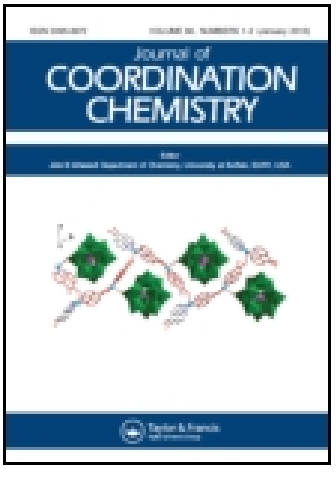

Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gcoo20

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Accepted author version posted online: 20 Jan 2014.Published online: 25 Feb 2014.

To cite this article: Ming-Lu Liu, Man Jiang, Kang Zheng, Yan-Tuan Li, Zhi-Yong Wu & Cui-Wei Yan (2014) Synthesis and structure of a new mononuclear copper(II) complex with 2,2′-bipyridine and picrate: molecular docking, DNA-binding, and in vitro anticancer activity, Journal of Coordination Chemistry, 67:4, 630-648, DOI: 10.1080/00958972.2014.884218

To link to this article: http://dx.doi.org/10.1080/00958972.2014.884218

Journal of Coordination Chemistry, 2014
Vol. 67, No. 4, 630–648, http://dx.doi.org/10.1080/00958972.2014.884218

Synthesis and structure of a new mononuclear copper(II) complex with 2,2′-bipyridine and picrate: molecular docking, DNA-binding, and *in vitro* anticancer activity

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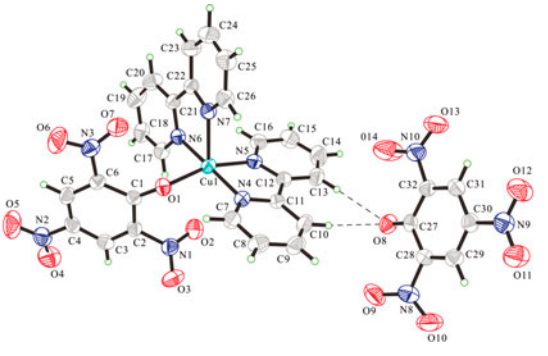
(*Received 29 September 2013; accepted 30 December 2013*)



A new monocopper(II) complex was synthesized and structurally characterized. The DNA-binding properties and *in vitro* anticancer activities of the complex were investigated.

A new mononuclear copper(II) complex identified as $[Cu(bpy)_2(pic)](pic)$, where bpy represents 2,2′-bipyridine and pic stands for picrate (pic), has been synthesized and characterized by elemental analysis, molar conductivity, IR and electronic spectral studies, and single-crystal X-ray diffraction. The crystal structure analysis reveals that copper(II) has a distorted square-pyramidal coordination geometry. The hydrogen bonding and π–π stacking interactions contribute to a 3-D supramolecular structure in the crystal. The DNA-binding properties of the copper(II) complex are investigated both theoretically and experimentally, revealing that the copper(II) complex interacts with herring sperm DNA (*HS*-DNA) by intercalation, and the molecular docking of the copper(II) complex with the self-complementary DNA duplex of sequence $d(ACCGACGTCGGT)_2$ facilitates the binding events. The *in vitro* anticancer activities suggest that the copper(II) complex is active against selected tumor cell lines. The influence of the complexation of pic in the mononuclear copper(II) complexes on DNA-binding properties and *in vitro* anticancer activities is discussed.

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Keywords: Crystal structure; Mononuclear copper(II) complex; DNA-binding property; Molecular docking; *In vitro* anticancer activity

1. Introduction

DNA is considered to be one of the main targets in the action of anticancer agents [1], and many compounds exert their anticancer effects through binding to DNA, thus changing the replication of DNA and inhibiting the growth of the tumor cells, which is the basis of designing new and more efficient anticancer drugs [2–4]. Thus, design, synthesis, and DNA-binding studies of metal complexes have been an active field of research with the aim of elucidating the site-specific recognition of DNA and to obtain information about designing and synthesizing new types of pharmaceutical molecules [2–9].

Some metal complexes have the ability to bind to DNA under physiological conditions, and can change the replication of DNA, thereby inhibiting the growth of tumor cells [1]. *Cis*-platin is a widely used metal-based anticancer drug targeting DNA. Although *cis*-platin is an effective drug against several types of cancers [10], the side-effects limit its activity and selectivity in cancer cells. The detailed molecular mechanism of the side-effects has been clarified, including covalent binding to DNA [1, 11]. Consequently, many researchers concentrate on development of new metal-based antitumor drugs, which bind DNA by non-covalent modes including groove binding, electrostatic effect, and intercalation. Among these noncovalent binding modes, intercalation has attracted special interest due to its various applications in cancer therapy and molecular biology [12]. The intercalating ability correlates not only with the planarity and donor types of the ligand but also with the coordination geometry of the metal center [13]. Both the type of metal and its valency play roles in deciding the binding of complexes to DNA [14]. Therefore, design and synthesis of DNA-binding metal complexes have focused on selection of metal ions and the design of ligands. Copper complexes have been extensively explored for their strong interactions with DNA and cytotoxic activities [14–18]. Especially significant progress has occurred in design, synthesis, and DNA-binding properties of mixed-ligand copper(II) complexes containing 2,2′-bipyridine (bpy) or 1,10-phenanthroline because copper(II) complexes are much more active in the presence of a nitrogen donor heterocyclic ligand [19]. Picrate (pic), due to both its versatile bonding with metal ions and its peculiar structure, involving phenolato and nitro groups that are noncoplanar with themselves and with the benzene ring, could be good candidates as mono-, di-, tri-, tetra- or penta-dentate ligands via phenolic oxygen, *ortho*-nitro oxygen, and *para*-nitro oxygen to build coordination networks [20–24]. All the above facts aroused our interest in the synthesis of new mononuclear copper(II) complexes with bpy and pic with a view towards evaluating their structures, DNA-binding, and anticancer activities.

We previously reported the structure and DNA-binding properties of a mononuclear copper(II) complex containing bpy and pic with formula of $[Cu(bpy)_2(CH_3OH)](pic)_2$ [25]. To evaluate and understand the influence of the complexation of pic groups in the mononuclear complexes on structure, DNA-binding properties, and cytotoxic activities, and to gain insight into the structure/activity relationship of this kind of mononuculear copper(II) complexes, it is necessary to synthesize a series of mononuclear ternary complexes of essentially the same chemical composition except for binding of coligands. In this article, we describe the synthesis and structure of a new mononuclear copper(II) complex with bpy and pic, $[Cu(bpy)_2(pic)](pic)$. *In vitro* anticancer activities and the

reactivity towards DNA of the mononuclear copper(II) complex have also been studied. The main results of the present investigation suggest complexation of pic in mononuclear complexes may play important roles in the DNA-binding properties and *in vitro* anticancer activities, thus indicating DNA-binding abilities and *in vitro* anticancer activities could be tuned through subtle differences in the chemical arrangement in these ternary copper(II) systems.

2. Experimental

2.1. *Materials and chemicals*

All chemicals used in the syntheses were of reagent grade and obtained commercially. Double distilled water was used to prepare buffers. Ethidium bromide (EB) and herring sperm DNA (*HS*-DNA) were purchased from Sigma Corp. and used as received.

