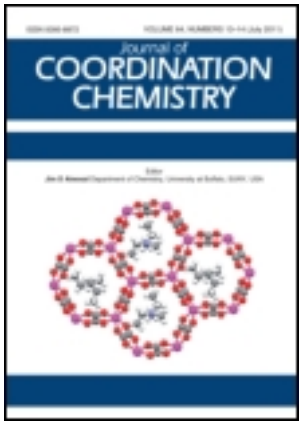

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Synthesis, urease inhibitory activities, and molecular docking studies of two Cu(II) complexes

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Two mononuclear copper(II) complexes, $[Cu(C_4H_3N_2O_2)_2 \cdot 4H_2O]$ (**1**) and $[Cu(C_{12}H_{11}N_2O_2Cl_2)_2]$ (**2**), were synthesized and structurally characterized by single-crystal X-ray analysis. The copper(II) adopts a square-planar environment in **1**, while the geometry in **2** can be described as distorted square-pyramidal. Complexes **1** and **2** were evaluated for their inhibitory activities against jack bean urease *in vitro* and both were found to have strong inhibitory activities comparable to that of acetohydroxamic acid. A docking simulation was performed to position **2** into the jack bean urease active site to determine the probable binding conformation.

Keywords: Cu(II) complexes; Molecular docking; Urease; Inhibitor; X-ray structure

1. Introduction

Urease (urea amidohydrolase; EC 3.5.1.5) is a nickel-dependent metalloenzyme that rapidly catalyzes hydrolysis of urea to form ammonia and carbamate [1–3]. Many microorganisms utilize urea as a source of nitrogen for augmentation. Urease plays an important role in nitrogen metabolism of plants during the germination process [4, 5]. The reaction catalyzed by urease may cause a pH increase and an accumulation of ammonia, which has important implications in medicine and agriculture. Some urease inhibitors have been reported such as hydroxamic acid derivatives, phosphorodiamidates, and imidazoles [6], but some are prevented from use *in vivo* because of their toxicity or instability. Thus, it is interesting to seek new urease inhibitors with good bioavailability and low toxicity.

Copper is an important life-required element. Due to their biological relevant behavior, many copper complexes with Schiff base have been reported [7–9]. In addition, metal complexes with carboxylates are among the most investigated compounds in the

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field of coordination chemistry [10–12]. Recently we focused our research on preparation of metal complexes with potential urease inhibitory activity [13, 14]. In this article we synthesized two Cu(II) complexes, $[Cu(C_4H_3N_2O_2)_2 \cdot 4H_2O]$ (**1**) and $[Cu(C_{12}H_{11}N_2O_2Cl_2)_2]$ (**2**), and investigated their inhibitory activities against jack bean urease. Docking simulation using AUTODOCK was performed to position **2** into the active site of jack bean urease to determine the probable binding conformation [15].

2. Experimental

2.1. *Materials and methods*

4-Imidazolecarboxylic acid, 3,4-dichlorophenylacetic acid, and 2-methylimidazole were purchased from Aldrich and used without purification. Elemental analyses for C, H, and N were carried out on a Perkin-Elmer 2400 analyser. X-ray crystallography was carried out using a Bruker SMART APEX II CCD diffractometer. All chemicals and reagents used in this study were of analytical grade. Jack bean urease (from jack beans, type III, 20990 unit per gram solid) and acetohydroxamic acid (AHA) were purchased from Sigma Aldrich Co. (St. Louis, MO, USA).

2.2. *Synthesis of 1*

Complex **1** was synthesized according to our previous work and its structure had been reported [16].

2.3. *Synthesis of 2*

A methanol solution (10 mL) of $CuCl_2 \cdot 2H_2O$ (171 mg, 1 mmol) in H_2O (2 mL) was added to a solution of 3,4-dichlorophenylacetic acid (410.1 mg, 2.0 mmol) in methanol (2 mL), followed by a methanol solution (2 mL) of 2-methylimidazole (164.2 mg, 2 mmol). The mixture was stirred for another 15 min at room temperature. After 1 week, black crystals of **2** were formed. The crystals were filtered off and dried in a vacuum desiccator containing anhydrous $CaCl_2$. Yield: 83% (based on Cu). Anal. Calcd for $Cu(C_{12}H_{11}N_2O_2Cl_2)_2$: C, 45.34; H, 3.49; N, 8.81. Found (%): C, 45.77; H, 3.88; N, 8.69.

