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Synthesis, DNA interaction and antimicrobial activities of copper (II) complexes with Schiff base ligands derived from kaempferol and polyamines

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ABSTRACT

Two novel copper (II) complexes **1** and **2** with Schiff base ligands derived from kaempferol and polyamines such as ethylenediamine and diethylenetriamine have been synthesized and characterized by elemental analysis, IR, UV–visible spectroscopy, ^{1}H NMR, molar conductance measurements and molecular modeling studies. The interactions of complexes with DNA have been studied by absorption spectra, viscosity measurements and gel electrophoresis under physiological conditions. The experimental results indicated that two complexes could bind to CT-DNA via an intercalative mode. Noticeably, cleavage DNA (pUC 19) activity of the complex **2** is stronger than that of complex **1**. The antimicrobial activities against *Escherichia coli* and *Staphylococcus aureus* of two complexes were evaluated by the minimum inhibition concentrations (MIC), which indicated that complex **2** possessed more active against *E. coli*.

Flavonoids are a group of naturally occurring compounds that are found in many high plants. Such compounds have evoked widespread interest in biological and pharmacological activities including antioxidant, anticancer, and antimicrobial, etc. [1–5]. Moreover, there has been rapid growth in the development of metal complexes of flavonoids that have better biological activity than flavonoids alone [6–10]. The studies of molecular targets have also revealed that DNA could be affected by flavonoids and their metal complexes [11–13].

Schiff base metal complex is a kind of attractive reagents due to specific activities of pharmacology and physiology. People pay great interest in synthesis, DNA interaction and biological activity of Schiff base metal complexes in recent years [14–20]. However, to the best of our knowledge, less attention was paid on the DNA interaction and biological activity of Schiff base metal complexes derived from flavonoids. In this work, we focus our attention on the synthesis, DNA binding and cleaving abilities of two copper (II) complexes with Schiff base ligands derived from kaempferol (3,3′,5,7-tetrahydroxyflavone) and different polyamines such as ethylenediamine and diethylenetriamine (Scheme 1). Furthermore, the antimicrobial activities were also investigated in vitro.

Schiff base ligands L_1 and L_2 were synthesized by a typical procedure. Polyamine (1 mmol) was added to kaempferol (0.578 g, 2 mmol) and refluxed in ethanol (10 mL) for 3 h. The yellow precipitate was filtered off, washed with ethanol, and dried. The structures of Schiff base ligands were confirmed by ^{1}H NMR, IR and elemental analysis, which were consistent with the proposed structures (Scheme 1B) [21,22]. Schiff base copper (II) complexes **1** and **2** were prepared by a following procedure. To a solution of copper (II) chloride dehydrate (0.017 g, 0.10 mmol) in ethanol (5 mL) was added a solution of the Schiff base ligands (0.10 mmol) in ethanol (100 mL) and sodium bicarbonate (0.032 g, 0.3 mmol) at room temperature for 6 h. The solution was filtered, and the filtrate was concentrated under reduce pressure. The resulting green solid complex was filtered off, washed with diethyl ether and dried in vacuo. We got the likely composition of complexes: $[C_{32}H_{22}CuN_2O_{10}]Cl_2\cdot 2H_2O$ (**1**) and $[C_{34}H_{27}CuN_3O_{10}]Cl_2\cdot H_2O$ (**2**) by IR, elemental analysis and molar conductivity [23,24].