2.2. *Physical measurements*

Carbon, hydrogen, and nitrogen elemental analyses were performed with a Perkin–Elmer elemental analyzer Model 240. Molar conductance was measured with a Shanghai DDS-11A conductometer. The infrared spectrum was recorded with KBr pellets in a Nicolet model Impact 470 FTIR spectrophotometer from 4000 to 400 cm^{-1}. The UV–Vis spectrum was recorded in a 1 cm path length quartz cell on a Cary 300 spectrophotometer. Fluorescence was tested on an Fp-750w fluorometer. A CHI 832 electrochemical analyzer (Shanghai CHI Instrument, Shanghai, China) in connection with a glassy carbon working electrode (GCE), a saturated calomel reference electrode, and a platinum wire counter electrode was used for electrochemical measurements. The GCE surface was freshly polished to a mirror prior to each experiment with 0.05 μm α-Al_2O_3 paste and then cleaned in water for 5 min. Viscosity measurement was carried out using an Ubbelohde viscometer immersed in a thermostatic water bath maintained at 298 K.

2.3. *Synthesis of [Cu(bpy)$_2$(pic)](pic)*

Into a methanol solution (5 mL) of $Cu(ClO_4)_2{\cdot}6H_2O$ (44.5 mg, 0.12 mM) was added dropwise, a solution (10 mL) of bpy (31.2 mg, 0.2 mM) stirred in the same solvent at room temperature. The vigorous stirring was continued until the mixture became limpid. It was then filtered to eliminate impurities. A water solution (10 mL) containing 2,4,6-trinitrophenol (45.8 mg, 0.2 mM) and piperidine (17.1 mg, 0.2 mM) was slowly added to the filtrate (containing $[Cu(bpy)_2(H_2O)]^{2+}$) with rapid stirring at room temperature. The color of the solution turned from blue to dark green immediately. After refluxing for *ca.* 2 h, the resulting solution was allowed to cool to room temperature, and green cube crystals of the copper(II) complex suitable for X-ray analysis were obtained by slow evaporation at room temperature. Yield: 0.0551 g (65%). Anal. Calcd for $CuC_{32}H_{20}N_{10}O_{14}$ (%): C, 46.19; H, 2.42; N, 16.83. Found (%): C, 46.02; H, 2.56; N, 16.62.

Table 1. Crystal data for $[Cu(bpy)_2(pic)](pic)$.

Formula	$CuC_{32}H_{20}N_{10}O_{14}$
Formula weight	832.12
Crystal system	Triclinic
Space group	*P-1*
a (Å)	7.5941(13)
b (Å)	13.698(2)
c (Å)	17.080(3)
α (°)	73.611(3)
β (°)	80.256(3)
γ (°)	78.878(3)
V (Å^3)	1660.0(5)
D(Calcd) [g cm^{-3}]	1.665
Z	2
μ (Mo Kα) (mm^{-1})	0.748
F(0 0 0)	846
Crystal size [mm]	0.06 × 0.12 × 0.35
Temperature (K)	296
Radiation [Å]	Mo Kα 0.71073
θ Range	1.74 – 26.14
Limiting indices	$-8 < h < 9$, $-15 < k < 16$, $-14 < l < 21$
Tot., Uniq. Data, R (int)	8965, 6509, 0.0291
Observed data [$I > 2\sigma(I)$]	4807
R, ωR_2, S	0.0474, 0.1107, 1.023
Max., av. shift/error	0.000, 0.000

Table 2. Selected distances (Å) and angles (°) for the complex.

Cu1–O1	1.990(2)	Cu1–N4	1.985(2)
Cu1–N5	2.035(2)	Cu1–N6	2.000(2)
Cu1–N7	2.198(2)		
O1–Cu1–N4	91.28(9)	O1–Cu1–N5	162.39(9)
O1–Cu1–N6	91.20(9)	O1–Cu1–N7	96.94(9)
N4–Cu1–N5	80.73(10)	N4–Cu1–N6	174.86(10)
N4–Cu1–N7	105.79(9)	N5–Cu1–N6	95.65(10)
N5–Cu1–N7	100.32(9)	N6–Cu1–N7	78.38(9)
O2–N1–C2–C3	117.2(3)	O5–N2–C4–C3	171.3(3)
O6–N3–C6–C1	−160.3(3)	O10–N8–C28–C29	−33.3(4)
O11–N9–C30–C31	179.1(3)	O13–N10–C32–C31	13.9(5)

2.4. *Determination of X-ray crystal structure*

Analysis of the single-crystal structure was carried out on a Bruker APEX area-detector diffractometer with graphite monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) at 296 K. The crystal structure was solved by direct methods followed by Fourier syntheses. Structure refinement was performed by full matrix least-squares on F^2 using SHELXL-97 [26]. All hydrogens were placed on calculated positions and refined as riding, with C–H bond lengths of 0.93 Å and Uiso(H) = 1.2 (carrier atoms). Crystal data, structural refinement parameters, and selected bond distances and bond angles are listed in tables 1 and 2, respectively.

2.5. In vitro *cytotoxic activity by SRB assays*

In vitro cytotoxic activities of copper(II) complexes together with *cis*-platin were evaluated against selected cell lines by using the sulforhodamine B (SRB) assays. All cells were

cultured in RPMI 1640 supplemented with 10% (v/v) fetal bovine serum, 1% (w/v) penicillin (104 U/mL), and 10 mg/mL streptomycin. Cell lines are maintained at 310 K in a 5% (v/v) CO_2 atmosphere with 95% (v/v) humidity. Cultures were passaged weekly using trypsin–EDTA to detach the cells from their culture flasks. The tested copper(II) complexes and *cis*-platin were dissolved in DMSO, respectively, and diluted to the required concentration with culture medium when used. The content of DMSO in the final concentrations did not exceed 0.1%. At this concentration, DMSO was found to be nontoxic to the cells tested. Rapidly growing cells were harvested, counted, and incubated at the appropriate concentration in 96-well microplates for 24 h. The copper(II) complexes and *cis*-platin dissolved in culture medium were then applied to the culture wells to achieve final concentrations ranging from 10^{-7} to 10^{-4} μM. Control wells were prepared by addition of culture medium without cells. The plates were incubated at 310 K in a 5% CO_2 atmosphere for 48 h. Upon completion of the incubation, the cells were fixed with ice-cold 10% trichloroacetic acid (100 mL) for 1 h at 277 K, washed five times in distilled water, and allowed to dry in air and stained with 0.4% SRB in 1% acetic acid (100 mL) for 15 min. The cells were washed four times in 1% acetic acid and air-dried. The stain was solubilized in 10 mM unbuffered Tris base (100 mL) and the OD of each well was measured at 540 nm on a microplate spectrophotometer. The IC_{50} values were calculated from the curves constructed by plotting cell survival (%) *versus* the tested copper(II) complexes and *cis*-platin concentration (μM).

