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# Effect of coordination sphere of the copper center and Cu—Cu distance on catechol oxidase and nuclease activities of the copper complexes

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**To explore the effect of Cu—Cu distance in the structure of copper complexes on their catechol oxidase and nuclease activity, six copper complexes with a similar coordination sphere but different Cu—Cu distances were synthesized and characterized with elemental analysis, single-crystal X-ray diffraction, molar conductivity measurements, IR and UV–visible spectroscopy. Complex 1 is a binuclear copper complex and complex 4 is a polynuclear complex with a Z-chain structure, as evidenced by their crystal structures. Complementary characterizations showed that complexes 2 and 3 have a similar binuclear structure to the complex 1; and complexes 5 and 6 are analogous to complex 4. The catechol oxidase activity of complexes 1–3 is quite akin to that of 4–6, suggesting that the catechol oxidase activity of the complexes was determined by the coordination environment of the copper center, when Cu—Cu distance is large. In contrast, DNA cleavage activity of the complexes 1, 2 and 3 are much higher than that of 4, 5 and 6, indicating that the planar ligand structure in the complexes 4, 5 and 6 is more critical than the copper coordination sphere and the Cu—Cu distance for their nuclease activity.** 

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**Keywords:** copper complexes; catechol oxidase; nuclease; intermetallic distance; coordination sphere

## Introduction

Metal complexes are being widely studied as model complexes of enzymes in understanding the roles of metal ions, reaction mechanisms of enzymes and geometries of metal centers, etc. For example, many copper complexes have been synthesized to mimic the catalytic reaction of catchol oxidase, and to investigate the reaction mechanism of nuclease.[1–8] Through extensive studies of the model complexes, it has been believed that the distance between two adjacent copper ions in catechol oxidase has a significant impact on its catalytic activity.[9–16] Ennio and co-workers have reported that binuclear copper complexes with two tridentate Schiff base ligands that have an intermetallic distance close to 2.9 Å show higher catechol oxidation activity than those mononuclear copper complexes or binuclear complexes with an intermetallic distance longer or shorter than 2.9 Å.[17] Many studies have also indicated that binuclear or multinuclear copper complexes showed higher catechol oxidase or chemical nuclease activities than those of mononuclear ones, which might be due to the synergistic interaction between the two copper centers.[1–3,5–8,18] On the other hand, recent studies have shown that some multinuclear copper complexes could cleave DNA with higher efficiency or selectivity than those of the mononuclear complexes.[19–21] A binuclear copper complex containing a bidentate ligand with two tris(2-pyridylmethyl) amine units covalently linked in their 5-pyridyl positions by a —$CH_2CH_2$— bridge is more efficient than its mononuclear complex in DNA cleavage.[19] In addition, it was also reported that the activities of DNA cleavage are different for binuclear metal complexes that have different intermetallic distances.[22,23] Hirota and co-workers found that a complex with copper(II) ion-bound CysGly dipeptides linked by an azobenzene shows better DNA cleavage activity in *cis* type (Cu—Cu distance ~8 Å) than that of *trans* type (Cu—Cu distance ~12 Å).[22] However, all these model complexes of catechol oxidase or nuclease have different Cu—Cu distances and different coordination spheres for copper centers; thus it is not easy to compare their catechol oxidase and nuclease activities in terms of Cu—Cu distance and structure of the complexes.

In this article, we designed and synthesized six Cu(II) complexes with *N*-(2-pyridylmethyl)-amino acids, *N*-(2-pyridylmethyl)-amino ethanol and their derivatives as ligands[24–27] (Fig. S1, supporting information) to understand the effect of intermetallic distance and complex structure on their catechol oxidase and nuclease activities. The copper complexes have two categories of structure – one is a binuclear structure and the other is a polynuclear Z-chain structure – but the copper centers in both cases have a very similar coordination sphere. We demonstrated that the catechol oxidase activities of the complexes are not determined by the Cu—Cu distance when it is above 3.4 Å, while, for the nuclease activities of the complexes with a similar coordination sphere, the planar structure feature is a more critical factor than the Cu—Cu distance.

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## Experimental

### Materials and Measurements

All reagents and organic solvents used in this study were reagent grade and used as received. Calf thymus DNA (CT-DNA) was purchased from Sigma Aldrich Company, and others from SinoPharm Chemical Reagent Co. Ltd. Solvents were dried according to the standard procedure and distilled prior to use.

Electronic absorption spectra of the catalytic products and reactions were recorded on a Cary 50 spectrophotometer (Varian, USA). $^1$H NMR spectra were recorded on an Avance 500 analyzer (Bruker, Germany). Elemental analyses were performed with an Elementar Vario ELIII analyzer (Germany) .Conductivity measurements were carried out with an HI8733 conductivity meter using methanol as solvent. X-ray crystallographic data of the compounds were collected on a SMART diffractometer (Bruker, USA) using Mo-$K_a$ radiation ($\lambda = 0.71073$ Å).