2.4. *Crystal structure determinations*

X-ray crystallographic data were collected on a Bruker SMART Apex II CCD diffractometer using graphite-monochromated Mo-Kα ($\lambda = 0.71073$ Å) radiation. The collected data were reduced using SAINT [17] and empirical absorption corrections were performed using SADABS [18]. The structures were solved by direct methods and refined against F^2 by full-matrix least-squares using SHELXTL version 5.1. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed in geometrically ideal positions and constrained to ride on their parent atoms.

The crystallographic data for the complexes are summarized in table 1. Selected bond lengths and angles are given in table 2.

2.5. *Measurement of inhibitory activity against jack bean urease*

Measurement of urease was carried out according to the literature report by Tanaka *et al.* [19]. Generally, the assay mixture, containing 25 μL of jack bean urease (100 mmol L^{-1} HEPES, pH 6.8) and 25 μL of the tested complexes of various concentrations (dissolved in DMSO : H_2O 1 : 1 (v/v)), was preincubated for 1 h at 37°C in a 96-well assay plate. After preincubation, 200 μL of 100 mmol L^{-1} HEPES buffer (pH 6.8) containing 500 mmol L^{-1} urea and 0.002% phenol red were added and incubated at 37°C [20]. The reaction time was measured by micro-plate reader (570 nm), which was required to produce enough ammonium carbonate to raise the pH of an

Table 1. Crystallographic data and experimental details for **2**.

Compound	**2**
Molecular weight	635.80
Crystal system	Monoclinic
Space group	*C2/c*
Unit cell dimensions (Å, °)	
a	12.9606(9)
b	9.3908(6)
c	21.5544(14)
α	90
β	91.5850(10)
γ	90
Volume (Å^3), *Z*	2622.4(3), 4
ρ_{Calcd} (g cm^{-3})	1.610
F(000)	1292
μ(Mo-Kα) (mm^{-1})	1.280
Crystal size (mm^3)	0.31 × 0.24 × 0.11
θ range for data collection (°)	2.68–28.25
Index range	$-15 \le h \le 15$; $-11 \le k \le 11$; $-26 \le l \le 26$
Type of scan	Multi-scan
Data/restraint/parameters	2574/0/170
R_{int}	0.0193
Goodness-of-fit on F^2	1.007
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0265$, $wR_2 = 0.0764$
$\Delta\rho_{max}$ and $\Delta\rho_{min}$ (e Å^{-3})	0.232 and −0.229

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

Cu(1)–N(1)	1.974(14)	Cu(1)–O(1)	1.987(12)
Cu(1)–N(1A)	1.974(14)	Cu(1)–O(1A)	1.987(12)
N(1)–Cu(1)–N(1A)	92.21(9)	N(1)–Cu(1)–O(1A)	90.61(6)
N(1)–Cu(1)–O(1)	173.08(5)	N(1A)–Cu(1)–O(1A)	173.08(5)
N(1A)–Cu(1)–O(1)	90.61(6)	O(1)–Cu(1)–O(1A)	87.30(8)

HEPES buffer from 6.8 to 7.7, the end-point being determined by the color of phenol red indicator [21].

2.6. *Docking simulations*

Molecular docking of the complex into the 3-D X-ray structure of jack bean urease structure (entry 3LA4 in the Protein Data Bank) was carried out using the AUTODOCK 4.0 software as implemented through the graphical user interface AutoDockTools (ADT 1.4.6) [22].

The graphical user interface AUTODOCKTOOLS was employed to setup the enzymes: all hydrogen atoms were added, Gasteiger charges were calculated, and non-polar hydrogen atoms were merged to carbons. The Ni initial parameters were set as $r = 1.170$ Å, $q = +2.0$, and van der Waals well depth of $0.100\,\text{kcal}\,\text{mol}^{-1}$ [23]. The 3-D structure of ligand molecules were saved in Mol2 format with the aid of the program MERCURY. The partial charges of Mol2 file were further modified by using the ADT package (version 1.4.6). The resulting files were saved as pdbqt files.

The AUTODOCKTOOLS program was used to generate the docking input files. In all docking a grid box size of $60 \times 60 \times 60$ points in x, y, and z directions was built, the maps were centered on Ni841 in the catalytic site of the protein. A grid spacing of 0.375 Å and a distances-dependent function of the dielectric constant were used for calculation of the energy map. Ten runs were generated by using Lamarckian genetic algorithm searches. Default settings were used with an initial population of 50 randomly placed individuals, a maximum number of 2.5×10^6 energy evaluations, and a maximum number of 2.7×10^4 generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen. The results of the most favorable free energy of binding were selected as the resultant complex structure.