2.6. *DNA-binding studies*

All experiments involving *HS*-DNA were performed in tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution (pH 7.23), prepared using deionized and sonicated triply distilled water. Solutions of DNA in Tris–HCl buffer gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of *ca.* 1.9, indicating that the DNA was sufficiently free from protein [27]. The concentration of the DNA solution was determined by UV absorbance at 260 nm. The molar absorption coefficient, ε_{260}, was taken as 6600 $M^{-1}\,cm^{-1}$. Stock solution of DNA was stored at 277 K and used after not more than four days. Concentrated stock solution of the copper(II) complexes was prepared by dissolving the complex in DMSO and diluted suitably with Tris–HCl buffer to required concentrations for all the experiments. The absorption spectral titration experiment was performed by keeping the concentration of the copper(II) complexes constant while varying *HS*-DNA concentration. An equal solution of *HS*-DNA was added to the copper(II) complexes solution and reference solution to eliminate absorbance of the DNA itself. In the EB fluorescence displacement experiment, 5 μL of the EB Tris–HCl solution (1 mM) was added to 1 mL of *HS*-DNA solution (at saturated binding levels) [28] and stored in the dark for 2 h. Then the solution of the copper(II) complexes was titrated into the DNA/EB mixture and diluted in Tris–HCl buffer to 5 mL to produce solutions with varied mole ratio of the copper(II) complexes to *HS*-DNA. Before measurements, the system was shaken and incubated at room temperature for 30 min. The fluorescence spectra bound to *HS*-DNA were obtained at excitation wavelength of 522 nm and an emission wavelength of 584 nm in the fluorometer. The electrochemical titration experiments were performed by keeping the concentration of the copper(II) complex constant while varying the DNA concentration using solvent of Tris–HCl buffer. All voltammetric experiments were performed in a single-compartment cell. The supporting electrolyte was Tris–HCl buffer solution. Solutions were deoxygenated by purging with nitrogen for 15 min prior to measurements; during measurements a stream of N_2 gas was passed over

the solution. The viscosity measurement was carried out using an Ubbelohde viscometer immersed in a thermostatic water bath maintained at 298 K. *HS*-DNA samples with approximately 200 base pairs in length were prepared by sonication in order to minimize complexities arising from DNA flexibility [29]. Flow times were measured with a digital stopwatch; each sample was measured three times, and an average flow time was calculated. Relative viscosities for *HS*-DNA in the presence and absence of the copper(II) complex were calculated from the relation $\eta = (t - t_0)/t_0$, where t is the observed flow time of DNA-containing solution and t_0 is the flow time of Tris–HCl buffer alone. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* binding ratio [30], where η is the viscosity of *HS*-DNA in the presence of the copper (II) complex and η_0 is the viscosity of DNA alone.

2.7. *Molecular docking studies*

Automated docking was used to determine the orientation of the mononuclear copper(II) complex binding to DNA. *HS*-DNA used in the experimental work was too large for current computational resources to dock, therefore, the structure of the DNA of sequence d $(CCGTCGACGG)_2$ (PDB id: 423D, a familiar sequence used in oligodeoxynucleotide study) obtained from the Protein Data Bank (www.rcsb.org/pdb) at a resolution of 1.60 Å was constructed in Autodock4 package to study the DNA-binding properties of the mononuclear copper(II) complex. MGL tools 1.5.4 with AutoGrid4 and AutoDock4 [31] were used to setup and perform blind docking calculations between the mononuclear copper(II) complex and the DNA of sequence $d(CCGTCGACGG)_2$. The parameters of Cu(II) were set as vdW radii of 0.96 Å and vdW well depth of 0.01 kcal/M [32]. The coordinates of the copper(II) complex were taken from the crystal structure as a CIF file and converted to PDB format using Mercury software [33].

Receptor (DNA) and the copper(II) complex files were prepared using AutoDock Tools. The heteroatoms including waters were deleted and polar hydrogens and Kollman charges were added to the receptor molecule. All other bonds were allowed to be rotatable. In the docking analysis, the binding site was assigned across all of the minor and major grooves of the DNA molecule, which was enclosed in a box with a number of grid points in $x \times y \times z$ directions, $123 \times 99 \times 101$ and a grid spacing of 0.375 Å. Lamarckian genetic algorithms, as implemented in Autodock4, were employed to perform docking calculations. All other parameters were default settings. All calculations were performed on an Intel Core 2 Duo, 2.0 GHz based machine running GNU/Linux as operating system. For each of the docking cases, the lowest energy docked conformation, according to the Autodock scoring function, was selected as the binding mode. Visualization of the docked pose has been done by using PyMOL (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC) molecular graphics program.

3. Results and discussion

3.1. *Synthetic route of the copper(II) complex*

The goal of the present work was to ascertain whether differences in the chemical arrangement and in the coordination environment of monocopper(II) complex with bpy and pic can affect DNA-binding and *in vitro* anticancer activities. In the course of preparing the copper (II) complex, the use of piperidine as base makes 2,4,6-trinitrophenol coordinate to copper

(II) ion through the deprotonated phenolic oxygen. In order to make pic coordinate to copper(II), a strategy of producing $[Cu(bpy)_2(H_2O)]^{2+}$ precursor was adopted by strictly regulating the mole ratio of copper(II) and bpy as well as the reaction time; water molecules with weak coordination in $[Cu(bpy)_2(H_2O)]^{2+}$ were substituted by pic. Elemental analyses indicate that reaction of $Cu(ClO_4)_2 \cdot 6H_2O$ with bpy and pic in *ca.* 1 : 2 : 2 ratio yielded [Cu $(bpy)_2(pic)](pic)$, in which one the pic group, as a monodentate ligand, is coordinated with copper(II).

3.2. *Characterization of the copper(II) complex*

The mononuclear copper(II) complex is soluble in H_2O, DMF, and DMSO to give stable solutions at room temperature, moderately soluble in methanol, ethanol and acetone, and practically insoluble in CCl_4, $CHCl_3$, Et_2O, and C_6H_6. In the solid state, the copper(II) complex is fairly stable in air. The molar conductivity value (125 S cm^2 M^{-1}) of the copper (II) complex in H_2O falls in the expected range for 1:1 electrolyte [34], indicating that only one pic is inside the metal coordination sphere, and thus the mononuclear copper(II) complex may stay intact as a cation $[Cu(bpy)_2(pic)]^+$ in solution. The structure of the mononuclear copper(II) complex was further characterized by the following spectroscopic and single-crystal structure X-ray analysis.

3.3. *IR spectra*

The IR spectrum (4000–400 cm^{-1}) provides some information regarding the mode of coordination in the copper(II) complex. Bands associated with ν(C=N) and ν(C=C) from the aromatic ring of bpy appear at 1492, 1473, and 1442 cm^{-1}, suggesting coordination to copper(II) [35]. While the O–H out-of-plane bending vibration of free Hpic at 1151 cm^{-1} disappears, and the vibration ν(C–O) of pic anion is shifted toward higher frequency 1274 cm^{-1}, indicating oxygen of the phenolate in pic coordinates with copper(II) [36]. Two broad and intense bands centered at 1306 and 1261 cm^{-1}, typical for a non-coordinated pic, are also observed. Thus, the above-spectral observations indicate that the present copper(II) complex has both ionic and coordinated pics, consistent with the molar conductivity measurement.

3.4. *Electronic spectra*

In order to obtain further structural information, the electronic spectrum of the copper(II) complex was recorded in the UV–Vis region using DMSO as the solvent. Spectra obtained for the copper(II) complex at different concentrations (1.0×10^{-4}–1.0×10^{-6} M) obey the Beer–Lambert law, indicating that the copper(II) complex stays intact at these concentrations. This is consistent with the molar conductance measurement. For the mononuclear copper(II) complex, two absorptions with varied intensity can be observed. The intense band at 229 nm should be attributed to inter-ligand (π–π^*) transition of bpy, and the less intense band at 312 nm is typical of charge transfer transition between pic and metal [37].

