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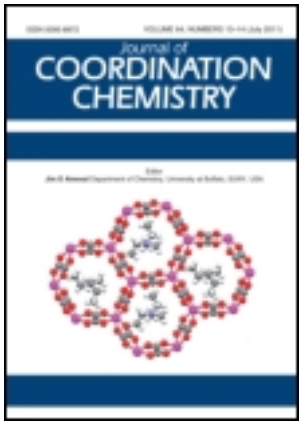

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Copper(II) complex based on a V-shaped ligand, 2,6- bis (2-benzimidazolyl)pyridine: synthesis, crystal structure, DNA-binding properties, and antioxidant activities

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Copper(II) complex based on a V-shaped ligand, 2,6-*bis*(2-benzimidazolyl)pyridine: synthesis, crystal structure, DNA-binding properties, and antioxidant activities

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A copper(II) complex based on a V-shaped ligand, 2,6-*bis*(2-benzimidazolyl)pyridine (bbp), has been synthesized and characterized by elemental analysis, molecular conductivity, ^{1}H NMR, IR, UV-Vis spectra, and X-ray single-crystal diffraction. The crystal structure of $[Cu(bbp)_2](pic)_2 \cdot 2DMF$ (pic = picrate) shows copper is six-coordinate forming a distorted octahedron. The interaction between Cu(II) complex and DNA was investigated by spectrophotometric methods and viscosity measurement. The experimental results suggest that the Cu(II) complex binds to DNA *via* intercalation. Antioxidant assay *in vitro* also shows that the Cu(II) complex possesses significant antioxidant activities.

Keywords: 2,6-*Bis*(2-benzimidazolyl)pyridine; Copper(II) complex; Crystal structure; DNA-binding property; Antioxidant

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1. Introduction

Benzimidazoles and their derivatives exhibit remarkable biological activities such as antitumor [1], antiviral [2], anticancer [3], antimicrobial [4], antiprotozoal [5], antihistaminic [6], antifungal [7], and anti-inflammatory or analgesic activities [8]. Since the discovery of the antitumor activity of cisplatin [9], transition metal complexes are used to bind and react at specific sequences of DNA in a search for novel chemotherapeutics and probing DNA, and for the development of highly sensitive diagnostic agents [10]. Copper is an essential element in biology with two relevant oxidation states +I and +II, which can be used in some superoxide dismutase enzymes to convert superoxide to dioxygen or peroxide [10g, 11]. Copper complexes have also been studied due to their DNA-binding activities, biologically accessible redox potential, and relatively high affinity for nucleobases [12, 13]. Design and synthesis of copper complexes with distinct biological and pharmaceutical features are suggested. For this reason, the V-shaped system 2,6-*bis*(2-benzimidazolyl)pyridine (bbp) was

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selected to chelate Cu(II), which may enhance the interaction of the metal center with DNA and have a positive impact in fields of diagnosis and therapy.

With biological activities, the free ligand bbp has been studied to focus on interactions of transition metal complexes with DNA [14]. In previous studies [15], the interactions of picrate Mn(II) and Ni(II) complexes based on bbp with DNA have been studied. In this article, we report the synthesis, crystal structure, DNA-binding properties, and antioxidant activities of a similar Cu(II) picrate complex. The DNA-binding properties of the title complex were investigated by spectrophotometric and viscosity measurements. In addition, the antioxidant activities of the complex were determined by the superoxide anion ($O_2^{-\bullet}$) and hydroxyl radical ($HO^{\bullet}$) scavenging methods *in vitro*.

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2. Experimental

2.1. *Materials and physical measurements*

Calf thymus DNA (CT-DNA), ethidium bromide (EB), nitroblue tetrazolium nitrate (NBT), methionine (MET), and riboflavin ($VitB_2$) were obtained from Sigma-Aldrich Co. (St. Louis, USA). Other reagents and solvents were of reagent grade obtained from commercial sources and used without purification. Tris-HCl buffer, Na_2HPO_4–NaH_2PO_4 buffer, and EDTA–Fe(II) solution were prepared using twice-distilled water. Stock solution of the complex was dissolved in DMF at 1×10^{-3} mol L^{-1}. Solution of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of 1.8–1.9, indicating that the DNA was sufficiently free of protein [16]. The stock solution of DNA (2.5×10^{-3} mol L^{-1}) was prepared in 5 mmol L^{-1} Tris-HCl/50 mmol L^{-1} NaCl buffer (pH = 7.2), stored at 4°C, and used in not more than 4 days. The DNA concentration was determined by measuring the UV absorption at 260 nm, taking the molar absorption coefficient (ε_{260}) of CT-DNA as 6600 (mol L^{-1})$^{-1}$ cm^{-1} [17].

Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer. IR spectra were recorded on a Bruker FT-IR Vertex 70 spectrometer from 4000 to 400 cm^{-1} using KBr pellets. Electrolytic conductance measurements were made with a DDS-11A conductivity bridge using a 10^{-3} mol L^{-1} solution in DMF at room temperature. ^{1}H-NMR spectra were obtained with a Mercury plus 400 MHz NMR spectrometer with TMS as internal standard and DMSO-d_6 as solvent.

