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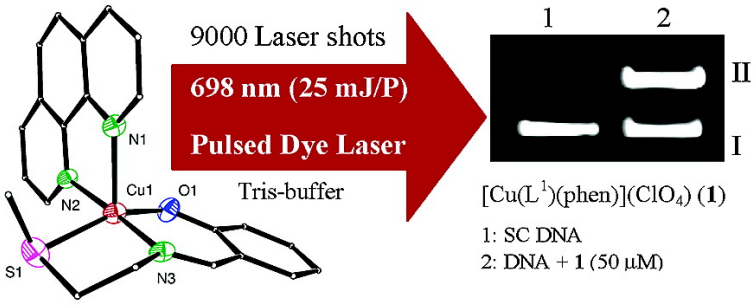
J. Am. Chem. Soc., **2003**, 125 (40), 12118-12124• DOI: 10.1021/ja036681q • Publication Date (Web): 12 September 2003



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Ternary Copper Complexes for Photocleavage of DNA by Red Light: Direct Evidence for Sulfur-to-Copper Charge Transfer and d−d Band Involvement

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Abstract: A new class of ternary copper(II) complexes of formulation $[Cu(L^n)B](ClO_4)$ (**1**−**4**), where HL^n is a NSO-donor Schiff base (HL^1, HL^2) and B is a NN-donor heterocyclic base viz. 1,10-phenanthroline (phen) and 2,9-dimethyl-1,10-phenanthroline (dmp), are prepared, structurally characterized, and their DNA binding and photocleavage activities studied in the presence of red light. Ternary complex $[Cu(L^3)(phen)](ClO_4)$ (**5**) containing an ONO-donor Schiff base and a binary complex $[Cu(L^2)_2]$ (**6**) are also prepared and structurally characterized for mechanistic investigations of the DNA cleavage reactions. While **1**−**4** have a square pyramidal (4 + 1) CuN_3OS coordination geometry with the Schiff base bonded at the equatorial sites, **5** has a square pyramidal (4 + 1) geometry with CuN_3O_2 coordination with the alcoholic oxygen at the axial site, and **6** has a square planar *trans*-CuN_2O_2 geometry. Binding of the complexes **1**−**4** to calf thymus DNA shows the relative order: phen ≫ dmp. Mechanistic investigations using distamycin reveal minor groove binding for the complexes. The phen complexes containing the Schiff base with a thiomethyl or thiophenyl moiety show red light induced photocleavage. The dmp complexes are essentially photonuclease inactive. Complexes **5** and **6** are cleavage inactive under similar photolytic conditions. A 10 μM solution of **1** displays a 72% cleavage of SC DNA (0.5 μg) on an exposure of 30 min using a 603 nm Nd:YAG pulsed laser (60 mJ/P) in Tris-HCl buffer (pH 7.2). Significant cleavage of **1** is also observed at 694 nm using a Ruby laser. Complex **1** is cleavage inactive under argon or nitrogen atmosphere. It shows a more enhanced cleavage in pure oxygen than in air. Enhancement of cleavage in D_2O and inhibition with sodium azide addition indicate the possibility of the formation of singlet oxygen as a reactive intermediate leading to DNA cleavage. The d−d band excitation with red light shows significant enhancement of cleavage yield. The results indicate that the phen ligand is necessary for DNA binding of the complex. Both the sulfur-to-copper charge transfer band and copper d−d band excitations helped the DNA cleavage. While the absorption of a red photon induces a metal d−d transition, excitation at shorter visible wavelengths leads to the sulfur-to-copper charge transfer band excitation at the initial step of photocleavage. The excitation energy is subsequently transferred to ground state oxygen molecules to produce singlet oxygen that cleaves the DNA.

Introduction

Metallointercalators play an important role in nucleic acids chemistry for their diverse applications such as foot printing, sequence specific binding and reactions, as new structural probes and therapeutic agents.[1−7] Transition metal complexes with their versatile structures, redox behavior and physicochemical properties are found to be useful as highly sensitive diagnostic agents as exemplified by the bleomycins or cis-platin in chemotherapeutic applications. The impetus in this area of non-porphyrinic chemistry draws from the pioneering work of Lippard and co-workers on the intercalative chemistry of platinum complexes as anticancer drugs.[8] The chemistry of metallointercalators has further been enriched from the significant contributions of Barton and co-workers on polypyridyl complexes of 3d−5d transition metals.[1,9] Other notable contributions have come from the work of Sigman and co-workers on copper−phenanthroline complexes.[6,10] Cleavage of DNA by metal complexes generally

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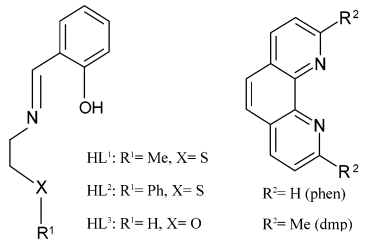
10.1021/ja036681q CCC: $25.00

follows three major pathways, viz. oxidative cleavage by abstraction of a sugar hydrogen atom, hydrolytic cleavage involving a phosphate group, and damage of DNA by oxidation of bases, primarily at the guanine site. Among several types of reactions, those cleaving DNA on photoactivation under physiological conditions have received considerable current attention[2] due to their utility in highly targeted chemotherapeutic applications over the other types cleaving DNA hydrolytically or in an oxidative manner in the presence of a reducing agent and/or H_2O_2.

Among the copper-based synthetic nucleases, bis(phen)copper complexes are directed toward the oxidative cleavage of DNA in the presence of a reducing agent.[4,6,7,10−12] There are also some reports of copper complexes cleaving DNA hydrolytically.[13,14] The chemistry of photoactive copper complexes is, however, virtually limited to only copper−porphyrin species.[5] It is thus highly desirable to develop the non-porphyrinic chemistry of copper complexes[15] for photodynamic therapeutic use. The copper−porphyrin complexes are known to be significantly less efficient in comparison to the free base due to significant quenching of the porphyrin $^3\pi\pi^*$ lifetime by the copper(II) center.[16,17] Again, the copper-based pseudo-nucleases are likely to show significantly different structural and photophysical properties in comparison to those of the polypyridyl complexes of 3d−5d transition metals reported by Barton and co-workers.[1,9]