These spectroscopic data of the mononuclear copper(II) complex are further supported by the determination of the crystal structure (*vide infra*).

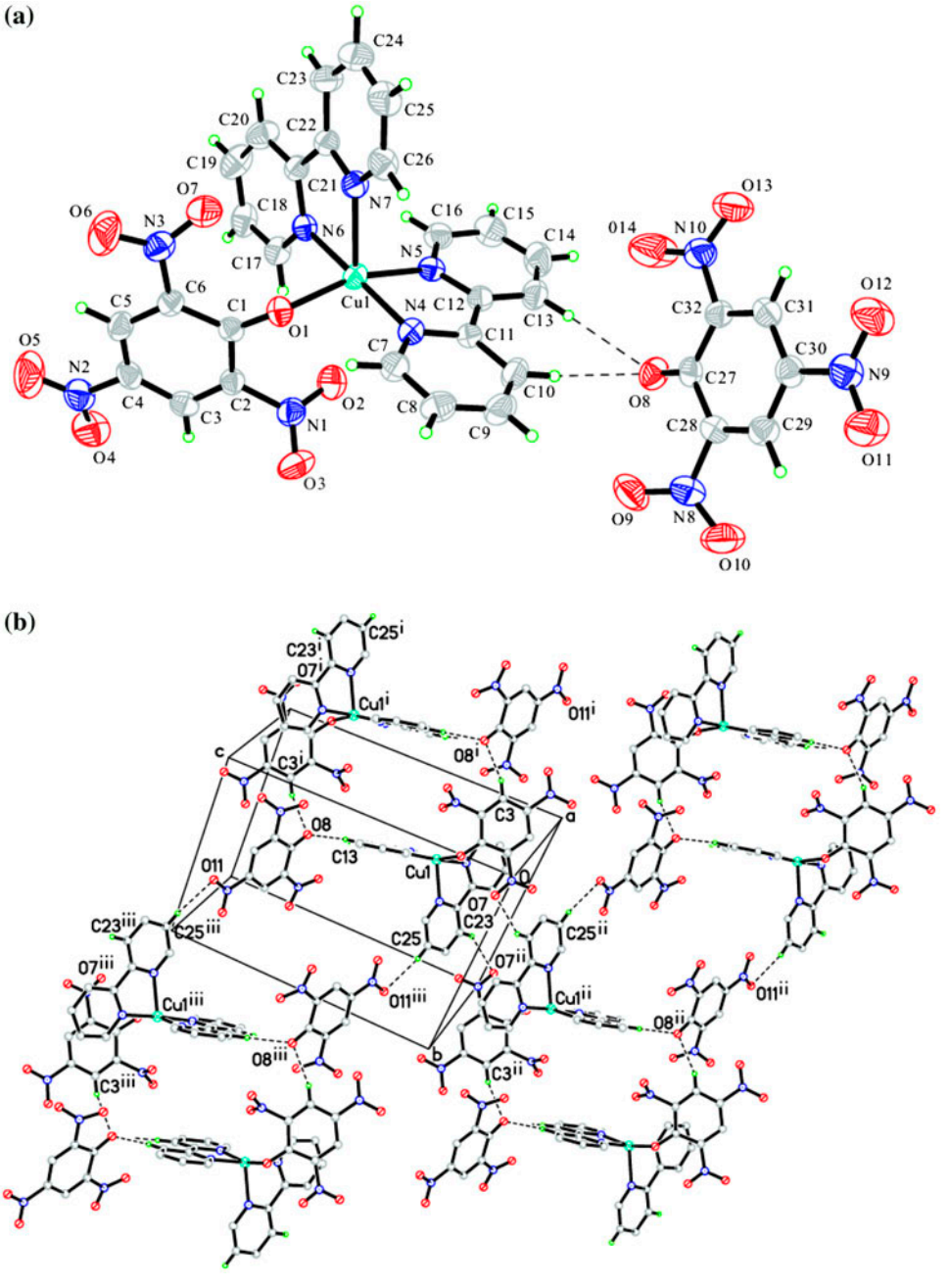

Figure 1. Crystal structure of $[Cu(bpy)_2(pic)](pic)$. (a) An ORTEP view of the copper(II) complex with the thermal ellipsoids at 50% probability level. Dashed lines indicate hydrogen bonds. (b) A 2-D hydrogen-bonding network parallel to the (1 2 2) plane. Symmetry codes: (i) $1-x, -y, 1-z$; (ii) $1-x, 1-y, -z$; (iii) $-x-1, 1-y, 1-z$. (c) The π–π stacking interactions among the layers. The two stacking complex are drawn with different styles for clarify. Symmetry codes: (iv) $-x, 1-y, 1-z$.

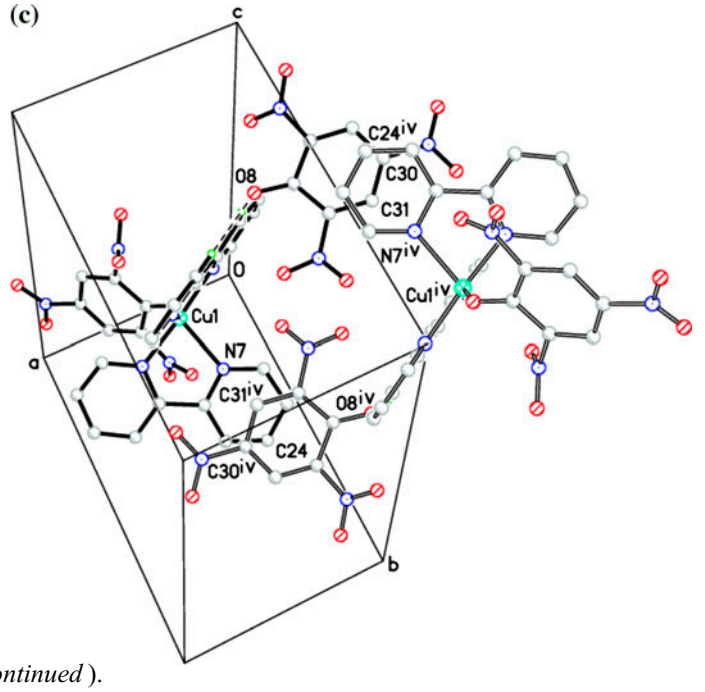

Figure 1. (*Continued*).

Table 3. Hydrogen bonding geometries (Å) of the complex.

D–H···A	D–H	H···A	D···A	D–H···A
C3–H3···O8^{i}	0.93	2.29	3.183	160.1
C10–H10···O8	0.93	2.33	3.256(4)	174.3
C13–H13···O8	0.93	2.41	3.340(4)	176.2
C23–H23···O7^{ii}	0.93	2.55	3.321(4)	140.6
C25–H25···O11^{iii}	0.93	2.49	3.403(4)	165.8

Note: Symmetry codes: (i) $1-x, -y, 1-z$; (ii) $1-x, 1-y, -z$; (iii) $-x-1, 1-y, 1-z$.