2.2. *DNA-binding experiments*

Electronic absorption spectra were taken on a Lab Tech UV Bluestar plus UV-visible spectrophotometer. According to the electronic absorption spectral method, the relative bindings of the complex to CT-DNA were studied in 5 mmol L^{-1} Tris-HCl/NaCl buffer (pH = 7.2). The sample solution was scanned from 200 to 500 nm.

Fluorescence spectra were recorded on a 970CRT spectrofluorophotometer. By gradually adding a certain amount of DMF solutions of the complex (5 μmol L^{-1}) step by step to the CT-DNA solution in Tris-HCl buffer (5 mmol L^{-1}, pH = 7.2) at 25°C, the emission intensity was recorded at an excitation of 337 nm. The sample solution was scanned from 350 to 600 nm.

EB-DNA competitive binding experiments were performed on a 970CRT spectrofluorophotometer. While gradually adding a certain amount of DMF solution of the complex to the EB-DNA solution in Tris-HCl buffer at 25°C, the fluorescence intensity was recorded at an excitation of 520 nm. The sample solution was scanned in the range of 550–750 nm.

Viscosity experiments were conducted on an Ubbelodhe viscometer immersed in a thermostated water-bath maintained at 25.0 ± 0.1°C. DNA samples approximately 200 bp in average length were prepared by sonication in order to minimize complexities arising from DNA flexibility [18]. Titrations were performed for the complex (1–10 μmol L^{-1}), and the complex was introduced into the CT-DNA solution (50 μmol L^{-1}) present in the viscometer. Data were presented as $(\eta/\eta_0)^{1/3}$ *versus* the ratio of the concentration of the compound to CT-DNA, where η is the viscosity of CT-DNA in the presence of the complex, and η_0 is the viscosity of CT-DNA alone. Viscosity values were calculated from the observed flow time of CT-DNA containing solutions corrected from the flow time of the buffer alone (t_0), $\eta = (t - t_0)/t_0$ [19].

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2.3. *Antioxidant assay*

Antioxidant activities involving the hydroxyl radical ($HO^{\bullet}$) and superoxide anion radical ($O_2^{-\bullet}$) were performed in a water-bath with a 722sp spectrophotometer.

The hydroxyl radical ($HO^{\bullet}$) in aqueous medium was generated through the Fenton reaction [20]. Aliquots of reaction mixture contained 1 mL 40 μg mL^{-1} safranin, 1 mL 1 mmol L^{-1} EDTA-Fe(II), 1 mL 3% aqueous H_2O_2, and the complex of various concentrations. The reaction mixtures were incubated at 37°C for 30 min in a water bath. Absorbance at 520 nm was measured and the scavenging effect for $HO^{\bullet}$ was calculated on the basis of $(A_i - A_0)/(A_{cv} - A_0) \times 100\%$. A_i was the absorbance in the presence of tested compound; A_0 was the absorbance in the absence of tested compound; A_c was the absorbance in the absence of tested compound, EDTA–Fe(II), H_2O_2.

The superoxide radical was investigated indirectly using the system of MET–$VitB_2$–NBT [21]. Aliquots of solution contained 0.5 mL 3.3×10^{-5} mol L^{-1} $VitB_2$, 1 mL 2.3×10^{-4} mol L^{-1} NBT, 1 mL 0.05 mol L^{-1} MET, and the complex of various concentrations prepared with 0.067 mol L^{-1} phosphate buffer (Na_2HPO_4–NaH_2PO_4, pH = 7.8). It was illuminated by a fluorescent lamp with a constant light intensity at 25°C. The optical absorbance (A) of the solution at 560 nm was measured with various illumination periods (t). Each solution was measured in two parallel tests. Scavenging percentage (%) was calculated according to the reported method [21b]. The activity (IC_{50}) is defined as the necessary concentration to scavenge 50% reduction of NBT.

2.4. *Synthesis*

2.4.1. Synthesis of 2,6-*bis*(2-benzimidazolyl)pyridine (bbp). The ligand bbp was synthesized according to the procedure reported by Addison and Burke [22]. m.p. > 300°C. The infrared spectra and ^1H NMR spectra of the bbp were almost consistent with the literature reports [22, 23]. IR (KBr pellet, cm^{-1}): 3185s (ν_{N-H}), 1600m (ν_{C-C}), 1573s ($\nu_{C=N}$), 1460s (ν_{C-N}), 1434s, 1319s, 1278s (ν_{C-N}), 1230m, 821m, 742vs ($\delta_{Ph(C-H)}$). ^1H NMR (400 MHz, DMSO-d_6, 298 K): $\delta = 13.02$ (s, 2H, N–H),

8.36–8.38 (d, $J = 7.59$ Hz, 2H, Py–H), 8.18–8.22 (t, $J = 7.46$ Hz, 1H, Py–H), 7.76–7.80 (m, $J = 7.83$ Hz 4H, Ph–H), 7.34–7.37 (m, 4H, Ph–H). UV-Vis (DMF): $\lambda = 300$, 345 nm. Λ_M (DMF, 297 K): 1.41 S cm^2 mol^{-1}.

