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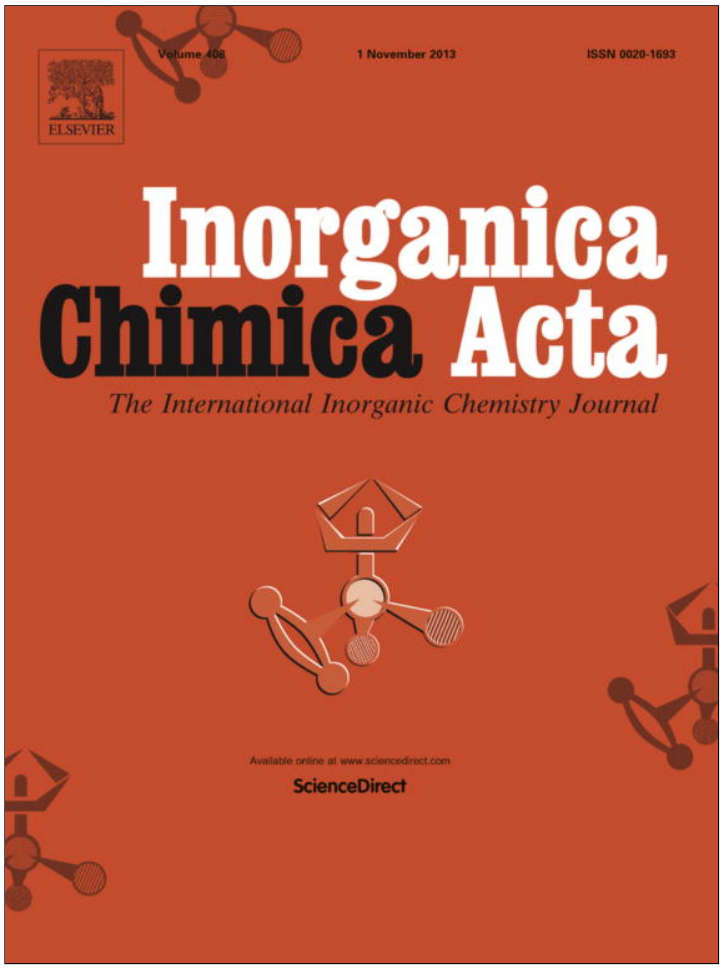

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Inorganica Chimica Acta 408 (2013) 46–52

Contents lists available at ScienceDirect

Inorganica Chimica Acta

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Synthesis, structure and urease inhibition studies of dimeric copper(II) complexes with a tridentate Schiff base ligand derived from tetrahydrofurfurylamine

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ARTICLE INFO

Article history:
Received 28 June 2013
Received in revised form 11 August 2013
Accepted 26 August 2013
Available online 3 September 2013

Keywords:
Copper(II) complexes
X-ray crystal structures
Molecular docking
Urease inhibitors
DFT calculations

ABSTRACT

Three new dimeric copper(II) complexes with general formula $Cu_2(L)_2X_2$, where L is a tridentate O,N,O-donor Schiff base ligand derived from tetrahydrofurfurylamine, have been synthesized and structurally characterized. X-ray single crystal studies reveal that complexes **1**, **2** and **3** possess a centrosymmetric dimeric core Cu_2X_2 (X = Cl, Br and SCN) and the copper(II) centers sit in slightly distorted square pyramidal coordination geometry (τ = 0.02–0.09). The urease inhibitory activities of the obtained copper(II) complexes have been tested *in vitro* against jack bean urease, and the results show that these copper(II) complexes exhibit potent inhibitory activities with IC_{50} ranges of 7.20–11.00 μM. Molecular docking analysis using DOCK program has also been conducted to gain further understanding of the inhibitory activities.

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1. Introduction

Urease (E.C. 3.5.1.5; urea amidohydrolase) is a nickel-dependent metalloenzyme that catalyzes the hydrolysis of urea into ammonia and carbamate in algae, fungi, bacteria and various plants [1–5]. Urease catalyzed hydrolysis of urea processes 10^{14} times faster than the uncatalyzed hydrolysis reaction. The reaction catalyzed by urease results in an abrupt overall pH increase, which is responsible for the negative effects of urease activity to human and animal health and agriculture production. For example, urease has shown to be an important virulent determinants to cause infection stones, pyelonephritis, hepatic coma, and urinary catheter encrustation in human and animal [6,7]. Besides, urease can severely decrease the efficiency of urea fertilizers to cause the release of large amounts of ammonia and further induce plant damage by ammonia toxicity and pH increase in soil. In this context, urease inhibition studies have attracted increasing attention to tackle the negative effects [8–11]. Moreover, urease inhibitory studies are important in elucidating the catalytic mechanism of urease which is still unclear as has been extensively discussed in a recent article [12]. It is of note that aetohydroxamic acid as a type of organic compounds has now be clinically used in the treatment for urinary tract infection [13]. However, the efficiency of currently available inhibitors is low and the full potential of urease inhibition has not yet been explored [14]. Therefore, the capability to control the rate of the enzymatic urea hydrolysis using urease inhibitors is an important goal to pursue.