3. Results and discussion

3.1. *Crystal structure description*

The crystal structure of diaquabis(imidazole-4-carboxylato)copper(II) dihydrate (**1**) has been studied and reported in our previous work [16]. In the crystal packing, the molecules are linked by weak intermolecular O(4)···O(1) [3.035 Å] and O(4)···O(2) [2.495 Å] interactions.

The structure of **2** is illustrated in figure 1. Copper(II) is in a distorted pyramid(tetrahedron) environment and is four-coordinate by two nitrogen atoms from two 2-methylimidazoles, and two oxygen atoms from two 3,4-dichlorophenylacetic acid ligands. The crystal structure of **2** drastically differs from that of **1**. Single crystal X-ray diffraction reveals that **2** crystallizes in the monoclinic space group $C2/c$ and has a tetranuclear structure with an inversion center. Copper is located 0.106(3) Å above the calculated N(1)–O(1)–N(1A) mean plane. The torsion angle N(1)–O(1)–N(1A)–O(1A) is 167.108°. This is due to a short contact Cu–O(1) [1.987(12) Å] between Cu(II) and O(1) of carboxylic acid residues of neighboring molecules (figure 1). The bond distance of Cu–N is 1.974(14) Å, and slightly shorter than the corresponding values of 1.999(3) Å

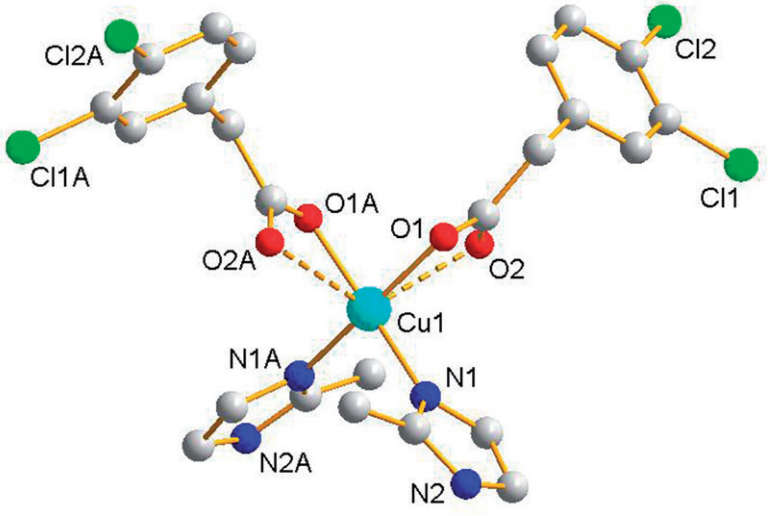

Figure 1. An ORTEP diagram showing molecular structure of **2**.; symmetry code A: $-x$, y, $1/2-z$.

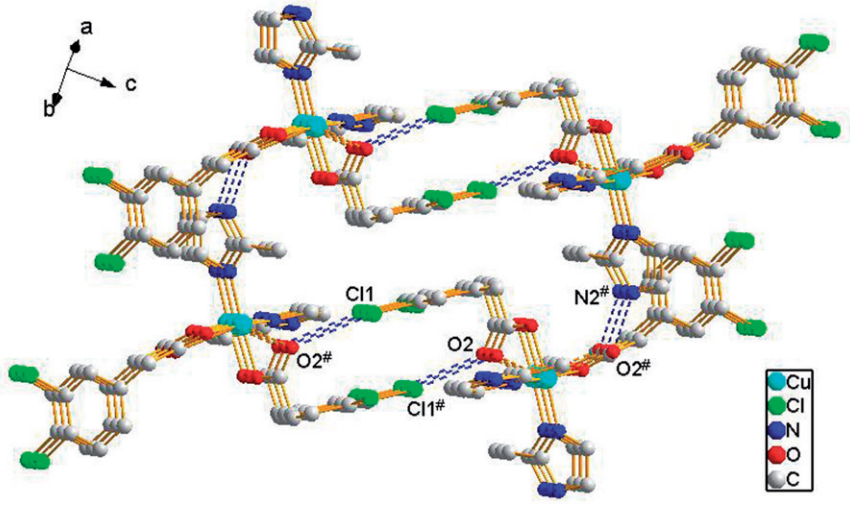

Figure 2. Molecular packing of **2** constructed through hydrogen bonds. All hydrogen atoms are omitted for clarity.

in **1**. The angles subtended at Cu(II) of the distorted-tetrahedral geometry (CuN_2O_2) are 90.61(6) and 173.08(5)°.