2.4.2. Preparation of [Cu(bbp)$_2$](pic)$_2$·2DMF. A solution of 2,6-*bis*(2-benzimidazolyl)pyridine (0.1557 g, 0.50 mmol) in 10 mL hot MeOH was mixed slowly with a solution of Cu(pic)$_2$ (0.1299 g, 0.25 mmol) in 5 mL MeOH. After 4 h of stirring, the resulting pale-yellow precipitate was collected by filtration, washed with MeOH and absolute Et_2O, and dried *in vacuo*. The dried precipitate was dissolved in DMF giving a yellow solution; green crystals suitable for X-ray diffraction studies were obtained by ether diffusion into DMF after several days at room temperature. Yield *ca* 55%. Anal. Calcd for $C_{56}H_{44}N_{18}O_{16}Cu$ ($Mr = 1288.63$) (%): C 52.20, H 3.44, N 19.57; Found (%): C 52.14, H 3.55, N 19.67. IR (KBr pellet, cm^{-1}): 3082s (ν_{N-H}), 1633m, 1610s ($\nu_{C=C}$), 1558m ($\nu_{C=N}$), 1494w, 1469m (ν_{C-N}), 1435w, 1363m, 1321s, 1273m (ν_{C-N}), 821w, 744s ($\delta_{Ph(C-H)}$). UV-Vis (DMF): $\lambda = 302$, 362, 402, 702(weak) (nm). Λ_M (DMF, 297 K): 81.04 S cm^2 mol^{-1}.

2.5. *Crystal structure determination*

A suitable single crystal was mounted on a glass fiber and intensity data were collected with a Rigaku *R* axis Spider diffractometer with graphitemonochromated Mo-Kα radiation ($\lambda = 0.71073$ Å) at 153 K. Data collection and cell refinement were performed with RAPID-AUTO [24]. Data reduction was carried out using CrystalStructure [25]. An empirical absorption correction was applied using ABSCOR [26]. The structure was solved by direct methods and refined by full-matrix least-squares against F^2 of data using SHELXTL software [27]. All hydrogen atoms were found in difference electron maps and were subsequently refined in a riding model approximation with C–H distances ranging from 0.93 to 0.96 Å, $U_{iso}(H) = 1.2$ or $1.5\,U_{eq}(C)$. A summary of parameters for data collection and refinement is given in table 1.

3. Results and discussion

The Cu(II) complex is remarkably soluble in polar aprotic solvents such as DMF, DMSO, and MeCN; slightly soluble in ethanol, methanol, ethyl acetate, and chloroform; insoluble in water, Et_2O, petroleum ether. The elemental analysis shows that the composition of the complex is [Cu(bbp)$_2$](pic)$_2 \cdot 2$DMF. The molar conductivity in DMF indicates that the complex is a 1 : 2 electrolyte [28].

3.1. *IR and electronic spectra*

IR spectra of the Cu(II) complex were analyzed in comparison with that of free bbp from 4000 to 400 cm^{-1}. The IR spectrum of free bbp shows characteristic absorptions of benzimidazole pyridine group at 1600, 1573, 1460, and 1278 cm^{-1} assigned to $\nu_{C=C}$, $\nu_{C=N}$, and ν_{C-N}, respectively [14f, 15, 29]. In free bbp, sharp bands of benzimidazole are

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Table 1. Crystal structure data for $[Cu(bbp)_2](pic)_2 \cdot 2DMF$.

Complex	$[Cu(bbp)_2](pic)_2 \cdot 2DMF$
Molecular formula	$C_{56}H_{44}N_{18}O_{16}Cu$
Molecular weight	1288.63
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions (Å, °)	
a	14.2347(4)
b	26.6382(6)
c	19.7220(4)
α	90
β	132.0610(10)
γ	90
Volume (Å^3), Z	5552.1(2), 4
Calculated density (Mg m^{-3})	1.542
$F(000)$	2652
Crystal size (mm^3)	$0.55 \times 0.28 \times 0.24$
θ range for data collection (°)	3.00–27.48
Limiting indices	$-18 \le h \le 18$; $-33 \le k \le 34$; $-25 \le l \le 25$
Reflections collected	52,232
Independent reflections	12,718 [R(int) = 0.0363]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	12,718/0/825
Goodness-of-fit on F^2	1.169
Final R indices [$I \ge 2\sigma(I)$]	$R_1 = 0.0408$, $wR_2 = 0.1005$
R indices (all data)	$R_1 = 0.0649$, $wR_2 = 0.1218$
Largest differences peak and hole (e Å^{-3})	0.567 and −0.818

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at 1573 cm^{-1} ($\nu_{C=N}$) and 1278 cm^{-1} (ν_{C-N}), but in the complex these bands shift to lower frequencies (1558, 1273 cm^{-1}), which can be attributed to the coordination of benzimidazole nitrogen to Cu(II). IR bands at 1328 ($\nu_{s(NO2)}$), 1544 cm^{-1} ($\nu_{as(NO2)}$) of the free picrate do not split but move slightly at 1317 ($\nu_{s(NO2)}$) and 1558 cm^{-1} ($\nu_{as(NO2)}$) in the complex, indicating that the picrate was not coordinated [30]. The bbp shows a well-defined band at 3185 cm^{-1} due to N–H stretching in the benzimidazole group, which can be found at 3086 cm^{-1} in the complex, indicating hydrogen bonds between N–H bonds and other atoms [30].