In our earlier studies, we have investigated the inhibition of jack bean urease by a series of Schiff base metal complexes and have studied their urease inhibitory potential [15–18]. An interesting observation is that the reported inhibitory activities are significantly influenced by ligand substituents, electronic configurations, the role of counterions, and the nature of metal center, whereas the full mechanism of the interaction is unclear yet. As a continuation of our work on Schiff base complexes as urease inhibitors, we report herein the jack bean urease inhibitory activity of three new dimeric copper(II) complexes of Schiff base ligand, namely $[Cu(L)(Cl)]_2$ (**1**), $[Cu(L)(Br)]_2$ (**2**) and $[Cu(L)(SCN)]_2$ (**3**). Ligand HL ($C_{16}H_{17}NO_2$) has been synthesized from the reaction of tetrahydrofurfurylamine with 2-hydroxy-1-naphthaldehyde (Scheme 1). A preliminary docking study is carried out using a DOCK program to gain further understanding of their inhibitory activities and DFT calculation is also performed to provide insight into the electronic structures of complexes **1**, **2** and **3**.

2. Experimental

2.1. Materials and methods

Urease (from jack beans, type III, activity 22 units/mg solid), HEPES (N-[2-hydroxy-ethyl] piperazine-N′-[2-ethanesulfonic

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http://dx.doi.org/10.1016/j.ica.2013.08.021

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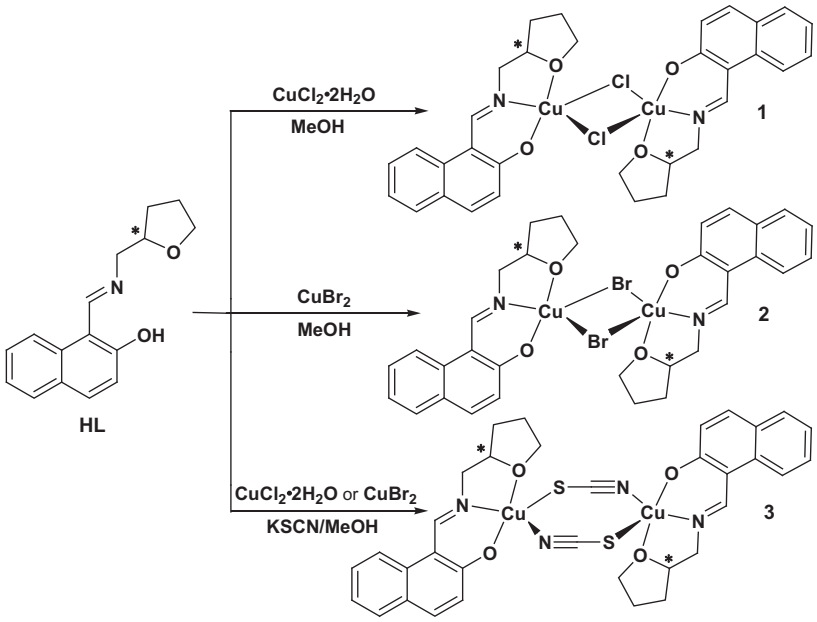

Scheme 1. General reaction scheme for the synthesis of dimeric copper(II) complexes.

acid]) buffer and urea (Molecular Biology Reagent) were purchased from Sigma. All other chemicals and solvents were purchased from Aldrich and used without further purification. Distilled water was used for all procedures. Elemental analyses (C, N, and H) were performed using an elementar vario EL *III* elemental analyzer. Infrared spectra were recorded using KBr pellets on a Nexus 870 FT-IR spectrophotometer in the 4000–400 cm^{-1} range. UV–Vis spectra were measured using DMSO–H_2O (1:1 v/v) solvents on a Shimadzu UV-160 A spectrophotometer in the 200–800 nm range. Mass spectrometry experiments were performed in the positive mode on mass spectrometer (amAzon SL, Bruker Daltonics, Bremen, Germany) equipped with an ESI source. The enzyme inhibitory activity data were obtained with a Bio-Tek Synergy™ HT microplate reader.

2.2. Preparation of dimeric copper(II) complexes *1*, *2* and *3*

2-Hydroxy-1-naphthaldehyde (0.34 g, 2 mmol) and tetrahydrofurfurylamine (0.20 g, 2 mmol) were dissolved in a methanol solution (35 mL) with stirring for 1 h to give an orange solution of Schiff base ligand HL. The reaction mixture was added to a methanol solution (10 mL) of $CuCl_2{\cdot}2H_2O$ (0.34 g, 2 mmol) or $CuBr_2$ (0.45 g, 2 mmol), respectively, and the brown mixture was stirred for another 10 min at room temperature before being filtered. The filtrate was kept in air for 7 days, yielding green block crystals of complexes **1** and **2**. The crystals were isolated, washed three times with distilled water and dried in a vacuum desiccator containing anhydrous $CaCl_2$.

2.2.1. $[Cu(L)Cl]_2$ (*1*)

Green solid, yield: 388 mg (55% based on Cu). *Anal.* Calc. for $C_{32}H_{32}Cl_2Cu_2N_2O_4$: C, 54.39; H, 4.56; N, 3.96. Found: C, 54.31; H, 4.62; N, 3.75%. IR (KBr, cm^{-1}): 3466, 3051, 2942, 2871, 1621, 1541, 1513, 1459, 1440, 1420, 1392, 1369, 1346, 1312, 1258, 1185, 1143, 1095, 1051, 1014, 986, 828, 873, 771, 747, 652, 580, 521, 474, 448, 424. UV–Vis [DMSO–H_2O (1:1 v/v), λ/nm (ε/M^{-1} cm^{-1})]: 252(24797), 306(9449), 316(9961), 382(6812), 396(6959). ESI-MS (positive ion mode, *m*/*z*): 316.8 for $[^1/_2M-Cl]^+$.