The photodynamic therapeutic process requires a photosensitizer, a visible light source of around 630 nm (red light), and oxygen to generate cytotoxic singlet oxygen.[18] Porphyrin-based photosensitizers are currently being used in photodynamic therapy (PDT).[19] The present work stems from our interest to design copper-based synthetic nucleases that will satisfy the basic requirements of drugs/agents used in PDT. Copper being a bioessential transition metal ion, its complexes with tunable coordination geometry in a redox active environment could find better application in the cellular level. We have recently communicated a ternary mono-phen copper(II) complex containing an NSO-donor Schiff base showing DNA cleavage on irradiation with visible light at 532 nm.[20] This observation has prompted us to study the nuclease activity of this class of complexes in detail using red light sources and to explore the mechanistic aspects of the cleavage activity for understanding the role of different ligands and copper in the photodynamic processes (Chart 1). In our efforts of designing copper complexes suitable for PDT, we have chosen planar chelating heterocyclic bases as groove binders to DNA and sulfur containing tridentate Schiff base ligands as photosensitizers. The rationale for choosing the sulfur containing Schiff base as an ancillary ligand is due to the fact that compounds containing thio or thione moieties generally show efficient intersystem crossing leading to the formation of singlet oxygen.[21,22] The significant aspect of this work is the observation of an efficient red light-induced cleavage of DNA by the ternary complexes containing a phen ligand and the Schiff base with a thiomethyl or thiophenyl moiety, thus making them potentially viable for photodynamic therapeutic studies. This work also provides the first direct evidence for the involvement of the d−d band in the photoexcitation processes resulting in the activation of a oxygen molecule that leads to DNA cleavage.

Chart 1. Ligands Used and the Formulations of the Copper(II) Complexes **1**−**6**



[Cu(L¹)(phen)](ClO₄) (**1**) [Cu(L²)(dmp)](ClO₄) (**4**)

[Cu(L¹)(dmp)](ClO₄) (**2**) [Cu(L³)(phen)](ClO₄) (**5**)

[Cu(L²)(phen)](ClO₄) (**3**) [Cu(L²)₂](**6**)

Experimental Section

Materials and Measurements. All reagents and chemicals were purchased from commercial sources and used without further purification. Solvents used for electrochemical and spectroscopic measurements were purified by standard procedures. The calf thymus DNA and supercoiled pUC19 DNA (cesium chloride purified) were purchased from Bangalore Genei (India). Agarose (molecular biology grade), distamycin, and ethidium bromide were from Sigma (USA). Tris-HCl buffer solution was prepared using deionized, sonicated triple distilled water. Schiff base HL^1 was prepared by initially reacting salicylaldehyde with 2-mercaptoethylamine hydrochloride (cysteamine hydrochloride) in a 1:1 molar ratio in MeOH in the presence of 1 equiv of NaOH and finally reacting the resulting Schiff base with NaOMe and MeI in methanol at 25 °C for 3 h. Schiff base HL^2 was prepared by condensation of $H_2NCH_2CH_2SPh$ with salicylaldehyde in a 1:1 molar ratio in MeOH. Schiff base HL^3 was obtained by reacting salicylaldehyde with $H_2NCH_2CH_2OH$ in MeOH. Heterocyclic bases 1,10-phenanthroline (phen) and 2,9-dimethyl-1,10-phenanthroline (dmp) were purchased from Aldrich, USA.

The elemental analyses were done using a Heraeus CHN-O Rapid instrument. The electronic, infrared, and EPR spectral data were obtained from Hitachi U-3400, Bruker Equinox 55, and Varian E-109 X-band spectrometers, respectively. Room temperature magnetic susceptibility data were obtained from a George Associates Inc.

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(Berkeley, CA) Lewis-coil force magnetometer using $Hg[Co(NCS)_4]$ as a standard. Electrochemical measurements were done at 25 °C on an EG & G PAR model 253 Versa Stat potentiostat/galvanostat with electrochemical analysis software 270 for cyclic voltammetric work using a three electrode setup comprising a glassy carbon working, platinum wire auxiliary, and a saturated calomel reference (SCE) electrode. Potassium chloride (0.1 M) was used as a supporting electrolyte for the electrochemical measurements in Tris-HCl buffer medium (pH, 7.2).

Preparation of $[Cu(L^1)B](ClO_4)$ (B = phen, 1; dmp, 2), $[Cu(L^2)(dmp)](ClO_4)$ (4), and $[Cu(L^3)(phen)](ClO_4)$ (5). Ternary copper(II) complexes **1**, **2**, **4**, and **5** were prepared by a general procedure in which a 0.2 g (0.5 mmol) quantity of dimeric copper(II) acetate hydrate in 10 mL of methanol was reacted with the heterocyclic base (1.0 mmol) while stirring at 25 °C for 0.5 h followed by the addition of the respective Schiff base (1.0 mmol) taken in 10 mL of MeOH. The reaction mixture was stirred for 1 h, and the product was isolated as a green solid in ~65% yield on addition of a methanolic solution of $NaClO_4$ (1.0 mmol). The solid was isolated, washed with cold methanol, and finally dried in vacuo over P_4O_{10}. Anal. Calcd for $C_{22}H_{20}N_3O_5SClCu$ (**1**): C, 49.16; H, 3.72; N, 7.82. Found: C, 49.09; H, 3.96; N, 7.59. FTIR, cm^{-1} (KBr phase): 3431w, 1627s, 1529w, 1448m, 1427m, 1317w, 1091vs, 849m, 626m (vs, very strong; s, strong; m, medium; w, weak). X-band EPR with $g_{||} = 2.21$ ($A_{||} = 156 \times 10^{-4}$ cm^{-1}) and $g_\perp = 1.99$ in DMF glass at 77 K; $\mu_{eff} = 1.97\mu_B$. Anal. Calcd for $C_{24}H_{24}N_3O_5SClCu$ (**2**): C, 50.93; H, 4.24; N, 7.43. Found: C, 50.64; H, 4.47; N, 7.21. FTIR, cm^{-1} (KBr phase): 3420w, 1627s, 1412m, 1086vs, 853w, 760m, 628s, 475w. X- band EPR with $g_{||} = 2.23$ ($A_{||} = 175 \times 10^{-4}$ cm^{-1}) and $g_\perp = 2.00$ in DMF glass at 77 K; $\mu_{eff} = 1.78\mu_B$. Anal. Calcd for $C_{29}H_{26}N_3O_5SClCu$ (**4**): C, 55.45; H, 4.14; N, 6.69. Found: C, 55.19; H, 4.09; N, 6.37. FTIR, cm^{-1} (KBr phase): 3437w, 1614vs, 1528w, 1445m, 1352w, 1081vs, 864w, 760m, 611w. X-band EPR with $g_{||} = 2.19$ ($A_{||} = 120 \times 10^{-4}$ cm^{-1}) and $g_\perp = 2.03$ in DMF glass at 77 K; $\mu_{eff} = 1.98\mu_B$. Anal. Calcd for $C_{21}H_{18}N_3O_6ClCu$ (**5**): C, 49.67; H, 3.55; N, 8.28. Found: C, 49.43; H, 3.81; N, 8.47. FTIR, cm^{-1} (KBr phase): 3461w, 1629vs, 1522m, 1311m, 1106vs, 837m, 751m, 718s, 617m, 423w. X-band EPR with $g_{||} = 2.18$ ($A_{||} = 130 \times 10^{-4}$ cm^{-1}) and $g_\perp = 2.03$ in DMF glass at 77 K; $\mu_{eff} = 2.05\mu_B$.