Electronic spectra of free bbp, picric acid, and the Cu(II) complex were recorded at 298 K in DMF. Free bbp shows two strong absorptions at 300 and 345 nm attributed to $\pi \to \pi^*$ transitions; picric acid shows four strong absorptions at 272, 327, 365, and 419 nm attributed to $\pi \to \pi^*$ and $n \to \pi^*$ transitions [31]. By comparison, the Cu(II) complex shows three strong absorption bands at 302, 362, and 402 nm attributed to $\pi \to \pi^*$ and $n \to \pi^*$ transitions and a weak absorption band at 702 nm attributed to $d \to d$ transition ($^2E_{2g} \to {}^2T_{2g}$). Therefore, it could be indicated that the ligand bbp coordinated to the metal center to form the Cu(II) complex.

3.2. *Description of the crystal structure of $[Cu(bbp)_2](pic)_2 \cdot 2DMF$*

Selected atomic distances and angles of $[Cu(bbp)_2](pic)_2 \cdot 2DMF$ are listed in table 2. The structure of $[Cu(bbp)_2](pic)_2 \cdot 2DMF$ with atom-numbering, as shown in figure 1, consists of a discrete $[Cu(bbp)_2]^{2+}$, two picrate anions, and two DMF molecules. The copper has distorted octahedral geometry with a N_6 ligand set with four N atoms

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

Cu(1)–N(10)	1.9873(17)	Cu(1)–N(5)	2.0674(17)
Cu(1)–N(8)	2.0335(17)	Cu(1)–N(3)	2.3736(18)
Cu(1)–N(6)	2.0344(17)	Cu(1)–N(1)	2.4138(18)
N(10)–Cu(1)–N(8)	78.80(7)	N(6)–Cu(1)–N(3)	88.84(7)
N(10)–Cu(1)–N(6)	78.93(7)	N(5)–Cu(1)–N(3)	75.33(6)
N(8)–Cu(1)–N(6)	157.65(7)	N(10)–Cu(1)–N(1)	96.28(7)
N(10)–Cu(1)–N(5)	169.88(7)	N(8)–Cu(1)–N(1)	89.85(6)
N(8)–Cu(1)–N(5)	96.74(7)	N(6)–Cu(1)–N(1)	94.56(7)
N(6)–Cu(1)–N(5)	105.56(7)	N(5)–Cu(1)–N(1)	74.49(6)
N(10)–Cu(1)–N(3)	114.16(7)	N(3)–Cu(1)–N(1)	149.43(6)
N(8)–Cu(1)–N(3)	98.43(6)		

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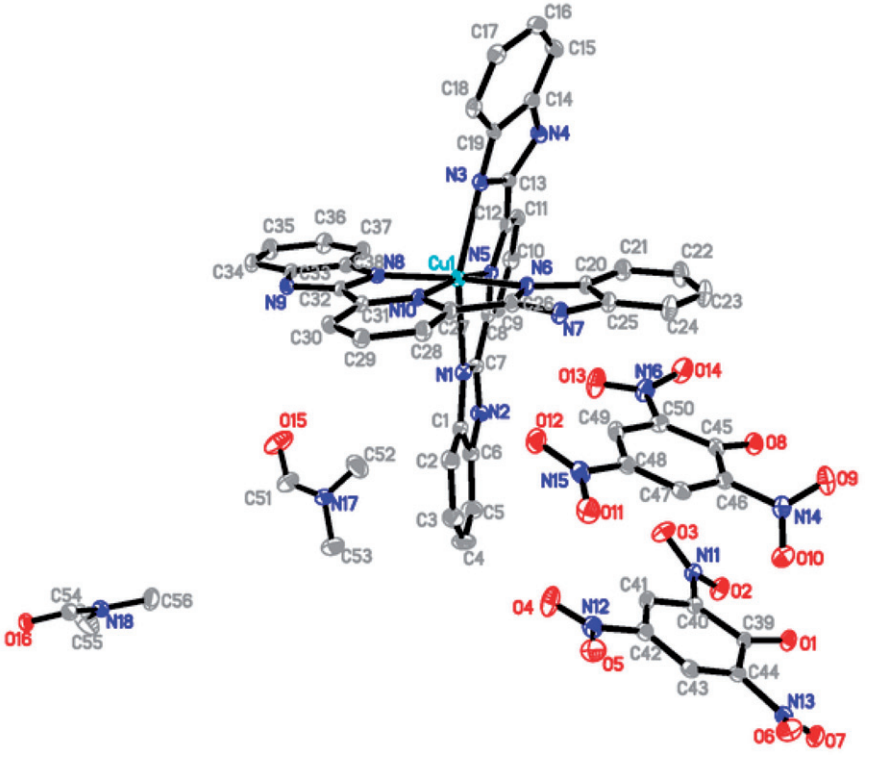

Figure 1. Molecular structure and atom numbering of $[Cu(bbp)_2](pic)_2 \cdot 2DMF$ with hydrogen atoms omitted for clarity.

