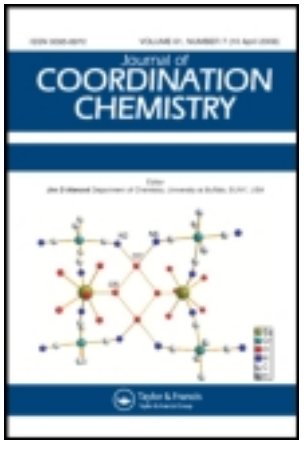

Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gcoo20

Synthesis, molecular docking, and activity of Schiff-base copper(II) complex with N-n-butylsalicylaldiminate as Helicobacter pylori urease inhibitor

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Published online: 28 Jan 2011.

To cite this article: Yong Ming Cui, Yuguang Li, Ying Jie Cai, Wu Chen & Hai Liang Zhu (2011) Synthesis, molecular docking, and activity of Schiff-base copper(II) complex with N-n-butylsalicylaldiminate as Helicobacter pylori urease inhibitor, Journal of Coordination Chemistry, 64:4, 610-616, DOI: 10.1080/00958972.2011.552110

To link to this article: http://dx.doi.org/10.1080/00958972.2011.552110

Journal of Coordination Chemistry
Vol. 64, No. 4, 20 February 2011, 610–616

Synthesis, molecular docking, and activity of Schiff-base copper(II) complex with *N*-*n*-butylsalicylaldiminate as *Helicobacter pylori* urease inhibitor

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(*Received 9 October 2010; in final form 22 November 2010*)

A Schiff-base copper(II) complex, *bis*(*N*-*n*-butylsalicylaldiminato)copper(II), was synthesized and its solid-state structure was determined by X-ray crystallography. Its inhibitory activity against *Helicobacter pylori* urease was evaluated *in vitro* and it showed strong inhibitory activity against urease ($IC_{50} = 0.95\,\mu M$), compared with acetohydroxamic acid ($IC_{50} = 42.47\,\mu M$) as a positive reference. A docking analysis using the AUTODOCK 4.0 program was performed to explain the potent inhibitory activity of the complex against urease.

Keywords: Copper(II) complex; Schiff base; Urease; Inhibitor; Molecular docking

1. Introduction

Transition metal complexes of Schiff bases derived from salicylaldehyde and its derivatives have become hot topics of contemporary research for potential applications, acting as single-molecule magnets [1], luminescent probes [2], and catalysts for specific DNA [3, 4] cleavage reactions. Therefore, Schiff-base transition metal complexes may be an untapped reservoir for drugs.

Urease, the first enzyme crystallized to be shown to possess nickel ions, is an important enzyme in both agriculture and medicine, rapidly catalyzing the hydrolysis of urea to form ammonia and carbamic acid [5, 6]. But the end-product of such decomposition also results in a pH increase, responsible for the negative effects of urease activity in human health, such as causing peptic ulcers, and stomach cancer [7]. Controlling the activity of urease through the use of inhibitors in order to counteract these negative effects is important. Recently, our group reported that some salen-type Schiff-base complexes possess inhibitory activities against xanthine oxidase and excellent antibacterial activities [8]. In addition, some transition metal complexes

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Journal of Coordination Chemistry
ISSN 0095-8972 print/ISSN 1029-0389 online
DOI: 10.1080/00958972.2011.552110

(M = Cu, Co, Ni, etc.) of such Schiff bases with potent inhibitory activities against urease were also studied [9]. In this article, we designed and synthesized a new copper(II) complex containing the Schiff-base ligand derived from condensation of salicylaldehyde with *n*-butylamine, and investigated its inhibitory activity against *Helicobacter pylori* urease. Docking simulation was performed to position the complex into the *H. pylori* urease active site to determine the probable binding conformation using AUTODOCK 4.0 [10].

2. Experimental

2.1. *Materials and methods*

Salicylaldehyde and *n*-butylamine were purchased from Aldrich and used without purification. Elemental analyses for C, H, and N were carried out on a Perkin-Elmer 2400 analyzer. X-ray crystallography was carried out using a Bruker SMART APEX II CCD diffractometer. All chemicals and reagents were of analytical grade. Protease inhibitors (Complete mini EDTA-free) were purchased from Roche Diagnostics GmbH (Mannhein, Germany) and brucella broth was from Becton-Dickinson (Cockeysville, MD, USA). Horse serum was from Hyclone (Utah, USA).