### Synthesis of Ligands

Ligands $L_1$–$L_6$ were known; they were characterized by comparing their proton NMR spectra (see supporting information) with those found in the literature.[24–27]

### Synthesis of Metal Complexes

*$Cu_2(L_1)_2Cl_2 2H_2O$ (**1**)*[24]

A methanol solution (10 ml) of $L_1$ (1 mmol, 0.18 g) was added to an aqueous solution (10 mL) of $CuCl_2.2H_2O$ (1 mmol, 0.17 g). The reaction mixture was stirred for 30 min and filtered. The filtrate was kept at room temperature for several days, yielding dark-blue crystals suitable for X-ray analysis. Yield 0.21 g (78%). IR (KBr, $cm^{-1}$) 3443 (O—H), 3216 (N—H), 1650, 1375 ($CO_2^-$); Anal. Calcd for $C_{18}H_{22}Cl_2Cu_2N_4O_4 \cdot 2H_2O$: C 36.49, N 9.46, H 4.42; found: C 35.93, N 9.03,H 4.66.

Complexes **2–6** were synthesized using a similar method, except the copper chloride dihydrate was replaced by the copper acetate monohydrate and copper perchloride hexahydrate for complexes **4** and **5**, respectively.

*$Cu_2(L_2)_2Cl_2$ (**2**)*[24]

Yield 0.16 g (62%). IR (KBr, $cm^{-1}$) 3236 (N—H), 1642, 1381 ($CO_2^-$); Anal. Calcd for $C_{16}H_{18}Cl_2Cu_2N_4O_4$: C 36.37, N 10.60, H 3.43; found: C 35.83, N 10.67, H 4.16.

*$Cu_2(L_3)_2Cl_4$ (**3**)*[26]

Yield 0.15 g (60%). IR (KBr, $cm^{-1}$) 3369 (O—H), 3204 (N—H); Anal. Calcd for $C_{16}H_{24}Cl_4Cu_2N_4O_2$: C 33.52, N 9.77, H 4.22; found: C 33.58, N 9.71, H 4.22.

*$Cu(L_4)(CH_3COO)$ (**4**)*

Yield 0.32 g (75%). IR (KBr, $cm^{-1}$) 3427 (O—H), 3180 (N—H), 1615, 1381 ($CO_2^-$); Anal. Calcd for $C_{17}H_{18}CuN_2O_5$: C 51.84, N 7.11, H 4.61; found: C 51.49, N 7.46, H 4.45.

*$Cu(L_5)ClO_4$ (**5**)*[27]

Yield 0.18 g (43%). IR (KBr, $cm^{-1}$) 3177 (N—H), 1608, 1383 ($CO_2^-$); Anal. Calcd for $C_{12}H_{13}ClCuN_4O_6$: C 35.30, N 13.72, H 3.21; found: C 35.44, N 13.77, H 4.06.

*$Cu(L_6)Cl$ (**6**)*[25]

Yield 0.29 g (82%). IR (KBr, $cm^{-1}$) 3117 (N—H), 1612, 1386 ($CO_2^-$); Anal. Calcd for $C_{15}H_{15}ClCuN_2O_2$: C 50.85, N 7.91, H 4.27; found: C 50.70, N 7.66, H 4.44.

### Catechol Oxidase Activity Assay

Reactivity studies were performed in a mixed-solvent composition of methanol and 50 mM phosphate buffer in pH 8.0 (volume ratio 6:4), at room temperature. The reaction proceedings were followed by UV–visible spectroscopy at 405 nm. The reaction rates were determined by UV–visible spectroscopy and the kinetic data of oxidation were analyzed using the Michaelis–Menten model.

### DNA Binding and Cleavage Activity

DNA binding experiments were performed in Tris–HCl/NaCl buffer (5 mM Tris, 50 mM NaCl, pH 7.2) using an aqueous solution of the complexes. CT-DNA in Tris–HCl buffer gave a ratio of UV absorbance at 260 and 280 nm of 1.9:1, indicating that the DNA was sufficiently free of protein. The concentration of CT-DNA was measured from its absorption intensity at 260 nm using the molar absorption coefficient value of 6600 $M^{-1}$ $cm^{-1}$.[28] UV–visible absorption titration experiments were conducted by adding CT-DNA solution to the complexes, keeping the concentration of complexes constant while the concentration of CT-DNA was varied. The binding constant $K_b$ was determined using the following equation:[29,30]

$$[DNA]/(\varepsilon_a - \varepsilon_b) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

where [DNA] is the concentration of DNA in base-pairs, $\varepsilon_a$, $\varepsilon_b$ and $\varepsilon_f$ are the extinction coefficients of complex bound to DNA at a definite concentration, the complex completely bound to DNA and the free complex in solution, respectively. The $K_b$ value is determined by the plot [DNA] / ($\varepsilon_a - \varepsilon_b$) versus [DNA].