(N1, N3, N6, and N8) from benzimidazole rings and other two (N5 and N10) from pyridine. The shortest Cu–N bond length is 1.9873(17) Å [Cu(1)–N(10)] and the longest is 2.4138(18) Å [Cu(1)–N(1)]. The bond angles range from 74.49(6)° [N(5)–Cu(1)–N(1)] to 114.16(7)° [N(10)–Cu(1)–N(3)] and from 149.43(6)° [N(1)–Cu(1)–N(3)] to 169.88(7)° [N(10)–Cu(1)–N(5)]. An equatorial plane is formed by N1, N3, N6, and N8, where the largest deviation from the mean plane is 0.526 Å and the Cu is out of this plane by

Table 3. Hydrogen bonds for the Cu(II) complex (Å and °).

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	∠(DHA)
N(2)–H(2A)···O(16)#1	0.88	1.90	2.773(2)	168.9
N(4)–H(4A)···O(8)#2	0.88	1.83	2.689(2)	163.1
N(4)–H(4A)···O(14)#2	0.88	2.43	2.971(2)	120.1
N(7)–H(7A)···O(15)#3	0.88	1.84	2.717(3)	173.6
N(9)–H(9A)···O(1)#4	0.88	1.97	2.804(2)	157.2
N(9)–H(9A)···O(7)#4	0.88	2.44	3.055(2)	127.4

Symmetry transformations used to generate equivalent atoms: #1 $-x+1$, $y+1/2$, $-z+1/2$; #2 $-x, -y+2, -z+1$; #3 $x, y, z+1$; #4 $-x+1, y-1/2, -z+3/2$.

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0.117 Å [32]. Axial positions are occupied by N(5) and N(10). The distance between the axial N5 and N10 and the equatorial plane are 1.945 Å and 2.081 Å, respectively. The bond angle [N(5)–Cu(1)–N(10)] is 169.88(7)°. Therefore, it reflects a relatively distorted coordination octahedron around Cu(II) [33].

The crystal structure of the complex is stabilized by hydrogen bonds, due to the picrate and DMF. Intermolecular N–H···O hydrogen bonds between bbp and the picrate anion, DMF solvent molecules are listed in table 3.

3.3. *DNA bonding mode and affinity*

3.3.1. Absorption spectroscopic studies. Electronic absorption spectra in DNA-binding studies is one of the most useful techniques. Intercalative drugs to DNA are characterized through absorption titrations, following the hypochromism and red shift associated with binding to the helix [34]. The absorption spectra of the Cu(II) complex in the absence and presence of CT-DNA (at constant concentration of the complex) are shown in figure 2. With increasing CT-DNA concentration, the hypochromism in the band at 342 nm reaches as high as 13.6% with a red shift of 4 nm. The absorption spectra suggest that Cu(II) complex has strong interaction with DNA [34].

To compare quantitatively the affinity of complexes toward DNA, the intrinsic binding constants K_b was determined from the spectral titration data using the following equation [19]:

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

where [DNA] is the concentration of DNA in base pairs, the apparent absorption coefficients ε_a, ε_f, and ε_b correspond to $A_{obsd}/[M]$, the extinction coefficient of the free compound, and the extinction coefficient of the compound when fully bound to DNA, respectively. In plots of $[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f)$ *versus* [DNA], K_b is given by the ratio of the slope to the intercept. From the $[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f)$ *versus* [DNA] plot (figure 2 inset), the binding constant K_b for the complex was estimated to be 1.06×10^5 $(\mathrm{mol\,L^{-1}})^{-1}$ ($R = 0.9968$ for ten points). Therefore, according to the reported DNA-intercalative ruthenium complexes (1.1×10^4–4.8×10^4 $(\mathrm{mol\,L^{-1}})^{-1}$) [34], the Cu(II) complex probably binds to DNA by intercalation, due to the large coplanar aromatic rings in the Cu(II) complex that facilitate intercalation to the base pairs of helical DNA.

