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# Synthetic analogue approach for the functional domains of copper(II) bleomycins and its DNA cleavage activity†

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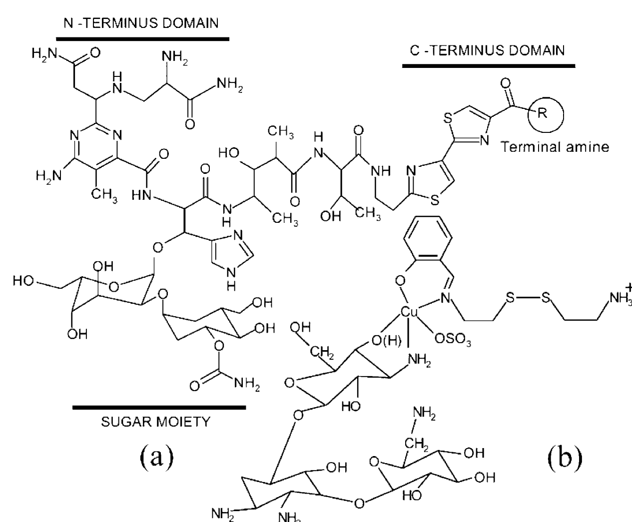
The dicopper(II) complex $[Cu_2(R'SSR)_2(SO_4)_2]$ (**1**), where R′SSR is a Schiff base, has been prepared from the reaction of $CuSO_4{\cdot}5H_2O$ with the Schiff base *N*,*N*′-1,1′-dithiobis(ethylenesalicylaldimine) ($H_2RSSR$) and structurally characterized by X-ray crystallography. The crystal structure of **1** shows two $\{Cu(R'SSR)\}^{2+}$ units linked by two sulfate ligands each showing a $\eta^3,\mu_2$-binding mode. The Cu · · · Cu distance is 4.562(2) Å with each copper having a square pyramidal (4 + 1) $CuNO_4$ coordination geometry. The monoanionic Schiff base R′SSR has a pendant cationic amine $-SCH_2CH_2NH_3^+$ group which is presumably formed from the hydrolysis of one imine bond of $H_2RSSR$. Complex **1** models the N- and C-terminus domains of bleomycins. The metal centers in **1** are essentially magnetically non-interacting giving a $-2J$ value of 3 $cm^{-1}$ with the singlet as the ground state. Using complex **1** as a precursor, ternary copper(II) complexes $[Cu(R'SSR)B(SO_4)]$ (**2**–**4**) are prepared, characterized and their DNA binding and cleavage properties studied (B: kanamycin A, **2**; 2,2′-bipyridine, **3**; 1,10-phenanthroline, **4**). IR spectral data suggest a square pyramidal (4 + 1) geometry for the one-electron paramagnetic ternary complexes with the sulfate bound to copper. The complexes are non-conducting in DMF but show conductivity in aqueous medium due to dissociation of the sulfate ligand. They bind to calf thymus DNA in the minor groove giving the relative order: **4** > **2** > **1** ~ **3** ($K_{app} = 5.4 \times 10^5$ $M^{-1}$ for **4**). The precursor complex **1** does not show any apparent chemical nuclease activity when treated with supercoiled (SC) DNA in the presence of 3-mercaptopropionic acid (MPA). The kanamycin A and phen adducts as such or generated under *in situ* reaction conditions using **1** and the ligand display efficient chemical nuclease activity in the presence of MPA, while the bpy species shows poor cleavage activity. The ternary kanamycin A complex presents the first synthetic model for three functional domains of bleomycins.

## Introduction

Bleomycins (BLMs) are the glycopeptide antitumor antibiotics that cleave DNA in an oxidative manner and are clinically used for the treatment of squamous cell and malignant lymphomas.[1–3] Bleomycins, in the presence of ferrous ion and molecular oxygen, cause sequence-selective DNA strand scission. The structure of bleomycins consists of three major domains playing different functional roles (Scheme 1). The N-terminus domain has binding sites for the metal, *viz.* iron or copper. Binding of molecular oxygen to the metal occurs in this domain. The C-terminus domain with a bithiazole unit and a cationic terminal amine moiety show DNA binding affinity and sequence selectivity. The carbohydrate domain facilitates cell permeability of BLMs and oxygen binding. Since activation of bleomycins requires a metal ion cofactor such as $Fe^{2+}$ (or $Cu^{2+}$ in the presence of dithiothreitol as reductant), the coordination chemistry of low molecular weight transition metal complexes as synthetic models assume paramount importance for gaining better insights into different structural and functional aspects of BLMs.