In the crystal packing, all mononuclear units are further linked by weak intermolecular N(2)–H(2A)···O(2) [2.786 Å] and Cl···O(2) [3.189 Å] hydrogen-bond interactions, as shown in figure 2. Complex **2** possesses a 3-D layered network with polygons formed by intrachain conformation of hydrogen bonds, the polygon units form a 14-membered ring. The ring is occupied by 10 carbons, one oxygen, and one

chloride from two 3,4-dichlorophenylacetic acid groups, respectively. Thus, the crystal structure of **2** is stabilized by moderate to weak hydrogen-bonding networks.

3.2. *Inhibitory bioactivity against urease*

The abilities of the ligands, Cu^{2+} and complexes in inhibiting urease were studied by the IC_{50} values of the material tested against jack bean urease. From table 3, the ligands as enzyme inhibitors had no ability to inhibit urease ($IC_{50} > 100\,mol\,L^{-1}$). Under the same conditions, both Cu(II) complexes had much stronger urease inhibitory activities with IC_{50} values of $4.76\,\mu mol\,L^{-1}$ for **1** and $2.42\,\mu mol\,L^{-1}$ for **2**, compared with that of the standard inhibitor AHA, as shown in table 3. The two copper(II) complexes exhibit stronger ability to inhibit urease, probably due to the strong Lewis acid properties of copper ions. This showed that inhibitory efficiency of the complex toward urease may be influenced not only by the transition metal ion but also by ligands. Complex **2** exhibits stronger ability to inhibit urease than **1** probably because of copper being in square-pyramidal coordination and chloride. The Cu(II) showed weaker inhibitory activity against urease than the complexes because the ligands strengthened inhibitory activity of the complexes. The results agree with the data of the AHA reported previously [24], but the IC_{50} of Cu(II) is higher than that of our previous report [25] and the literature [26], caused by the different urease.

3.3. *Molecular docking study*

In the X-ray structure available for the native jack bean urease (entry 3LA4 in the Protein Data Bank), the two nickels were coordinated by His136, His138, Kcx219, His248, His274, Asp362, and water [27], while in AHA-inhibited jack bean urease (entry 3LA4 in the Protein Data Bank), these water molecules were replaced by AHA [28]. In order to give an explanation of the good activity observed, molecular docking of **2** into the active site of jack bean urease was performed on the binding model based on the jack bean urease structure (3LA4.pdb). The binding model of **2** with urease is depicted in figure 3 and the enzyme surface model is shown in figure 4. All amino acid residues which had interactions with urease are shown. In comparison to Ni complex-*Helicobacter pylori* urease complex previously reported [25], in the binding model of the complex with urease, one hydrogen bond was formed. The amino hydrogen of **2** formed hydrogen bond with oxygen of Gln635, as shown in figure 4 (length of the hydrogen

Table 3. Inhibition of jack bean urease by **1**, **2**, ligands, and Cu(II).

Tested materials	IC_{50} ($mol\,L^{-1}$)
4-imidazolecarboxylic acid	>100
3,4-dichlorophenylacetic acid	>100
2-methylimidazole	>100
Cu^{2+}	8.62 ± 0.23
Complex **1**	4.76 ± 0.21
Complex **2**	2.42 ± 0.13
AHA	62.52 ± 0.16