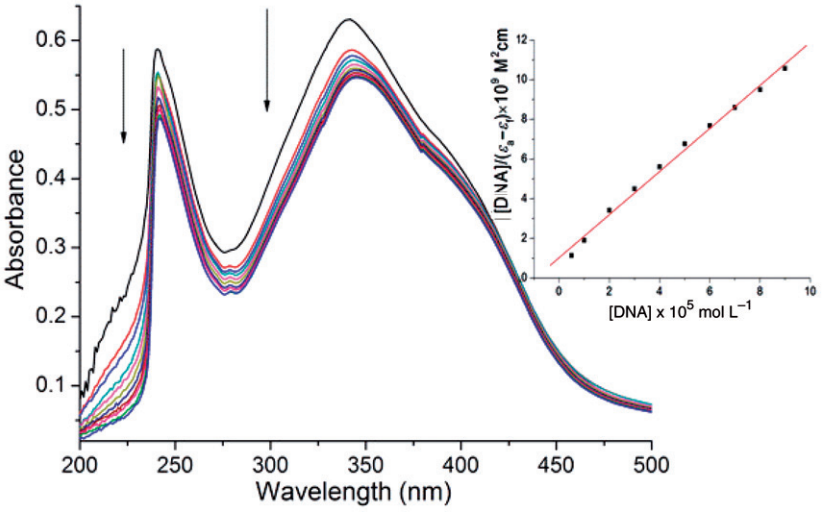

Figure 2. Absorption spectra of the Cu(II) complex in the absence and presence of increasing amounts of CT-DNA. [Cu(II) complex] = 10 μmol L^{-1}, [CT-DNA] = 0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 μmol L^{-1}. Arrows show the absorbance changes upon increasing the DNA concentration. Inset: plots of [DNA]/($\varepsilon_a - \varepsilon_f$) *vs*. [DNA] for the titration of DNA with complex; ■, experimental data points; solid line, linear fitting of the data.

3.3.2. Fluorescence spectroscopic studies. In the absence of DNA, the Cu(II) complex can emit fluorescence in DMF-Tris buffer at room temperature, with a maximum at 409 nm. As shown in figure 3, the fluorescence intensity of the complex is quenched steadily with increasing concentration of the CT-DNA. The Cu(II) complex may intercalate the guanine base of DNA to result in the photoelectron transfer from the guanine base of DNA to the excited metal-to-ligand charge transfer (MLCT) state of the complex [35].

According to the Stern–Volmer equation [36]:

$$F_0/F = 1 + K_{sv}[Q]$$

where F_0 is the emission intensity in the absence of the quencher, F is the emission intensity in the presence of the quencher, K_{sv} is the quenching constant, and [Q] is the quencher (CT-DNA) concentration. The shape of the Stern–Volmer plots can be used to characterize the quenching as being predominantly dynamic or static. The Stern–Volmer quenching plots from the fluorescence titration data are shown in figure 3 inset. The fluorescence quenching constant (K_{sv}) of the complex is 9.50×10^3 $(\text{mol L}^{-1})^{-1}$ ($R = 0.9743$ for eight points). The behavior of Cu(II) complex is in good agreement with the Stern–Volmer equation, which provides further evidence that the Cu(II) complex binds to DNA.

3.3.3. EB-DNA competitive binding studies. In order to further study the binding of the complex with DNA, competitive binding was carried out. Relative binding to CT-DNA was studied by the fluorescence spectral method using EB bound CT-DNA solution in Tris-HCl/NaCl buffer (pH = 7.2). As a typical indicator of intercalation, EB

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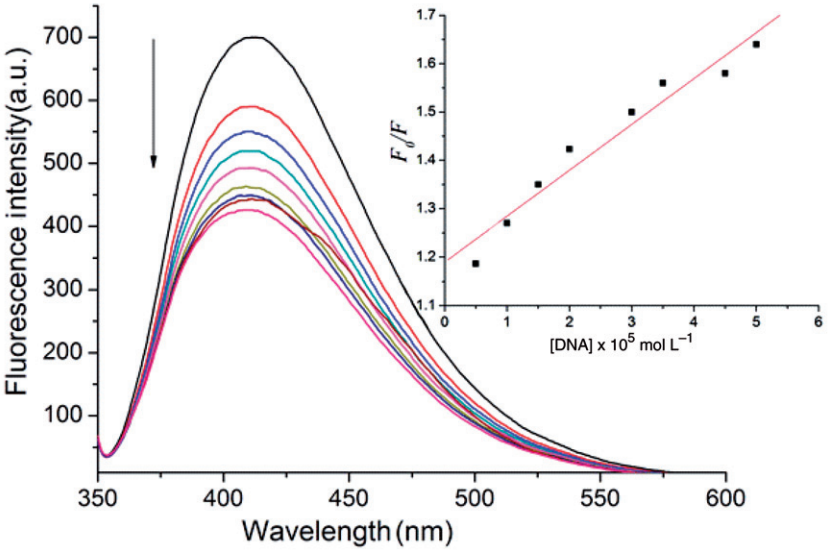

Figure 3. Fluorescence spectra of the DMF solution of the Cu(II) complex in Tris-HCl buffer upon the addition of CT-DNA. [Cu(II) complex] = 10 μmol L^{-1}, [CT-DNA] =0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 μmol L^{-1}. Arrow shows the intensity changing upon increasing the CT-DNA concentrations. A Stern–Volmer quenching plot of the title complex inset in its own fluorescence spectra with increasing concentrations of CT-DNA.

is a weakly fluorescent compound. But in the presence of DNA, emission intensity of EB is greatly enhanced because of its strong intercalation between adjacent DNA base pairs [37].

Competitive binding of the title complex to CT-DNA resulted in the displacement of bound EB with quenching of the bound EB, and as a consequence the emission intensity of EB decreased. In competitive binding experiment, a 10 μL of CT-DNA solution was added to a 10 μL of an EB buffer solution (pH = 7.2) and the fluorescence intensity was measured using the excitation wavelength of 520 nm resulting in an emission at about 600 nm at room temperature.