DNA cleavage activity was carried as follows. The complex and supercoiled PSICOR-GFP DNA (0.5 μg) in 50 mM Tris 18 mM/NaCl pH 7.2 buffer were first mixed together. The mixture was then incubated at 37 °C for 4 h. The reaction was quenched by adding 2 μl of a loading buffer solution (0.05% bromophenol blue, 1% SDS and 50% glycerol, pH 8.0) and then subjected to electrophoresis on 0.6% agarose gel containing 50 μg ethidium bromide (EB) in 40 ml TBE buffer (89 mM Tris, 89 mM boric acid and 2 mM EDTA, pH 8.3) at 80 V for approximately 1 h. The cleavage efficiency was measured by determining the ability of the complex to convert the supercoiled DNA (band I) to nicked circular form (band II) or linear DNA (band III). Agarose gel electrophoresis was carried out with DYY-6C electrophoresis apparatus (Liuyi Instrumental Co., China). The gels were visualized and digitized with a Tanon-2500 gel image analysis system and analyzed using Tanon Gis software.

## Results and Discussion

### Structures of the Complexes

The crystallographic data and details of the refinement of the crystal structures of compounds **1** and **4** are summarized in Table 1.The selected bond distances and angles are listed in Table 2. The crystal structures of the **1** and **4** are shown in Fig. 1.

There are two copper centers in the crystal structure of complex **1**. Each Cu(II) ion is coordinated by two nitrogen atoms from

**Table 1.** Crystallographic parameters for complexes **1** and **4**

| | **1** | **4** |
|---|---|---|
| Empirical formula | $C_9H_{12}ClCuN_2O_3$ | $C_{18}H_{21}CuN_2O_6$ |
| Formula weight | 295.20 | 424.91 |
| Temperature (K) | 293(2) | 293(2) |
| Wavelength (Å) | 0.71073 | 0.71073 |
| Crystal system | Monoclinic | Orthorhombic |
| Space group | *C*2/*c* | $P2_12_12_1$ |
| *a* (Å) | 15.764(3) | 7.8575(16) |
| *b* (Å) | 11.433(2) | 12.817(3) |
| *c* (Å) | 12.781(3) | 19.047(4) |
| *β* (Å) | 99.88(3) | 90 |
| Volume (Å$^3$) | 2269.4(8) | 1918.1(7) |
| *Z* | 8 | 4 |
| Calculated density (Mg m$^{-3}$) | 1.728 | 1.471 |
| Absorption coefficient (mm$^{-1}$) | 2.152 | 1.175 |
| *F*(000) | 1200 | 800 |
| Crystal size (mm) | 0.20 × 0.20 × 0.20 | 0.20 × 0.20 × 0.20 |
| Theta range for data collection (°) | 3.24–25.01 | 3.04–25.01 |
| Reflections collected / unique [$R_{int}$] | 6582 / 2004 [0.0640] | 11546 / 3375 [0.0477] |
| Completeness to theta (%) | 99.7 | 99.7 |
| Data/restrains / parameters | 2004 / 0 / 149 | 3375 / 0 / 248 |
| Goodness-of-fit on $F^2$ | 1.271 | 1.118 |
| Final *R* indices | $R_1 = 0.0696$, $wR_2 = 0.1319$ | $R_1 = 0.0381$, $wR_2 = 0.0815$ |
| *R* indices (all data) | $R_1 = 0.0977$, $wR_2 = 0.1409$ | $R_1 = 0.0432$, $wR_2 = 0.0835$ |
| Largest diff. peak and hole (eÅ$^{-3}$) | 0.774 and −0.528 | 0.287 and −0.209 |

**Table 2.** Selected bond lengths (Å) and bond angles (°) for complexes **1**and **4**

| | Bond length | | | Bond angle | | |
|---|---|---|---|---|---|---|
| **1** | Cu(1)—O(2) | 1.966(4) | O(2)—Cu(1)—N(1) | 164.98(19) | O(2)—Cu(1)—N(2) | 82.11(17) |
| | Cu(1)—O(2)# | 2.750(4) | N(1)—Cu(1)—N(2) | 82.96(19) | O(2)—Cu(1)—Cl(1) | 94.18(13) |
| | Cu(1)—N(1) | 1.990(5) | N(1)—Cu(1)—Cl(1) | 100.77(15) | N(2)—Cu(1)—Cl(1) | 176.22(14) |
| | Cu(1)—N(2) | 2.011(4) | O(2)—Cu(1)—O(2)# | 87.77(13) | N(1)—Cu(1)—O(2)# | 90.43(16) |
| | Cu(1)—Cl(1) | 2.265(2) | N(2)—Cu(1)—O(2)# | 89.48(18) | Cl(1)—Cu(1)—O(2)# | 91.15(11) |
| **4** | Cu(1)—O(3) | 1.935(2) | O(3)—Cu(1)—O(1) | 94.85(10) | O(3)—Cu(1)—N(1) | 97.18(11) |
| | Cu(1)—N(1) | 1.982(3) | O(1)—Cu(1)—N(1) | 164.39(11) | O(3)—Cu(1)—N(2) | 172.49(12) |
| | Cu(1)—N(2) | 2.004(3) | O(1)—Cu(1)—N(2) | 83.26(10) | N(1)—Cu(1)—N(2) | 83.53(12) |
| | Cu(1)—O(1) | 1.949(2) | O(3)—Cu(1)—O(2)# | 94.45(10) | O(1)—Cu(1)—O(2)# | 97.34(10) |
| | Cu(1)—O(2)# | 2.311(2) | N(1)—Cu(1)—O(2)# | 91.61(11) | N(2)—Cu(1)—O(2)# | 93.00(10) |

