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# Synthesis, characterization and studies on DNA-binding of a new Cu(II) complex with $N^1$,$N^8$-bis(l-methyl-4-nitropyrrole-2-carbonyl)triethylenetetramine

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**Abstract**

A new Cu(II) complex of $CuLCl_2$ (here, L = $N^1$,$N^8$-bis(1-methyl-4-nitropyrrole-2-carbonyl)triethylenetetramine) had been synthesized and characterized. The structure of the complex was investigated with density functional theory (DFT) calculations. DNA-binding of the Cu(II) complex and its effects on tumor cell viability were firstly studied. The interactions between the complex and calf thymus DNA had been investigated using UV spectra, fluorescent spectra, viscosity and CV (cyclic voltammetry). The cleavage reaction on plasmid DNA has been monitored by agarose gel electrophoresis. The experimental results show that the mode of binding of the complex to DNA is classical intercalation and the complex can cleave pBR322 DNA. The effects of the CuL on cell viability were tested using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) dye assay and the results indicate that the CuL had certain effect on cancer cells.


*Keywords:* $N^1$,$N^8$-Bis(1-methyl-4-nitropyrrole-2-carbonyl)triethylenetetramine; Cu(II) complex; Calf thymus DNA

## 1. Introduction

Numerous control mechanisms in the living cell are based on the recognition and interaction between biological activity molecules and biomacromolecules [1,2]. The design of synthetic ligands that read the information in the DNA duplex has been a central goal at the interface of chemistry and biology [3]. Syntheses of DNA-binding molecules, such as triplex-forming oligonucleotide [4–7], peptide nucleic acid [8,9], oligosaccharide [10] and oligopeptide [11,12], have been exploited. Polyamides containing *N*-methylpyrrole and *N*-methylimidazole amino acids have attracted considerable attention on the part of synthetic and biological groove of predetermined DNA sequences with high affinity and specificity [13–16]. Since these polyamides can permeate living cell membranes, they have the potential to control specific gene expression [17,18]. The principal rules developed by Dervan et al. are that antiparallel pairing of Py/Im (Py = *N*-methylpyrrole and Im = *N*-methylimidazole) targets a C,G base pair, Im/Py targets a G,C base pair [19,20], and Py/Py is degenerate, recognizing either an A,T or T,A base pair [21,22].

Furthermore, many useful complexes can be created by polyamides coordinating with other various molecules. The complex $[Mg_2(dien)Cl(OH)]Cl_2 \cdot 2H_2O$ (dien = diethylene triamine) was found to have high cleavage activity to DNA by Ren et al. [23]. Polyamine complex with glucoses, synthesized by Liu et al., has great potential in curing congenital genetic and acquired immunity disease because it can transfer nucleic acid medicines safely, innocuously and high efficiently [24,25].

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Up to now, study on the spin equilibrium [26], subchronic toxicity [27] and Ion flotation [28]of triethylenetetramine metal complexes have been reported but there are few reports about the interaction between triethylenetetramine metal complexes and DNA. In this paper, the new compounds $N^1$,$N^8$-bis(1-methyl-4-nitro-pyrrole-2-carbonyl) triethylenetetramine and its Cu(II) complex have been synthesized and characterized. The structure of the complex was investigated with density functional theory (DFT) calculations. And the interaction of the Cu(II) complex with calf thymus DNA has been firstly investigated using UV spectra, fluorescent spectra, viscosity, CV (cyclic voltammetry) and molecular modeling. The cleavage reaction on plasmid DNA has been monitored by agarose gel electrophoresis. Toxicity was assessed using the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl- tetrazolium bromide dye (MTT) assay. The aim is to discover a new DNA-binding reagent. The results should be valuable in understanding the mode of the complex with DNA as well as laying a foundation for the rational design of novel, powerful agents for probing and targeting nucleic acids. These can also provide valuable information for exploiting and developing drugs and their application in the field of molecular biology. Thus, new chemical nuclease and anticancer medicines may be exploited and developed.

## 2. Experimental

### 2.1. Materials

Calf thymus DNA and Plasmid DNA PBR322 was obtained from Sigma. Ligand was synthesized with an efficient combination of the chloroform reaction. Its structure was identified by MS, elemental analysis, FTIR, $^1$H and $^{13}$C NMR. All other chemicals were of analytical reagent grade and used without further purification. Human bile duct cancer cell line QBC 939 was obtained from the Cell Bank of Chinese Academy of Science (Shanghai, PR China).