3.5. *Crystal structure of [Cu(bpy)$_2$(pic)](pic)*

As shown in figure 1(a), the complex consists of a mononuclear $[Cu(bpy)_2(pic)]^+$ and a free pic. The cation contains a five-coordinate copper(II) in a distorted square-pyramidal $\{CuN_4O\}$ geometry with τ value [38] of 0.208. The two bpy ligands afford three nitrogens (N4–N6), together with a phenol oxygen (O1) of pic, to make up the basal plane. The other nitrogen (N7) occupies the apex of the square-pyramid with an elongated Cu–N bond (2.198(2) Å, table 2). At the opposite position of atom N7 is a nitro oxygen (O2), which does not coordinate to the central copper(II) with the long Cu1···O2 distance (2.737(3) Å).

Besides static electrifications between free pic and $[Cu(bpy)_2(pic)]^+$, there are nonclassical C–H···O hydrogen bonding and π–π stacking interactions in the crystal. The free pic anions link $[Cu(bpy)_2(pic)]^+$ cations to form a chain extended along the direction $[2\ \bar{1}\ 0]$. Then these chains are joined to a 2-D network parallel to the face (1 2 2) by hydrogen bonds between bpy and coordinated pic [figure 1(b), table 3]. Among the hydrogen bonding layers, there is an offset π–π stacking interaction between a complex cation at $-x, 1-y,$

$1-z$ (iv) [figure 1(c)]. The pyridine ring containing N4 and the benzene ring at (*iv*) has a dihedral angle of 14.07(15)° and a center-to-center distance of 4.0230(19) Å. The angle between the center-to-center vector and the normal to the N4-pyridine ring is 24.58°. Separations to the N4-pyridine of $C30^{iv}$ and $C31^{iv}$ are 3.534(4) and 3.332(4) Å, respectively.

Compared with our previously reported $[Cu(bpy)_2(CH_3OH)](pic)_2$ [25], with similar chemical composition, the main difference is the binding of pic, although the two mononuclear copper(II) complexes crystallized in triclinic with the same *P-1* space group. In $[Cu(bpy)_2(CH_3OH)](pic)_2$, the two free pic involve nonclassical hydrogen bonds with neighboring bpy molecules and bridge these complexes into a 1-D chain. In the present copper(II) complex, one pic is coordinated with copper(II) center to form a mononuclear copper(II) complex, $[Cu(bpy)_2(pic)](pic)$. These copper(II) complexes were further joined by the C–H···O hydrogen bonds and the π–π stacking interactions to construct a 2-D network. Furthermore, these differences of the structure have also affected DNA-binding properties and *in vitro* anticancer activities.

3.6. *DNA-binding studies*

DNA is the primary target for many metal-based drugs, and many metal complexes exhibit their anticancer effects through binding to DNA. Therefore, investigations of the interactions of DNA with metal complexes are important in the development of new anticancer drugs. For this reason, the binding behaviors of the monocopper(II) complex toward DNA are studied both theoretically and experimentally with the aid of different techniques.

3.6.1. Molecular docking of the complex with DNA duplex of sequence d $(ACCGACGTCGGT)_2$. Molecular docking technique has played important roles in understanding the drug-DNA interactions for rational drug design and discovery, as well as in the mechanistic study by placing a small molecule into the binding site of the target specific region of DNA mainly in a noncovalent fashion [39]. Molecular docking studies of the copper(II) complex with the DNA duplex of sequence $d(CGCGAATTCGCG)_2$ dodecamer were performed to predict the chosen binding site along with the preferred orientation of complex inside the DNA groove. According to the docking results, listed in table 4, the binding affinity of the copper(II) complex with the DNA indicates that van der Waal interactions are dominant over electrostatic interactions. Generally, the lower the relative binding energy, the more potent the binding affinity between DNA and target molecules. Thus, the negative values of the binding energy of docked the copper(II) complex suggest that the present complex reasonably binds to DNA. As shown in figure 2, the energy-minimized docked pose obtained for copper(II) complex shows that the best possible conformation of the interaction is through bpy ring and pic ring inside the DNA minor groove, which is mainly sta-

Table 4. Molecular docking results of the complex.

Binding energy (kcal/M)	Inter-mol energy (kcal/M)	Vdw_hb_desolv energy (kcal/M)	Electrostatic energy (kcal/M)	Total internal energy (kcal/M)	Torsional energy (kcal/M)
−7.78	−8.33	−9.07	0.74	0.34	0.55

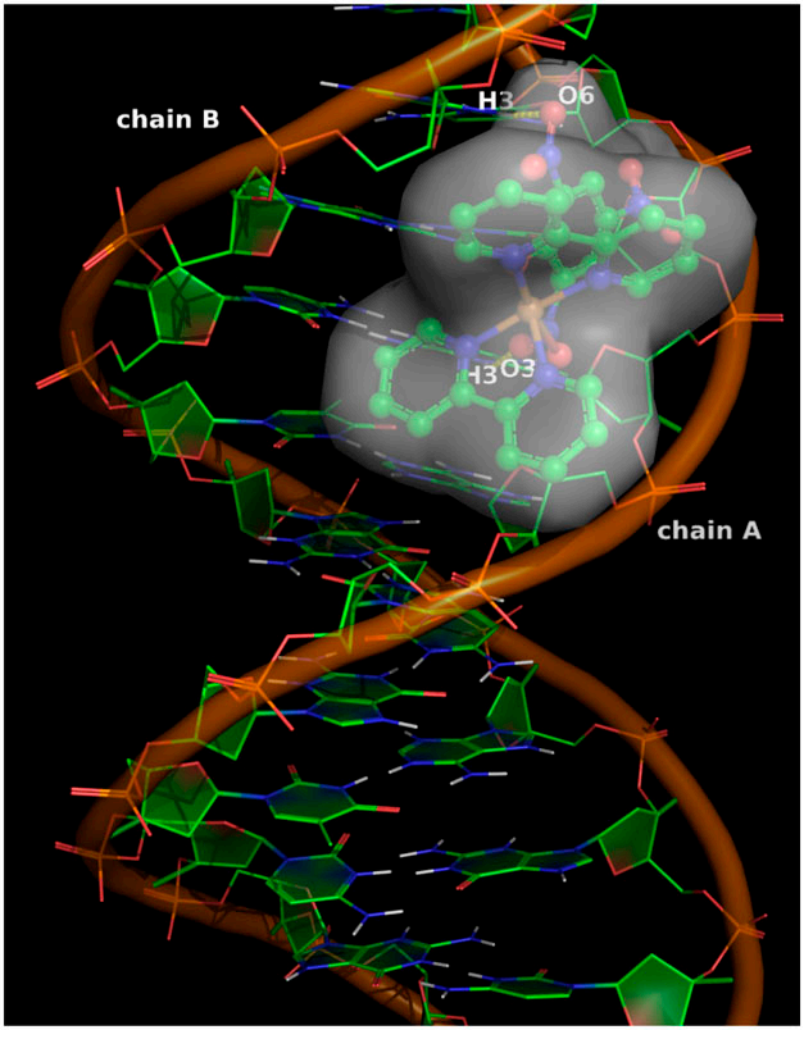

Figure 2. Molecular docking of the copper(II) complex with the DNA duplex of sequence d $(CGCGAATTCGCG)_2$ dodecamer. Wireframe model of the DNA with the complex (ball and stick) and showing the hydrogen bond formation with bond length. The copper(II) complex is docked in the minor groove of the DNA showing interaction with both chain A and chain B.