the secondary amine and pyridine of one ligand, and one coordinated oxygen atom from carboxylate groups of the ligand. With one chlorine atom, a four-coordinating planar copper center was formed. The two copper ions were bridged by two oxygen atoms from the carboxylate groups of two ligands, as shown in Fig. 1. The $Cu_2O_2$ core is also in one plane, which is perpendicular to the plane of the $CuN_2OCl$ as revealed by the bond angles listed in Table 2. The distance between two copper centers is 3.4 Å, which is larger than that of native catechol oxidase and its many binuclear model copper complexes.[10,17,31] The Cu—O length in the copper coordination plane is 2.0 Å, and 2.7 Å for the axial bond; the latter is much longer than that in many copper complexes,[10,31] suggesting that this Cu—O is a weak bond.

Compared to the structure of complex **1**, X-ray crystal structural analysis indicates that complex **4** has poly-copper centers with a Z-chain conformation (Fig. 1). The copper centers are bridged by the two oxygen atoms of carboxylate groups from one ligand. Each copper ion is coordinated by five atoms: two nitrogen atoms that are from the secondary amine and pyridine of one ligand, and two coordinated oxygen atoms from the carboxylate groups of the two ligands, with the other oxygen atom from acetate, forming a $N_2O_3$ pentahedron structure, with one bridge oxygen in the apex and the other four forming a plane. However, the acetate is totally out of the plane, and the phenyl ring is almost perpendicular to the copper-centered plane. The Cu—O distances in the plane are both 1.9 Å, while the axial Cu—O is 2.3 Å, which is shorter than that in complex **1**. However, because complex **4** has a Z-chain conformation, the neighboring copper-centered planes are not parallel as that in **1**; the Cu—Cu distance in **4** is thus much longer than that of **1** (5.3 Å vs. 3.4 Å). A similar complex has reported in which the copper centers in that complex were bridged by chlorine atoms instead of the oxygen atoms of carboxylate groups as in **4**.[24,32,33]

In spite of many attempts, we were unable to obtain single crystals of **2**, **3**, **5** and **6**. Hence the molar conductance of complexes **1–6** was measured to further identify their structures. According to

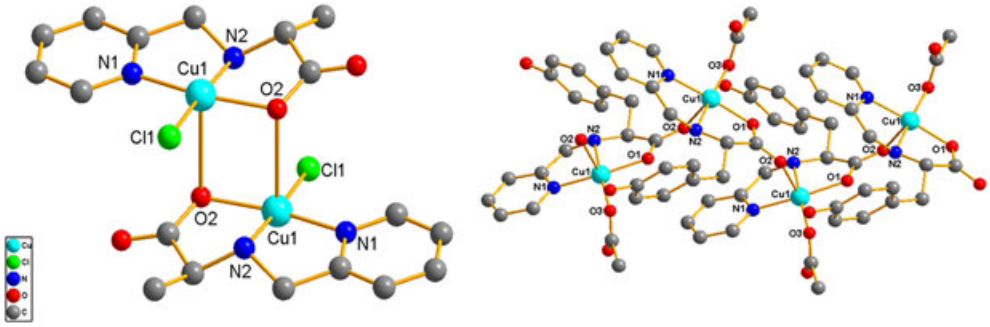


**Figure 1.** Crystal structures of complexes **1** and **4**.

the literature, for 1:2 electrolytes in methanol solution at room temperature the conductance is roughly 160–220 s $cm^2$ $mol^{-1}$; for 1:1 electrolytes the conductance is 80–115 s $cm^2$ $mol^{-1}$; for non-electrolytes, conductance is below 80 s $cm^2$ $mol^{-1}$.[34] Under the same conditions, the molar conductance of complexes **1–6** in methanol are 55, 67, 68, 38, 56 and 29 s $cm^2$ $mol^{-1}$, respectively, suggesting that the six complexes are all non-electrolytes, and the anions of the complexes are all coordinated to the copper centers. On the basis of the ligand similarity in complexes **2**, **3** and **1** and in **5**, **6** and **4**, the crystal structures of complexes **1** and **4**, elemental analysis and their molar conductance, the structures of **2**, **3**, **5** and **6** were proposed as shown in Fig. 2. Similar UV–visible spectra of complexes **1–6** further support the proposed structures (Fig. S2, supporting information). There is an absorbance band at 256 nm for the all complexes, which contributes to the pyridine group in the ligand. The absorption of **5** at 256 nm is larger than the others, which may be due to the imidazole group in the ligand. The bands at ~690 nm are assigned to copper d–d transition in octahedral geometry structure.[10,17]

