.968(3)	N(3)–Cu(1)–N(2)	177.37(14)
Cu(1)–N(3)	1.953(3)	N(3)–Cu(1)–N(1)	102.86(14)
Cu(1)–N(4)	2.158(4)	N(3)–Cu(1)–N(4)	78.74(15)
Cu(1)–S(1)	2.599(2)	N(3)–Cu(1)–S(1)	94.47(11)
Cu(1)–S(2)	2.7051(17)	N(3)–Cu(1)–S(2)	81.78(11)
N(1)–Cu(1)–N(4)	102.62(13)	N(4)–Cu(1)–S(1)	95.84(10)
N(1)–Cu(1)–S(1)	156.74(9)	N(4)–Cu(1)–S(2)	158.13(10)
N(1)–Cu(1)–S(2)	91.27(9)	S(1)–Cu(1)–S(2)	75.82(5)
N(2)–Cu(1)–N(1)	79.69(13)		
N(2)–Cu(1)–N(4)	100.16(14)		
N(2)–Cu(1)–S(1)	83.25(11)		

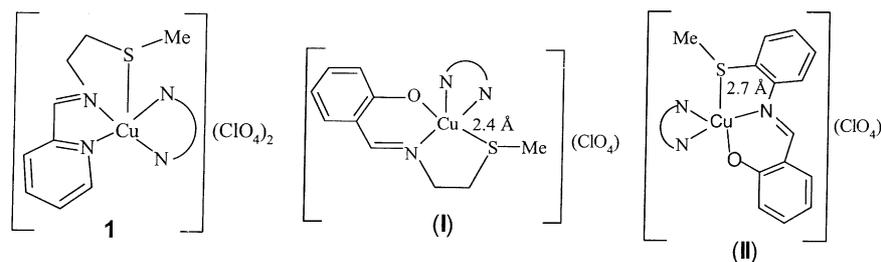
**Figure 3.** ORTEP diagram of the cationic complex in **1** showing atom-numbering scheme and thermal ellipsoids at 50% probability level.

and two lattice perchlorate anions. In the binary structure, the copper atom is bonded to two Schiff-base ligands each showing tridentate chelating N_2S -binding mode giving a six-coordinate meridional pseudo-octahedral structure. The thiomethyl moieties are *cis* to each other. The Cu–S bonds of 2.6 and 2.7 Å are unusually long when compared to known ternary copper(II) complexes having similar metal-bound thiomethyl moiety bound at the basal plane like in structure (**I**) (scheme 2).^{29,31,52} This bond is of particular importance as a shorter Cu–S bond length is expected to show enhanced photosensitization effect leading to higher metal-assisted DNA photocleavage activity. It is likely that in presence of strong Cu–N (pyridine) and Cu–N (imine) bonds of ≈ 2.15 and

1.96 Å, the Cu–S bond becomes long due to steric constraints imposed by two 5-membered rings of the {CuL} unit. In comparison, the presence of six-member rings in structures (**I**) and (**II**) of the {Cu (salicylaldimine moiety)} reduces the steric strain considerably in (**I**) and (**II**) (scheme 2). The long Cu–S bond of ~ 2.7 Å in (**II**) is due to the presence of sulphur at the elongated axial site of the five coordinate structure. In **1**, the imine nitrogen atoms are essentially *trans* to each other. The N(1)–Cu(1)–S(1) and N(4)–Cu(1)–S(2) angles of $\approx 157^\circ$ deviate significantly from the *trans* geometrical arrangement. This is again due to the steric constraints of the five-membered chelate rings. The structural features of **1** suggest this complex to be sterically unstable and we are able to generate the ternary complexes **2** and **3** on stoichiometric reactions using N,N-donor heterocyclic bases phen and dpq.

2.3 DNA binding property

Absorption and fluorescence spectral techniques have been used to determine the relative binding propensity of the complexes to CT DNA (table 2, figure 4). The intrinsic binding constant values, obtained by absorption spectral method, vary as: **3** > **2** > **1**. The dpq complex with its extended aromatic quinoxaline ring shows efficient DNA-binding ability. The phen complex is relatively a poor binder to DNA. Earlier studies on *bis*-phen or *bis*-dpq copper complexes have shown that the complexes bind DNA in the minor groove.^{30,40–42} A similar mode of binding is expected for complexes **2** and **3**. Complex **1** shows poor DNA binding propensity due to the absence of any planar ligand moieties for intercalative and/or groove binding to DNA. The dicationic complexes are, in addition, expected to have electrostatic inter-



Scheme 2. A comparison of the structural features in **1** and reported ternary copper(II) complexes **(I)** and **(II)** having similar metal-bound thiomethyl moiety.