Table 5. Hydrogen bonding interactions involving the energy-minimized docked poses of d(ACCGAC GTCGGT)$_2$ with the complex.

Acceptor group (Y–H)	Donor group *Z*	Distance (Å)
H3(G4)(DNA-chain A)	O3(complex)	1.840
H3(G23)(DNA-chain B)	O6(complex)	1.954

bilized by hydrogen bonding, particularly involving O3 of the copper(II) complex to H3 of G4 from chain A and O6 of the copper(II) complex to H3 of G23 from chain B. These observations are further supported by hydrogen bonding interactions involving the

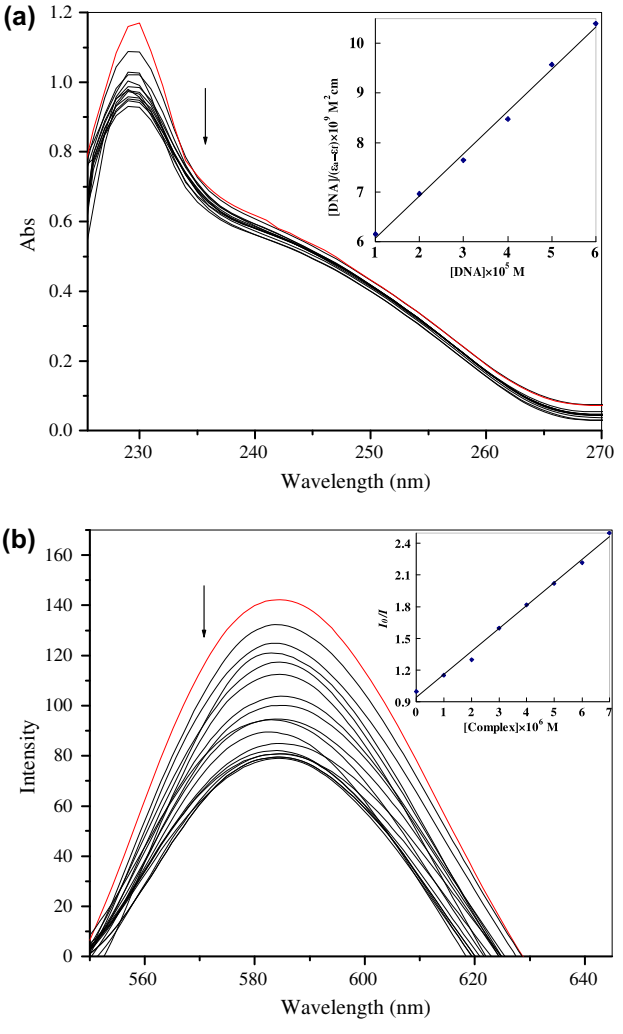

Figure 3. The absorption and emission spectra of the copper(II) complex in the absence (red line) and presence of *HS*-DNA. (a) Electronic absorption spectra of the complex in Tris–HCl buffer upon titration of *HS*-DNA. Arrow indicates the direction of change upon increase of the DNA concentration. Inset: plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ *vs.* [DNA] for the absorption titration of *HS*-DNA with the complex. (b) Emission spectra of *HS*-DNA-EB system upon titration of the complex. Arrow shows the change upon increasing complex concentration. Inset: plot of I_0/I *vs.* [complex] for the titration of the complex to *HS*-DNA-EB system (see http://dx.doi.org/10.1080/00958972.2014.884218 for color version).

energy-minimized docked poses of the DNA duplex of $d(ACCGACGTCGGT)_2$ with the copper(II) complex, as shown in table 5.

These results of molecular docking were further validated experimentally by the following spectroscopic and electrochemical titrations, as well as viscosity measurements.

3.6.2. Electronic absorption titration. Electronic absorption spectroscopy is an effective method to examine the binding modes and magnitudes of metal complexes with DNA.

Hypochromism and bathochromism are associated with the binding of the metal complexes to the DNA helix, due to intercalation involving a strong stacking interaction between the aromatic chromophore of the complexes and the base pairs of DNA [40]. The absorption spectra of the copper(II) complex in the absence and presence of *HS*-DNA are given in figure 3(a). As shown in this figure, when titrated by *HS*-DNA, the transition bands of the copper(II) complex at 229 nm exhibit hypochromism of 10.1% with slight red-shifts of 3 nm at a ratio of [DNA]/[complex] of 10. These spectral characteristics indicate that there is intercalation between the copper(II) complex and *HS*-DNA [41], which can be rationalized by the following two reasons. First, when the copper(II) complex intercalates the base pairs of *HS*-DNA, the π^*-orbital of the intercalated ligands in the copper(II) complex can couple with the π-orbital of the base pairs of *HS*-DNA, thus decreasing the π–π^* transition energy in the copper(II) complex and leading to the bathochromism. Second, the coupling π^*-orbital is partially filled by electrons, decreasing the transition probabilities, and concomitantly resulting in hypochromism.

To quantitatively evaluate the binding magnitude of the copper(II) complex with *HS*-DNA, the intrinsic binding constant (K_b) was determined from a plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA] using the following equation [42]:

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

where [DNA] is the concentration of DNA, ε_a, ε_f, and ε_b correspond to the extinction coefficients, respectively, for each addition of *HS*-DNA to the copper(II) complex, for the free copper(II) complex and for the copper(II) complex in the fully bound form. From the plot [inset in figure 3(a)] of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA], the binding constant K_b is given by the ratio of the slope to the intercept. The intrinsic binding constant K_b for the copper(II) complex was $9.8 \times 10^4\ \mathrm{M}^{-1}$ ($R = 0.9969$ for six points). The K_b value of the present copper (II) complex is lower than that observed for a classical intercalator, such as EB-DNA [43], and higher than that of some mono- [18, 25] and bi-copper(II) complexes containing bpy ligands [16, 17], which can be attributed to their structures.

3.6.3. Fluorescence titration. The present copper(II) complex can bind to *HS*-DNA by intercalation from the electronic absorption studies, but the binding mode needed to be further proved. Therefore, EB fluorescence displacement experiments were also employed. The intrinsic fluorescence intensity of DNA is very low, and that of EB in Tris–HCl buffer is also not high because of quenching by solvent. However, when DNA is added, the fluorescence intensity of EB is enhanced due to its intercalation into the DNA. If metal complexes can intercalate into DNA, the binding sites of DNA for EB will be reduced, and hence the fluorescence intensity of EB will be quenched [44]. Thus, EB can be used to probe the interaction of the metal complexes with DNA. In our experiments, as illustrated in figure 3(b), the fluorescence intensity of EB bound to *HS*-DNA at 584 nm shows a notable decrease with the increasing concentration of the copper(II) complex, indicating that some EB molecules were replaced with the copper(II) complex, resulting in fluorescence quenching of EB. These observations are characteristic of intercalation [45].