To further identify their structures, the complexes were also characterized by IR spectroscopy. The IR bands of the complexes are given in Table 3. The bands in the 3117–3236, 1608–1650 and 1375–1386 $cm^{-1}$ regions were ascribed to $\nu_{N-H}$, $\nu_{as}(CO_2^-)$ and $\nu_s(CO_2^-)$ vibrations, respectively. There is an extra band at 3443 $cm^{-1}$ of complex **1**, which may be assigned to $\nu_{O-H}$ vibrations of water molecules. To further determine the speculation, we performed IR measurement for complex **1** after rigorous drying, and similar IR data was obtained (Fig. S3, supporting information). We therefore believe it is from water molecules. This observation is consistent with the element analysis result that there is a water molecule in complex **1**. The IR spectrum of **2** is similar to that of **1** except that no band in the 3443 $cm^{-1}$ region was observed. The bands in the 3369 and 1038 $cm^{-1}$ regions may be ascribed to $\nu_{O-H}$ and $\nu_{C-O}$ vibrations of the hydroxyl group in **3**. This suggests that the alcoholic hydroxyl group in **3** is unionized, which is consistent with the results of molar conductance and element analysis. The IR spectra of **5** and **6** are similar to that of **4**, except for the band in the 3427 $cm^{-1}$ region ascribed to $\nu_{O-H}$ vibrations of the phenolic hydroxyl group in **4**. The similarity in the IR spectra clearly indicates that the structures of complexes **2** and **3** are similar to that of **1**, and **5** and **6** are similar to that of **4**. The bands in the 1082, 941 and 615 $cm^{-1}$ regions were ascribed to $ClO_4^-$ vibrations for complex **5**. These bands indicate that perchlorate ion is coordinated in complex **5**.

## Catechol Oxidase Activity

Catechol oxidase activities of complexes **1–6** were first investigated using 3,5-di-*tert*-butylcatechol (3,5-DTBC) as a substrate, because its oxidation product 3,5- di-*tert*-butyl-*o*-quinone (3,5-DTBQ) is stable and shows strong absorption at 405 nm ($\varepsilon = 1900$ $M^{-1}$ $cm^{-1}$). Thus the catechol oxidase activity of the complexes can be evaluated by detecting the change in absorption at 405 nm of 3,5-DTBQ. All the complexes showed similar catechol oxidase activities under the same reaction conditions as shown in Fig. 3. With reaction processing, the substrate–catalyst adduct breaks down, resulting in a decrease in absorption. At the same time, the product starts to form, leading to absorption recovery. The kinetics of 3,5-DTBC oxidation by the complexes were determined by plots of the initial reaction rates versus substrate concentration. Figure 4 displays the