BLM–DNA interactions have been studied using various analogues/conjugates to probe specific roles played by each domain.[1–6] In comparison, synthetic model iron or copper complexes are few and they do not model all the domains of the elephantine structure of BLMs.[7–11] The structural and chemical complexities associated with BLMs have been addressed by Mascharak and co-workers in a series of papers on synthetic model iron and copper complexes.[7,8] The complexes generally model the metal binding N-terminus domain. Hertzberg and Dervan have reported a model iron complex with a proposed structure that mimics the N- and C-terminus domains.[9] They suggested that methidiumpropyl-EDTA in presence of $Fe^{2+}$ forms an iron–EDTA complex with a pendant cationic DNA binding site and the complex cleaves DNA in an oxidative manner. Using a similar synthetic analogue approach, we are successful in forming ternary copper(II) complexes containing carbohydrate or heterocyclic base (B) using a precursor copper(II) complex $[Cu_2(R'SSR)_2(SO_4)_2]$ (**1**) that has a pendant arm with a cationic amine moiety. Complex **1** has been structurally characterized by X-ray crystallography. Our proposed kanamycin A bound ternary copper(II) complex[12] is unique in modeling three functional domains of BLMs (Scheme 1). The significant result of this study is the observation of efficient DNA




**Scheme 1** (a) Bleomycins with three functional domains. (b) Proposed ternary structure of the kanamycin A copper(II) complex (**2**).

† Electronic supplementary information (ESI) available: Variable temperature magnetic susceptibility data (Table S1), $\chi_M T$ *vs.* $T$ plot and unit cell packing diagram of **1** (Figs. S1, S2). See http://www.rsc.org/suppdata/dt/b4/b414639e/

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cleavage activity of the kanamycin A complex in the presence of mercaptopropionic acid (MPA).

Ternary copper(II) complexes [Cu(R′SSR)B($SO_4$)] (**2**–**4**) are prepared for the DNA binding and cleavage studies from the reaction of **1** with kanamycin A or heterocyclic bases such as 2,2′-bipyridine (bpy) and 1,10-phenanthroline (phen) (B: kanamycin A, **2**; bpy, **3**; phen, **4**). Our choice of **1** as a precursor is based on its novel structural features showing the presence of a cationic DNA-binder arm making it a potential synthetic model for the N- and C-terminus domains of BLMs. Herein we report the synthesis, structure and DNA cleavage properties of **1**–**4**.

## Experimental

### Materials

All reagents and chemicals were procured from commercial sources and used without further purifications. Solvents used were purified by standard procedures.[13] Calf thymus (CT) DNA and supercoiled (SC) pUC19 DNA (caesium chloride purified) were purchased from Bangalore Genie (India). Agarose (molecular biology grade) and ethidium bromide (EB) were from Sigma (USA). The disulfide Schiff base ligand ($H_2$RSSR) was prepared by a literature procedure using cysteamine hydrochloride (Lancaster, UK) and salicylaldehyde (Aldrich, USA).[14]

### Physical measurements

The elemental analyses were done using a Thermo Finnigan Flash EA 1112 CHNSO analyser. The IR, electronic, and fluorescence spectral data were obtained from Bruker Equinox 55, Hitachi U-3400, and Perkin Elmer LS-50B spectrometers, respectively. Conductivity measurements were made using Control Dynamics Conductivity Meter. 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 consisting of a glassy carbon working, platinum wire auxiliary and saturated calomel reference electrode. Variable temperature magnetic susceptibility data in the temperature range 18–300 K were obtained for polycrystalline samples using a George Associates Inc. Lewis-coil-force magnetometer system (Berkeley, CA) equipped with a closed-cycle cryostat (Air Products) and a Cahn balance. Hg[Co(NCS)$_4$] was used as a standard. Experimental susceptibility data were corrected for diamagnetic contributions and temperature independent paramagnetism. The corrected molar magnetic susceptibilities for the dicopper(II) complex were theoretically fitted by the modified Bleaney–Bowers expression based on the isotropic form of the Heisenberg–Dirac–van Vleck (HDvV) model giving $\boldsymbol{H} = -2J\boldsymbol{S}_1\boldsymbol{S}_2$, where $S_1 = S_2 = \frac{1}{2}$.[15,16] The susceptibility equation used for fitting was: $\chi_{Cu} = [Ng^2\beta^2/kT][3 + \exp(-2J/kT)]^{-1}(1 - \rho) + (Ng_1{}^2\beta^2/4kT)\rho + N_\alpha$, where $\rho$ is the fraction of monomeric impurity.