### 2.2. Methods

Carbon, nitrogen and hydrogen analyses were determined using a Perkin–Elmer 240C elemental analyzer. $^1$H NMR and $^{13}$C NMR spectra were measured on a Bruker DRX-300 spectrometer in $CDCl_3$ and DMSO-$d_6$ solution, with TMS as the internal standard. IR spectra were recorded on a Shimadzu FT-IR-8300 instrument using KBr discs in the 400–4000 cm. Cyclic voltammetry was carried out on a CHI660B electrochemical workstation (CH Instruments, Shanghai, China) connected with a three-electrode cell at room temperature. A glass carbon (GC) working (3 mm in diameter) and a Pt wire counter electrode were employed. The reference electrode is Ag/AgCl (saturated). Solution was prepared by dissolving the complex in DMF and 0.1 M $NaClO_4$ was used as supporting electrolyte. Absorbance spectra recorded on a Hewlett–Packard HP-8453 Chemstation spectrometer, UV–Vis spectrometer was employed to check DNA purity ($A_{260}$:$A_{280} > 1.80$) and concentration ($\varepsilon = 6600\ M^{-1}\,cm^{-1}$ at 260 nm). Fluorescence measurements were made with a Perkin–Elmer Ls-50B spectrophotometer equipped with quartz curettes of 1 cm path length at room temperature. The excitation and emission slit widths were 10 nm. Plasmid DNA cleavage products were analyzed with a UVP GDS8000 complete gel documentation and analysis system. The conductance measurement was carried out on a DDS-307 conductivity gauge (Jingke Rex, Shanghai, China). Solutions of $CuCl_2$ ($1.314 \times 10^{-3}$ M) and $CuLCl_2$ ($1.039 \times 10^{-3}$ M) were prepared by dissolving $CuCl_2 \cdot 2H_2O$ and $CuLCl_2$ in water, respectively.

### 2.3. Synthesis of the ligand

Compounds 1, 2 and 3 (Fig. 1) were prepared according to the literature methods [29]. Compound 1: yellowish liquid, b.p. 116.2–117.3 °C. Compound 2: yellowish solid, m.p. 65–66 °C. $^1$H NMR ($CDCl_3$): 3.98 (s (singlet), 3H), 6.23 (m (multiplet), $J = 1.7$ Hz, 1H), 6.98 (s, 1H), 7.50 (t (triplet), $J = 1.7$ Hz, 1H). Compound 3: white solid, m.p.138.9–140 °C. TLC (7:2 benzene/ethyl acetate) $R_f$ 0.7; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.56 (d (double), 1H, $J = 1.62$ Hz), 7.80 (d, 1H, $J = 1.92$ Hz), 3.42 (s, 3H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 173.668, 135.117, 133.443, 121.467, 117.200, 95.410, 39.499; IR(KBr): 1694, 1516, 1423, 1314, 1183, 1113, 998, 750.

#### 2.3.1. Synthesis of $N^1$, $N^8$-bis (1-methyl-4-nitropyrrole-2-carbonyl) triethylenetetramine (compound **4**)

*N*-Methyl-2-(trichloroacetyl)-4-nitropyrrole (10.02 g, 36.9 mmol) was dissolved in DMF (60 mL) with stirring and cooled to 0 °C on an ice bath for 20 min. To this solution was added dropwise triethylenetetramine (2.71 g, 18.5 mmol) dissolved in DMF (20 mL) with stirring at 0 °C for 30 min, and the mixture was further stirred at 0 °C for 2 h. The reaction was then allowed to proceed at room temperature for 2 h. To the resulting solution was poured 200 mL water to give a yellow precipitate. The precipitate was filtered and residue washed with THF ($3 \times 10$ mL), then recrystallized from hot THF and dried in vacuo. Yield was 5.84 g (77%). m.p. 174.6–175.2 °C. Anal. Calc. for $C_{18}H_{26}N_8O_6$: C, 48.00; N, 24.88; H, 5.82. Found: C, 47.93; N, 24.81; H, 5.78. $^1$H NMR (300 MHz, DMSO-$d_6$, $\delta$, ppm): 8.37 (t, $J = 5.76$ Hz, 2H, H7), 8.13 (d, $J = 1.65$ Hz, 2H, H5), 7.44 (d, $J = 1.92$ Hz, 2H, H3), 3.91 (s, 6H, H1), 3.28 (t, $J = 6.32$ Hz, 4H, H8), 2.65 (t, $J = 6.31$ Hz, 4H, H9), 2.60 (s, 4H, H11), 2.00 (m, $J = 7.08$ Hz, 2H, H10). $^{13}$C NMR (300 MHz, DMSO-$d_6$, $\delta$, ppm), C6 160.8, C4 138.7, C5 132.7, C2 128.2, C3 118.6, C11 49.1, C9 48.6, C8 39.8, C1 31.5. IR (KBr): $\nu$(N—H) 3386.5, $\nu$(N—H) 3278.8, $\nu$(Py C—H) 3128.3, $\nu$(C—H) 2939.3, $\nu$(C—H) 2839.0, $\nu$(C=O) 1651.0, $\nu$(N—H) 1558.4, $\nu$(C=C) 1527.5, $\nu$(C=C)1504.4, $\nu$(N—$CH_3$ N—C) 1419.5, $\nu$(C—$NO_2$ C-N)1315.4, $\nu$(CO—NH C—N)1276.8,