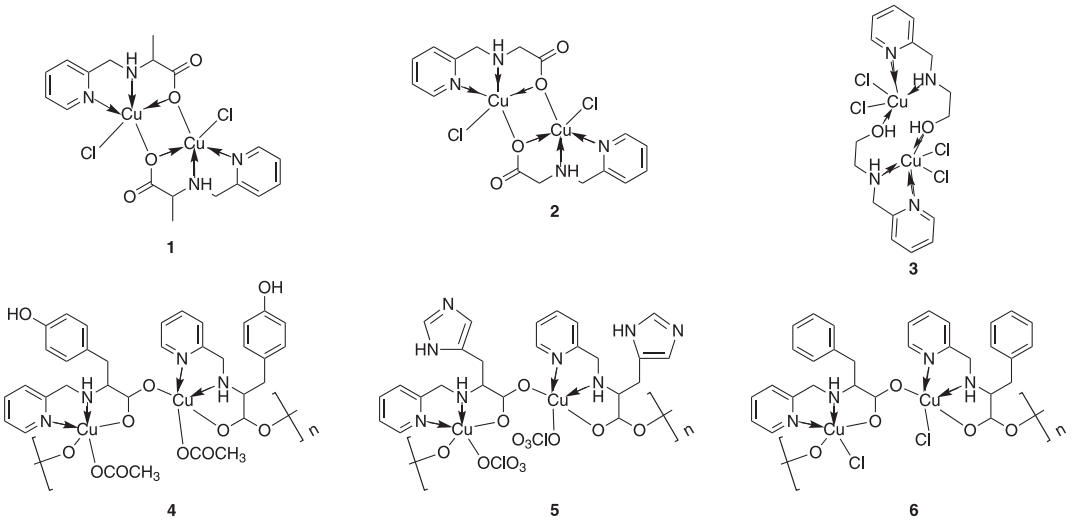


**Figure 2.** Structures of the complexes **1-6**.

**Table 3.** IR absorption bands ($cm^{-1}$) for complexes **1–6**

| Compound | $\nu_{O-H}$ ($cm^{-1}$) | $\nu_{N-H}$ ($cm^{-1}$) | $\nu_{as}$ ($CO_2^-$) ($cm^{-1}$) | $\nu_s$ ($CO_2^-$) ($cm^{-1}$) | $\nu_{C-O}$ ($cm^{-1}$) |
|---|---|---|---|---|---|
| **1** | 3443 | 3216 | 1650 | 1375 | |
| **2** | | 3236 | 1642 | 1381 | |
| **3** | 3369 | 3204 | | | 1038 |
| **4** | 3427 | 3180 | 1615 | 1381 | |
| **5** | | 3177 | 1608 | 1383 | |
| **6** | | 3117 | 1612 | 1386 | |

dependence of initial rate on the concentration of 3,5-DTBC catalyzed by complex **1** as an example. The maximum velocity ($V_{max}$), catalytic constant ($K_{cat}$), Michaelis binding constant ($K_m$) and catalytic efficiency parameter ($K_{cat}/K_m$) of the six complexes were evaluated and the data are summarized in Table 4. All six complexes showed comparable activity. When compared in terms of the copper center, complexes **1–3** showed a slightly higher activity, which may due to their binuclear structure, since many works reported that the catechol oxidase activity of binuclear copper complexes is higher than that of mononuclear copper complexes.[35] Although the Cu—Cu distance of complexes **1–3** is shorter than that of complexes **4–6**, the distance is still much larger than that of the native enzyme (the Cu—Cu distance in catechol oxidase met form is 2.9 Å); hence the activity is relatively lower than the many reported binuclear compounds and catechol oxidase itself.[17,36] The Cu—Cu distance in complexes **4–6** is much larger and thus they can be regarded as a mononuclear complex in the catalysis. It is likely that the copper centers in these six complexes actually act as a mononuclear complex; thus their activity is comparable. Taking these results together, the activity of the binuclear and polynuclear copper complexes is determined by the coordination sphere of the copper center instead of the Cu—Cu distance when it is longer than 3.4 Å. This result is consistent with the literature in that the activity will be lower if the Cu—Cu distance is larger than 4.0 Å.[10] However, the key issue regarding the activity of these complexes is whether they still use their binuclear or polynuclear core structures as catalytic species in solution. It was reported in the literature that oxygen-bridged binuclear copper complex could maintain its binuclear state in solutions of different pH.[37] To clarify this question, the mass spectra of

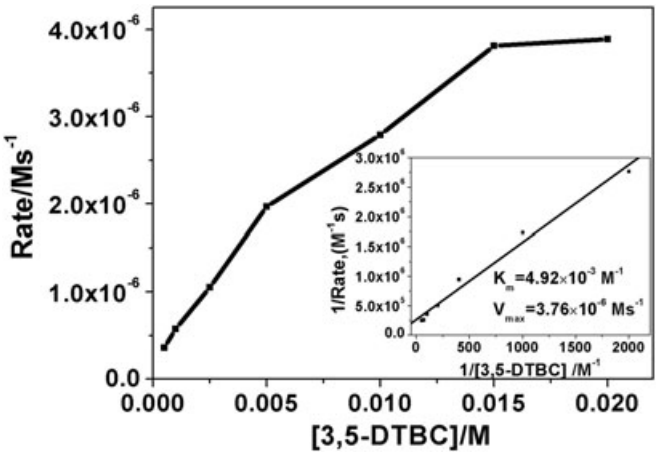


**Figure 4.** Plot of reaction rate versus concentration of 3,5-DTBC catalyzed by complex **1**. Inset shows the reciprocal Lineweaver–Burk plot.

some binuclear and polynuclear compounds were recorded. The *m/z* 519.3 peak was observed in complex **1** assigned as a $(Cu_2(L_1)_2Cl)^+$ species; similarly, in complex **2**, a binuclear species $Cu_2(L)_2Cl)^+$ was observed at *m/z* 491.2. Similarly for polynuclear complex **6**, a binuclear and trinuclear species were observed at *m/z* 745.8, and 1113.7, respectively, corresponding to $[Cu_2L_2(OH)]^+$ and $[Cu_3(HL_2)_2L_2(H_2O)]^+$. These results revealed that binuclear complexes **1** and **2** maintained their binuclear structure intact in solution, and that polynuclear complex **6** was in a polynuclear state in solution also.

## Nuclease Activity

The affinity to DNA of the six copper complexes was first compared. Upon the addition of CT-DNA to complexes **1–6**, a decrease in the molar absorption of the π–π* absorption band of the complexes was observed, indicating that binding of the complexes to DNA occurred. The binding constants ($K_b$) of all the complexes are summarized in Table 5; the binuclear complexes **1–3** exhibit a slightly higher affinity to DNA, possibly because their planar structure that may partially intercalate with DNA. The small difference in DNA affinity for the six complexes also suggested that electrostatic attraction between the complexes and DNA may play a major role in their DNA binding.