2.2.2. $[Cu(L)Br]_2$ (*2*)

Green solid, yield: 461 mg (58% based on Cu). *Anal.* Calc. for $C_{32}H_{32}Br_2Cu_2N_2O_4$: C, 48.31; H, 4.05; N, 3.52. Found: C, 48.25; H, 4.00; N, 3.42%. IR (KBr, cm^{-1}): 3453, 3049, 2941, 2871, 1621, 1540, 1513, 1458, 1438, 1419, 1391, 1367, 1344, 1310, 1257, 1185, 1142, 1094, 1050, 1013, 985, 915, 874, 827, 747, 652, 579, 520, 476, 446, 424. UV–Vis [DMSO–H_2O (1:1 v/v), λ/nm (ε/M^{-1} cm^{-1})]: 252(25443), 306(9987), 316(10490), 382(7106), 396(7270). ESI-MS (positive ion mode, *m*/*z*): 316.8 for $[^1/_2M-Br]^+$.

To a methanol solution (35 mL) of Schiff base ligand HL (2 mmol), was added a solution of $CuCl_2{\cdot}2H_2O$ (0.34 g, 2 mmol) and potassium thiocyanate (KSCN) (2 mmol) in methanol (10 mL) or a solution of $CuBr_2$ (0.45 g, 2 mmol) and KSCN (2 mmol) in methanol (10 mL), respectively, and the mixture was filtered upon 10 min stirring at room temperature. The filtrate was kept in air for 7 days, yielding black block crystals of complex **3**. The crystals were isolated, washed three times with distilled water and dried in a vacuum desiccator containing anhydrous $CaCl_2$.

2.2.3. $[Cu(L)(SCN)]_2$ (*3*)

Black solid, yield: 345 mg (46% based on Cu). *Anal.* Calc. for $C_{34}H_{32}Cu_2N_4O_4S_2$: C, 54.31; H, 4.28; N, 7.45. Found: C, 54.22; H, 4.20; N, 7.41%. IR (KBr, cm^{-1}): 3437, 3066, 2939, 2094, 1620, 1539, 1505, 1453, 1435, 1413, 1394, 1360, 1306, 1250, 1183, 1138, 1093, 1050, 984, 864, 836, 775, 748, 654, 581, 520, 467, 446, 420. UV–Vis [DMSO–H_2O (1:1 v/v), λ/nm (ε/M^{-1} cm^{-1})]: 252(24285), 306(9335), 316(9754), 382(6426), 396(6598). ESI-MS (positive ion mode, *m*/*z*): 316.9 for $[^1/_2M-(SCN)]^+$ and 692.0 for $[M-(SCN)]^+$.

2.3. Crystal structure determinations

X-ray crystallographic data were collected on a Bruker SMART Apex II CCD diffractometer using graphite-monochromated Mo Kα (λ = 0.71073 Å) radiation [19,20]. The collected data were reduced using the SAINT program, and empirical absorption corrections were performed using the SADABS program. The structures were solved by direct methods and refined against F^2 by full-matrix least-squares methods using the SHELXTL version 6.1. All of the non-hydrogen atoms were refined anisotropically. All other hydro-

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gen atoms were placed in geometrically ideal positions and constrained to ride on their parent atoms. The crystallographic data for dimeric copper(II) complexes **1**, **2** and **3** are summarized in Table 1. Selected bond lengths and angles are given in Table 2.

2.4. DFT calculations

Spin density and electrostatic potential for the complexes were calculated in methanol phase with a single point calculation at a density functional theory (DFT) level using the B3LYP exchange–correlation functional. Considering both the calculation cost and the accuracy, the LACVP** basis set was used, which corresponds to the "double-ξ" basis set with polarization functions 6-31G(d,p) [21] for N, O, Cl, Br and S, whereas for Cu the pseudo potential LanL2DZ [22] was used. The atomic coordinates were obtained from the X-ray structures. All calculations were done with the GAMESS suite of codes [23].

2.5. Measurement of jack bean urease inhibitory activity

The measurement of urease activity was carried out according to the literature method reported by Tanaka [24]. Generally, the assay mixture, containing 25 μL of jack bean urease (12 kU/L) and 25 μL of the test complexes with a concentration range of 1–50 μM (dissolved in DMSO:H_2O = 1:1 v/v), was pre-incubated for 1 h at 37 °C in a 96-well assay plate. After pre-incubation, 200 μL of 100 mM HEPES buffer [25] (pH 6.8) containing 500 mM urea and 0.002% phenol red was added and incubated at 37 °C. The reaction was measured with a micro plate reader (570 nm), where increase of the pH value from 6.8 to 7.7 for the HEPES buffer is determined by the color change of the Phenol Red indicator [26].