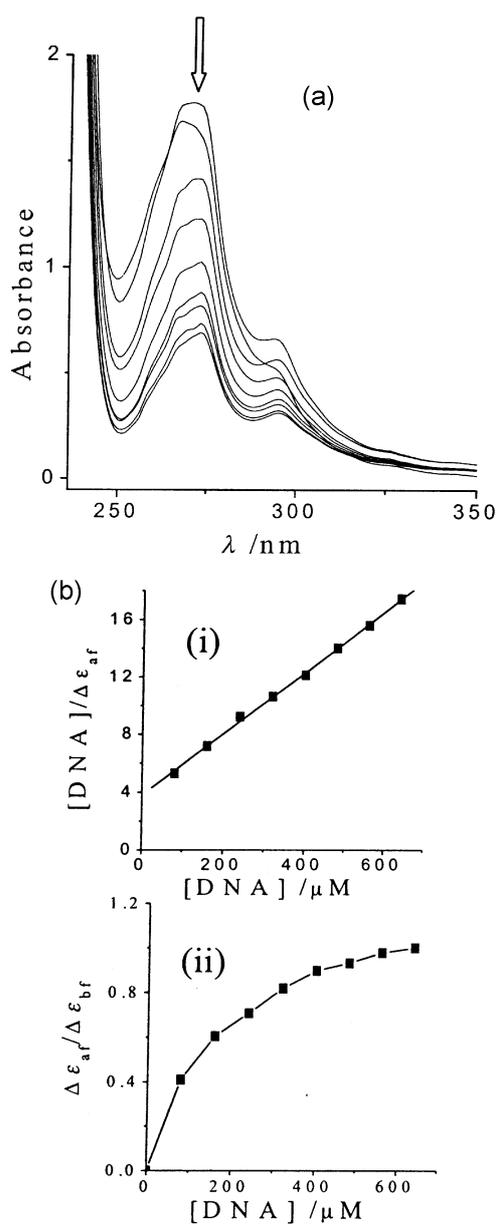


Figure 4. (a) Absorption spectral changes on addition of CT DNA to the solution of **3** (shown by arrow). (b) Plots of $[DNA]/(\Delta\epsilon_{af})$ vs $[DNA]$ (i) and $\Delta\epsilon_{af}/\Delta\epsilon_{bf}$ vs $[DNA]$ (ii), where $\Delta\epsilon_{af} = (e_a - e_f)$ and $\Delta\epsilon_{bf} = (e_b - e_f)$.

action with the double-stranded DNA. The apparent binding constant values of the complexes, obtained from ethidium bromide displacement assay by fluorescence spectral method, show similar binding order as observed from the absorption spectral studies.

2.4 DNA cleavage activity

The oxidative DNA cleavage activity of the complexes was studied by gel electrophoresis using supercoiled (SC) pUC19 DNA (0.5 mg) in Tris-HCl buffer (pH, 7.2). Selected DNA cleavage data are given in table 4 and the gel diagrams are shown in figure 5. The complexes **1–3** (33 nM in 18 ml volume) show 46, 68 and 71% cleavage of the SC DNA respectively, on 1 h exposure at 365 nm. The greater cleavage efficiency of the ternary complexes compared to that of the binary complex **1** is due to their efficient DNA-binding ability. Control experiments using Schiff base or the dpq ligand alone does not show any significant cleavage of SC DNA even on longer exposure time. The results indicate the important role of metal in these photo-induced DNA cleavage reactions. The complexes show the presence of a charge-transfer band near 450 nm. It is likely that the photo-cleavage at 365 nm involves photo-excitation of the charge-transfer band leading to the formation of an excited singlet state that through the triplet state activates molecular oxygen to form reactive singlet oxygen species. Control experiments show that the singlet oxygen quencher sodium azide significantly inhibits the cleavage reaction, while the hydroxyl radical scavenger DMSO has no apparent effect on the cleavage process. The formation of singlet oxygen is further supported by the enhancement of percent SC DNA cleavage in D₂O solvent in which singlet oxygen is known to have longer triplet state life time.⁵³ The oxidative DNA cleavage by singlet oxygen is likely to proceed via oxidation of guanine nucleobase.^{2,54} The cleavage activity of **2**

Table 4. Selected DNA (SC pUC19 DNA, 0.5 mg) cleavage data^a of complexes 1–3 in Tris-buffer (pH 7.2).

Reaction conditions	<i>I</i> (nm)	<i>t</i> (h)	[Complex] (mM)	Form-I (%)	Form-II (%)
DNA control	365	1.5	–	93	7
DNA + 1	365	1.0	33	54	46
DNA + 2	365	1.0	33	32	68
DNA + 3	Dark	1.5	–	91	9
DNA + 3	365	1.0	33	29	71
DNA + 3 in D ₂ O (14 mL)	365	1.0	33	10	90
DNA + NaN ₃ (38 mM) + 3	365	1.0	33	89	11
DNA + DMSO (4 mL) + 3	365	1.0	33	34	66
DNA + L (66 mM)	356	1.0	–	92	8
DNA + dpq (33 mM)	365	1.5	–	88	12
DNA control	632.8	1.0	–	98	2
DNA + 1	632.8	1.0	33	97	3
DNA + 2	632.8	1.0	33	95	5
DNA + 3	632.8	1.0	33	77	23