DNA cleavage activity of the complexes was then determined by monitoring the conversion of the supercoiled DNA (band I) to nicked DNA (band II) and linear DNA (band III) using agarose gel electrophoresis. Under the same reaction conditions, six complexes exhibit different levels of DNA cleavage activity (Fig. 5). Complexes **1** and **2** show higher DNA cleavage activity: only 20% and 18% supercoiled DNA, respectively, remained unreacted, while for complexes **4**, **5** and **6** more than 50% of the supercoiled DNA was left. Notably, complex **3** exhibits very low DNA cleavage activity, possibly because the oxygen atoms coordinated

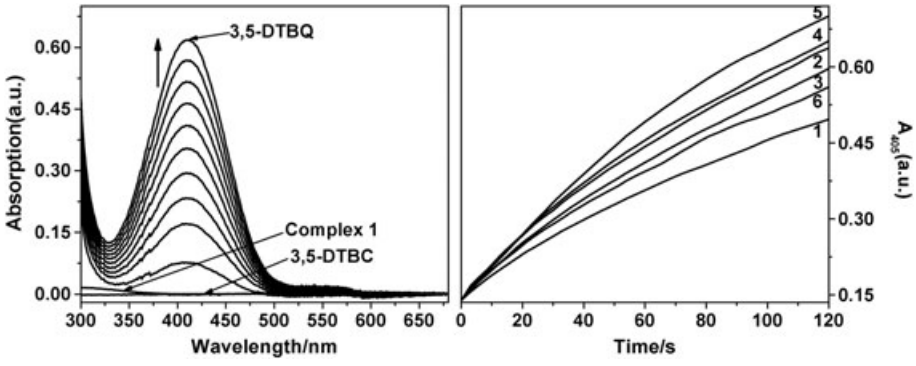


**Figure 3.** (a) Oxidation of substrate 3,5-DTBC (5 mM) by the complex 1 (0.1 mM in mononuclear entity). The spectra were recorded after each 5 min interval as a function of the reaction time upon addition of 3,5-DTBC. (b) Time course of 3,5-DTBC (20 mM) oxidation by complexes **1–6** (0.1 mM in mononuclear entity) at room temperature in phosphate buffer (pH 8.0) monitored at 405 nm.

**Table 4.** Kinetic parameters of complexes **1–6**

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $K_m$ (M) | $4.92\times10^{-3}$ | $1.03\times10^{-2}$ | $5.68\times10^{-3}$ | $1.42\times10^{-2}$ | $2.57\times10^{-2}$ | $9.30\times10^{-3}$ |
| $V_{max}$ (M $s^{-1}$) | $3.76\times10^{-6}$ | $7.68\times10^{-6}$ | $6.88\times10^{-6}$ | $8.33\times10^{-6}$ | $1.48\times10^{-5}$ | $5.01\times10^{-6}$ |
| $K_{cat}$ ($s^{-1}$) | $3.76\times10^{-2}$ | $7.68\times10^{-2}$ | $6.88\times10^{-2}$ | $8.33\times10^{-2}$ | $1.48\times10^{-1}$ | $5.01\times10^{-2}$ |
| $K_{cat}/K_m$ ($M^{-1}$ $s^{-1}$) | 7.64 | 7.45 | 12.1 | 5.87 | 5.76 | 5.39 |

**Table 5.** DNA binding constant ($K_b$) of complexes **1–6**

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $K_b\times10^6$ $M^{-1}$ | 2.00 | 3.76 | 3.66 | 1.93 | 2.39 | 1.34 |

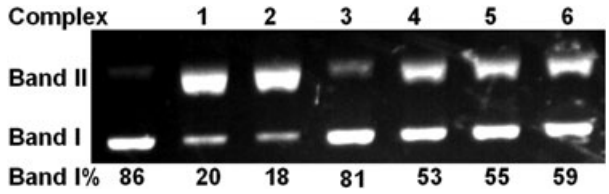


**Figure 5.** Gel electrophoresis diagram of the DNA cleavage (38 μM bp) by complexes **1–6** (1 mM) in pH 7.2, 50 mM Tris, 18 mM NaCl buffer at 37°C for 4 h.

to two copper(II) ions in **3** are from hydroxyl groups, instead of the carboxylic group as in the complexes **1** and **2**; thus the copper center is not in a planar structure as in complex **1**, which needs to be further confirmed by its crystal structure. We also found that the DNA cleavage activity of the complexes is concentration dependent. Figure S4 (supporting information) displays the DNA cleavage with different concentrations of complex **1**. With the concentration of **1** increasing, cleavage of the supercoiled DNA was more complete. At a concentration of 2.5 mM, only 7% of the supercoiled DNA was left. Figure S5 (supporting information) shows that the DNA cleavage activity of complexes is also time dependent. With increasing time, the DNA cleavage was more complete. After 5 h of incubation, 78% of the supercoiled DNA was converted to the nicked DNA. However, the concentration of the complexes used in the cleavage experiment (in millimoles in the mononuclear entity) is much higher compared to the complexes reported in the literature, in which only micromoles were necessary.[38]