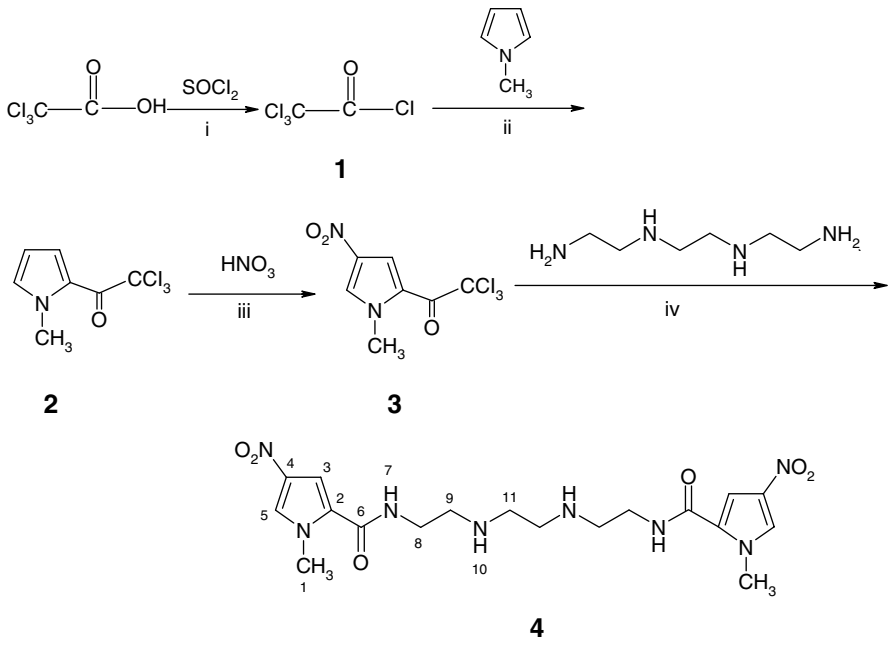



Fig. 1. The synthetic route of the ligand. (i) DMF, 100 °C; (ii) anhydrous ethyl ether; (iii) acetic anhydride, fuming $HNO_3$, −40 °C; (iv) DMF, 0 °C.

$\nu(CH_2—NH\ C—N)$ 1068.5, $\nu(C—NO_2\ C—N)$ 848.6, $\nu(N—H)$ 813.9, $\nu(N—H)$752.2, $\nu(NO_2)$ 705.9, $\nu(NO_2)$ 601.7. HRMS: calculated for $C_{18}H_{26}N_8O_6$ 450.4552; found 450.4556.

### 2.4. Synthesis of the complex

$CuCl_2 \cdot 2H_2O$ (0.137 g, 0.805 mmol) dissolved in 5 mL of water was added to a stirring solution of the ligand (0.362 g, 0.805 mmol) in 100 mL of DMF. The solution turned azure immediately and was allowed to stir at room temperature overnight. The reaction mixture was evaporated to remove DMF in vacuum. The residue was dissolved in 50 mL of methanol and then the solution was filtered. Placed the filtrate in refrigerator overnight and green precipitate was formed. The precipitate was collected and washed three times with water. Recrystallization from hot methanol and further drying in vacuum afforded a green solid. Anal. Calc. for $CuLCl_2$ (here, $L = N^1$, $N^8$-bis(1-methyl-4-nitropyrrole-2-carbonyl) triethylenetetramine): C, 36.93; N, 19.15; H, 4.44. Found: C, 36.89; N, 19.11; H, 4.42. $^1$H NMR (300 MHz, DMSO-$d_6$, $\delta$, ppm): 8.35 (t, $J = 5.76$ Hz, 2H, H7), 8.18 (d, $J = 1.65$ Hz, 2H, H5), 7.55 (d, $J = 1.92$ Hz, 2H, H3), 4.10 (s, 6H, HI), 3.37 (t, $J = 6.32$ Hz, 4H, H8), 3.11 (t, $J = 6.31$ Hz, 4H, H9), 2.95 (s, 4H, H11). $^{13}$C NMR (300 MHz, DMSO-$d_6$, $\delta$, ppm), C6 168.6, C4 138.8, C5 132.8, C2 131.4, C3 119.6, C11 53.1, C9 51.6, C8 42.8, C1 31.6. IR (KBr): $\nu(N—H)$ 3385.8, $\nu(N—H)$ 3244.0, $\nu$(Py C—H)3127.4, $\nu(C—H)$ 2938.8,$\nu(C—H)$ 2837.4, $\nu(C{=}O)$ 1642.9, $\nu(N—H)$ 1549.7, $\nu(C{=}C)$ 1527.5, $\nu(C{=}C)$ 1501.5, $\nu(N—CH_3\ N—C)$ 1418.5, $\nu(C—NO_2\ C—N)$ 1312.5, $\nu(CO—NH\ C—N)$ 1267.1, $\nu(CH_2—NH\ C—N)$ 1060.8, $\nu(C—NO_2\ C—N)$ 847.7, $\nu(N—H)$ 812.0, $\nu(N—H)$ 750.3, $\nu(NO_2)$ 708.8, $\nu(NO_2)$ 591.1. HRMS: calculated for $C_{18}H_{26}N_8O_6Cl_2Cu$ 584.9069; found 584.9060. Conductance: $CuCl_2$: 260 s cm$^2$ mol$^{-1}$, $CuLCl_2$: 135 s cm$^2$ mol$^{-1}$.