2.2. *Synthesis of the title complex ([Cu(L)$_2$])*

Salicylaldehyde (24 mg, 0.2 mmol) and *n*-butylamine (15 mg, 0.2 mmol) were dissolved in an aqueous methanol solution (5 mL). The mixture was stirred for 5 min to give an orange solution, which was added to a methanol solution (2 mL) of $Cu(NO_3)_2 \cdot 3H_2O$ (24 mg, 0.1 mmol). The mixture was stirred for another 5 min at room temperature to give a celadon solution and then filtered. The filtrate was exposed to air for 7 days, forming black block crystals. The crystals were isolated, washed three times with distilled water, and dried in a vacuum desiccator containing anhydrous $CaCl_2$. Yield: 69%. Anal. Calcd for $C_{22}H_{28}CuN_2O_2$: C, 63.52; H, 6.78; N, 6.73. Found: C, 63.46; H, 6.86; N, 6.65%.

2.3. *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 [11] and empirical absorption corrections were performed using SADABS [12]. The structures were solved by direct methods and refined against F^2 by full-matrix least-squares using the SHELXTL version 5.1. All nonhydrogen atoms were refined anisotropically. All hydrogens were placed in geometrically ideal positions and constrained to ride on their parent atoms. The crystallographic data for the compound are summarized in table 1. Selected bond lengths and angles are given in table 2.

Table 1. Crystal data for the complex.

Empirical formula	$C_{22}H_{28}CuN_2O_2$
Formula weight	416.00
Crystal system	Monoclinic
Space group	$P2_1/n$
Unit cell dimensions (Å, °)	
a	10.9193(8)
b	7.3503(6)
c	14.7002(8)
α	90.00
β	119.628(4)
γ	90.00
T (K)	291(2)
Volume (Å^3), Z	1025.58(13), 2
Calculated density (g cm^{-3})	1.347
$F(0\,0\,0)$	438
μ(Mo-Kα) (mm^{-1})	1.083
Data/restraint/parameters	2549/0/125
Goodness-of-fit on F^2	1.008
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0307$, $wR_2 = 0.0777$

Table 2. Selected bond lengths (Å) and angles (°) in the complex.

Cu1–O1	1.8795(13)	O1–Cu1–N1	91.58(5)
Cu1–O1$^{\#a}$	1.8795(13)	O1$^{\#a}$–Cu1–N1$^{\#a}$	91.58(5)
Cu1–N1	2.0070(13)	O1–Cu1–N1$^{\#a}$	88.42(5)
Cu1–N1$^{\#a}$	2.0070(13)	O1$^{\#a}$–Cu1–N1	88.42(5)
N1–C7	1.290(2)		
N1$^{\#a}$–C7$^{\#a}$	1.290(2)		

Symmetry code: $^{\#a}$ $1-x$, $-y$, and $-z$.

2.4. *Measurement of inhibitory activity against urease*

Helicobacter pylori (ATCC 43504; American Type Culture Collection, Manassas, VA) was grown in brucella broth supplemented with 10% heat-inactivated horse serum for 24 h at 37°C under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2), as previously described [13].

The method of preparation of *H. pylori* urease was followed by Mao *et al.* [14]. Briefly, 50-mL broth cultures (2.0×10^8 CFU mL^{-1}) were centrifuged ($5000 \times g$, 4°C) to collect the bacteria, and after washing twice with phosphate-buffered saline (pH 7.4), *H. pylori* precipitate was stored at −80°C. *H. pylori* was returned to room temperature, and after addition of 3 mL of distilled water and protease inhibitors, sonication was performed for 60 s. Following centrifugation ($15{,}000 \times g$, 4°C), the supernatant was desalted through Sephadex G-25 column (PD-10 columns, Amersham Pharmacia Biotech, Uppsala, Sweden). The resultant crude urease solution was added to an equal volume of glycerol and stored at 4°C until use in the experiment. The mixture, containing 25 µL (4 U) of *H. pylori* urease and 25 µL of the test compound, was preincubated for 3 h at room temperature in a 96-well assay plate. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [15].