### Synthesis

**Preparation of [$Cu_2$(R′SSR)$_2$($SO_4$)$_2$] (1).** The precursor complex **1** was prepared as a green crystalline solid in ~55% yield (220 mg) from the reaction of $CuSO_4 \cdot 5H_2O$ (0.25 g, 1.0 mmol) with the Schiff base *N*,*N*′-1,1′-dithiobis(ethylenesalicylaldimine) ($H_2$RSSR, 0.36 g, 1.0 mmol) in $CH_2Cl_2$–MeOH (12 cm$^3$, 9 : 1 v/v) at 25 °C for 1 h magnetic stirring in dark under nitrogen atmosphere. The product, obtained on slow concentration of the solution, was washed with methanol and finally dried in vacuum over $P_4O_{10}$ (Found: C, 31.8; H, 3.7; N, 6.9, $C_{22}H_{32}Cu_2N_4O_{10}S_6$ (**1**) requires C, 31.7; H, 3.9; N, 6.7%). Conductivity ($\Lambda_M/\Omega^{-1}$ cm$^2$ mol$^{-1}$) in $H_2O$ = 390.[17] UV-vis in $H_2O$ [$\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$)]: 631 (90), 321 (3850), 269 (8250), 237 (12300), 218 (14200). IR (KBr phase, cm$^{-1}$): 2930w, 1632s, 1542m, 1475m, 1452m, 1402w, 1349w, 1315w, 1200s, 1144vs, 1130vs, 1115vs, 1052w, 1000m, 936m, 912w, 760m, 656w, 620m, 586w, 486w, 456w (vs, very strong; s, strong; m, medium; w, weak). $\mu_{eff}$ per Cu = 2.0 at 300, 1.9 $\mu_B$ at 18 K [theoretical fit: $-2J = 3$ cm$^{-1}$, $g = 2.18$, $g_1 = 2.2$, $\rho = 0.004$].

**Synthesis of [Cu(R′SSR)B($SO_4$)] (B: kanamycin A, 2).** The ternary copper(II) kanamycin A adduct was prepared from reaction of **1** (1.0 mmol, 0.83 g) with kanamycin A (2.0 mmol, 1.16 g) in water (20 cm$^3$) at 25 °C on magnetic stirring for 12 h. The solution was filtered and the filtrate on slow concentration gave brownish-green solid which was isolated, washed with aqueous methanol (1 : 1 v/v) and dried in vacuum over $P_4O_{10}$ giving ~62% (0.55 g) yield (Found: C, 39.1; H, 3.9; N, 9.5, $C_{29}H_{37}CuN_6O_{16}S_3$ (**2**) requires C, 39.3; H, 4.2; N, 9.5%). Conductivity ($\Lambda_M/\Omega^{-1}$ cm$^2$ mol$^{-1}$) in DMF–$H_2O$ (1 : 4 v/v) = 145. UV-vis in DMF [$\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$)]: 630 (175), 369 (1090), 310 (3970). IR (KBr phase, cm$^{-1}$): 3337br, 3054w, 1622s, 1538m, 1465m, 1450m, 1400m, 1345w, 1315m, 1282w, 1205m, 1145vs, 1109vs, 1000m, 939m, 906w, 756m, 613s, 483w, 453w (br, broad).