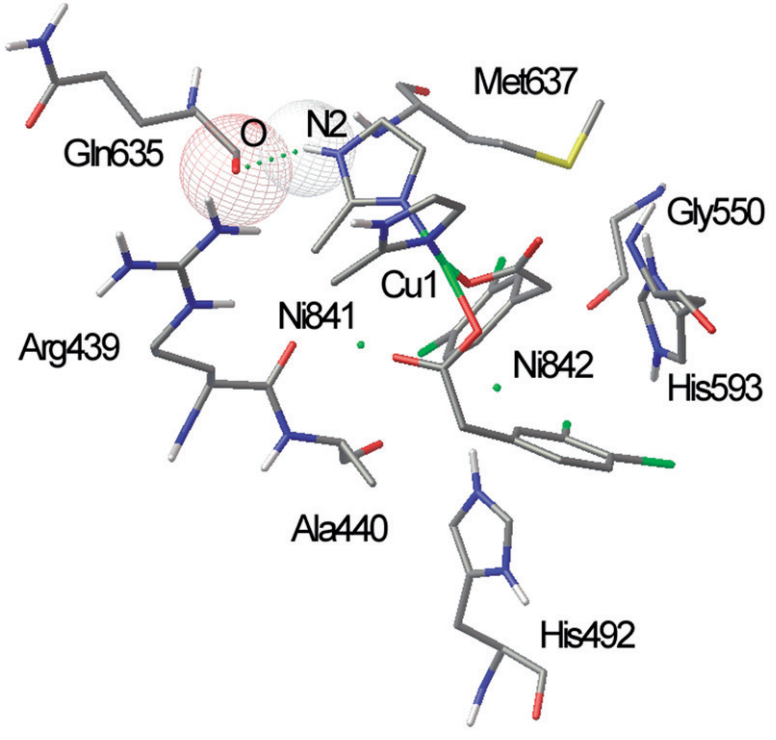

Figure 3. Complex **2** is bound into jack bean urease (entry 3LA4 in the Protein Data Bank). The dotted line shows the hydrogen bond.

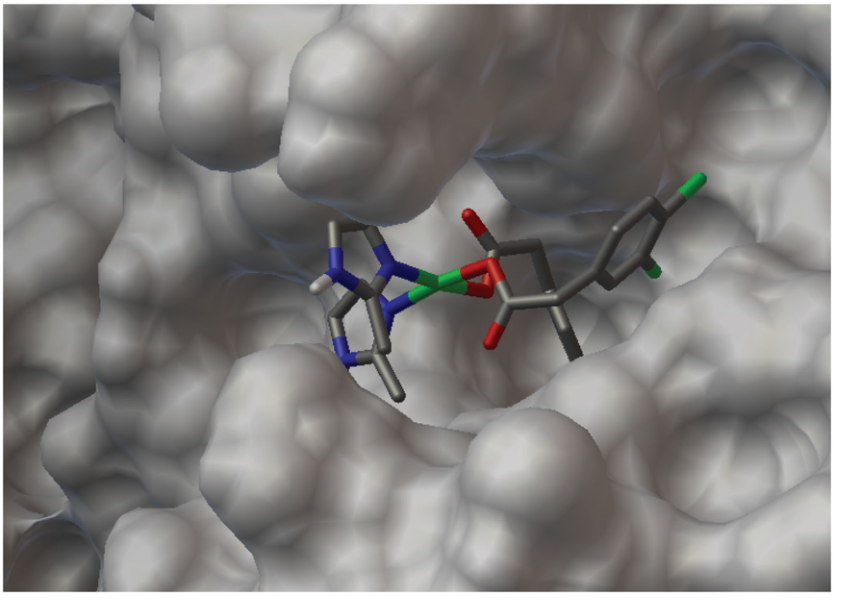

Figure 4. The enzyme surface model of **2** – jack bean urease complex.

bond: $O_{Gln635}\ldots N–H_{complex\mathbf{2}} = 2.167$ Å; angle of the hydrogen bond: $O_{Gln635}\ldots N–H_{complex\mathbf{2}} = 155.845°$). Moreover, the complex might form hydrophobic interaction with Ala440 of urease. The urease inhibitory property may be attributed to the above hydrogen bond and hydrophobic interaction formed between the copper complex and jack bean urease.

4. Conclusion

This study describes the syntheses, X-ray crystal structure, and inhibitory enzyme activity of Cu(II) complexes. The complexes exhibit ability to inhibit urease, although their ligands had no ability to inhibit urease ($IC_{50} > 100\,\mu mol\,L^{-1}$). Docking simulation was performed to position **2** into the jack bean urease active site to determine the probable binding conformations, and the results indicated that the complex was a potent inhibitor of jack bean urease. Detailed investigations are continuing to study the mechanisms of the inhibitory activity reported here.

Supplementary material

Crystallographic data in CIF format for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre No. 834972 for $[Cu(C_4H_3N_2O_2)_2\cdot 4H_2O]$ (**1**) and No. 834973 for $[Cu(C_{12}H_{11}N_2O_2Cl_2)_2]$ (**2**). Copies of this information can be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336 033; Email: deposit@ccdc.cam. ac.uk or www: http://www.ccdc.cam.ac.uk).

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