The interactions between Schiff base ligands and Cu (II) were studied by absorption spectra. Kaempferol absorbed with maxima at 372 nm (band I) and 269 nm (band II). Band I is related to ring B (cinnamoyl system) and band II to ring A (benzoyl system) [25,26]. Absorption spectra of Schiff base ligand L_1 in the ethanol solution with different concentration of $CuCl_2$ are shown in Fig. 1A. Schiff base ligand L_1 exhibits two strong absorption bands at 367 nm (band I) and 267 nm (band II). The intensity of Schiff base ligand L_1 (band I) decreased gradually with addition of Cu (II) to the solution, a new absorbance peak appeared at 429 nm. The results indicated that Schiff base ligand could form complex with Cu (II). The appeared new peak at 429 nm suggested that Cu (II) had bond to 3-hydroxyl of ring B and C═N of ring C [10–12]. Band I bathochromic shift can be explained by the interaction of Cu (II) with the 3-hydroxyl group of ring B resulting in electronic redistribution between Schiff base ligand and Cu (II) to become an extended π-bonding system. However, the absorbance peak of complex at 429 nm disappeared, and the new peak at 336 nm appeared when the complex was dissolved in Tris–HCl buffer solution (pH = 7.4) containing ethanol (10%). The result demonstrated the presence of one new complex form. When followed by UV–visible spectra,

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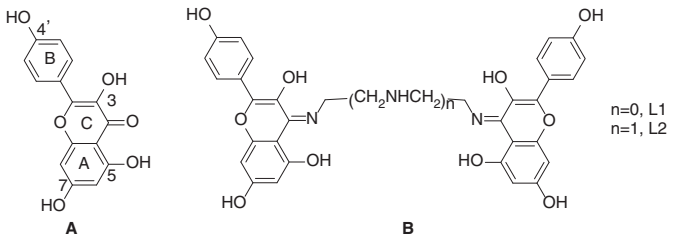

Scheme 1. Kaempferol (A) and Schiff base ligands derived from kaempferol and different polyamines (B).

complexation of Cu (II) by Schiff base ligand L_1 in Tris–HCl buffer solution (pH = 7.4) was investigated and shown in Fig. 1B. The intensity of two bands of Schiff base ligand L_1 decreased gradually with addition of Cu (II), and a new absorbance peak appeared at 336 nm. Hypsochromic shift of band I and disappearance of band II of Schiff base ligand L_1 can be explained by the interaction of Cu (II) with the 5-hydroxyl group of ring A and C═N of ring C. These different approaches drew the conclusion that the first site occupied by Cu in Schiff base ligand L_1 in the ethanol solution is 3-hydroxyl of ring B as the 3-hydroxyl has a more acidic proton, and also because of the presence of a strong intramolecular hydrogen bond between 5-hydroxyl and C═N, it seems difficult for 5-hydroxyl to take part to the formation of complex, whereas the destruction of intramolecular hydrogen bond by addition of Tris–HCl buffer solution (pH = 7.4) can be advantageous to form stable complex by the coordination of Cu (II) to 5-hydroxyl of ring A [22]. Absorption spectra of the interaction of Cu (II) with Schiff base ligand L_2 were similar to those of the interaction of Cu (II) with Schiff base ligand L_1 (Fig. 2).

Since no single crystals suitable for X-ray determination could be isolated, structural information for complexes **1** and **2** was also obtained from the B3LYP/6-311G** optimization calculations. As shown in Fig. 3 theory calculation result demonstrated that the coordination of Cu (II) to 5-hydroxyl of ring A could be more stable than that of 3-hydroxyl of ring B.

The mode of the two complexes bound to DNA was investigated by absorption spectra. Generally, hypochromism is observed in the absorption spectra of small molecules if they intercalate into DNA base pairs [27]. The absorption spectra were obtained by titration of 50 μM complexes in Tris–HCl buffer solution (pH = 7.4) with increasing concentration of DNA, as shown in Fig. 4. The absorption bands of complexes **1** and **2** at 336 nm exhibited hypochromism of about 31% and 18%, respectively. The hypochromism suggested that complexes may bind to DNA by an intercalative mode. The absorption spectra of complexes had almost not changed when the ratio of [DNA]/[complex] was 1.8. The results showed that the DNA-binding reached