Upon addition of the title complex, the emission intensity of EB-bound CT-DNA solution decreases, indicating that the complex competes with EB to bind with DNA (figure 4). In addition, the extent of quenching of the fluorescence intensity can give a measure for the binding affinity of the complex with CT-DNA. According to the classical Stern–Volmer equation mentioned above, [Q] is the quencher (the Cu(II) complex) concentration. The fluorescence quenching constant (K_{sv}) of the title complex is 1.44×10^4 (mol L^{-1})$^{-1}$ (figure 4 inset, $R = 0.9950$ for 14 points). The quenching plot illustrates that the quenching of EB-bound CT-DNA by Cu(II) complex agrees well with the linear Stern–Volmer equation. Thus the Cu(II) complex interacts with DNA by intercalation, releasing some free EB from the EB-bound CT-DNA complex system, consistent with the above absorption and fluorescence spectral results.

3.3.4. Viscosity studies. Photophysical techniques are widely used to explore the binding of metal complexes and DNA, but do not give sufficient clues to support a

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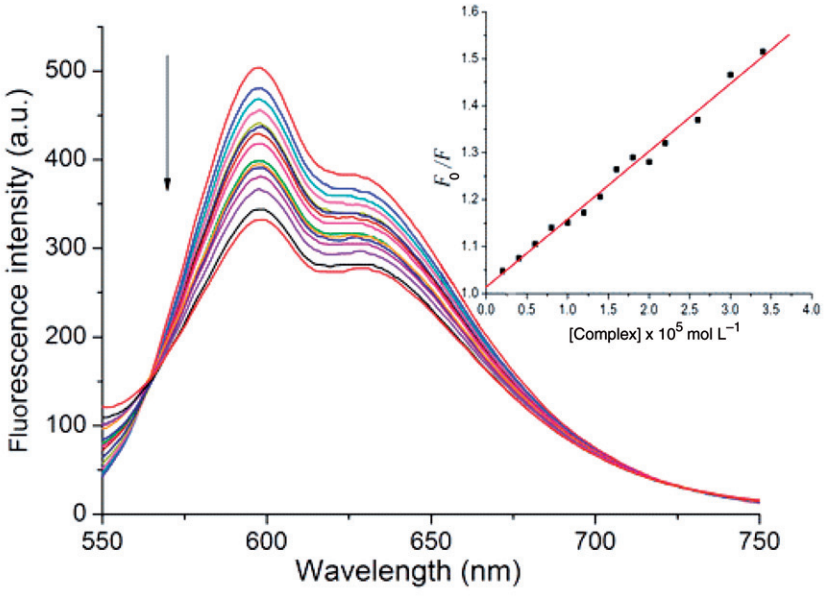

Figure 4. Fluorescence spectra of the binding of EB to DNA in the absence and presence of increasing amounts of the Cu(II) complex, $\lambda_{ex} = 520$ nm, [EB] = 8.8 μmol L^{-1}, [CT-DNA] = 10 μmol L^{-1}. [Cu(II) complex] = 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 26, 28, 32 μmol L^{-1}. Arrow shows the absorbance changes upon increasing the CT-DNA concentration. The Stern–Volmer quenching plot of F_0/F *vs.* [Complex] for the titration of EB bound to CT-DNA by the Cu(II) complex; ■, experimental data points; solid line, linear fitting of the data.

binding model. Therefore, viscosity measurements were carried out to further clarify the interaction with DNA. Hydrodynamic measurements that are sensitive to length change (i.e., viscosity and sedimentation) are regarded as the least ambiguous and most critical tests of a binding model in solution in the absence of crystallographic structural data [38, 39]. With increasing amounts of the complex, the viscosities of DNA increase steadily. The values of $(\eta/\eta_0)^{1/3}$ were plotted against [Complex]/[DNA] (figure 5). In classical intercalation, the DNA helix lengthens as base pairs are separated to accommodate the bound ligand leading to increased DNA viscosity whereas a partial, nonclassical ligand intercalation causes a bend (or kink) in DNA helix reducing its effective length and thereby its viscosity [39].

The effects of the Cu(II) complex on the viscosity of CT-DNA is shown in figure 5. The viscosity of CT-DNA increases steadily with the increment of the complex and further illustrates that the Cu(II) complex intercalates with CT-DNA. The viscosity experiments confirm the mode of the complex intercalation into DNA base pairs already established through absorption and fluorescence spectral titration studies.

3.4. *Antioxidant activities*

According to the literature [40], some transition metal complexes exhibit antioxidant activity. We therefore conducted an investigation to explore whether the Cu(II) complex has antioxidant activities.

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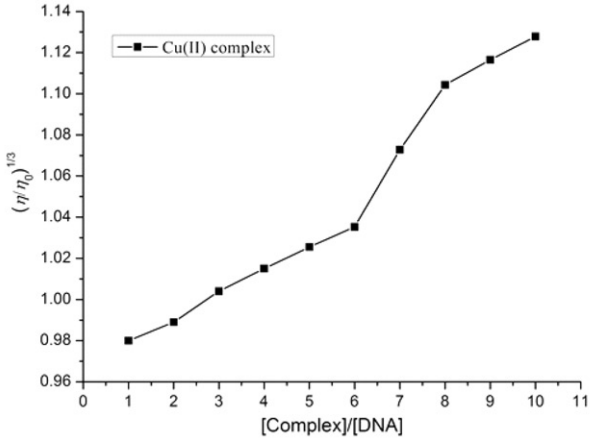

Figure 5. Effects of increasing amounts of $[Cu(bbp)_2]^{2+}$ (■) on the relative viscosities of CT-DNA at 25 ± 0.1°C; [DNA] = 50 μmol L^{-1}.