Preparation of $[Cu(L^2)(phen)](ClO_4)$ (3). Complex **3** was prepared by reacting a 0.38 g (1.0 mmol) quantity of $Cu(ClO_4)_2 \cdot 6H_2O$ in 10 mL of MeOH with 0.26 g (1.0 mmol) of HL^2 under stirring for 30 min at 25 °C. The resulting solution was then treated with phen (0.2 g, 1.0 mmol) taken in 10 mL of MeOH. The reaction mixture was stirred for 1 h, and the product was isolated as a green solid in ~70% yield on slow evaporation of the solvent at an ambient temperature. Anal. Calcd for $C_{27}H_{22}N_3O_5SClCu$ (**3**): C, 54.09; H, 3.67; N, 7.01. Found: C, 53.83; H, 3.95; N, 6.86. FTIR, cm^{-1} (KBr phase): 3434w, 1605vs, 1510s, 1418s, 1394m, 1311w, 1105vs, 846w, 760m, 718m, 617m. X-band EPR with $g_{||} = 2.18$ ($A_{||} = 150 \times 10^{-4}$ cm^{-1}) and $g_\perp = 2.04$ in DMF glass at 77 K; $\mu_{eff} = 1.99\mu_B$.

Preparation of $[Cu(L^2)_2]$ (6). This complex was prepared by reacting 0.5 g (1.0 mmol) of copper(II) perchlorate hexahydrate with 0.7 g (2.0 mmol) of the Schiff base HL^2 in CH_2Cl_2. The reaction mixture was stirred for 0.5 h, and the product was isolated as a brownish green solid in 85% yield on evaporation of the solvent. Anal. Calcd for $C_{30}H_{28}N_2O_2S_2Cu$ (**6**): C, 62.55; H, 4.87; N, 4.87. Found: C, 62.78; H, 5.02; N, 5.01. FTIR, cm^{-1} (KBr phase): 2951m, 1605s, 1531w, 1436s, 1311w, 1254w, 1168w, 834w. X-band EPR with $g_{||} = 2.25$ ($A_{||} = 163 \times 10^{-4}$ cm^{-1}) and $g_\perp = 2.02$ in DMF glass at 77 K; $\mu_{eff} = 1.75\mu_B$.

Solubility and Stability. The complexes are moderately soluble in common organic solvents such as MeCN, CH_2Cl_2, DMF, and MeOH; less soluble in water; and insoluble in hydrocarbons. They are stable in the solid as well as in the solution phase.

X-ray Crystallography. Single crystals of **1**, **2**, and **5** were obtained by slow evaporation of aqueous methanolic solutions of the complexes. Single crystals of **4** and **6** were grown by a diffusion technique by layering hexane on the top of the dichloromethane solution of the complexes. Intensity data for **1**·0.5MeOH, **2**, and **4**−**6** were obtained from a Bruker SMART APEX CCD diffractometer, equipped with a fine focus 1.75 kW sealed tube Mo Kα X-ray source, with increasing ω (width of 0.3° per frame) at a scan speed of 18, 15, 10, 12, and 4 s/frame, respectively. The intensity data were corrected for absorption.[23] Structures were solved and refined with SHELX programs.[24] The hydrogen atoms, either located or placed in the fixed positions, were refined using a riding model. All non-hydrogen atoms were refined anisotropically. Crystal structures of **1** and **2** showed the presence of two molecules in the crystallographic asymmetric unit giving different trigonality parameter (τ) values,[25] possibly due to the crystal packing effect. Perspective views of the molecules are obtained by ORTEP.[26]

DNA Binding and Cleavage Experiments. The concentration of calf thymus (CT) DNA was determined from the absorption intensity at 260 nm with an ϵ value of 6600 M^{-1} cm^{-1}.[27] Relative binding of the ternary complexes to CT DNA with respect to the bis-phen copper(II) complex, used as a standard, was studied by fluorescence spectral method using ethidium bromide (EB) bound CT DNA solution in Tris-HCl/NaCl buffer (pH, 7.2). The fluorescence intensities at 601 nm (510 nm excitation) of EB with an increasing amount of the ternary complex concentration were recorded. Ethidium bromide was nonemissive in Tris-buffer medium due to fluorescence quenching of the free EB by the solvent molecules. 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 calf thymus DNA resulted in the displacement of bound EB, and as a consequence, the emission intensity decreased. The relative order of binding was obtained from the comparison of the slopes of the lines in the fluorescence intensity versus complex concentration plot. The cleavage of DNA was studied by agarose gel electrophoresis. For the cleavage reactions in the presence of a reducing agent, supercoiled pUC19 DNA (6 μL, 0.5 μg) in 50 mM tris-(hydroxymethyl)methane-HCl (Tris-HCl) buffer (pH, 7.2) containing 50 mM NaCl was treated with the metal complex (40 μM) and 3-mercaptopropionic acid (MPA, 5 mM) under dark conditions followed by dilution with the Tris-HCl buffer to a total volume of 18 μL. For photocleavage studies, the reactions were carried out under illuminated conditions using a UV source at 312 nm (96 W) or visible monochromatic light source at 532 nm (125 W, mercury vapor lamp) and 632 nm (250 W halogen lamp). The flash photolytic cleavage experiments were carried out using a pulsed Nd:YAG pumped laser Dye laser system (Spectra Physics, 10 Hz, 5−7 ns) and a pulsed Ruby laser (Lumonics, $^1/_6$ Hz, 20 ns) under dark conditions. The laser light at 532 nm was generated by frequency-doubling of the Nd:YAG laser fundamental. Light at 560, 603, 640, 662, and 698 nm was generated using various dyes (Rhodamine 590 chloride for 560 nm; Rhodamine 640 for 603 nm; DCM for 640 and 662 nm; LDA 698 for 698 nm) in the dye laser pumped by the 532 nm output from the YAG laser. It was possible to generate high power up to 60 mJ/P at 603 nm. The dye used for 660 nm could give power only up to 20 mJ/P. Radiation at 632.8 nm was generated in a low output (3 mW) CW He−Ne laser (Scientifica-Cook Ltd make, U. K.). In each experiment, sample DNA and the complex were premixed before being exposed to the laser light in the dark. After exposure to the laser light, each sample was incubated for 1 h at 37 °C and analyzed for the photocleaved products using gel electrophoresis as discussed below. For the laser power dependence studies, several quartz plates at the Brewster's angle were stacked on