The conclusion can be drawn from comparing the IR spectra of the ligand and the complex, which shows that the Cu(II) complex had been formed through Cu(II) coordinating two N atoms of NH groups and two O atoms of C=O groups. The ligand is hard dissolvent in water while the complex is easily dissolved in water, which indicates the complex may be ionization coordination. It can be confirmed from comparing the conductance of $CuCl_2$ and the $CuLCl_2$ complex that the $CuLCl_2$ complex should be 1:1 model. So the structure of the $CuLCl_2$ complex can be conferred as Fig. 2.

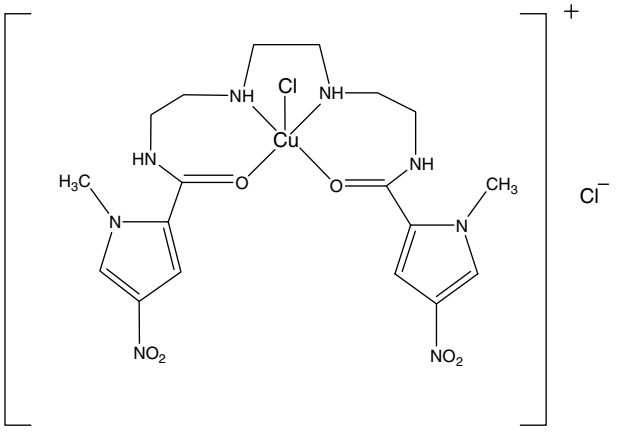



Fig. 2. The conferred structure of the complex based on the experimental results.

### 2.5. Computational details

The DFT calculations were carried out by the unrestricted Hartree-Fock method using the Gaussian 03 system of programs [30]. The optimization of various possible starting structures were carried out at B3LYP/3-21g [31–33] level and imaginary frequencies were analyzed at the same theoretical level. The binding mode between the complex and DNA was studied by molecular modeling methods with Insight II program package in SGI workstation.

In accordance with the experimental results, only a non-symmetrical structure (Fig. 3) with pentacoordinate copper center was confirmed to be a minimum during the calculation process. Other structures (such as those with tetracoordinate or hexacoordinate copper center) were proved to be transition states (TS) or higher order saddle points. As shown in Fig. 3, the copper atom had a slightly distorted square-based pyramidal pentacoordination. The lengths of coordination bonds are labeled in the figure. It is worthy of note that, in this structure, the two pyrrole rings were perpendicular to each other.

### 2.6. $[CuL]^{2+}$–DNA interaction studies

CT DNA (40 mg) was dissolved in 10 mL of 0.1 mol NaCl solution and keep at 4 °C for a few days. A little above solution was took out and diluted to a certain volume. $A_{260}$, $A_{280}$ were determined on UV–Vis spectra and $A_{260}/A_{280}$ should be between the ranges of 1.8–2.0. Then, the concentration of CT DNA could be calculated from $\varepsilon = 6600\ M^{-1} \cdot cm^{-1}$.

The Cu(II) complex was dissolved in water at a concentration of $1.00 \times 10^{-4}$ M. The absorption titrations were performed by keeping the concentration of CT DNA ($1.5 \times 10^{-4}$ M) constant while varying the complex concentration (0–$6 \times 10^{-6}$ M). The absorption was recorded after each addition of the complex. The fluorescent spectra ($\lambda_{ex} = 520$ nm) were also recorded at room temperature. All solutions were allowed to equilibrate thermally for about 30 min before measurements were made.

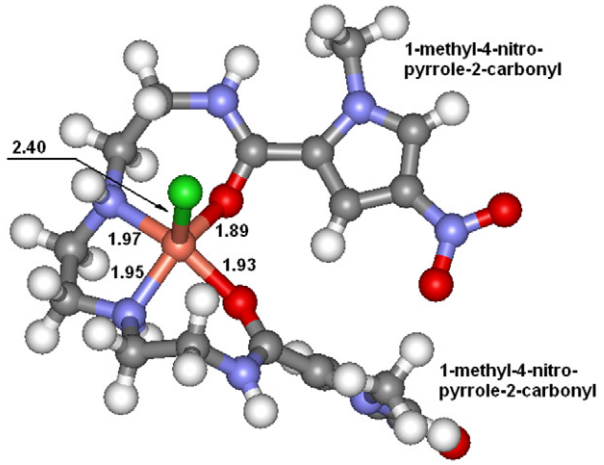



Fig. 3. The optimized structure of the copper (II) complex with lengths of five coordination bonds. The croci ball stands for copper atom, the green ball for chlorine, the red balls for oxygens, the blue balls for nitrogens, the grey balls for carbons and the white balls for hydrogens. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The cleavage of pBR322 DNAby Cu(II) complex was carried out with 10 μL reaction mixture containing of 10 mM Tris–HCl (pH 7.5 containing 5 mM NaCl) buffer, varying concentrations of complex, 0.5μL of pBR322 (0.5 μg/μL). After mixing, the DNA solutions were incubated at 37 °C for 4 h. The reactions were quenched by the addition of EDTA and bromophenol blue. The gel was stained with EB (ethidium bromide) for 0.5 h after electrophoresis, and then photographed.