2.5. *Docking simulations*

Molecular docking of the complex into the 3-D X-ray structure of *H. pylori* urease (entry 1E9Z 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) [16].

The graphical user interface AUTODOCKTOOLS was employed to set up the enzymes: all hydrogens were added, Gasteiger charges were calculated and nonpolar hydrogens were merged to carbons. The Ni initial parameters are set as $r = 1.170$ Å, $q = +2.0$, and van der Waals well depth of 0.100 kcal mol^{-1} [17]. The 3-D structure of ligand molecule was saved in Mol2 format with the aid of the program MERCURY. The partial charges of Mol2 file were further modified using the ADT package (version 1.4.6), so that the charges of the nonpolar hydrogens assigned to the atom to which the hydrogen was attached. The resulting file was saved as pdbqt file.

The AUTODOCKTOOLS program was used to generate the docking input files. In all dockings, a grid box size of $60 \times 60 \times 60$ points in x, y, and z directions was built, the maps were centered on N1 of the Kcx 219 in the catalytic site of the protein. A grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant were used for the calculation of the energy map. Ten runs were generated 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*

Single crystal X-ray diffraction reveals that the complex crystallizes in the monoclinic system with space group $P2_1/n$. The crystal structure of *bis*(N-*n*-butylsalicylaldiminato)copper(II) is shown in figure 1, as a mononuclear copper complex of Schiff base derived from condensation of *n*-butylamine with salicylaldehyde. The copper(II) is four-coordinate one with two nitrogens and two oxygens from two bidentate ligands in the usual *trans* arrangement. The copper(II) lies on a center of inversion with a square-planar geometry and axial positions are vacant. Analogous square-planar copper(II) species were previously reported [18, 19]. Table 1 summarizes the unit-cell parameters and details of data collection for the crystallographic studies of the complex. Selected bond lengths and angles from the complex are given in table 2.

3.2. *Inhibitory bioactivity against urease*

The ability against urease of the Schiff-base ligand HL, Cu^{2+} and the complex has been studied by IC_{50} values of the material (25 μL, 100 μg) tested against *H. pylori* urease (25 μL, 10 kU L^{-1}) using urea (500 mmol L^{-1}) in HEPES buffer (0.2 mL, 100 mmol L^{-1}; pH = 6.8). On reaction with *H. pylori* urease in the presence of phenol red, HL has no

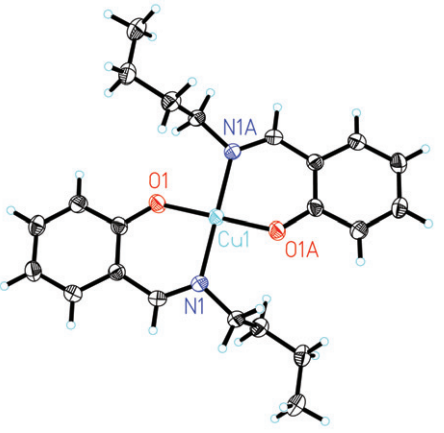

Figure 1. An ORTEP diagram showing the molecular structure of the complex. Thermal ellipsoids are shown at 30% probability level. Symmetry code A: $1-x$, $-y$, and $-z$.

Table 3. Inhibition of *H. pylori* urease by the complex, Schiff-base ligand, and Cu(II) ion.

Tested materials	IC_{50} (μM)
HL	>100
Cu^{2+}	1.35 ± 0.13
Complex ($Cu(C_{11}H_{14}NO)_2$)	0.95 ± 0.04
Acetohydroxamic acid	42.47 ± 0.19

ability to inhibit urease ($IC_{50} > 100\,\mu M$). This indicates the Schiff-base ligand has less influence on the activity of *H. pylori* urease. Under the same condition, both Cu(II) ion and its Schiff-base complex showed potent urease inhibitory activities, compared with that of the standard inhibitor acetohydroxamic acid which had IC_{50} values of 42.47 μM, as shown in table 3. The results agree with those reported previously [20, 21].