2.6. Docking simulations

Molecular docking of the inhibitor with the three-dimensional structure of jack bean urease (entry 3LA4 in the Protein Data Bank) was carried out using the DOCK 4.2 program suite [27–29]. The docking procedure for copper(II) complexes **1**, **2** and **3** with the enzyme active site of jack bean urease was performed as described previously by our group [15–18]. The graphical user interface AutoDockTools (ADT 1.4.5) was performed to setup an interaction of the inhibitor with enzyme, where all hydrogen atoms were added. Gasteiger charges were calculated and nonpolar hydrogen atoms were merged to carbon atoms. The Ni initial parameters were set as r = 1.170 Å, q = +2.0, and van der Waals well depth of 0.100 kcal/mol [30]. As performed by the graphical user interface AutoDockTools, the catalytic center and the peripheral anionic site of the target protein were scanned to evaluate the modeled binding mode of the inhibitor–urease complex. The flexible docking of the ligand structures was done by the Lamarckian genetic algorithm (LGA), searching for favorable bonding conformations of the ligands at the sites of the target protein.

Table 1
Crystal data for dimeric copper(II) complexes **1**, **2** and **3**.

Complex	**1**	**2**	**3**
Empirical formula	$C_{32}H_{32}Cl_2Cu_2N_2O_4$	$C_{32}H_{32}Br_2Cu_2N_2O_4$	$C_{34}H_{32}Cu_2N_4O_4S_2$
Molecular weight	706.58	795.50	751.84
Crystal system	monoclinic	monoclinic	triclinic
Space group	$P2_1/c$	$P2_1/c$	$P\bar{1}$
a (Å)	8.2004(8)	8.4436(11)	7.3872(6)
b (Å)	25.154(2)	25.326(3)	8.9620(8)
c (Å)	7.7887(7)	7.9660(11)	12.2167(10)
α (°)	90.00	90.00	82.2990(10)
β (°)	115.4950(10)	116.080(2)	86.1870(10)
γ (°)	90.00	90.00	73.3890(10)
T (K)	291(2)	291(2)	291(2)
V (Å^3)	1450.2(2)	1530.1(4)	767.71(11)
Z	2	2	2
$\rho_{calc.}$ (g cm^{-3})	1.618	1.727	1.626
F(000)	724	796	386
μ (Mo K$_\alpha$) (mm^{-1})	1.693	4.041	1.569
Data/restraints/parameters	2846/0/166	2968/0/190	2968/0/208
Goodness-of-fit (GOF) on F^2	1.018	1.020	1.004
Final R_1, wR_2 [$I > 2\sigma(I)$]	0.0531, 0.1163	0.0448, 0.1455	0.0497, 0.1104

Table 2
Selected bond lengths [Å] and angles [°] for dimeric copper(II) complexes **1**, **2** and **3**.

1 (i: 1 − x, 1 − y, 2 − z)			
Cu1–O1	1.891(3)	Cu1–Cl1	2.275(3)
Cu1–O2	2.031(3)	Cu1–Cl1^i	2.790(3)
Cu1–N1	1.946(3)	Cu1–Cu1^i	3.644(3)
O1–Cu1–N1	91.97(12)	O1–Cu1–Cl1	94.24(8)
O1–Cu1–O2	168.89(12)	O2–Cu1–Cl1	92.0(7)
N1–Cu1–O2	80.23(12)	Cl1–Cu1–Cl^i	88.6(3)
2 (i: 1 − x, 2 − y, −z)			
Cu1–O1	1.887(4)	Cu1–Br1	2.410(8)
Cu1–O2	2.026(3)	Cu1–Br1^i	2.942(3)
Cu1–N1	1.942(4)	Cu1–Cu1^i	3.764(3)
O1–Cu1–N1	92.20(15)	O1–Cu1–Br1	94.0(3)
O1–Cu1–O2	171.01(15)	O2–Cu1–Br1	91.84(9)
N1–Cu1–O2	80.58(14)	Br1–Cu1–Br1^i	91.2(3)
3			
Cu1–O1	1.899(3)	Cu1–N1	1.925(3)
Cu1–O2	2.051(3)	Cu1–N2	1.944(4)
O1–Cu1–N1	92.84(13)	N1–Cu1–O2	81.09(13)
O1–Cu1–O2	170.98(13)	N1–Cu1–N2	169.78(16)
O1–Cu1–N2	94.95(14)	N2–Cu1–O2	90.38(14)

3. Results and discussion

3.1. IR and UV–Vis analysis

Infra-red and UV–Vis spectroscopic studies have been performed for the obtained complexes (Figs. S2 and S3, Supporting information). The proposed assignments in the infrared spectra for dimeric copper(II) complexes **1**, **2** and **3** are based on the results reported in literature [31–33]. The strong absorption at *ca.* 1621 cm^{-1} of the IR spectra for complexes **1**, **2** and **3** is assigned to the stretching vibration of the C=N groups, while the medium intensity bands in the region of 581–420 cm^{-1} come from the stretching of ν(Cu–N) and ν(Cu–O) in complexes **1**, **2** and **3**, respectively. Significantly, the IR spectrum of **3** shows a strong absorption at 2094 cm^{-1} attributed to the stretching vibration of the thiocyanato group. The electronic spectra for complexes **1**, **2** and **3** were obtained under the assay conditions (DMSO/H_2O, 1:1 v/v). Complex **1**, **2** or **3** exhibits an intense higher-energy band at 252 nm representing an intra ligand charge transfer ($\pi \rightarrow \pi^*$). The formation of complexes **1**, **2** or **3** in DMSO–H_2O mixture is supported by the characteristic charge-transfer bands at 306 and 316 nm, and also by the evolution of a shoulder in the near UV region centered at 382 and 396 nm, originated from a charge transfer [34]. However, the bands associated with $d \rightarrow d$ transitions were not detected in the electronic spectra for complexes **1**, **2** and **3**, probably due to the intensity of the charge transfer and intraligand transitions [35].