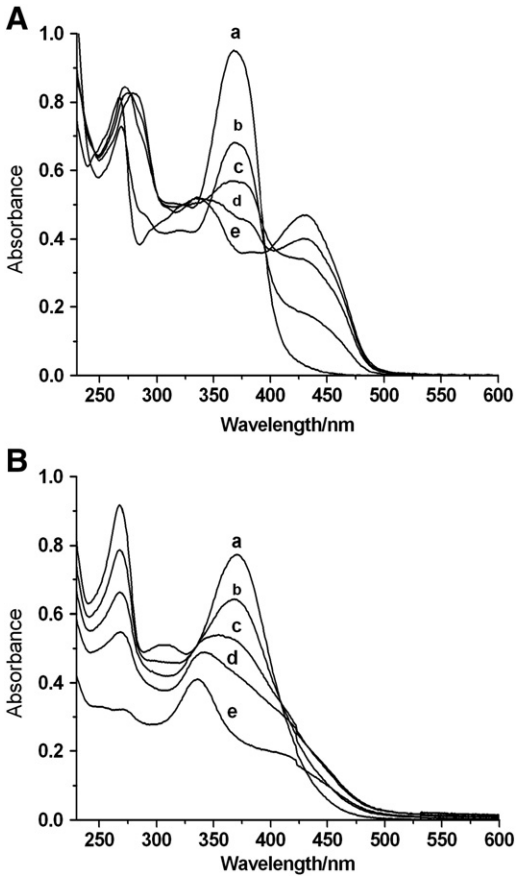

Fig. 1. Absorption spectra of Schiff base ligand L_1 in ethanol (A) and Tris–HCl buffer solution (pH = 7.4) containing ethanol (10%) (B) in the presence of Cu (II), respectively. The molar ratios $[CuCl_2]/[L_1] = 0$ (a), 0.25 (b), 0.50 (c), 0.75 (d), 1.0 (e).

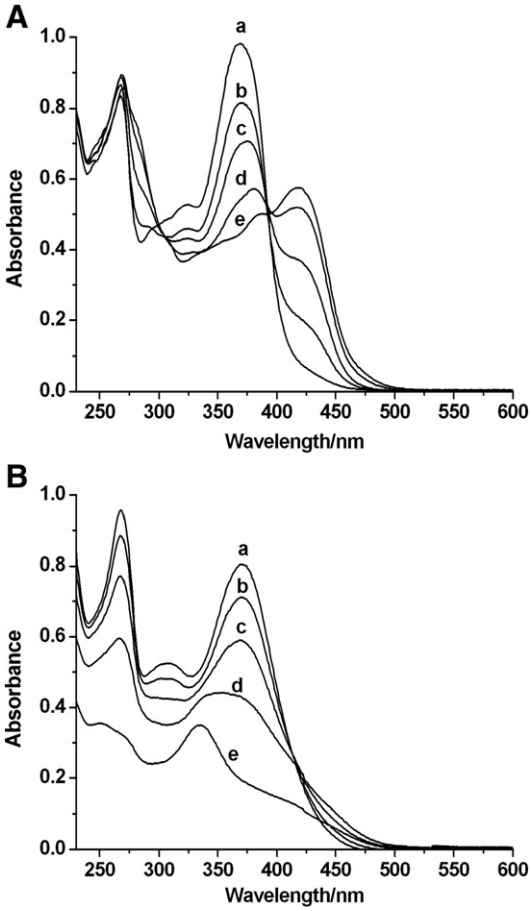

Fig. 2. Absorption spectra of Schiff base ligand L_2 in ethanol (A) and Tris–HCl buffer solution (pH = 7.4) containing ethanol (10%) (B) in the presence of Cu (II), respectively. The molar ratios $[CuCl_2]/[L_2] = 0$ (a), 0.25 (b), 0.50 (c), 0.75 (d), 1.0 (e).