^a*I*, excitation wavelength; *t*, exposure time; form-I and form-II are SC and NC forms of DNA respectively

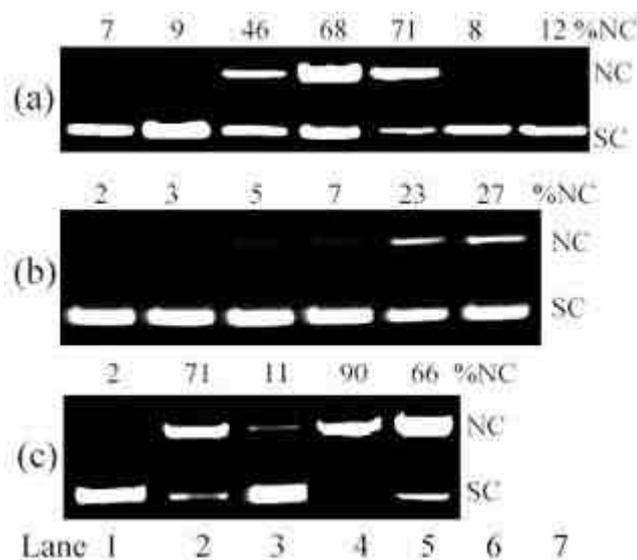


Figure 5. (a) Gel diagram showing the photo-cleavage of SC pUC19 DNA (0.5 mg) by 1–3 (33 mM) in DMF–Tris buffer medium on 1 h exposure at 365 nm. Complexes 2 and 3 are *in situ* generated by reacting 1 with the bases phen or dpq in a 1 : 1 molar ratio. Lane 1, DNA control (1 h); lane 2, DNA + 3 (in dark); lane 3, DNA + 1; lane 4, DNA + 2; lane 5, DNA + 3; lane 6, DNA + L (66 mM); lane 7, DNA + dpq (33 mM). (b) Gel diagram showing the photo-cleavage of SC pUC19 DNA (0.5 mg) by 1–3 (17 mM) in DMF–Tris buffer medium on irradiation with CW He–Ne laser of 632.8 nm wavelength. Lane 1, DNA control (1 h); lane 2, DNA + 1 (1 h); lane 3, DNA + 2 (1 h); lane 4, DNA + 2 (2 h); lane 5, DNA + 3 (1 h); lane 6, DNA + 3 (2 h). (c) Gel electrophoresis diagram of the control experiments using SC DNA (0.5 mg), 3 (33 mM), and other additives at 365 nm for an exposure time of 1 h. Lane 1, DNA Control; lane 2, DNA + 3; lane 3, DNA + NaN₃ (38 mM) + 3; lane 4, DNA + D₂O (14 mL) + 3; lane 5, DNA + DMSO (4 mL) + 3.

and 3 at 365 nm is essentially the same although 3 is expected to show significantly higher cleavage activity than 2 based on its greater DNA binding propensity. This could be due to the weak nature of the visible ~450 nm band.

We have also studied the photo-induced DNA cleavage activity of the complexes using a CW He–Ne laser at 632.8 nm. This wavelength is ideally suited as complex 1 has a *d–d* band at 631 nm and the anticancer drug Photofrin[®] is known to be active at 630 nm. While complexes 1 and 2 are cleavage-inactive at 632.8 nm, the dpq complex 3 showing 23% of DNA cleavage is less active in comparison to its analogous ternary copper(II) complexes having short Cu–S bond lengths (scheme 2).^{29,31} The red light-induced photocleavage of 3 is, however, similar to that observed for the ternary species (II) with a Cu–S bond length of ~2.7 Å. The results indicate the necessity of a strong Cu–S bond in the ternary structure along with the absence of any *p*-conjugation between the thiomethyl and imine moieties for observing efficient red light-induced DNA cleavage.

4. Conclusion

A new binary six-coordinate copper(II) complex containing tridentate Schiff-base ligand with CuN₄S₂ coordination in meridional geometry is prepared and structurally characterized by X-ray crystallography. The complex shows *cis* disposition of two thio-methyl moieties having significantly long Cu–S distances. Complex 1 is found to be a poor binder and cleaver of DNA in the absence of any planar ligand

moiety needed for intercalative and/or groove binding of DNA. Using complex **1** as a precursor, two new ternary copper(II) complexes having the sulphur-containing Schiff-base as photosensitizer and phen or dpq as DNA binder are generated under *in situ* reaction conditions. The ternary complexes display significantly enhanced DNA binding and photo-induced DNA cleavage activity in comparison to **1** owing to the formation of singlet oxygen as the reactive species in a type-II process. The steric constraints imposed by two five-membered chelate rings in the {CuL} moiety results in significant lengthening of the Cu–S bond. A weak Cu–S bond considerably reduces the photosensitizing effect of the Schiff-base ligand. The results show the importance of the Cu–S bond length on the photo-induced DNA cleavage activity and its significance in designing non-porphyrinic transition metal complexes for PDT applications.

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