3.2. Crystal structure description

Three new dimeric copper(II) complexes with general formula $[Cu(L)X]_2$, where L acts as a tridentate Schiff base ligand with

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O,N,O-donor sets and X is Cl (**1**), Br (**2**) and SCN (**3**), were obtained from the reaction of equivalent amount of Schiff base ligand HL with copper(II) source. The X-ray crystal structures of complexes **1**, **2** and **3** are shown in Figs. 1, S1 and 2. Selected bond distances and bond angles for the structures of complexes **1**, **2** and **3** are listed in Table 2. X-ray diffraction reveals that complexes **1** and **2** crystallize in a monoclinic system with a space group $P2_1/c$ (No. 14), while complex **3** crystallize in a triclinic system with a space group $P\bar{1}$ (No. 2). All three complexes possess centrosymmetric Cu_2X_2 dimeric units (X = Cl, Br and SCN).

Complexes **1** and **2** have two five-coordinated $Cu(II)O_2NX_2$ centers, with Cu(II) coordinated to O,N,O-donor sets from a tridentate Schiff base ligand L and the remaining coordination sites are occupied by two bridging halogen X atoms (X = Cl and Br in **1** and **2**, respectively). Due to the presence of an inversion center in complexes **1** and **2**, the halo-bridged dimeric Cu_2X_2 units are strictly planar (see Figs. 1 and S1). The Cu···Cu distances in **1** and **2** are 3.644(3) and 3.764(3) Å, respectively, which are comparable to those in analogous compounds [36,37]. The coordination geometry around each copper center in complexes **1** and **2** can be described as slightly distorted square pyramidal, as exemplified by its corresponding τ values (τ = 0.02 in **1** and 0.09 in **2**). In complex **1**, the apical Cu–Cl bond length is 0.51 Å, longer than the basal one (Table 2), and the Cu–Cl–Cu^i bridging angle is 91.4(3)° (^{i}symmetry code: $1-x, 1-y, 2-z$). These two characteristic numbers are practically identical with those for the chloro-bridged dimeric copper(II) complexes [38,39]. However, the Cu–Br apical bond in **2** is 0.53 Å, longer than the basal one and the Cu–Br–Cu^i bridging angle is 88.8(3)° (^{i}symmetry code: $1-x, 2-y, -z$). Interestingly, the discrete dimeric molecule of complexes **1** and **2** is further linked by two intramolecular C16–H···$O1^{\#}$ hydrogen bonds between a tetrahydrofurfuryl group and an adjacent phenolato oxygen atom as depicted in Figs. 1 and S1. The C16···$O1^{\#}$ distances and C16–H···$O1^{\#}$ angles in **1** are 3.221(5) Å and 151.0(5)°, respectively, while 3.353(6) Å and 154.0(6)°, respectively in **2**, where # refers to the different L ligand. Besides, the dimeric molecules of **1** were further linked into a one-dimensional (1D) ladder chain by the intermolecular C12–H···$O1^{\#}$ hydrogen bonds between a CH_2 group bound to the imino moiety of L ligand and an adjacent phenolato oxygen atom (C12···$O1^{\#}$ = 3.532(5) Å), as depicted in Fig. 3.

In contrast to **1** and **2**, complex **3** shows a thiocyanato–bridged dinuclear copper(II) center with the Cu···Cu distance of ca. 5.7 Å (Fig. 4). In the centrosymmetric dimeric units of **3**, copper atom is five-coordinated in the form of a slightly distorted square pyramidal geometry (τ = 0.02), with the basal atoms being O1, N1 and O2 from the tridentate Schiff base ligand L and N2 from the bridging thiocyanato ligand. The apical $S1^i$ atom from the other centrosymmetrically related thiocyanato bridge is used to complete the

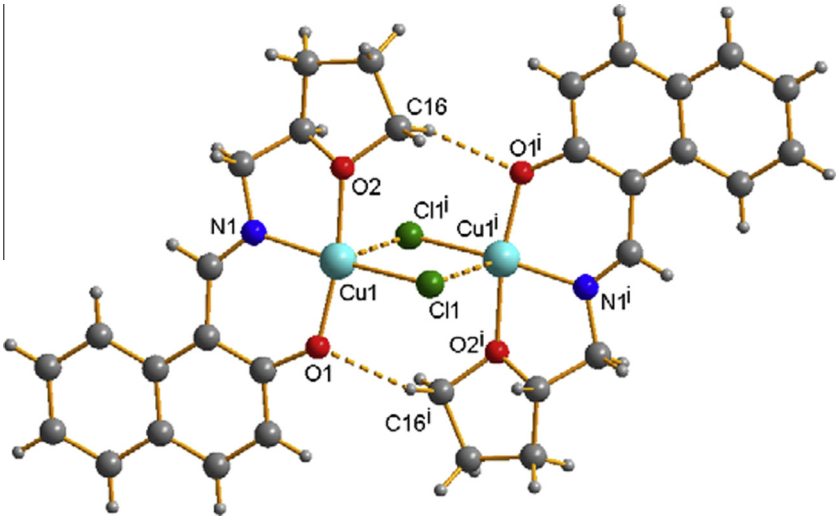

Fig. 1. *Ball-and-stick* representation of molecular structure of **1** (symmetry code: $^i1-x, 1-y, 2-z$), atoms are shown as sphere of arbitrary diameter.