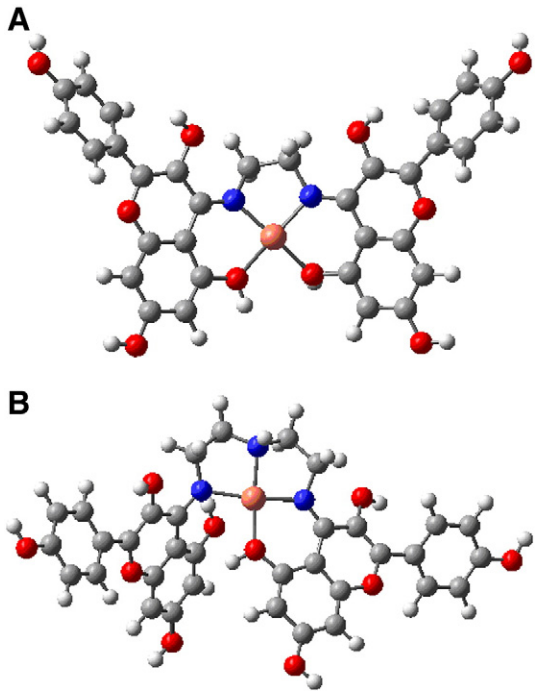

Fig. 3. The optimized structure of the complex **1** (A) and **2** (B). The pink ball stands for copper atom, the red ball stands for oxygen atom, the blue balls for nitrogens, the gray balls for carbons and the white balls for hydrogens.

saturation. In order to compare the binding abilities of the complexes to DNA, the binding constant, K_b, was calculated from the spectroscopic titration data using the equation [28]:

$$[\mathrm{DNA}]/\left(\varepsilon_a-\varepsilon_f\right) = [\mathrm{DNA}]/\left(\varepsilon_b-\varepsilon_f\right) + 1/\mathrm{K_b}\left(\varepsilon_b-\varepsilon_f\right)$$

Where ε_a,ε_b and ε_f are the apparent, bound, and free extinction coefficients respectively. Fit the plot of $[\mathrm{DNA}]/(\varepsilon_a-\varepsilon_f)$ vs. [DNA], the K_b was obtained from the ratio of the slope to the *Y* intercept.

The binding constants obtained for complexes **1** and **2** are 2.9×10^4 and 1.3×10^4 M^{-1}, respectively. From the binding constant values, it is clear that the complexes are moderate binders and complex **1** shows a stronger binding ability towards DNA.

Spectroscopic data are necessary, but not sufficient to support a binding mode. Viscosity experiment is an effective tool to study the binding mode of complexes to DNA in the absence of crystallographic structural data and NMR. A classical intercalative mode causes a significant increase in viscosity of DNA solution due to increase in separation of base pairs at intercalation site and hence an increase in overall DNA length. However, a partial and/or nonclassical intercalation of complex may bend (or kink) the DNA helix, resulting in the decrease in its effective length with a concomitant decrease in its viscosity [29,30]. The effects of complexes on the viscosity of CT-DNA solution were given in Fig. 5. It can be seen that the relative viscosity of DNA increased with the addition of complexes. The results further revealed that the complexes could bind to DNA by intercalation mode.

Besides the above methods, interactions between the copper (II) complexes and DNA were also investigated by the cleavage assay of plasmid DNA (pUC 19). The cleavage of the plasmid DNA was analyzed by monitoring the conversion of supercoiled circular DNA (form I) to nicked DNA (Form II). The amounts of strand scission were assessed by agarose gel electrophoresis [31].

Firstly we compared the cleavage abilities of the complexes at a concentration of 225 μM and an incubation time of 6 h. Fig. 6 showed