To understand quantitatively the binding strength of the copper(II) complex with *HS*-DNA, the linear Stern–Volmer equation is employed [46]:

$$I_0/I = 1 + K_{sv}[Q] \quad (2)$$

where I_0 and I represent the fluorescence intensities in the absence and presence of quencher, respectively. [Q] is the concentration of quencher. K_{sv} is a linear Stern–Volmer quenching constant. As inserted in figure 3(b), in the quenching plot of I_0/I *versus* [complex], K_{sv} is given by the ratio of the slope to intercept. The K_{sv} value for the copper(II) complex is 8.5×10^4 ($R = 0.9934$ for eight points). Thus, based on experiment evidence observed in the EB fluorescence displacement experiment, we conclude that the present copper(II) complex can bind to *HS*-DNA through intercalation, which is in agreement with that derived by electronic absorption spectra measurements.

If we compare K_b ($9.8 \times 10^4\ M^{-1}$) and K_{sv} (8.5×10^4) of the present copper(II) complex with those of our previously reported analogous copper(II) complex $[Cu(bpy)_2(CH_3OH)](pic)_2$ (K_b, $5.6 \times 10^4\ M^{-1}$; K_{sv}, 5.3×10^4) [25], we find that the K_b and K_{sv} values of the present mononuclear copper(II) complex are both higher than those previously reported, indicating that the present copper(II) complex can bind to *HS*-DNA more strongly by intercalation. From the structural viewpoint of the two mononuclear copper(II) complexes, the main chemical species (Cu^{2+} ion, bpy, and pic) in the two monocopper(II) complexes are identical. The main difference between the two complexes is the binding of pic to copper (II) although both have two pic groups. In $[Cu(bpy)_2(CH_3OH)](pic)_2$, the two pic groups are outside the metal coordination sphere, increasing the steric hindrance between the positive charge $[Cu(bpy)_2(CH_3OH)]^{2+}$ and the negative phosphate backbone of *HS*-DNA, and decreasing the binding affinity of the complex $[Cu(bpy)_2(CH_3OH)](pic)_2$ toward the DNA. However, in $[Cu(bpy)_2(pic)](pic)$, one pic is coordinated with copper(II), decreasing the electron density, hence reinforcing the binding ability between copper(II) complex and DNA, and consequently stabilizing the system formed by DNA and the present monocopper(II) complex. This increases the binding affinity between the present copper(II) complex and *HS*-DNA. According to these analyses, we may deduce that the difference in the binding pic in this kind of mononuclear complexes may have an effect on DNA-binding, as revealed by the different binding affinities.

We also compared the DNA-binding properties of the present copper(II) complex with those of previously reported mono- [47–51], di- [52–54] and tetra-copper(II) [55, 56] complexes, as well as 1-D copper(II) coordination polymers [57, 58] with various ligands, and we find that the modes and affinities of DNA-binding of all these copper(II) systems correlate not only with the planarity and donor types of their ligands but also with the coordination geometry of the copper(II) centers, suggesting that the interactions of these systems towards DNA may be tuned by changing the nature of the ligands. Such strategy should be valuable in understanding the binding mechanisms between the metal complexes and DNA, and designing new metal-based drugs targeting DNA.

3.6.4. Electrochemical titration. Cyclic voltammetry applied to study the interaction between metal complexes and DNA provides a useful complement to the previously used spectral studies. Here, it is employed to further understand the nature of DNA-binding of the copper(II) complex [figure 4]. In the absence of *HS*-DNA (red line), the copper(II) complex shows a couple of waves corresponding to Cu(II)/Cu(I) with the cathodic (E_{pc}) and anodic peak potential (E_{pa}) being –0.245 and –0.081 V, respectively. Separation of the anodic and cathodic peaks (ΔE_p) is found to be 0.164 V. The formal potential of the Cu(I)/Cu(II) couple in free form ($E_f^{0'}$) taken as the average of E_{pc} and E_{pa} is –0.163 V. In the presence of *HS*-DNA (blue line) with $R = 4$ (R = [DNA]/[complex]) the voltammetric peak current decreased, indicating that there exists interaction between the copper(II)

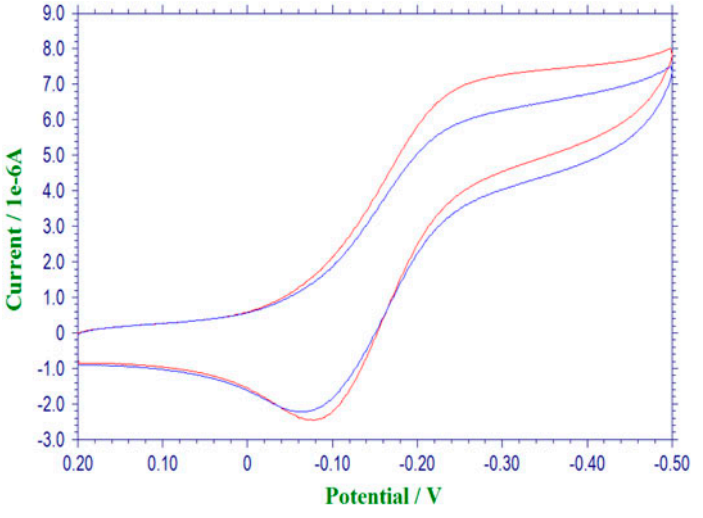

Figure 4. Cyclic voltammograms of the complex in the absence (red line) and presence (blue line) of *HS*-DNA (see http://dx.doi.org/10.1080/00958972.2014.884218 for color version).

complex and *HS*-DNA [59]. The drop of the voltammetric current in the presence of *HS*-DNA may be attributed to slow diffusion of the copper(II) complex bound to *HS*-DNA. The E_{pc} and E_{pa} are –0.230 and –0.063 V, respectively. The peak-to-peak separation becomes larger with $\Delta E_p = 0.167$ V, suggesting that in the presence of *HS*-DNA the electron-transfer becomes less reversible for the copper(II) complex. The formal potential of the Cu(I)/Cu(II) couple in binding ($E_b^{0'}$) is –0.147 V. The $E_b^{0'}$ value of the copper(II) complex is shifted towards positive region by 0.016 V, indicating that the copper(II) complex could bind intercalatively to *HS*-DNA [60]. This result is in agreement with the above-spectral observations.

Separation between $E_b^{0'}$ and $E_f^{0'}$ can be used to estimate the ratio of binding constants for the reduced and oxidized forms to DNA using the following equation [60]:

$$E_b^{0'} - E_f^{0'} = 0.059 \log[K_{Cu(I)}/K_{Cu(II)}] \quad (3)$$

where $K_{Cu(I)}$ and $K_{Cu(II)}$ are the binding constants of Cu(I) and Cu(II) forms to DNA, respectively. The ratio of constants for the binding of Cu(I) and Cu(II) to *HS*-DNA was estimated to be 1.87 for the copper(II) complex, suggesting that the reduced form of the copper (II) complex interacts more strongly than the oxidized one. Thus, the electrochemical results are in agreement with the above-spectral studies, which reinforce the conclusion that the copper(II) complex can bind to *HS*-DNA in an intercalation mode.