3.3. *Molecular docking study*

The binding model of Schiff-base copper(II) complex and the enzyme active site of urease are depicted in figure 2. All amino acid residues which had interactions with *H. pylori* urease are shown in the figure. In the binding model, hydroxy of Phe218 of the urease protein forms a hydrogen bond with oxygen of the complex (length of the hydrogen bond: Phe218 $O\text{–}H\cdots O_{complex} = 2.412$ Å; angle of the hydrogen bond: Phe218 $O\text{–}H\cdots O_{complex} = 144.9^\circ$). Moreover, the complex may form hydrophobic interactions with Leu196 and Ile220 of urease. The urease inhibitory property possessed by the complex may be attributed to the above hydrogen bond and hydrophobic interactions formed with *H. pylori* urease. The docking calculations reveal that complex has lower free energy of binding ($-12.57\,kcal\,mol^{-1}$) than that of AHA

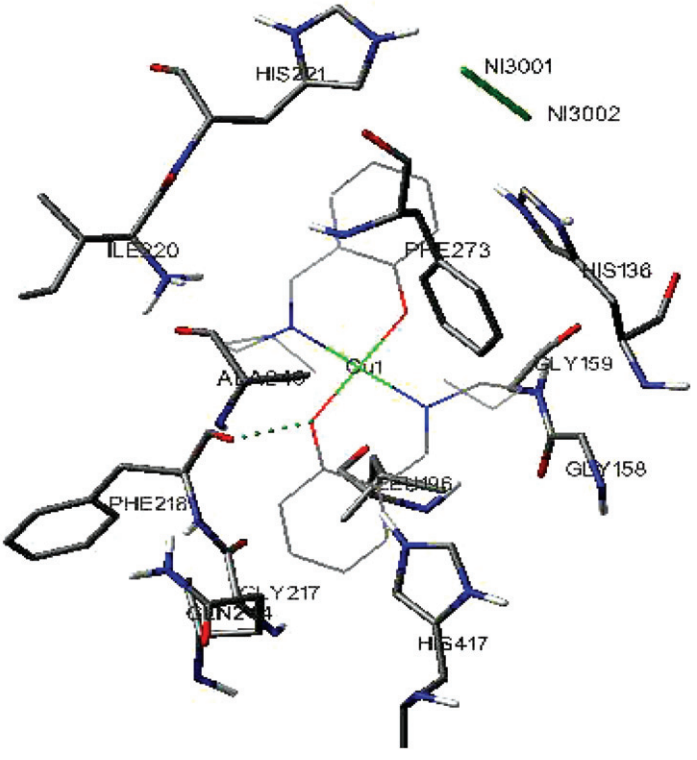

Figure 2. $(Cu(C_{11}H_{14}NO)_2)$ bound into *H. pylori* urease (entry 1E9Z in the Protein Data Bank). The dotted line shows the hydrogen bond.

$(-10.07\,kcal\,mol^{-1})$, which may also explain the excellent inhibitory activity of the complex against *H. pylori* urease.

4. Conclusions

This article describes the synthesis, X-ray crystal structure, and inhibitory enzyme activity of the mononuclear copper complex of a Schiff base, *bis*(N-*n*-butylsalicylaldiminato)copper(II). Although its Schiff-base ligand HL has no ability to inhibit urease ($IC_{50} > 100\,\mu M$), the complex exhibits ability to inhibit urease. Docking simulation was performed to position the complex into the *H. pylori* urease active site to determine the probable binding conformation and the result indicated that the complex was a potent inhibitor of *H. pylori*. Detailed investigations are continuing to study the mechanisms of the inhibitory activity.

Supplementary material

Crystallographic data in CIF format for the structural analysis have been deposited with the Cambridge Crystallographic Data Center No. 761918 for the complex.

These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336 033; Email: deposit@ccdc.cam.ac.uk).

Acknowledgments

This study was financed by grant from National Natural Science Foundation of China (50978208), Hubei National Science Foundation (2009CDA022), and Education Office of Hubei Province (D20091704 and Q20101610).

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