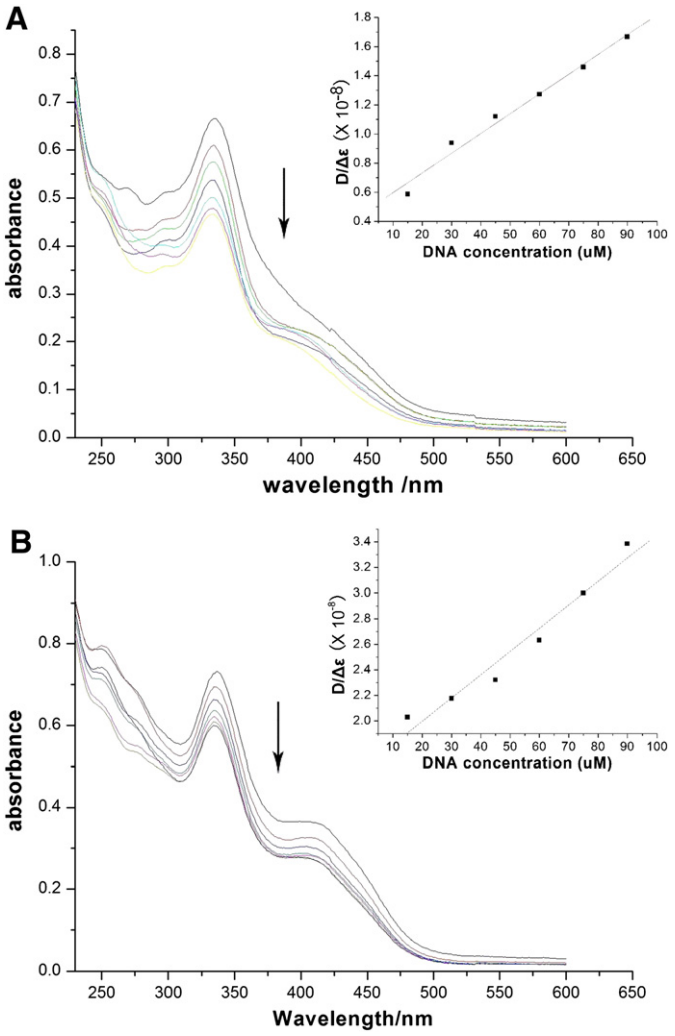

Fig. 4. Absorption spectra of copper complexes **1** (A) and **2** (B) in the presence of DNA in Tris–HCl buffer solution (pH = 7.4). [complex] = 50 μM, [DNA] = 0, 15, 30, 45, 60, 75, 90 μM, respectively. The arrow shows the intensity changes on increasing DNA concentration.

the relative cleavage efficiency of complexes **1** and **2**. It is obvious that complex **2** showed the stronger cleavage ability to DNA (Lane 3). Therefore, our subsequent efforts focus on the reactivity of complex **2**.

The cleavage of DNA by different concentrations of complex **2** was studied for incubation time of 6 h. The amount of nicked DNA (Form II) was observed in agarose gel electrophoresis diagram increased in accord with the changed trend of the concentration of complex **2** in the reaction system (Fig. 7). Increasing the concentration of complex **2** in the order of 75, 150, 225 and 300 μM resulted in 40%, 55%, 81% and 96% of nicked DNA, respectively.

Minimum inhibition concentration (MIC) is the lowest concentration of an antimicrobial complex that will inhibit the visible growth of microorganisms after overnight incubation. The MIC of Schiff base copper (II) complexes and kaempferol (as a comparison drug) was tested against bacterial strains by tube double dilution method. Ciprofloxacin was used as the control. The MIC of ciprofloxacin, kaempferol, complexes **1** and **2** was found to be 21, 100, 104 and 54 μM for *Escherichia coli*, whereas the MIC of ciprofloxacin, kaempferol, complexes **1** and **2** was found to be 11, 120, 126 and 164 μM against *Staphylococcus aureus*, respectively. The results indicated that the ciprofloxacin was highly effective against the studied bacteria. Moderate activity was observed for kaempferol, complexes **1** and **2**, and complex **2** possessed better antimicrobial activity for *E. coli* than kaempferol and complex **1**.

