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STRUCTURE AND UREASE INHIBITORY ACTIVITY OF COPPER(II) COMPLEX WITH (*E*)-3-(2,3-DIHYDROBENZO[b][1,4]DIOXIN-6-yl)ACRYLIC ACID

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A new caffeic acid derivative ((*E*)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylic acid, HL^1)) is synthesized from caffeic acid, followed by the preparation of a Cu(II) complex, $[Cu_2(L^1)_4DMSO_2]\cdot 2DMSO$. The structure is determined by single crystal X-ray diffraction, and the urease inhibitory activity of the complex is studied. The results show that IC_{50} of the complex is 0.56 μmol/L, which is superior to positive control acetohydroxamic acid (AHA, IC_{50} was 10.95 μmol/l), i.e., the complex has strong inhibitory activity towards urease.

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Keywords: caffeic acid, copper(II) complex, crystal structure, urease inhibitory.

INTRODUCTION

Urease (urea amidohydrolase; E.C.3.5.1.5) is a nickel-containing metalloenzyme that rapidly catalyzes the hydrolysis of urea to form ammonia and carbamate [1, 2]. Urease is widely distributed in a variety of algae, bacteria, fungi, and plants. The reaction catalyzed by urease may cause an accumulation of ammonia and accompanying pH elevation, which has important negative implications in medicine and agriculture [3-6]. The gram-negative bacterium *Helicobacter pylori* is associated with severe gastric pathologies, including peptic ulcer, chronic active gastritis and gastric cancer. This microorganism is able to invade and colonize human stomach, directly interacting with gastric epithelial cells mainly because of its high urease activity. Already in 1994, *H.pylori* was classified as a type I carcinogen for humans by the IARC/WHO [7]. Urease inhibitors have a very important status in the treatment of the infections caused by urease producing bacteria [8]. There are many different kinds of urease inhibitors but they usually have strong side effects [9]. Therefore, it is necessary to search for new urease inhibitors with low toxicity and good bioavailability. Transition metals have biological activity in many biochemical processes [10]. Generally used transition metals include cobalt, zinc, nickel, copper etc. They act as cofactors in some important enzymatic and organ functions, resulting in increased activities of these enzymes [11]. Complexes can effectively reduce the side effects of the transition metal, and have become a hot spot in research. In recent years, some metal complexes have been reported to show anticancer [12-16] and urease inhibitory activity [17-21].

Caffeic acid is an important natural antioxidant [22, 23], and has been widely applied in food, pharmaceuticals, and cosmetics industries due to its biological or physiological activity as antioxidant [24], antitumor agent [25], antibiotic [26],

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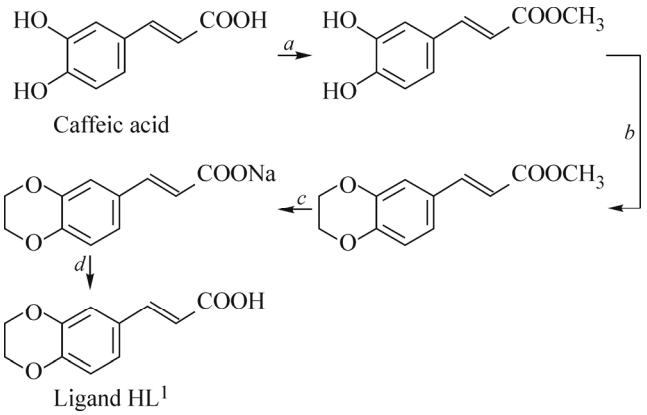
and so on. However, caffeic acid has low solubility and stability in common solvent systems, which limits its application. Caffeic acid derivatives can increase its solubility in hydrophobic media. The action of caffeic acid and its derivatives is multiple, including removal of free radicals, metal ion chelating, inhibition of special enzyme ACTS to reduce the formation of free radicals and lipid peroxide [27, 28]. Therefore, it acts as antioxidant to prevent the oxidation of fat and oily food spoilage, and peroxide damage and disease, such as cancer, diabetes, coronary heart disease, etc [29]. Thus, study of caffeic acid and its derivatives for food and other fields is important.

In this paper, we report the synthesis of a caffeic acid derivative ((*E*)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylic acid) and a new Cu(II) complex with this derivative as ligand as well as its urease inhibitory activity.