3.6.5. Viscosity measurements. Optical photophysical probes are necessary, but not sufficient clues to support a binding mode of metal complex to DNA. Further clarification of the interaction mode between the copper(II) complex and *HS*-DNA is carried out by viscosity measurements to explore the DNA-binding mode assessed from the above spectral and electrochemical studies. As it is sensitive to the changes in the length of DNA molecule, viscosity measurement is the least ambiguous and the most critical means to

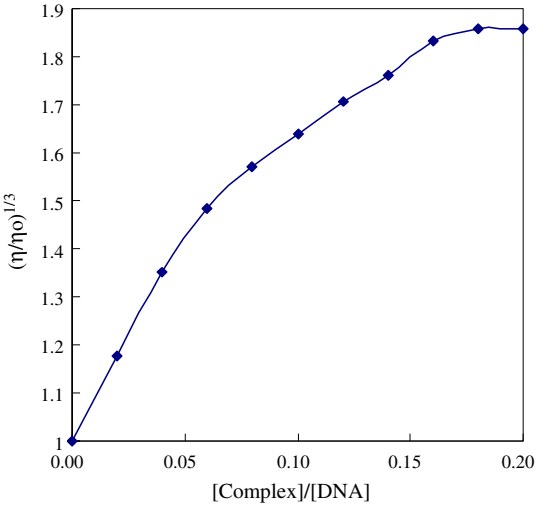

Figure 5. Effect of the increasing amount of the complex on the relative viscosity of *HS*-DNA at 298 K, [DNA] = 0.1 mM.

study the binding mode of metal complexes with DNA in solution, and provides reliable arguments for intercalative-binding [61, 62]. In classical intercalation the DNA helix lengthens as base pairs are separated to accommodate the bound ligand leading to increased DNA viscosity. Conversely, in groove binding and electrostatic mode, the length of the helix is unchanged with no apparent alteration in DNA viscosity [62]. As shown in figure 5, with increasing concentration of the copper(II) complex the relative viscosity of *HS*-DNA increases steadily, which further proved that the copper(II) complex binds to DNA by intercalation, and are consistent with the foregoing conclusions obtained from spectral and electrochemical titrations.

These positive results of DNA-binding investigations obtained both theoretically and experimentally for the copper(II) complex prompt us to explore *in vitro* anticancer activity against some cancer cells.

3.7. In vitro *anticancer activity studies*

To explore potential antitumor activities of the present copper(II) complex and our previously reported $[Cu(bpy)_2(CH_3OH)](pic)_2$ [25], the *in vitro* anticancer assays of the two copper(II) complexes against three cancer cell lines human hepatocellular carcinoma SMMC-7721, human lung adenocarcinoma A549, and human liver carcinoma Hep G2,

Table 6. The *in vitro* anticancer activity of the complexes.

	IC_{50} values (μM)			
Complexes	SMMC-7721	A549	Hep G2	L02
$[Cu(bpy)_2(pic)](pic)$	8.8 ± 0.7	10 ± 1	4.2 ± 0.4	390 ± 20
$[Cu(bpy)_2(CH_3OH)](pic)_2$	20 ± 1	23 ± 1	26 ± 1	360 ± 10
Cis-platin	3.5 ± 0.2	5.6 ± 0.4	10.4 ± 0.9	200 ± 10

along with the human normal cell line hepatocyte L02 by the SRB assay method were conducted, and *cis*-platin was used as a positive control to assess the cytotoxicity of the tested complexes. The results were analyzed by cell inhibition expressed as IC_{50} values, which are listed in table 6. The present copper(II) complex possessed the most potent inhibitory effect against Hep G2 cell line, and the inhibitory activity (IC_{50} value) is about two times the well-known anticancer drug (*cis*-platin). Furthermore, the IC_{50} values of the two monocopper(II) complexes for the other cancer cell lines are much higher than those of *cis*-platin, suggesting that the cytotoxic activities of the two copper(II) complexes are less than that of *cis*-platin. However, inhibition of cell proliferation produced by the two copper(II) complexes on the same batch of cell lines is still rather active. Particularly, the IC_{50} values of the two copper(II) complexes against the human normal cell line hepatocyte L02 are above 360 μM, indicating that these copper(II) complexes act very specifically on the selected tumor cell lines.

On comparison of the IC_{50} values of the two monocopper(II) complexes, IC_{50} values of the present copper(II) complex are lower than $[Cu(bpy)_2(CH_3OH)](pic)_2$ [25], indicating better activities than the latter under identical experimental conditions. This may be attributed to complexation of pic ligand in the present copper(II) complex considerably reducing the polarity of the metal ion due to the partial sharing of its positive charge with the donor groups. Such complexation could enhance the lipophilic character of the complex, and further lead to the increasing hydrophobic forces of DNA interaction [63], which subsequently favors permeation through the lipid layers of the cell membrane. We conclude that complexation may be the major cause of the enhanced effect on the viability of cancer cells. The order of the *in vitro* anticancer activities of the two copper(II) complexes to the selected cancer cell lines is in accord with their DNA-binding abilities, implying that the anticancer activities of the two copper(II) complexes may be related to, or originate from, their ability to intercalate the base pairs of DNA. In other words, the two copper(II) complexes might target DNA, leading to cell death. DNA-binding and *in vitro* anticancer activities could be tuned through varying the binding of some ligands in these mononuclear copper(II) complexes. This strategy opens vast perspectives in understanding the anticancer activities and DNA-binding behaviors of this kind of metal complexes.

4. Conclusion

To get insight into the structure/activity relationship of mononuclear ternary copper(II) complexes, a new copper(II) complex with bpy and pic, $[Cu(bpy)_2(pic)](pic)$, has been synthesized and structurally characterized by crystal X-ray diffraction. The noncovalent interactions of the copper(II) complex with DNA are investigated both theoretically and experimentally, revealing that it can interact with *HS*-DNA through intercalation, and the molecular docking of the copper(II) complex with the self-complementary DNA duplex of sequence $d(ACCGACGTCGGT)_2$ facilitates the binding events. The *in vitro* anticancer activities suggest that the copper(II) complex is active against the selected tumor cell lines. Particularly, the inhibitory effect against Hep G2 cell line (IC_{50} value) of the present copper (II) complex is about two times larger than *cis*-platin, indicating the copper(II) complex may be a candidate for DNA-binding reagent, as well as laying the foundation for the rational design of new DNA probes. Compared to our previously reported copper(II) complex with the same chemical composition (Cu^{2+}, bpy, and pic), complexation of the pic

group results in interesting differences in the space configuration and the electron density distribution, thus enhancing the DNA-binding abilities and *in vitro* anticancer activities of the present copper(II) complex. The *in vitro* anticancer activities of these mononuclear copper(II) complexes are in accord with their DNA-binding abilities, implying that the anticancer activities may be associated with their ability to intercalate the base pairs of the DNA. The DNA-binding abilities and the *in vitro* anticancer activities could be tuned through varying the chemical arrangement in these mononuclear copper(II) systems. Such strategy should be valuable in understanding the relationship of DNA-binding behaviors and anticancer activities and lay a foundation for the rational design of powerful agents for probing and targeting nucleic acids, and providing important insight into the field of DNA interactions.

Supplementary material

CCDC 929000 contains the supplementary crystallographic data for this article. Copies of this information can be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44 1233 336 033; E-mail: deposit@ccdc.cam.ac.uk).

Funding

This project was supported by the National Natural Science Foundation of China [grant numbers 51273184 and 81202399]; the Program for Science and Technology of Shandong Province [2011GHY11521]; the Natural Science Foundation of Qingdao City [grant numbers 11-2-4-1-(9) gch, 12-1-3-52-(1)-nsh 12-1-4-16-(7)-jch].

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