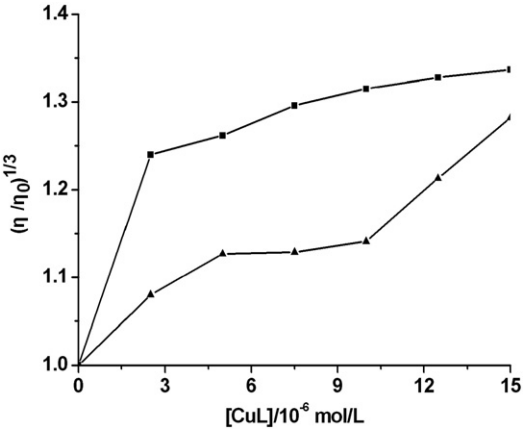

Fig. 5. Effects of increasing amount of copper complexes **1** (■) and **2** (▲) on the viscosity of CT-DNA, [DNA] = 50 μM.

In summary, the Schiff base copper (II) complexes **1** and **2** derived from kaempferol and polyamines were synthesized and characterized. The interactions of complexes with DNA were studied by UV spectra, viscosity and gel electrophoresis under physiological conditions. The results indicate that complexes **1** and **2** are capable of binding DNA by an intercalative mode and cleaving DNA. Moreover, complex **2** shows the considerably high cleavage DNA abilities. In addition, the antimicrobial activities of kaempferol, complexes **1** and **2** were evaluated by the minimum inhibition concentrations (MIC) and the results indicated that complex **2** exhibited more antimicrobial activity against *E. coli*. These studies reveal that the complexes can be used as potential chemotherapeutic agents.

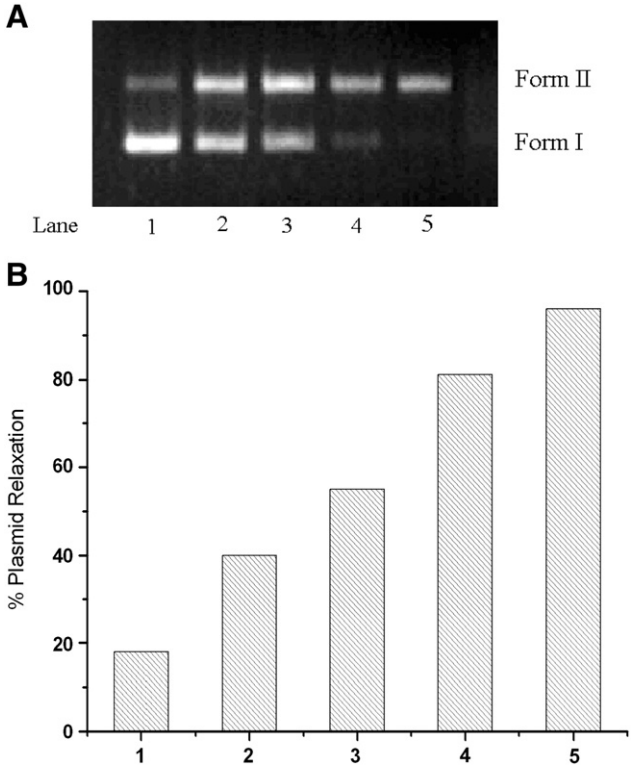

Fig. 7. Effect of concentration of complex **2** on the cleavage of pUC19 DNA (10 μg/mL) in a Tris–HCl buffer (100 mM, pH 7.4) at 37 °C for 6 h. (A) Agarose gel electrophoresis diagram: lane 1, DNA control; lanes 2–5, [complex **2**] = 75, 150, 225 and 300 μM. (B) Quantitation of % plasmid relaxation (Form II %) relative to plasmid DNA per lane.