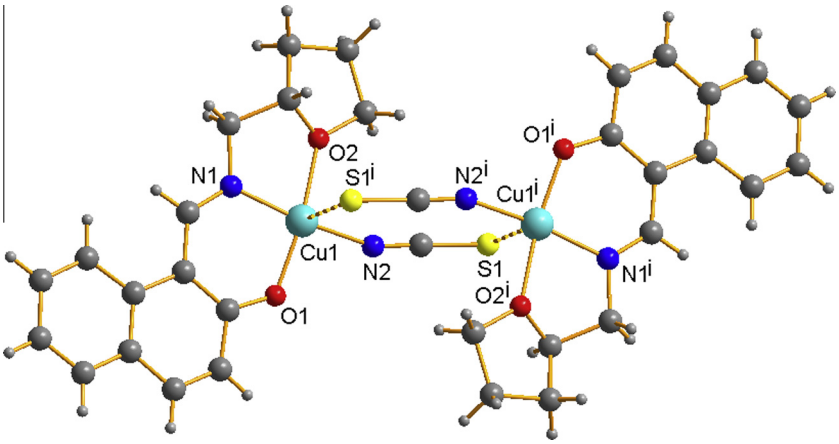

Fig. 2. *Ball-and-stick* representation of molecular structure of **3** (symmetry code: $^i1-x, 1-y, 2-z$), atoms are shown as sphere of arbitrary diameter.

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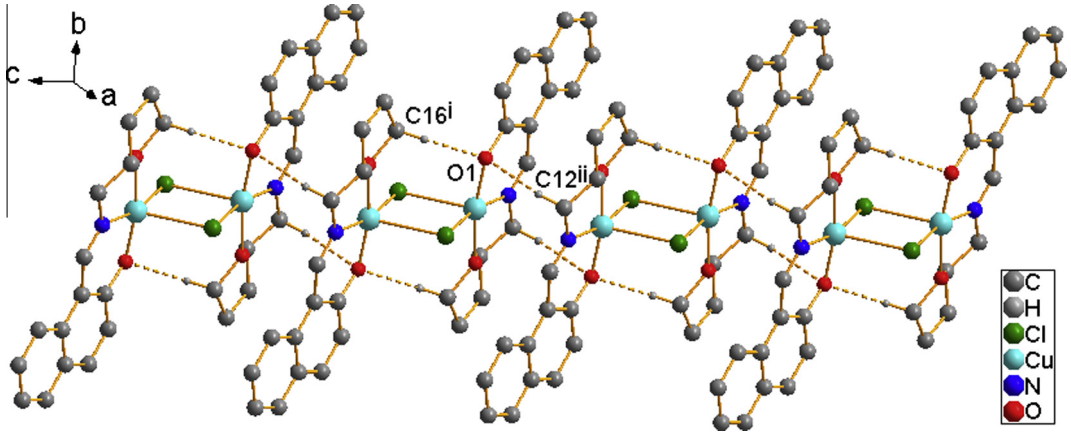

Fig. 3. A 1D chain structure of **1**, formed by intermolecular C–H···O hydrogen bonds (symmetry codes: $^{i}1-x, 1-y, 2-z$; $^{ii}1-x, 1-y, 1-z$).

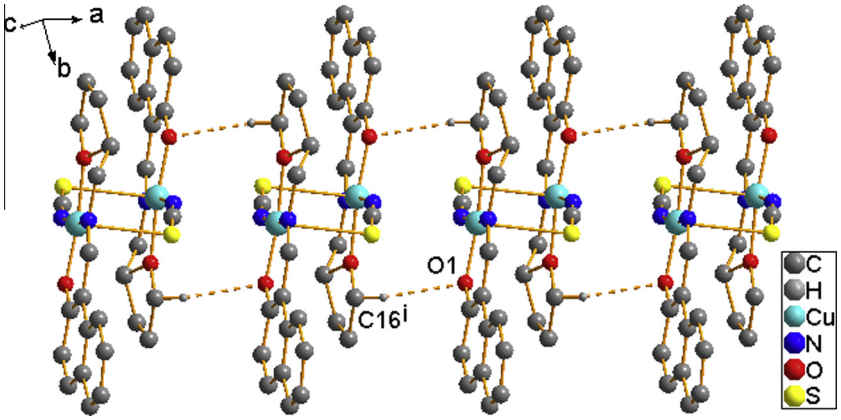

Fig. 4. A 1D chain structure of **3**, formed by intermolecular C–H···O hydrogen bonds (symmetry code: $^{i}-x, 1-y, 2-z$).