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Table 1. Spectral and Cyclic Voltammetric Data for the Complexes **1**−**6**

no.	complex	IR,[a] cm^{-1} ν(ClO_4)	λ,[b] nm (ϵ, M^{-1} cm^{-1}) d–d	λ,[b] nm (ϵ, M^{-1} cm^{-1}) CT	$E_{1/2}$,[c] V (ΔE_p, mV)
1	[Cu(L^1)(phen)](ClO_4) (**1**)	1091	662 (120)	394 (1290),[d] 374 (1530)	−0.15 (220)
2	[Cu(L^1)(dmp)](ClO_4) (**2**)	1086	643 (110)	457 (1280), 369 (3800)	−0.05 (480)
3	[Cu(L^2)(phen)](ClO_4) (**3**)	1105	619 (200)	392 (2000), 347 (2600)	−0.03 (140)
4	[Cu(L^2)(dmp)](ClO_4) (**4**)	1081	680 (195)	377 (2900), 339 (3800)	−0.04,[e] 0.46[f]
5	[Cu(L^3)(phen)](ClO_4) (**5**)	1106	641 (130)	369 (4900)	−0.18 (182)
6	[Cu(L^2)$_2$] (**6**)		657 (130)	383 (1880), 326 (3600)	−0.31,[e] 0.03[f]

[a] In KBr phase. [b] In MeCN. [c] Corresponds to Cu(II)/Cu(I) couple at scan rate 50 mV s^{-1} in DMF−Tris-buffer (1:4 v/v)/0.1 M KCl. $E_{1/2} = 0.5(E_{pa} + E_{pc})$. $\Delta E_p = E_{pa} - E_{pc}$, where E_{pa} and E_{pc} are anodic and cathodic peak potentials, respectively. [d] Additional broad peak near 476 nm (ϵ, ~93 M^{-1} cm^{-1}) [e] Broad E_{pc}. [f] E_{pa} with a reduced current height.

the light path in front of the sample and the power was measured by a laser power meter.

The inhibition reactions for the chemical nuclease reactions were carried out under dark conditions by adding reagents (distamycin, 75 μM; DMSO, 4 μL; mannitol, 85 μM; ethanol, 4 μL) prior to the addition of the complexes. The inhibition reactions for the photonuclease studies were carried out at 532 nm using reagents (NaN_3, 90 μM; DMSO, 4 μL; mannitol, 85 μM; ethanol, 4 μL; distamycin 75 μM) prior to the addition of the complex. For the D_2O experiment, this solvent was used for dilution to 18 μL. Eppendorf and glass vials were used for the UV and visible light experiments, respectively, at 25 °C in a dark room. The samples after incubation for 1 h at 37 °C in a dark chamber were added to the loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol (3 μL), and the solution was finally loaded on 0.8% agarose gel containing 1.0 μg/mL ethidium bromide. Electrophoresis was carried out in a dark chamber for 3 h at 40 V in TAE (Tris-acetate EDTA) buffer. Bands were visualized by UV light and photographed. The extent of DNA 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.[28] Cleavage reactions for mechanistic investigations using NaN_3, pure oxygen, or inert atmosphere were also carried out using an Nd:YAG laser at 603 nm.

Results and Discussion

General Aspects and Crystal Structures. Our major objective in the synthesis of five ternary (**1**−**5**) complexes and one binary (**6**) copper(II) complex is to explore the mechanistic aspects of the photocleavage reactions using red light. Complexes **1**−**4** have NSO-donor Schiff bases with a thiomethyl or thiophenyl moiety. First, to explore the effect of the heterocyclic base on the nuclease activity, we have prepared **1** and **2** using planar heterocyclic DNA binding ligands phen and dmp in the presence of a common Schiff base HL^1. Again, to investigate the role of the Schiff base on the photonuclease activity, phen complexes **1** and **3** having two different NSO-donor Schiff bases are synthesized. While the Schiff base HL^1 has a thiomethyl moiety, HL^2 has a thiophenyl group. The photosensitizing abilities of two thio moieties are expected to vary. For a comparative study related to the role of sulfur in the photocleavage reaction, we have prepared a ternary complex **5** containing a ONO-donor Schiff base and a phen ligand. To probe the importance of the ternary structure and the heterocyclic base on the photonuclease activity, a binary complex [Cu(L^2)$_2$] (**6**) is prepared and its nuclease activity studied along with the known binary species, viz. [Cu(phen)$_2$(H_2O)](ClO_4)$_2$.

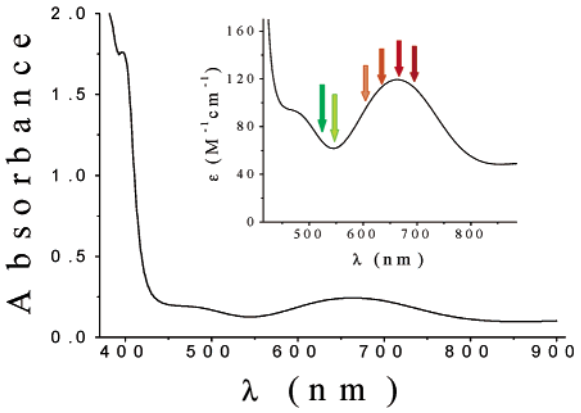

Figure 1. Electronic spectrum of **1** in MeCN showing different wavelengths used for pulsed laser irradiations (by arrow).