### 2.7. MTT assay

Toxicity was assessed using the tetrazolium salt 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) dye assay. The CuL was initially diluted in phosphate buffered saline and then storing it at 4 °C until use. Serial dilutions of the stock solution were prepared in the culture medium in 96-well microtiter plates (Costar). CuL at the increasing concentrations (1 nM–1 μM) was added to cell cultures ($5 \times 10^4$ cells/well) for 24 h without renewal of the medium. At the end of the designed reaction time, the culture medium was replaced by 200 μL medium containing 0.5 mg/mL MTT and the plates were incubated for 4 h at 37 °C. The medium was then removed and replaced with 100 μL DMSO to solubilize the converted purple dye in culture plates. Finally, the products were evaluated by measuring the optical density for each well at 595 run, using a microplate reader (Bio-rad 550). Inhibition index (II%) was calculated according to the following equation: $II(\%) = (1 - T/C) \times 100\%$, where $T$ and $C$ represent the mean optical density of the treated group and vehicle control group, respectively. Experiments were performed at least three times.

## 3. Results and discussion

DNA-binding studies are important for the rational design and construction of new and more efficient drugs targeted to DNA [34]. A variety of small molecules interact reversibly with double-stranded DNA, primarily through three modes: (i) electrostatic interactions with the negative-charged nucleic sugar–phosphate structure, which are along the external DNA double helix and do not possess selectivity; (ii) binding interactions with two grooves of DNA double helix; and (iii) intercalation between the stacked base pairs of native DNA. Heterocyclic dyes, such as ethidium, anthracyclines, phenothiazines and acridine derivatives interact through intercalation with the planar, aromatic group stacked between base pairs [35–37]. So as to explore the mode of the Cu(II) complex binding to DNA, the experiments as follow have been carried out.

### 3.1. Fluorescence spectroscopic studies

#### 3.1.1. Effect of complex on the fluorescence spectra of DNA–EB complex

In order to investigate the mode of the Cu(II) complex binding to DNA, the competitive binding experiment has carried out. The fluorescent emission of EB (2 μmol $L^{-1}$) bound to DNA (20 μmol $L^{-1}$) in the absence and the presence of complex is shown in Fig. 4. EB is a conjugate planar molecule. Its fluorescence intensity is very weak, but it is greatly increased when EB is specifically intercalated into the base pairs of double-stranded. When EB is free from DNA, the fluorescence of DNA–EB complex is quenched evidently. Therefore EB can be used as a probe for DNA structure detection [38].The CuL does not shows itself appreciable fluorescence in the spectral region studied, either free or bound to DNA, and does not quench the fluorescence of EB in the absence of DNA under the conditions of our experiments. The emission band at 590 nm of the DNA–EB system decreased in intensity on increasing the Cu(II) complex concentration. Since intercalated EB is the only fluorescent species, the observed fluorescence decrease indicates that the complex can replace EB inside the DNA cavities. Such a characteristic change is often observed in the intercalative DNA interaction [39].

#### 3.1.2. Scatchard plots

To get a better insight into the nature of complex-DNA-binding, we have carried out a fluorescence study of EB to DNA in the presence of a competing metal complex. The characteristics of the binding of EB to DNA can be expressed by Scatchard equation [38].

$$r/C_f = K(n - r)$$

Here, $r$ is the ratio of bound EB to total nucleotide concentration; $C_f$ is the concentration of free EB, $n$ is the number of binding sites per nucleic acid and $K$ is the intrinsic binding constant for EB. Fluorescence Scatchard plots for the binding of EB to CT DNA ($2.5 \times 10^{-6}$ M) in the presence of CuL are given in Fig. 5. The $K$ change indicates that the complex binds to DNA by a competitive inhibition [40]. This is due to the insertion of the planar pyrrole ring into DNA, blocking potential intercalation sites of EB and competing for the intercalative binding sites with EB.

### 3.2. UV spectroscopic studies

"Hyperchromic effect" and "hypochromic effect" are the spectra features of DNA concerning its double-helix structure [41]. This spectral change process reflects the corresponding changes of DNA in its conformation and structures after the drug bound to DNA. Hypochromism results from the contraction of DNA in the helix axis, as well as from the change in conformation on DNA, while hyperchromism results from the damage of the DNA double-helix structure [41]. Fig. 6 shows that the absorption spectra of DNA increase on increasing the Cu(II) complex concentration. This is a typical "hyperchromic effect". Therefore, the result indicates the damage of the DNA double-helix structure after the complex bound to DNA.