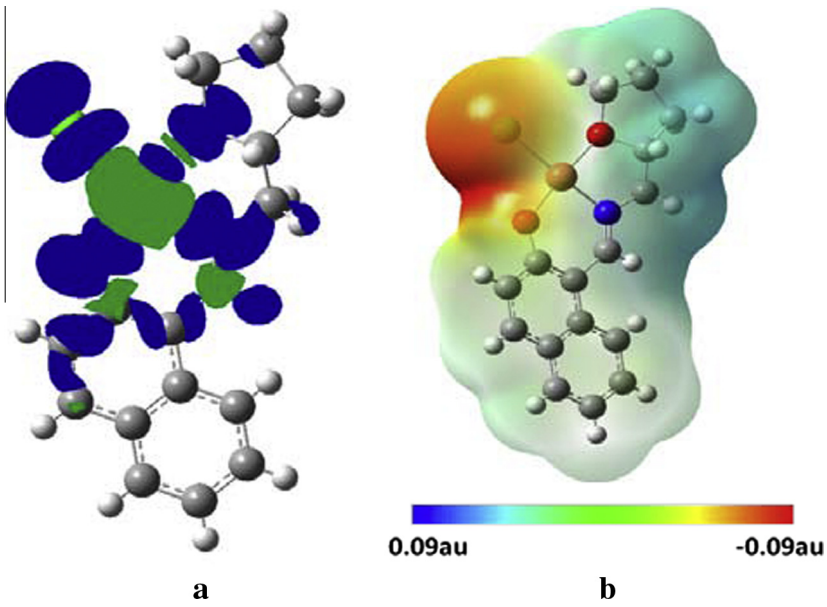

Fig. 5. (a) B3LYP/LACVP** surface of spin density for **1**; (b) Electrostatic potential surface for **1** (the electron density isosurface is 0.0004 electrons/au^3).

coordination [Cu1–S1^{i} 2.983(2) Å] (^{i}symmetry code: $1-x, 1-y, 2-z$), resulting the formation of eight-membered rings (Cu(μ-SCN)$_2$Cu) in complex **3**. It should be noted that only a few examples of the double thiocyanato-bridged dimers with five-coordinate copper(II) centers have been reported [33]. The solid state structure of **3** shows that the dimeric molecules are connected into a

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Table 3
Uncorrected and CP-corrected interaction energies of complexes **1**, **2** and **3** (ΔE/kJ mol^{-1}).

complex	ΔE_{tot}	BSSE	ΔE_{CP}
1	−59.21	8.92	−50.27
2	−43.05	6.03	−37.02
3	−48.62	7.87	−40.75

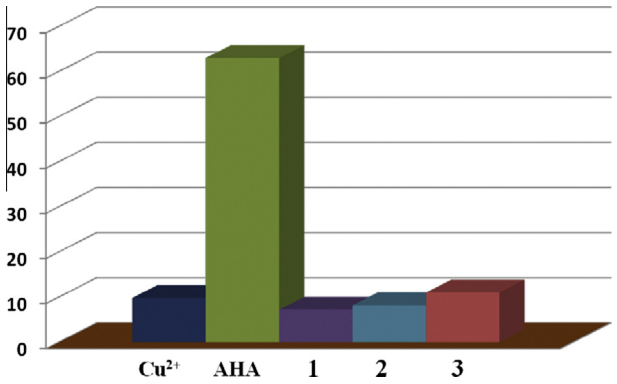

Fig. 6. Histogram of IC_{50} values for complexes **1**, **2** and **3**.

one-dimensional (1D) ladder chain by the intermolecular C16–H···O1$^{\#}$ hydrogen bonds between a tetrahydrofurfuryl group and an adjacent phenolato oxygen atom (C16···O1$^{\#}$ = 3.460(5) Å), as depicted in Fig. 4.

3.3. Theoretical calculations

The atomic charge calculations give a description of the location of the electron density of dimeric copper(II) complex **1** as depicted in Fig. 5. The calculated spin density indicates that the unpaired electrons are mainly localized on the $CuClNO_2$ moiety in **1** (Fig. 5a). The presence of spin density on the first coordination sphere of copper atom (NO_2Cl) is owing to a delocalization process. The plots of the electrostatic potentials for complex **1** are shown in Fig. 5b, where the blue zone and the red zone represent the poorest electron density region and the richest one, respectively. The red zone encompasses phenolate oxygen atoms and chlorine atoms of the $CuClNO_2$ moiety in **1**. Therefore the surfaces of electrostatic potential and spin density suggest that if any interactions exist between two monomeric copper(II) molecules of **1**, this would occur through the planar $CuClNO_2$. Herein the electronic structure of complex **1**, presented in particular by density of states diagrams, has been correlated with its ability to inhibit jack bean urease. Besides, the binding energies of dimeric copper(II) complexes **1**, **2** and **3** are calculated to evaluate their stability (Table 3). Interaction energies were corrected for the basis set superposition error (BSSE) in all the complexes as follows: **1** > **3** > **2**. Such bonding behavior has also been observed in analogous dimeric copper(II) complexes reported in literature [40].