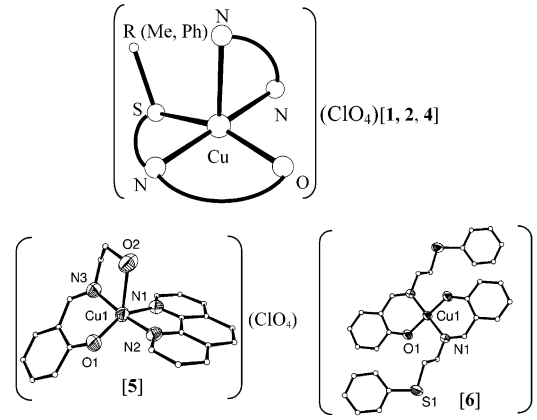

Figure 2. A representative view of the structures of **1**, **2**, and **4** with a CuN_3OS core along with the perspective views of **5** having a CuN_3O_2 unit and the square planar complex **6**.

The complexes **1**−**6** are characterized from the analytical and physicochemical data (Table 1). They exhibit a d−d band near 650 nm in the electronic spectra (Figure 1). An intense band near 390 nm with a weak shoulder around 480 nm is assignable to the sulfur-to-copper(II) charge transfer (LMCT) transition.[29] Complexes **1**−**5** are redox active, and the Cu(II)/Cu(I) couple is observed near −0.1 V (vs SCE) in DMF-Tris buffer medium.[30,31] All the complexes except **3** have been structurally characterized (Figure 2). Crystal structures of **1**, **2**, and **4** consist of a discrete monomeric cationic complex containing a tridentate NSO-donor Schiff base and a didentate NN-donor heterocyclic base (phen or dmp) along with a noncoordinating perchlorate anion. These complexes are structurally similar having the Schiff

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(29) Kita, T.; Miura, I.; Nakayama, N.; Kawata, T.; Kano, K.; Hirota, S.; Kodera, M. *J. Am. Chem. Soc.* **2001**, *123*, 7715.

base bonded at the basal plane and the heterocyclic base displaying axial−equatorial bonding. The crystal structure of $[Cu(L^3)(phen)](ClO_4)$ (**5**) shows a CuN_3O_2 core in which the phenoxo oxygen and the imine nitrogen atoms of the Schiff base occupy the basal plane while the alcoholic oxygen atom binds at the axial site. The binary complex $[Cu(L^2)_2]$ (**6**) has a square planar structure with a trans geometry in a CuN_2O_2 core. The sulfur atoms in this structure do not show any bonding with the metal ion.

DNA Binding and Photoinduced Cleavage. The binding of the complexes to calf thymus DNA has been studied by fluorescence spectral technique using emission intensity of ethidium bromide (EB). The bis-phen complex of copper(II) has been used as a standard to determine the relative binding propensities of the complexes. It is observed that the monocationic complexes having phen as a heterocyclic base show greater binding to CT DNA than dmp complexes. The steric constraint imposed by the methyl groups at 2 and 9 positions in planar dmp seem to reduce the binding ability of this heterocyclic base to the CT DNA. Among the phen complexes, the one with a thiophenyl group displays greater binding propensity than the one with a thiomethyl moiety. It is likely that the phenyl group could make favorable contact(s) in the DNA groove.[6,11] The binary complex **6** shows poor binding to DNA. The DNA cleavage activity of the complexes has been studied under different reaction conditions. The complexes are nuclease inactive in the absence of any reducing agent when the reactions are carried out in a dark chamber. In the presence of mercaptopropionic acid (5 mM) as a reducing agent, the complexes (40 μM) containing phen ligands show efficient cleavage of supercoiled pUC19 DNA (0.5 μg), while the dmp species remain nuclease inactive. The results suggest that the DNA binding of the complexes utilizing the planar heterocyclic base phen is a necessity for observing any nuclease activity among this class of ternary complexes.[32]

The utility of the complexes **1**−**6** in photoinduced DNA cleavage reactions is initially probed using monochromatic UV radiation of 312 nm in the absence of mercaptopropionic acid. The phen complexes with a CuN_3OS core are found to be photonuclease active.[33] The results indicate the necessity of the phen as well as the NSO-donor Schiff base in the ternary structure for observing the photonuclease activity. Among the phen complexes with a CuN_3OS core, **1** with a thiomethyl group shows greater cleavage activity than that of **3** with a thiophenyl group. The photonuclease activities of **1** and **3** have been studied using different visible light sources such as a mercury vapor (532 nm, 125 W), a halogen lamp (632 nm, 250 W), and a CW laser (632.8 nm, 3 mW). A 150 μM quantity of complex **1** shows 53% cleavage of SC DNA on irradiation at 632 nm for 60 min using a halogen lamp. A similar experiment using CW laser (3 mW) of 632.8 nm displays significantly higher cleavage. The photonuclease activities of **1** and **3** have been studied in detail using a pulsed dye laser at different wavelengths (Table 2, Figure 3). A 10 μM quantity of **1** shows 72% cleavage of SC DNA for an exposure time of 30 min at 603 nm (60 mJ/P power). Complex **3** displays cleavage activity at higher concentration and with more exposure time. The laser setup used for the experiments is displayed in Figure 4. The observed photocleavage activity of **1** compares well with those reported for the porphyrin species, viz. PHOTOFRIN, an anticancer drug, which is known to cleave DNA at 630 nm.[18,19] A significant observation of this study is the efficient cleavage activity of complex **1** at 698 nm using the pumped dye laser or at 694 nm using a Ruby laser source.

Table 2. Photocleavage Data of SC pUC19 DNA (0.5 μg) by $[Cu(L^1)(phen)](ClO_4)$ (**1**) and $[Cu(L^2)(phen)](ClO_4)$ (**3**) Using an Nd:YAG Pulsed Laser

Sl no.	reaction condition	complex (μM)	λ (nm)	laser peak power (mJ/P)	exposure time (min)	form-I, %	form-II, %
1	DNA control		532	40	30	95	5
2	DNA + **1**	50	532	10	15	74	26
3	DNA + **1**	50	532	15	15	65	35
4	DNA + **1**	50	532	20	15	48	52
5	DNA + **3**	50	532	30	15	93	7
6	DNA + **3**	100	532	30	30	50	50
7	DNA + **3**	100	532	40	30	45	55
8	DNA control		603	50	30	92	8
9	DNA + **1**	10	603	40	30	45	55
10	DNA + **1**	10	603	50	30	37	63
11	DNA + **1**	10	603	60	30	28	72
12	DNA + **1**	50	603	20	15	75	25
13	DNA + **1**	50	603	40	15	45	55
14	DNA + **1**	50	603	40	30	22	78
15	DNA + **1**	50	603	50	30	13	87
16	DNA + **1**	50	603	60	30	2	98
17	DNA + **3**	50	603	30	15	93	7
18	DNA + **3**	100	603	30	30	39	61
19	DNA + **3**	100	603	40	30	30	70