**Synthesis of [Cu(R′SSR)B($SO_4$)] (B: bpy, 3; phen, 4).** Complexes **3** and **4** were prepared by following a general procedure in which the heterocyclic base (2.0 mmol: 0.31 g bpy or 0.4 g phen) was reacted with an aqueous solution (20 cm$^3$) of **1** (1.0 mmol, 0.83 g). The solution was magnetically stirred for 5 h at 25 °C. The solution was filtered. The product as a green solid was isolated on slow concentration of the filtrate on a rotary evaporator. The solid was washed with aqueous ethanol (1 : 1 v/v) and dried in vacuum over $P_4O_{10}$ (Yield: 74%, 0.42 g for **3**; 68%, 0.4 g for **4**). Characterization data for **3**: Found: C, 44.3; H, 4.0; N, 9.6, $C_{21}H_{24}CuN_4O_5S_3$ requires C, 44.1; H, 4.2; N, 9.8%. Conductivity ($\Lambda_M/\Omega^{-1}$ cm$^2$ mol$^{-1}$) in DMF–$H_2O$ (1 : 4 v/v) = 140. UV-vis in DMF [$\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$)]: 611 (150), 371 (830), 274 (2300). IR (KBr phase, cm$^{-1}$): 3700br, 3037w, 1618s, 1595m, 1565w, 1532m, 1470m, 1442s, 1320m, 1242w, 1116vs, 1022m, 980w, 906w, 769s, 730m, 660w, 620s, 470w, 456w. Characterization data for **4**: Found: C, 46.2; H, 3.7; N, 9.5, $C_{23}H_{24}N_4CuO_5S_3$ requires C, 46.3; H, 4.0; N, 9.4%. Conductivity ($\Lambda_M/\Omega^{-1}$ cm$^2$ mol$^{-1}$) in DMF–$H_2O$ (1 : 4 v/v) = 135. UV-vis in DMF [$\lambda_{max}$/nm ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$)]: 666 (105), 372 (1820), 318 (1350), 268 (3260). IR (KBr phase, cm$^{-1}$): 3740br, 3050w, 1621s, 1541m, 1515m, 1473m, 1450m, 1424m, 1348m, 1330w, 1147vs, 1103vs, 1040w, 987w, 912w, 855s, 760m, 722s, 616s.

### Crystal structure determination of 1

**Crystal data.** $C_{22}H_{32}Cu_2N_4O_{10}S_6$, $M_r$ = 831.96, green rectangular (0.50 × 0.32 × 0.28 mm), monoclinic, space group $P2_1/c$, $a$ = 12.771(7), $b$ = 15.664(9), $c$ = 8.544(5) Å, $\beta$ = 105.971(9)°, $U$ = 1643.2(16) Å$^3$, $Z$ = 2, $D_c$ = 1.682 g cm$^{-3}$, $\mu$ = 1.732 mm$^{-1}$, min./max. transmission = 0.48/0.64, $2\theta_{max}$ = 50°, $\lambda$(Mo-K$\alpha$) = 0.71073 Å, $T$ = 293(2) K.

**Data collection and processing.** Crystals of **1** were obtained from the mother-liquor on slow concentration. Intensity data were measured in frames with increasing $\omega$ (width of 0.3° frame$^{-1}$, scan speed of 12 s frame$^{-1}$) on a Bruker SMART APEX CCD diffractometer. The data were corrected for absorption.[18]

**Structure solution and refinement.** The structure was solved and refined with SHELX system of programs.[19] The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were located from the difference Fourier maps and were refined isotropically. The final full-matrix least-squares refinement converged to $R1$ = 0.0506, $wR2$ = 0.1162 for 2895 reflections with $I > 2\sigma(I)$ and 263 parameters [$R1$ (all data) = 0.0652; $wR2$ = 0.1229 (all data)], weighting scheme: $w = 1/[\sigma^2(F_o{}^2) + (0.063P)^2 + 0.7566P]$, where $P = [F_o{}^2 + 2F_c{}^2]/3$. The goodness-of-fit and the largest difference peak are 1.142

and 0.625, respectively. The perspective view of the complex was obtained using ORTEP.[20]

CCDC reference number 236447.

See http://www.rsc.org/suppdata/dt/b4/b414639e/ for crystallographic data in CIF or other electronic format.

### DNA-binding and cleavage experiments

The concentration of the calf thymus DNA (125 μM) was obtained from its absorption intensity at 260 nm with a known $\varepsilon$ value of 6600 dm$^3$ mol$^{-1}$ cm$^{-1}$.[21] The binding of the complexes **1**–**4** to calf thymus (CT) DNA has been studied by fluorescence spectral method using the emission intensity of ethidium bromide (EB). The apparent binding constant ($K_{app}$) value for the phen complex was estimated from the equation: $K_{EB}[EB] = K_{app}[complex]$ using the $K_{app}$ value of EB as $10^7$ M$^{-1}$.[22]