3.4. Inhibitory bioactivity against urease

The activity to inhibit jack bean urease of the three new dimeric copper(II) complexes of Schiff base ligand derived from tetrahydrofurfurylamine was screened in this work (Fig. 6). Compared with acetohydroxamic acid (AHA) co-assayed as a standard urease inhibitor agent (IC_{50} = 63.00 μM), the dimeric copper(II) species **1**, **2** and **3** display good inhibitory activity with the respective IC_{50} values of 7.20, 8.20 and 11.00 μM. Complex **1** exhibits slightly higher inhibitory activity against jack bean urease than **2** probably as a result of the difference on electron withdrawing of the halogen ligands. In contrast, complex **3** shows weak urease inhibitory activity due to the steric hindrance of the thiocyanato ligand. Controlled experiments show that the Schiff base ligand HL is inactive against jack bean urease (IC_{50} > 100 μM), however, free Cu^{2+} shows a strong urease inhibitory activity with the IC_{50} value of 10.85 μM. Similar to the inhibition by other heavy metal ions [41–43], the urease inhibitory activity of copper(II) ion is probably due to its interactions with the urease active site [44,45].

3.5. Molecular docking study

The binding models of the monomeric species of complexes **1**, **2** and **3** with jack bean urease have been simulated using a Dock program to study the structure–activity relationship [27–29]. Only monomeric complexes **1**, **2** and **3** are considered herein in order to get the possibly identical manner as their real conformation in biofluid solutions. The analogous chemistry has also been described for those of the dimeric copper(II) complexes, presenting the monomer–dimer conversions [46,47]. The results reveal that the complex molecules are well put into the active pocket of jack bean urease (Fig. 7). Additional interactions have been established in a variety of conformations because of the flexibility of the tridentate O,N,O-donor Schiff base ligand L and the amino acid residues of jack bean urease. The optimized cluster (20 occurrences) has been ranked by energy level in the best conformation of inhibitor–urease modeled structures. The binding energies of the amino acid residues with the corresponding copper(II) species **1**, **2** and **3** exhibit −5.83, −5.93 and −5.75 kcal/mol, respectively, while their lowest intermolecular energies is −6.38, −6.48 and −6.57 kcal/mol, respectively. Besides, some hydrophobic interactions have also been found in the corresponding inhibitor–urease complex.

As depicted in Fig. 7a and b, complexes **1** and **2** can form two hydrogen bonds with the amino-acid residues His593 and His492. The hydrogen-bonding distances of $NH_{His593}\cdots N1_{complex}$ and $NH_{His492}\cdots O2_{complex}$ are 3.297(2) and 2.719(2) Å for **1** and

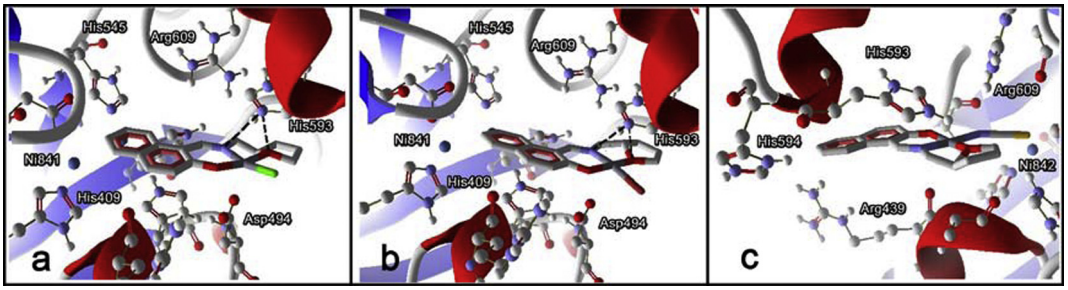

Fig. 7. Modeled structures of urease–inhibitor complexes for **1**, **2** and **3**. Hydrogen bonds are presented as dark dotted lines.

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3.187(2) and 2.971(2) Å for **2**, respectively, while the hydrogen-bonding angles of $NH_{His593}\cdots N1_{complex}$ and $NH_{His492}\cdots O2_{complex}$ are 145.9(2)° and 118.4(2)° for **1** and 153.6(2)° and 123.9(2)° for **2**, respectively. In contrast, complex **3** forms no hydrogen bonds with the amino-acid residues (Fig. 7c). Additionally, the nickel to copper distances between the two nickel atoms of the urease active site and the respective copper atoms of the obtained copper(II) complexes have been calculated as 9.44 and 7.95 Å for **1**, 9.82 and 9.13 Å for **2** and 6.07 and 6.10 Å for **3**, respectively. The results further demonstrate the difference of their urease inhibitory activities.

4. Conclusions

The present paper has reported the synthesis, crystal structures and urease inhibition properties of three dimeric copper(II) complexes containing a tridentate O,N,O-donor Schiff base ligand derived from tetrahydrofurfurylamine. The dimeric molecules of complexes **1**, **2** and **3** present two five-coordinated copper(II) centers, each of which exists in a square pyramidal geometry. The obtained copper(II) complexes **1**, **2** and **3** are potent urease inhibitors with activities comparable to the other copper(II) complexes of Schiff bases reported in our earlier work. DFT studies provide insight into their electronic structures and docking studies have been performed to gain further understanding of their inhibitory activities. Detailed investigations are continuing in our lab to study the mechanisms of urease inhibitory activity reported herein.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (grants 21101122 and 21277106) and China Postdoctoral Science Foundation (grant 20100481108).

Appendix A. Supplementary material

CCDC 889142–889144; contains the supplementary crystallographic data for dimeric copper(II) complexes **1**, **2** and **3**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2013.08.021.

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