Mechanistic aspects of the photocleavage reaction have been studied at 532 nm (mercury vapor lamp, 125 W) and 603 nm (Nd:YAG laser) irradiation using different reagents (Figure 5). The minor groove binder complex **1** is nuclease inactive under argon or nitrogen atmosphere. This indicates the necessity of oxygen in the cleavage reactions. Enhancement of cleavage in D_2O or pure oxygen and inhibition in the presence of azide anion or histidine suggest the possible involvement of singlet oxygen (O_2, $^1\Delta_g$) as the reactive species. In the presence of pure oxygen, the extent of cleavage increases by 18% compared to that in air. This indicates that the presence of oxygen is necessary for the cleavage to be effective. Addition of KI, DMSO, mannitol, or ethanol does not show any significant effect on the photocleavage activity. The photoinduced DNA cleavage data indicate the role of 1,10-phenanthroline as a binder to DNA, while the NSO-donor Schiff base acts as a photosensitizer. Photoexcitation of the phen complexes with a CuN_3OS core

(30) Santra, B. K.; Reddy, P. A. N.; Nethaji, M.; Chakravarty, A. R. *Inorg. Chem.* **2002**, *41*, 1328.
(31) Santra, B. K.; Reddy, P. A. N.; Nethaji, M.; Chakravarty, A. R. *J. Chem. Soc., Dalton Trans.* **2001**, 3553.
(32) Complex **6** is nuclease inactive under similar experimental conditions. The cleavage efficiency of **3** with a thiophenyl group is found to be more than **1** having a thiomethyl moiety. This may be related to the greater binding ability of **3** to DNA than **1**. Complex **5** with an ONO-donor Schiff base exhibits chemical nuclease activity. Mechanistic investigations reveal minor groove binding of the complexes and possible involvement of hydroxyl radical or reactive copper-oxo species in the cleavage reactions as the complexes do not show any significant DNA cleavage activity in the presence of inhibiting reagents such as DMSO, mannitol, ethanol, and the minor groove binder distamycin.
(33) When SC DNA (0.5 μg) is irradiated in the presence of the complexes **1**−**6** with a wavelength of 312 nm for 10 min, the phen complexes (80 μM) having NSO-donor Schiff bases show significant cleavage activity. The dmp complexes do not show any photonuclease activity. Similarly, the phen complex **5** containing ONO-donor Schiff base, the binary Schiff base complex **6**, and the bis(phen)copper(II) complex are cleavage inactive on photoirradiation. Control experiments using the Schiff base ligands alone or other copper(II) compounds such as $CuCl_2 \cdot 2H_2O$, $[Cu(phen)_2(H_2O)](ClO_4)_2$, and [Cu(TPP)] ($H_2TPP$ = tetraphenylporphyrin) do not show any apparent cleavage activity on photoirradiation at 638.2 nm using He−Ne CW laser.

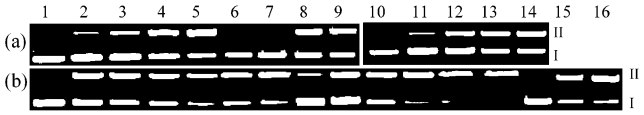

Figure 3. (a) DNA cleavage activity of **1** and **3** using an Nd:YAG pulsed laser of wavelength 532 nm (lanes 1−9) and of **1** at 560 nm (lanes 10−14). Lane 1, DNA control (20 mJ/P, 15 min); lane 2, DNA + **1** (50 μM, 5 mJ/P, 15 min); lane 3, DNA + **1** (50 μM, 15 mJ/P, 15 min); lane 4, DNA + **1** (50 μM, 18 mJ/P, 15 min); lane 5, DNA + **1** (50 μM, 20 mJ/P, 15 min); lane 6, DNA + **3** (50 μM, 10 mJ/P, 15 min); lane 7, DNA + **3** (50 μM, 20 mJ/P, 15 min); lane 8, DNA + **3** (100 μM, 30 mJ/P, 30 min); lane 9, DNA + **3** (100 μM, 40 mJ/P, 30 min); lane 10, DNA control (50 mJ/P, 15 min); lane 11, DNA + **1** (50 μM, 20 mJ/P, 15 min); lane 12, DNA + **1** (50 μM, 30 mJ/P, 15 min); lane 13, DNA + **1** (50 μM, 40 mJ/P, 15 min); lane 14, DNA + **1** (50 μM, 50 mJ/P, 15 min). (b) DNA cleavage activity of **1** and **3** using an Nd:YAG pulsed laser of wavelength 603 nm. Lane 1, DNA control (50 mJ/P, 30 min); lane 2, DNA + **1** (10 μM, 40 mJ/P, 15 min); lane 3, DNA + **1** (10 μM, 40 mJ/P, 30 min); lane 4, DNA + **1** (10 μM, 50 mJ/P, 15 min); lane 5, DNA + **1** (10 μM, 50 mJ/P, 30 min); lane 6, DNA + **1** (10 μM, 60 mJ/P, 25 min); lane 7, DNA + **1** (10 μM, 60 mJ/P, 30 min); lane 8, DNA + **1** (50 μM, 20 mJ/P, 15 min); lane 9, DNA + **1** (50 μM, 20 mJ/P, 30 min); lane 10, DNA + **1** (50 μM, 40 mJ/P, 15 min); lane 11, DNA + **1** (50 μM, 40 mJ/P, 30 min); lane 12, DNA + **1** (50 μM, 60 mJ/P, 25 min); lane 13, DNA + **1** (50 μM, 60 mJ/P, 30 min); lane 14, DNA + **3** (50 μM, 30 mJ/P, 15 min); lane 15, DNA + **3** (100 μM, 30 mJ/P, 30 min); lane 16, DNA + **3** (100 μM, 40 mJ/P, 30 min).

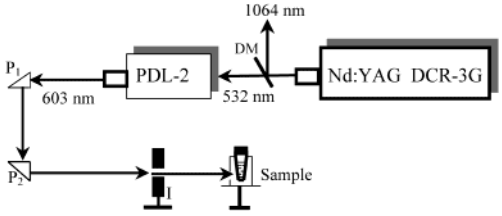

Figure 4. Pulsed laser setup for the photoirradiation of complexes **1** and **3** (PDL, pulsed dye laser; first, P_1, and second, P_2, 90° prisms; I, iris; DM, dichroic mirror).