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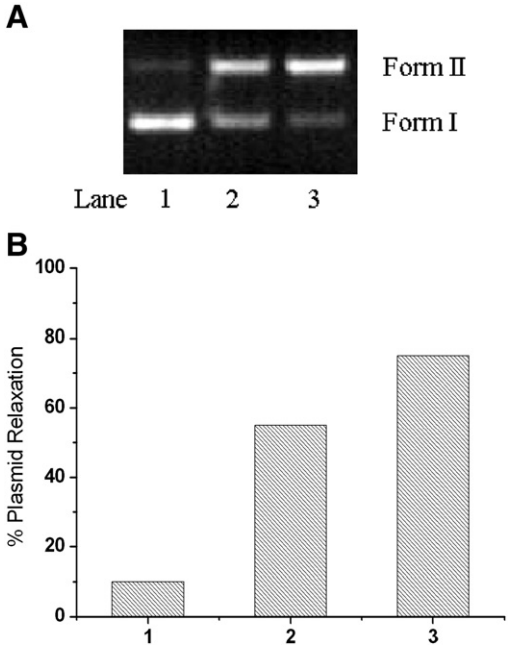

Fig. 6. Effect of different copper (II) complexes (225 μM) on the cleavage reactions of pUC 19 DNA (10 μg/mL) in a Tris–HCl buffer (100 mM, pH 7.4) at 37 °C for 6 h. (A) Agarose gel electrophoresis diagram: lane 1, DNA control; lane 2, complex **1**; lane 3, complex **2**. (B) Quantitation of % plasmid relaxation (Form II %) relative to plasmid DNA per lane.

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[21] **L_1**: IR (KBr, cm^{-1}): 3291, 3115, 1644, 1570, 1501, 1309, 1173, 838. ^1H NMR (600 MHz, DMSO, TMS) δ (ppm): 2.63–2.66 (m, 4H), 6.05 (s, 2H), 6.26 (s, 2H), 6.89–6.91(d, 4H), 8.02–8.03 (d, 4H). Anal. Calcd. For $C_{32}H_{24}N_2\ O_{10}\cdot 2H_2O$: C, 60.76; H, 4.46; N, 4.43%. Found: C, 60.35; H, 4.56; N, 4.38%.
[22] **L_2**: IR (KBr, cm^{-1}): 3318, 3116, 1659, 1569, 1505, 1312, 1177, 823. ^1H NMR (600 MHz, DMSO, TMS) δ (ppm): 2.61–2.70 (m, 8H), 5.99 (s, 2H), 6.20 (s, 2H), 6.88–6.89(d, 4H), 8.02–8.03 (d, 4H). Anal. Calcd. For $C_{34}H_{29}N_3\ O_{10}\cdot 2H_2O$: C, 60.44; H, 4.92; N, 6.22%. Found: C, 60.15; H, 4.96; N, 6.16%.
[23] **complex 1**: IR (KBr, cm^{-1}): 3253, 1602, 1364, 1268, 1172, 1091, 838, 651. Conductance: 181 S cm^2 mol^{-1} in DMF solutions. Anal. Calcd. for $[C_{32}H_{22}CuN_2O_{10}]Cl_2\cdot 2H_2O$: C, 50.24; H, 3.43; N, 3.66%. Found: C, 49.03; H, 3.65; N, 3.74%.
[24] **complex 2**: IR (KBr, cm^{-1}): 3249, 1603, 1384, 1268, 1172, 1091, 836, 648. Conductance: 174 S cm^2 mol^{-1} in DMF solutions. Anal. Calcd. for $[C_{34}H_{27}CuN_3O_{10}]Cl_2\cdot H_2O$: C, 50.54; H, 3.87; N, 5.20%. Found: C, 50.02; H, 3.70; N, 5.40%.
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[31] Plasmid DNA (pUC 19) cleavage activity of complexes was monitored by using agarose gel electrophoresis. In a typical experiment, supercoiled DNA (pUC 19) (10 μg/mL, 10 μL) in Tris–HCl (100 mM, pH 7.4) was treated with different concentrations of complexes, followed by dilution with the Tris–HCl buffer to a total volume of 30 μL. The samples were then incubated at 37 °C for 6 h, and loaded on a 0.7% agarose gel containing 1.0 μg/mL ethidium bromide. Electrophoresis was carried out at 40 V for 30 min in TAE buffer and run in duplicate. Bands were visualized by UV light and photographed followed by the estimation of the intensity of the DNA bands using a Gel Documentation System.