### 3.3. Viscosity study

Hydrodynamic method, such as determination of viscosity, which is exquisitely sensitive to the change of length of DNA, may be the most effective means studying the binding mode of complexes to DNA in the absence of X-ray crystallographic or NMR structural data [42]. To further confirm the interaction mode of the Cu(II) complex with DNA, a viscosity study was carried out (Fig. 7). The viscosity measurement is based on the flow rate of a DNA solution through a capillary viscometer. The specific viscosity contribution ($\eta$) due to the DNA in the presence of a

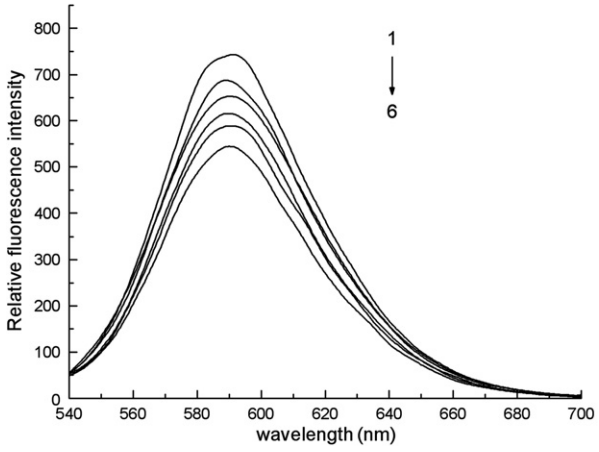



Fig. 4. Fluorescence emission spectra (excited at 520 nm) of EB-DNA complex in the absence (1) and presence (2–6) of increasing concentrations of the complex (2 mM, 1 μL per scan). Arrow shows that the intensity changes upon increasing.

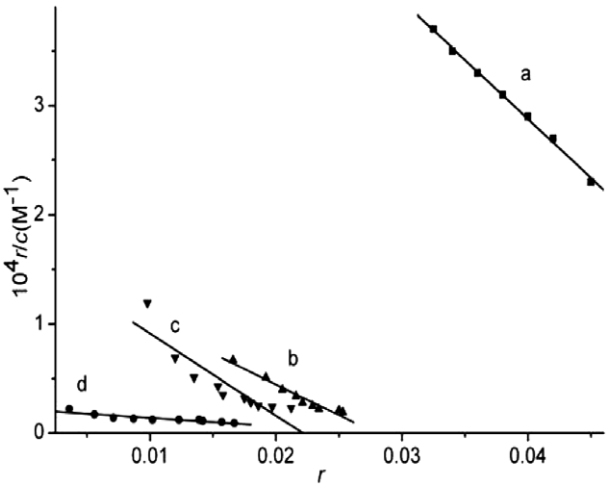



Fig. 5. Fluorescence Scatchard polts of the binding of EB to CT DNA in the absence (a) and the presence (b–d) of increasing concentrations of the complex ([CuL]/[DNA] = 0.25, 0.5, 1.0, respectively (from up to down)) $C_{DNA} = 2.5 \times 10^{-6}$ M.

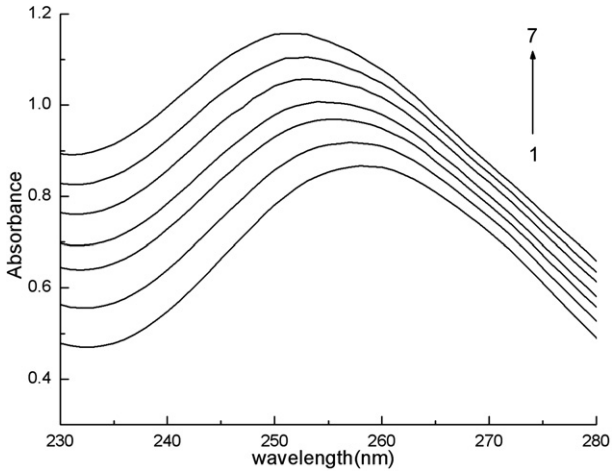



Fig. 6. Absorption spectra of calf thymus DNA (1, $1.5\times10^{-4}$ M) in tris–HCl buffer upon addition of CuL (2–7, 1, 2, 3, 4, 5, $6\times10^{-6}$ M, respectively). Arrow shows that the absorbance changes with increasing CuL concentration.

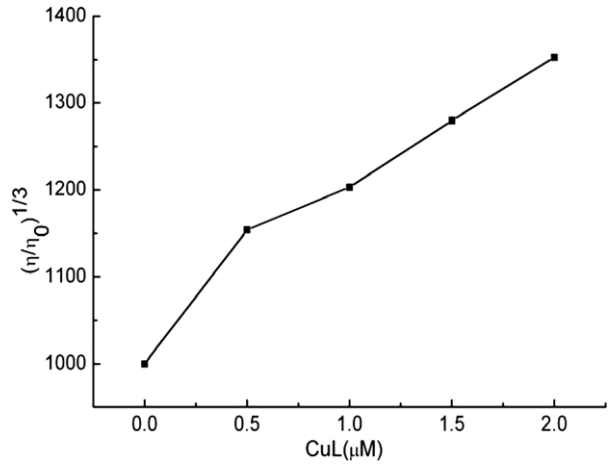


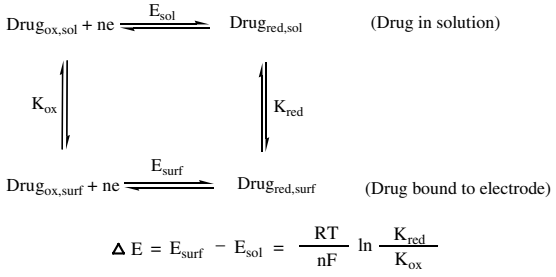

Fig. 7. Effect of the increasing concentration of CuL on the relative viscosity DNA $c$(CuL) = 0, 0.5, 0.10, 0.15, $0.20\times10^{-3}$ mM.