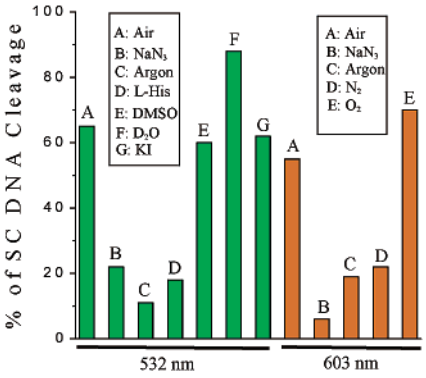

Figure 5. Percentage of SC pUC19 DNA cleavage upon irradiation with a mercury vapor lamp (125 W) of 532 nm for 10 min and Nd:YAG pulsed laser of 603 nm for 15 min (40 mJ/P) in the presence of complex **1** having concentrations of 80 and 50 μM, respectively, at different reaction conditions. The control experiments in the absence of **1** show a cleavage of 5% at 532 nm and 8% at 603 nm of SC DNA.

seems to sensitize the thio alkyl/aryl group of the complexes to an excited state followed by an efficient energy transfer to the triplet state which presumably activates oxygen from its stable triplet (O_2, $^3\Sigma_g^-$) to the highly toxic singlet (O_2, $^1\Delta_g$) state. Although we have not made any attempt to isolate the cleaved products, photocleavage of nucleic acids is known to involve an initial oxidative reaction involving either a nucleobase or a sugar residue. Among the four nucleobases, guanine is most susceptible for oxidation by singlet oxygen for its low oxidation potential, and guanine-selective photocleavage reactions are

Table 3. Photocleavage Data[a] of SC pUC19 DNA (0.5 μg) in the Presence of **1** on Irradiation with Wavelengths at the d−d Band Using an Nd:YAG Pulsed Laser of 560, 640, 662, and 698 nm

Sl no	reaction condition	complex (μM)	λ (nm)	pulse laser power (mJ/P)	exposure time (min)	form-I, %	form-II, %
1	DNA control		560	50	15	92	8
2	DNA + **1**	50	560	20	15	80	20
3	DNA + **1**	50	560	50	15	45	55
4	DNA control		640	35	15	98	2
5	DNA + **1**	50	640	20	15	59	41
6	DNA + **1**	50	640	35	15	32	68
7	DNA control		662	20	15	94	6
8	DNA + **1**	50	662	5	15	75	25
9	DNA + **1**	50	662	15	15	58	42
10	DNA + **1**	50	662	20	15	48	52
11	DNA control		698	25	15	98	2
12	DNA + **1**	50	698	15	15	66	34
13	DNA + **1**	50	698	20	15	54	46
14	DNA + **1**	50	698	25	15	43	57

[a] A similar experiment using complex **5** (50 μM) having phen as DNA binder and ONO donor ancillary Schiff base shows no apparent photocleavage on irradiation with a pulsed laser of 662 nm (20 mJ/P) for 15 min (form-I, 90%; form-II, 10%).

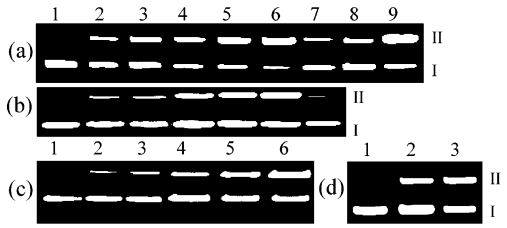

Figure 6. Gel diagrams showing the effect of d−d band on photosensitizations at different wavelengths. (a) DNA cleavage activity of **1** (50 μM) using pulsed Nd:YAG laser of wavelength 640 nm. Lane 1, DNA control (35 mJ/P, 30 min); lane 2, DNA + **1** (30 mJ/P, 10 min); lane 3, DNA + **1** (30 mJ/P, 15 min); lane 4, DNA + **1** (30 mJ/P, 20 min); lane 5, DNA + **1** (30 mJ/P, 25 min); lane 6, DNA + **1** (30 mJ/P, 30 min); lane 7, DNA + **1** (15 mJ/P, 15 min); lane 8, DNA + **1** (20 mJ/P, 15 min); lane 9, DNA + **1** (35 mJ/P, 15 min). (b) DNA cleavage activity of **1** and **5** (50 μM) using an Nd:YAG pulsed laser source of wavelength 662 nm. Lane 1, DNA control (20 mJ/P, 15 min); lane 2, DNA + **1** (5 mJ/P, 15 min); lane 3, DNA + **1** (10 mJ/P, 15 min); lane 4, DNA + **1** (13 mJ/P, 15 min); lane 5, DNA + **1** (15 mJ/P, 15 min); lane 6, DNA + **1** (20 mJ/P, 15 min); lane 7, DNA + **5** (20 mJ/P, 15 min). (c) DNA cleavage activity of **1** (50 μM) using an Nd:YAG pulsed laser source of wavelength 698 nm. Lane 1, DNA control (25 mJ/P, 15 min); lane 2, DNA + **1** (5 mJ/P, 15 min); lane 3, DNA + **1** (10 mJ/P, 15 min); lane 4, DNA + **1** (15 mJ/P, 15 min); lane 5, DNA + **1** (20 mJ/P, 15 min); lane 6, DNA + **1** (25 mJ/P, 15 min). (d) DNA cleavage activity of **1** using a pulsed Ruby laser of wavelength 694 nm. Lane 1, DNA control (80 mJ/P, 30 min); lane 2, DNA + **1** (10 μM, 80 mJ/P, 30 min); lane 3, DNA + **1** (50 μM, 60 mJ/P, 30 min). The percentage SC DNA cleavage for lanes 1−3 are 3%, 42%, and 50%, respectively.

known to occur with porphyrin bases and their metal complexes used in PDT.[2,34]

The laser wavelengths for cleavage experiments were chosen based on the absorption spectrum of the complex (Figure 1). Several wavelengths (603, 632.8, 640, 662, and 698 nm) under the d−d transition band were selected to probe the involvement of the long wavelength band in the photocleavage activity (Table 3, Figures 3 and 6). The wavelengths at 532 and 560 nm were chosen since they are near the midpoint of the weak d−d band (λ_{max}, 661.5 nm) and the strong ligand-to-metal (S-to-Cu) charge transfer band peaking at around 390 nm. The absorption

(34) Croke, D. T.; Perrouault, L.; Sari, M. A.; Battioni, J. P.; Mansuy, D.; Hélène, C.; Le Doan, T. *J. Photochem. Photobiol. B* **1993**, *18*, 41.