The DNA cleavage activity of the complexes was studied by agarose gel electrophoresis. Supercoiled pUC19 DNA (0.8 μl, ~500 ng) in Tris-HCl buffer (50 mM, pH 7.2) containing NaCl (50 mM) was treated with the complex taken in DMF in the presence or absence of additives. The oxidative DNA cleavage by the complex was studied in the presence of 3-mercaptopropionic acid (MPA) as a reducing agent. The sample was incubated for 1 h at 37 °C, added loading buffer (25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol (3 μl)) and loaded on 0.8% agarose gel containing 1.0 μg ml$^{-1}$ EB. Electrophoresis was carried out at 40 V for 2.0 h in Tris-acetate EDTA (TAE) buffer. Bands were visualized by UV light and photographed. The cleavage efficiency was measured by determining the ability of the complex in relaxing the SC DNA to its nicked circular (NC) form. The proportion of DNA in the SC and NC form after electrophoresis was estimated quantitatively from the intensities of the bands using UVITEC Gel Documentation System with due correction of the low level of NC present in the original sample and the low affinity of EB binding to SC compared to NC and linear forms of DNA.[23] Control experiments were carried out in the dark to detect any hydrolytic cleavage of DNA. Religation experiments were carried out to exclude the possibility of hydrolytic cleavage. In these experiments, the NC DNA, obtained from the hydrolytic cleavage reaction, was recovered from the agarose gel using a gel extraction kit and this was followed by addition of 5X ligation buffer and T4 DNA ligase (1 μl, 4 units). The solution was incubated for 10 h at 16 °C prior to gel electrophoresis.[24] In the inhibition reactions, the additive such as distamycin or DMSO was added initially to the SC DNA and incubation was done for 15 min at 37 °C prior to the addition of the complex and MPA.[25] Considering the good solubility of **1** in water, the DNA binding and cleavage reactions of the adducts **2**–**4** were studied under *in situ* conditions in which complex **1** was treated with the ligand (B) in a Tris buffer medium.

## Results and discussion

### Synthesis and general aspects

Ternary copper(II) complexes [Cu(R′SSR)B($SO_4$)] (**2**–**4**) are prepared by reacting the dimeric precursor [$Cu_2$(R′SSR)$_2$($SO_4$)$_2$] (**1**) with kanamycin A or a heterocyclic base such as 2,2′-bipyridine (bpy) and 1,10-phenanthroline (phen) (B: kanamycin A, **2**; bpy, **3**; phen, **4**) (Scheme 2). Our choice of **1** as a precursor is based on its novel structural features showing the presence of a cationic amine pendant arm which is expected to show good DNA binding ability. Complex **1** is prepared from a reaction of $CuSO_4 \cdot 5H_2O$ with the Schiff base *N*,*N*′-1,1′-dithiobis(ethylenesalicylaldimine) ($H_2$RSSR) (Scheme 2). The complexes **1**–**4** are characterized by analytical and spectral methods. Complex **1** is structurally characterized by X-ray crystallography.

The crystal structure of **1** shows the dimeric nature of the complex in which two {Cu(R′SSR)}$^{2+}$ units are bridged by two sulfates (Fig. 1). The Cu···Cu distance is 4.562(2) Å and each copper atom has a square pyramidal (4 + 1) $CuNO_4$ coordination geometry with the sulfate atom O(2) as the axial ligand. The pendant $-SCH_2CH_2NH_3^+$ group is presumably formed from the hydrolysis of one imine bond of $H_2$RSSR. The disulfide moiety does not show any apparent interaction with the metal atoms. The phenolato oxygen atom O(1), the terminal amine group and the uncoordinated oxygen atom O(5) of the sulfate are involved in hydrogen bonding interactions in the solid state. Selected bond parameters for **1** are given in Table 1.

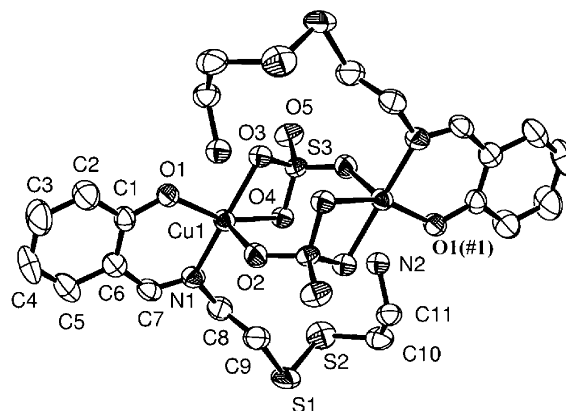
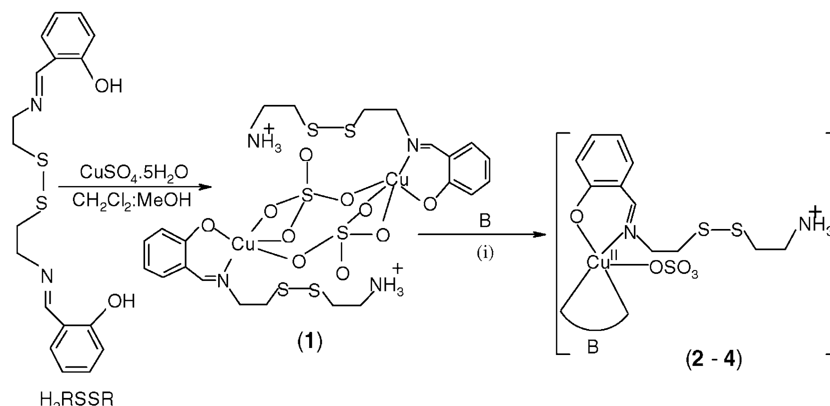