binding agent was obtained. The results indicate that the absence and the presence of the metal complex have a marked effect on the viscosity of the DNA. The specific viscosity of the DNA sample increases obviously with the addition of the complex. The viscosity studies provide a strong argument for intercalation [43,44]. The viscosity increase of DNA is ascribed to the intercalative binding mode of the drug because this could cause the effective length of the DNA to increase [45,46]. In essence, the length of the linear piece of B-form DNA is given by the thickness of the base pairs that are stacked along the helix axis in Van der Waals contact with each other. Introducing another aromatic molecule into the stack therefore increases the length. So, we think the viscosity increase of the DNA caused by the addition of the complex can provide further support for the intercalative mode of the Cu(II) complex.

### 3.4. CV study

Electrochemical investigation of drug–DNA interactions can provide a useful complement to other methods and yield information about the mechanism of interaction and the conformation of adduct [47].

Native DNA is not reducible at the GC electrode because the stability of the intact double helix makes the reducible bases inaccessible to the electrode. In DMF, the modified GC electrode with DNA causes all of the peak currents of CuL to decrease considerably. Additionally, the peak potentials, $E_{pc}$, and $E_{pa}$, both shifted to more positive values, shown in Fig. 8. We think that CuL intercalates into the base pairs of DNA by the pyrrole planar [48]. Because of the intercalation, CuL is not readily accessible to the electrode, thus causing the peak currents of the CV waves to diminish greatly. Moreover, the obvious positive shifts of peak potentials also indicate that this interaction mode may be intercalation between CuL and DNA [49]. The $E_{pc}$ and $E_{pa}$ are −0.053 and 0.404 V. The half-wave potentials $E_{sol}$ taken as $(E_{pc}+E_{pa})/2$, is 0.176 V. On CT DNA/GC redox peak potentials have positive shifts. The $E_{pc}$, $E_{pa}$ and $E_{surf}$ are 0.0515, 0.438 and 0.245. According to the $\Delta E$ ($E_{surf}-E_{sol}$) value, it can be evaluated that is the ratio of binding constants of the interaction of oxidation state and reduction state of the complex with DNA [47,50,51].



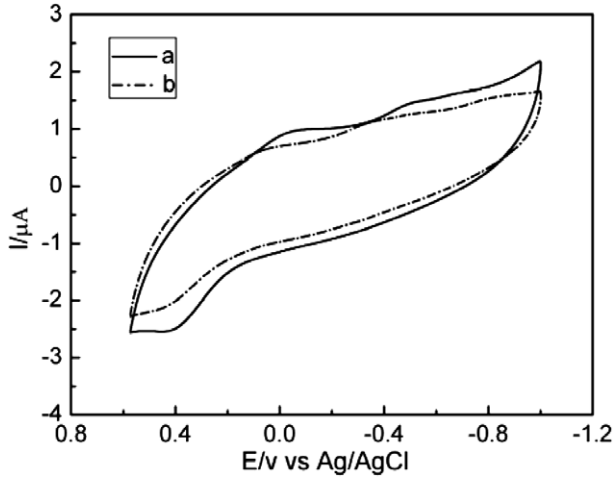




Fig. 8. Cyclic voltammogram of 0.174 mM CuL (a) in the absence and (b) in the presence of DNA (0.30 mM nucleotide phosphate). Supporting electrolyte, 100 mM $NaClO_4$ in DMF, Sweep rate, 100 mV/s.

$$\Delta E = E_{surf} - E_{sol} = \frac{RT}{nF} \ln \frac{K_{red}}{K_{ox}}$$

Here $K_{red}$ and $K_{ox}$ are the surface binding constants of the reduction state and oxidation state of the complex binding to DNA. As a result, the ratio of the $K_{red}/K_{ox}$ is 14.7, which implies that the interaction between reduction state of the complex and DNA is much stronger than its oxidation state.

### 3.5. Molecular modeling

To give a clear observation of binding pattern between the complex and DNA, we optimized an intercalating interaction model of them in insight II package. As shown in Fig. 9, a pyrrole ring of the complex has inserted into the DNA base stack and the other parts stay at the major groove. It can be seen in the model that the copper center is not at the middle location between two side chains, but near one of them, which facilitates the further cleavage reactions.