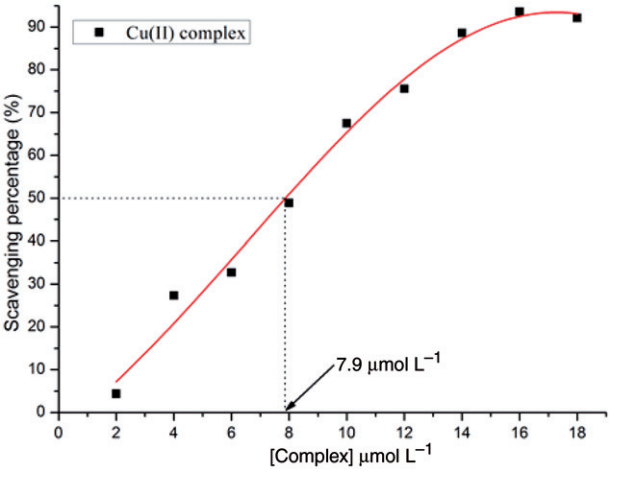

Figure 6. A plot of scavenging percentage (%) *vs.* concentration of the Cu(II) complex (■) on hydroxyl radical.

3.4.1. Hydroxyl radical scavenging activity. We compared the abilities of the copper complex to scavenge hydroxyl radicals with those of known natural antioxidants mannitol and vitamin C, using the same method as reported in [41]. The 50% inhibitory concentration (IC_{50}) of mannitol and vitamin C are about 9.6×10^{-3} and 8.7×10^{-3} $(\text{mol L}^{-1})^{-1}$, respectively. According to the antioxidant experiments, the IC_{50} value of Cu complex is 7.9×10^{-6} $(\text{mol L}^{-1})^{-1}$ (figure 6), implying that the Cu(II) complex exhibits scavenging activity. We suggest that the mechanism of action of Cu(II) complex involves redox of copper (Cu^{2+}/Cu^{+}) [42].

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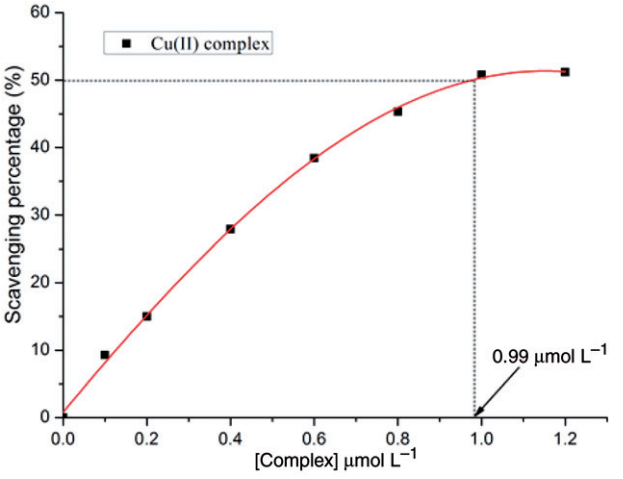

Figure 7. A plot of scavenging percentage (%) *vs.* concentration of the Cu(II) complex (■) on superoxide radical.

3.4.2. Superoxide radical scavenging activity. As another assay of antioxidant activity, superoxide radical ($O_2^{-\bullet}$) scavenging activity has been investigated. The Cu(II) complex has good superoxide radical scavenging activity.

The Cu(II) complex shows an IC_{50} value of 0.99 μmol L^{-1} (figure 7), which indicates that it has potent scavenging activity for superoxide radical ($O_2^{-\bullet}$). This indicates that the Cu(II) complex exhibits good superoxide radical scavenging activity and may be an inhibitor (or a drug) to scavenge superoxide radical ($O_2^{-\bullet}$) *in vivo* which needs further investigation.

4. Conclusions

In this work, a new Cu(II) complex with 2,6-*bis*(2-benzimidazolyl)pyridine has been synthesized and characterized. The crystal structure of $[Cu(bbp)_2](pic)_2 \cdot 2DMF$ is six-coordinate, adopting a distorted octahedral geometry. The DNA-binding suggests that the Cu(II) complex binds to DNA through intercalation, due to the large coplanar aromatic rings in the title complex that facilitate intercalation into base pairs of double helical DNA. The Cu(II) complex can be considered as a potential drug to eliminate hydroxyl radical ($OH^{\bullet}$) and an inhibitor (or a drug) to scavenge superoxide radical ($O_2^{-\bullet}$). These findings indicate that the Cu(II) complex has many potential applications for the development of nucleic acid molecular probes and new therapeutic reagents for diseases.

Supplementary material

Crystallographic data (excluding structure factors) for the structure in this article has been deposited with the Cambridge Crystallographic Data Center as supplementary

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publication CCDC 825917. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK.

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