**Fig. 1** An ORTEP view of [$Cu_2$(R′SSR)$_2$($SO_4$)$_2$] (**1**) with atoms showing 50% probability thermal ellipsoids and the numbering scheme.

We have probed the formation of the ternary complexes in solution by electronic spectral and cyclic voltammetric studies. The conductivity value of **1** in $H_2O$ suggests the formation of two monomeric units in solution.[17] The two near UV bands




**Scheme 2** The reaction pathways for the synthesis of [Cu(R′SSR)($SO_4$)]$_2$ (**1**) and ternary complexes (B; kanamycin A, **2**; bpy, **3**; phen, **4**). *Reagents*: (i) solvent: $H_2O$ (**2**); $H_2O$–EtOH 5 : 1 v/v (**3**, **4**).

**Table 1** Bonding parameters for $[Cu_2(R'SSR)_2(SO_4)_2]$ (**1**)

| Bond | Length | Angle | Value |
|---|---|---|---|
| Cu(1)–O(1) | 1.889(3) | O(1)–Cu(1)–N(1) | 95.59(14) |
| Cu(1)–O(2) | 2.306(3) | O(1)–Cu(1)–O(3) | 90.35(11) |
| Cu(1)–O(3) | 2.020(3) | N(1)–Cu(1)–O(3) | 168.49(13) |
| Cu(1)–O(4) | 2.025(3) | O(1)–Cu(1)–O(4) | 156.29(12) |
| Cu(1)–N(1) | 1.923(4) | N(1)–Cu(1)–O(4) | 100.73(13) |
| S(1)–S(2) | 2.053(3) | O(3)–Cu(1)–O(4) | 70.83(11) |
| S(1)–C(9) | 1.823(6) | O(1)–Cu(1)–O(2) | 91.56(12) |
| S(2)–C(10) | 1.805(6) | N(1)–Cu(1)–O(2) | 97.66(13) |
| S(3)–O(2) | 1.455(3) | O(3)–Cu(1)–O(2) | 92.01(11) |
| S(3)–O(3) | 1.487(3) | O(4)–Cu(1)–O(2) | 103.07(12) |
| S(3)–O(4) | 1.518(3) | | |
| S(3)–O(5) | 1.444(3) | | |
| N2 · · · O(1)[a] | 2.767(6) | | |
| N2 · · · O(5)[b] | 2.729(6) | | |

[a] $-x + 1, -y + 2, -z + 1$. [b] $x, -y + \frac{1}{2} + 1, z - \frac{1}{2}$.

at 360 and 320 nm for a solution of **1** in water disappear on addition of the ligand and a new band is observed at 380 nm for kanamycin A and 389 nm for phen (Fig. 2). Cyclic voltammetric experiments show a cathodic peak for **1** at −0.62 V with an anodic counterpart at −0.04 V *vs.* SCE at 50 mV $s^{-1}$ in $H_2O$–0.1 M KCl. Addition of kanamycin A to **1** causes a shift of the cathodic peak to −1.04 V with a reduced current and no corresponding anodic response. The voltammetric responses of the ternary species are significantly different from those of $CuSO_4$ in the presence or absence of kanamycin A. The ternary structure of complexes are found to be stable in water even on addition of ten-fold excess quantity of kanamycin A or heterocyclic base (bpy, phen). Although the formation of bis(phen)copper(II) species is a possibility from the reaction of **1** with excess phen, the cyclic voltammetric measurements rule out any such complex formation as the voltammetric response is not characteristic of this binary complex.[26] The one-electron paramagnetic ($\mu_{eff} \sim 1.8\ \mu_B$) complexes **2**–**4** are non-electrolytic in DMF but show 1 : 1 electrolytic behavior in aqueous DMF (4 : 1 v/v).