### 3.6. Electrophoretic analysis

The cleavage reaction on plasmid DNA can be monitored by agarose gel electrophoresis. When circular plasmid DNA is subject to electrophoresis, relatively fast migration will be observed for the intact supercoil form (Form I, CCC form). If scission occurs on one strand (nicking), the supercoiled will relax to generate a slower-moving open circular form (Form II, OC form). If both strands are cleaved, a linear form (Form III) that migrates between Form I and Form II will be generated [52]. Fig. 10 shows the results of cleaving superhelical pBR322 DNA in the presence of varying concentrations of the CuL. The results indicate that the CCC form of pBR322 DNA diminishes gradually, whereas the OC form increases with the increase of concentration of the CuL and the linear form is also produced. When Rt goes up to 0.40 (lane 5), the CCC form almost completely converts to the OC form and linear form. Therefore, the result here indicates that the CuL can bind to and cleave DNA efficiently.

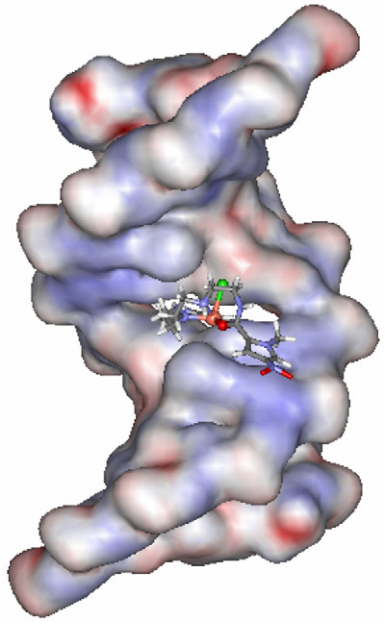


Fig. 9. The binding mode between the titled complex and oligodeoxynucleotide.

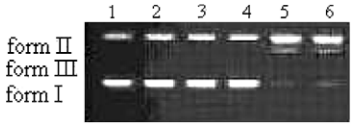



Fig. 10. Results of electrophoresis of pBR322 DNA in the presence of varying concentrations of CuL 1–6, Rt: 0, 0.10, 0.20, 0.30, 0.40, 0.50, respectively. Form I is supercoiled DNA (CCC form), form II is nicked circular form DNA (OC form) and form III is linear DNA.

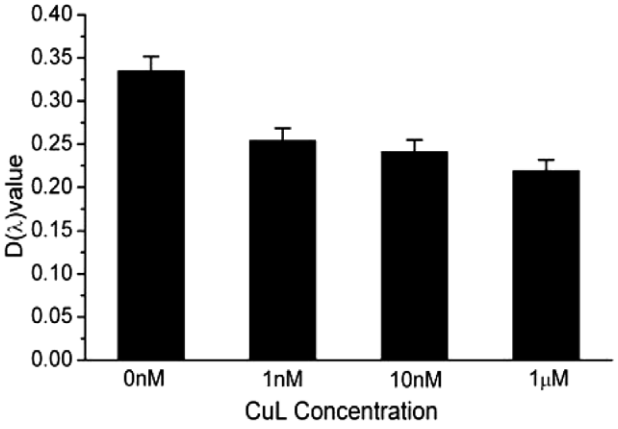



Fig. 11. The effect of CuL on cell death measured by the MTT assay on QBC 939 cells over incubation periods of up to 24 h.

### 3.7. MTT assay

The effects of the CuL on cell viability were tested using the MTT assay. Application of 1 nM CuL to QBC 939 for 24 h resulted in a decrease (II% = 24.2%) in the percentage of cell viability when compared with controls. But the maximal effect was II% = 34.6% with 1 μM (Fig. 11). These results indicate that the CuL had certain effect on cancer cells but may not be undivided to QBC 939. Further studies will be needed to understand the selectivity of the CuL on other cells. The detailed results will be reported in our future works.

## 4. Conclusions

In summary, a new Cu(II) complex had been synthesized and characterized by elemental analyses, $^{1}H$ NMR and $^{13}C$ NMR, IR and HRMS. The experiment-postulated structure of the complex was confirmed by DFT calculations. The interactions between the complex and calf thymus DNA had been investigated using UV spectra,

fluorescent spectra, viscosity and CV (cyclic voltammetry). Furthermore, the binding mode between the complex and DNA was studied by molecular modeling. The cleavage reaction on plasmid DNA had been investigated by agarose gel electrophoresis. Remarkably, our results show that the complex is a new species that could bind DNA with intercalation mode and could cleave DNA. Thus, the triethylenetetramine copper complexes were found out a new kind of chemical nuclease. Furthermore, the effects of the CuL on cell viability were tested using the MTT assay and the results indicated that the CuL had certain effect on cancer cells but may not be undivided to QBC 939. Further studies will be needed to understand the selectivity of the CuL on other cells. The detailed results will be reported in our future works.

## 5. Abbreviations

| | |
|---|---|
| CT DNA | calf-thymus DNA |
| CV | cyclic voltammetry |
| DFT | density functional theory |
| DMSO | dimethyl sulfoxide |
| EB | ethidium bromide |
| EDTA | ethylenediaminetetraacetic acid |
| HRMS | high resolution mass spectrum |
| Im | *N*-methylimidazole |
| MTT | 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide |
| Py | *N*-methylpyrrole |
| TMS | tetramethyl silicon |
| Tris | tris-(hydroxymethyl) aminomethane |
| UV–Vis | UV–visible |

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