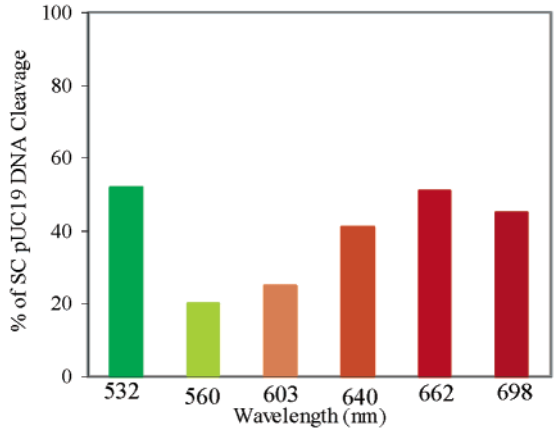

Figure 7. Photocleavage activity of complex **1** after 9000 laser shots at 20 mJ/pulse at different wavelengths using an Nd:YAG PDL system.

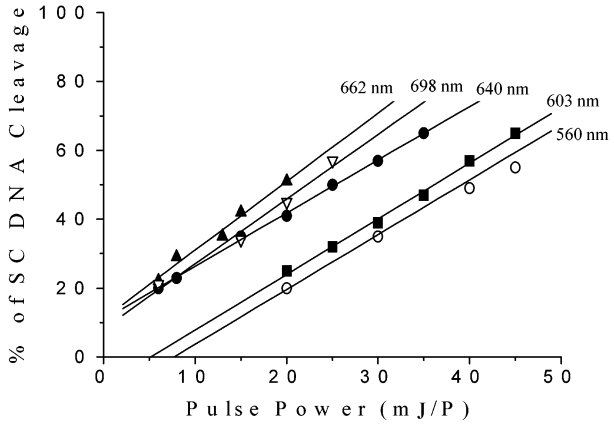

Figure 8. Variation of percentage DNA cleavage with laser power at different wavelengths using an Nd:YAG PDL system.

coefficient at 532 and 560 nm is significant but much lower than that at ≥600 nm. If only the d−d band is involved in the initial step of the photocleavage mechanism at these two wavelengths, we would expect no or very little cleavage of the DNA. However, at 532 nm where the absorption is mostly due to the tail of the LMCT band, 52% cleavage is observed. In fact, the observation that the extent of cleavage is the same as that at 662 nm under similar conditions leads us to believe that the LMCT band is also contributing to the initial step of photocleavage of the DNA (Figure 7). Initially the photon from the laser is believed to excite the metal d−d transition and the LMCT transition in the complex, which then transfers the energy to a ground state oxygen molecule and excites it to the $^1\Delta_g$ state.

The variation of the percentage cleavage of SC DNA with laser power has been studied at several visible wavelengths, and the results are shown in Figure 8. The plots show linear behavior, and the slopes of the straight lines at different wavelengths are similar within experimental errors. The percent cleavage increasing linearly with laser power indicates that the initial photoexcitation step is a single photon process. The extent of cleavage at different wavelengths under similar conditions using a laser power of 20 mJ/P follows the absorption spectral pattern closely (Figure 7). In fact, this strengthens our claim that both the Cu(II) d−d band and sulfur-to-metal charge transfer band are involved in the primary step of the photocleavage process.

Conclusions

A series of ternary copper(II) complexes containing tridentate Schiff bases and didentate heterocyclic bases and a binary copper(II) complex have been prepared and structurally characterized, and their photoinduced nuclease activities studied under laser light illumination. The ternary copper(II) complexes containing phen and NSO-donor Schiff base ligands show red light induced photonuclease activity. Mechanistic investigations reveal the role of phen as a minor groove binder to DNA and the NSO-donor Schiff base as a photosensitizer. Photoinactivity of the complexes under inert atmosphere, inhibition with azide addition, and enhancement of cleavage in D_2O or pure oxygen suggest the involvement of singlet oxygen as a reactive species. The pulsed laser photolysis data show significant cleavage of DNA at 603 nm using a 10−50 μM quantity of the complex **1**. This complex also shows significant cleavage near 700 nm and generally satisfies the basic requirements for a sensitizer to be useful in photodynamic therapy. Consequently, this class of ternary copper(II) complexes offer scopes for studies related to clinical investigations along with the currently used porphyrin derivatives and their metal complexes in PDT. This work presents the first direct evidence for a dual involvement of the sulfur-to-copper charge transfer (LMCT) and the d−d band in the photosensitization process leading to an efficient DNA cleavage activity near 660 nm.

Acknowledgment. The authors thank the Department of Science and Technology, Government of India, for the financial support (SP/S1/F01/2000) and for the CCD diffractometer facility. We are thankful to Mr. P. A. N. Reddy for the crystallographic studies and the Alexander von Humboldt Foundation, Germany, for donation of an electroanalytical system. We also thank the Convener, Bioinformatic Center, and the Chairman, Department of Chemical Engineering, of our Institute for database search and the Ruby Laser equipment, respectively.

Supporting Information Available: Selected crystallographic data, bond distances and angles for **1**, **2**, and **4**−**6** (Tables S1−S3); trigonality parameter values for **1**, **2**, **4**, and **5** (Table S4); chemical nuclease data in the presence of mercaptopropionic acid (Table S5); photonuclease data at 312 nm (Table S6); photonuclease data at 532, 632, and 632.8 nm using a mercury vapor lamp (125 W), halogen lamp (250 W), and CW laser (3 mW), respectively (Table S7); cleavage data in the presence of different inhibiting reagents (Table S8); ORTEP diagrams with 50% probability thermal ellipsoids for the complexes **1**, **2**, and **4**−**6** with atom labeling schemes (Figures S1−S5); the DNA binding plots for **1**−**6** (Figures S6 and S7); gel diagram showing chemical nuclease activity in the presence of mercaptopropionic acid (Figure S8); gel diagram displaying photocleavage data at 312 nm (Figure S9); gel diagram exhibiting photonuclease activity at 532 and 632 nm (Figure S10); gel diagrams showing inhibition reactions (Figure S11); gel diagram showing control experiments using $CuCl_2 \cdot 2H_2O$, bis(phen)copper(II), and Cu-(TPP) (Figure S12); full list of bond distances and bond angles, anisotropic thermal parameters, and hydrogen atom coordinates for complexes **1**, **2**, and **4**−**6** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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