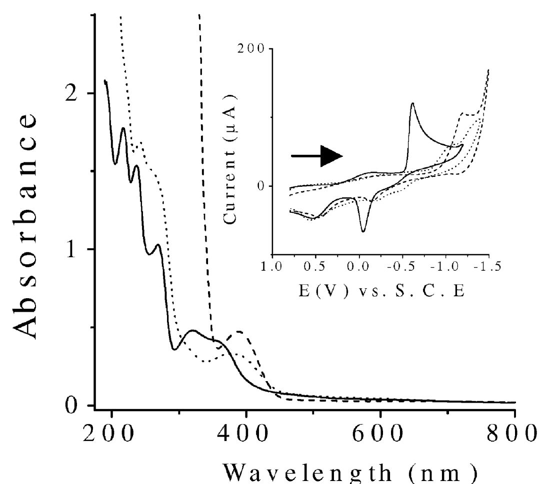



**Fig. 2** UV-Vis spectra of **1** before (—) and after addition of kanamycin A (· · ·) or phen (---) in water. The inset shows the cyclic voltammograms of **1** in $H_2O$–0.1 M KCl (—) and after addition of kanamycin A (· · ·) or phen (---) at a scan rate of 50 mV $s^{-1}$

Complex **1** shows three IR bands for the sulfate which displays a $\eta^3,\mu_2$-binding mode to the metal centers. We have also observed two well resolved IR bands for the sulfate ligand in **2** and **4** indicating its bonding to the metal atom. We propose that the sulfate binding could be at the elongated axial site considering its labile nature in aqueous medium. The complexes display a visible band in the spectral range 611–666 nm. This band is assignable to the d–d transition. While complex **1** shows good solubility both in water and DMF, complexes **2**–**4** are soluble in DMF. Variable temperature magnetic susceptibility data in the range 18–300 K show the essentially paramagnetic nature of the metal atoms in **1**. The theoretical fit of the data shows a $-2J$ value of 3 $cm^{-1}$ indicating the non-interacting nature of the spins in the absence of any superexchange pathway(s) involving the sulfate ligands.

### DNA binding and cleavage studies

The propensity of binding of **1**–**4** to calf thymus (CT) DNA has been studied by fluorescence spectral method using the emission intensity of ethidium bromide (EB). The DNA binding plot gives the relative order: **4** > **2** ≥ **1** ≈ **3** ($K_{app}$: 5.4 × $10^5$ $M^{-1}$ for **4**) (Fig. 3). Complex **1** alone is cleavage inactive when reacted with supercoiled (SC) pUC19 DNA and MPA as a reductant, but it cleaves DNA efficiently on addition of kanamycin A or phen under *in situ* reaction conditions (Fig. 4). Similar cleavage efficiency is observed using adducts **2** and **4** in the presence of MPA. A 5 µM solution of **1** cleaves SC DNA (~500 ng) to the extent of ~90% on addition of 30 µM phen or 50 µM kanamycin A and 5 mM MPA.

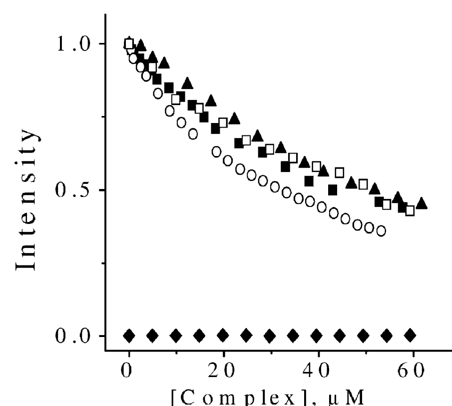



**Fig. 3** The emission intensity of 303 µM calf thymus DNA-bound ethidium bromide (1.3 µM) at different complex concentrations in 50 mM Tris-HCl buffer (pH, 7.2) at 25 °C on addition of $[Cu_2(R'SSR)_2(SO_4)_2]$ (**1**) (□) alone and in the presence of bpy (▲), phen (○) and kanamycin A (■). The emission intensities of the ethidium bromide in the absence of CT DNA but at various concentrations of complex **4** are also shown (◆).