The cleavage mechanism by these complexes could be through an oxidative pathway as in many copper complexes, since no

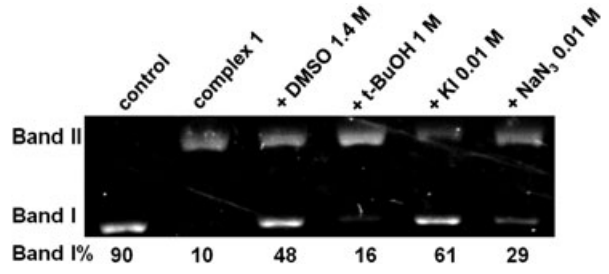


**Figure 6.** Gel electrophoresis diagram of the DNA cleavage (38 μM bp) by complex **1** (2.5 mM) in the presence of various additives with an incubation time of 4 h.

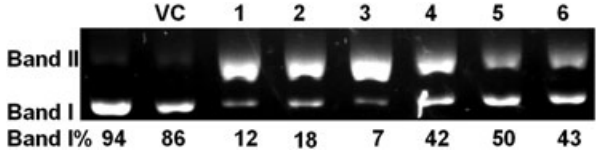


**Figure 7.** Gel electrophoresis diagram of the DNA cleavage (38 μM bp) by complexes **1–6** (50 μM) in the presence of VC (50 μM) in 50 μM Tris/18 mM NaCl buffer at pH 7.2, 37°C, with an incubation time of 30 min.

oxygen or reducing agents were included during the experiment. This assumption was supported by cleavage experiments in the presence of various additives, including DMSO (1.4 M), *t*-BuOH (1 M), KI (10 mM), $NaN_3$ (10 mM) (Fig. 6). Hydroxyl radical scavengers DMSO, *t*-BuOH and KI show significant inhibition of cleavage activity, and the singlet oxygen scavenger $NaN_3$ also slightly inhibited the reaction. The results indicate that diffusible hydroxyl radicals and singlet oxygen were involved in DNA cleavage by the complexes.

The oxidative cleavage pathway was further supported by including a reductant – ascorbic acid (VC) – in the cleavage experiments. Figure 7 indicates that all complexes showed higher cleavage activity in the presence of VC compared to its absence (Fig. 5). It is interesting that complex **3** showed almost no DNA cleavage activity without VC, while it exhibited similar DNA cleavage activity to complexes **1** and **2** in the presence of VC. The higher DNA cleavage activity of **1** and **2** may be partially contributed by the planar structures of their copper centers, as shown in Fig. 1. The time dependence of the DNA cleavage activity of complexes with ascorbic acid was also investigated, as shown in Fig. S6 (supporting information). With increasing time the cleavage was more complete, as expected.

Taking these results together, the nuclease activity of the binuclear copper complexes is higher than that of the polynuclear Z-chain complexes, though they are all through an oxidative mechanism. The Cu—Cu distances in these six complexes are likely too large to have a synergetic effect like that in the native enzyme, while they are too short to allow each copper center to bind to one DNA helix at the same time; thus Cu—Cu distance is not a decisive factor for their difference in nuclease activity. The activity difference thus may be contributed partially by the copper-centered planar structure. As seen in the crystal structure of complex **1**, the copper center and its coordinated atoms are in an almost perfect plane, whereas in complex **4** the acetate is totally out of the copper-centered plane. In addition, the Cu—O distance (axial bond) in the binuclear complexes is much longer than the regular Cu—O (oxygen atom from carboxylate) distance (2.7 Å vs. 1.9 Å), whereas in polynuclear Z-chain complexes the distance is closer to the regular length (2.3 Å vs. 1.9 Å). The larger Cu—O distance makes binuclear complexes **1–3** more flexible in interacting with DNA, while a smaller Cu—O bridge distance in polynuclear Z-chain complexes **4–6** will have a larger steric hindrance in their interaction with DNA.

## Conclusions

In summary, six new Cu(II)complexes with *N*-(2-pyridylmethyl)-amino acids or N-(2-pyridylmethyl)-amino ethanol as ligands were synthesized. All the complexes exhibit catechol oxidase and nuclease activity. The catechol oxidase activity of the binuclear complexes **1–3** is quite similar to that of the **4–6**, suggesting that once the Cu—Cu distance is larger there is no synergetic interaction between Cu centers; thus the activity was determined mainly by its coordination environment. The nuclease activities of the six complexes follow the order **1**, **2** and **3** $>$ **4**, **5** and **6**. Although the DNA cleavage by these complexes is all through the oxidation pathway, the better copper-centered plane and longer bridge Cu—O bond in the binuclear complexes compared to that in polynuclear Z-chain complexes make binuclear complexes easier to interact with DNA, and thus show higher nuclease activity.

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