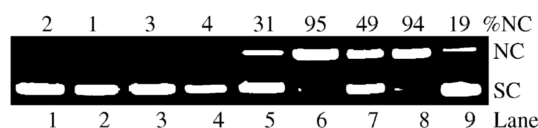



**Fig. 4** Gel electrophoresis diagram showing the cleavage of SC DNA (0.5 µg) by **1** on addition of the kanamycin A or heterocyclic base and MPA (5 mM): lane 1, DNA control; lane 2, DNA + **1** (40 µM) + MPA; lane 3, DNA + kanamycin A (50 µM) + MPA; lane 4, DNA + $CuSO_4·5H_2O$ (50 µM) + kanamycin A (300 µM) + MPA; lane 5, DNA + **1** (5 µM) + kanamycin A (10 µM) + MPA; lane 6, DNA + **1** (5 µM) + kanamycin A (50 µM); lane 7, DNA + **1** (5 µM) + phen (10 µM) + MPA; lane 8, DNA + **1** (5 µM) + phen (40 µM) + MPA; lane 9, DNA + **1** (10 µM) + bpy (100 µM) + MPA.

We have probed different aspects of the DNA binding and cleavage reactions from control experiments using various additives (Fig. 5). The reaction of copper sulfate with kanamycin A in the presence of MPA shows no apparent cleavage of SC DNA. Similarly, no cleavage of DNA occurs by $Cu^{2+}$ (aq) or kanamycin A alone in presence of MPA. Distamycin addition is found to inhibit the cleavage activity of the ternary complexes suggesting minor groove directing nature of **1**–**4** similarly to BLMs. A similar inhibition of DNA cleavage is observed in the presence of hydroxyl radical scavengers such as DMSO indicating an oxidative cleavage pathway involving hydroxyl radicals. Although the true identity of the reactive

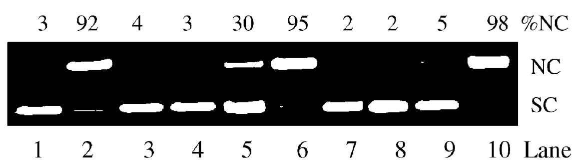



**Fig. 5** Gel electrophoresis diagram for control experiments using SC DNA (0.5 μg), **1** (5 μM), MPA (5 mM for all lanes except lane 5 for which the concentration was 2.5 mM) and other reagents in presence of kanamycin A (50 μM) or phen (40 μM): lane 1, DNA control; lane 2, DNA + **1** + kanamycin A + MPA; lane 3, DNA + DMSO (2 μL) + **1** + kanamycin A + MPA; lane 4, DNA + distamycin (40 μM) + **1** + kanamycin A + MPA; lane 5, DNA + **1** + phen + MPA (2.5 mM); lane 6, DNA + **1** + phen + MPA (5 mM); lane 7, DNA + DMSO (2 μL) + **1** + phen + MPA; lane 8, DNA + ethanol (2 μL) + **1** + phen + MPA; lane 9, DNA + distamycin (40 μM) + **1** + phen + MPA; lane 10, NC form (obtained from DNA + **1** + kanamycin A + MPA) after treatment with 4 units of T4 DNA ligase.

oxygen species is yet to be conclusively established, it could be either free hydroxyl radical or a metal bound oxo or hydroxo moiety effecting hydrogen abstraction from the deoxyribose sugar moiety.[1,27]

The oxidative nature of the DNA cleavage is evidenced from the T4 ligase experiments which show no religation of the nicked circular DNA to its original SC form thus excluding the possibility of any hydrolytic cleavage. The disulfide bond of the Schiff base R′SSR is found to be stable under reducing conditions in the presence of excess MPA as the control experiments show enhancement of DNA cleavage efficiency of the ternary complex on increasing the concentration of MPA.

## Conclusions

In conclusion, the ternary kanamycin A complex, obtained from a dicopper(II) precursor containing a pendant cationic amine moiety, exemplifies the first potential model for three functional domains of BLMs. While the precursor complex is cleavage inactive, the ternary complexes containing kanamycin A and phen show efficient oxidative DNA cleavage in the presence of MPA. Binding of kanamycin A to copper significantly enhances the DNA cleavage activity of **2** in comparison to the precursor or its bpy adduct thus mimicking the facilitating role of the sugar unit in the DNA cleavage activity of BLMs. The higher cleavage efficiency of the phen complex in comparison to **1**–**3** could be related to the efficient DNA groove binding ability of this planar heterocyclic base.

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