EXPERIMENTAL

General methods and materials. Unless otherwise stated, all solvents were of reagent grade quality and purchased commercially. All chemicals were also commercially available and used without further purification. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer.

Synthesis of HL^1 ((*E*)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylic acid). HL^1 was synthesized from caffeic acid and 1,2-dibromoethane (Scheme 1). The steps from caffeic acid to sodium (*E*)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylate acid include esterification, cyclization, hydrolysis and acid precipitation.



Scheme 1. Synthesis of the ligand HL^1. Reagents and conditions: (*a*) MeOH, H_2SO_4, reflux, 8 h; (*b*) $Br(CH_2)_2Br$, K_2CO_3, acetone, reflux, 24 h; (*c*) NaOH, H_2O, reflux, 12 h; (*d*) HCL, washing, drying

For etherification, caffeic acid (0.25 mol, 45 g) was dissolved in methanol (350 ml) with stirring, in the presence of concentrated sulfuric acid as catalyst. The mixture was stirred for about 20 h at 63 °C to produce a clear solution. The solution was poured into 400 ml ethyl acetate and 200 ml water. The ethyl acetate was evaporated to give yellow powdered caffeic acid methyl ester.

Caffeic acid methyl ester (0.02 mol, 3.88 g) and 1,2-dibromoethane (0.06 mol, 5.2 ml) were dissolved in acetone (100 ml) with stirring, using K_2CO_3 (0.06 mol, 8.28 g) as acid binding agent. The mixture was stirred at 56 °C. After the reaction had been completed, the insoluble substance was removed by filtration, the solvent was evaporated to leave a brown oily matter. The oily matter was stirred for about 20 h at 80 °C in the presence of NaOH (0.12 mol, 4.8 g) and H_2O (100 ml). The oily matter dissolved to give to a brown solution. The pH value of solution was adjusted to 1 and free acid precipitated. The white precipitate was isolated by filtration and washed with H_2O until the pH reached the value of 7.0, then dried. The white powder was the (*E*)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylic ligand (HL^1). After keeping it in the methanol

solution for 7 days, colorless block shaped single crystals of HL^1 suitable for structure determination were obtained at the bottom of the vessel. Yield 74%. *Anal.* Calc. for $C_{11}H_{10}O_4$: C 64.08, H 4.85, O 31.07%. Found: C 64.03, H 4.77, O 31.02%.

Preparation of $[Cu_2(L^1)_4DMSO_2]\cdot 2DMSO$. The complex was prepared by addition of a water solution (10 ml) of (*E*)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylic acid (2 mmol, 0.412 g) to a water solution (10 ml) of NaOH (2 mmol, 0.08 g) followed by addition of $CuSO_4$ (1 mmol, 0.16 g). After stirring for 1 h at room temperature, the solution was filtered to leave a green precipitate. It was dissolved in a minimal volume of DMSO. Keeping the solution in a refrigerator (4 °C) for 15 days yielded green block-shaped single crystals of the complex suitable for the structure determination at the bottom of the vessel. Crystals were isolated by filtration and washed with cold H_2O and then dried. Yield 75%. *Anal.* Calc. for $C_{52}H_{60}Cu_2O_{20}S_4$: C 49.55, H 4.80, O 25.39%. Found: C 49.51, H 4.68, O 25.32%.

Crystal structure determination. Single crystal X-ray diffraction data were collected at 150(2) K on a Nonius Kappa CCD diffractometer with graphite monochromated MoK_α radiation (λ = 0.71073 Å). The data were processed by using DENZO [30]. The structure was solved by direct methods implemented in SIR-97 [31] and refined by a full-matrix least squares procedure based on F^2 with SHELXL-97 [32]. All of atoms were refined anisotropically except the hydrogen atom. Hydrogen atoms bound to O were first found in the Fourier map and then fixed at their ideal positions. The detailed crystallographic data were summarized in Table 1.

Measurement of inhibitory activity against jack bean urease. *Jack bean* urease was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The measurement of urease activity was carried out according to the literature [33, 34]. Generally, the assay mixture, containing 25 μl of jack bean urease (10 kU/l) and 25 μl of the tested samples (complex, also the parent ligands and metal salts) of various concentrations (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 preincubation, 200 μl of 100 mM HEPES (N-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid]) buffer pH = 6.8 containing 500 mM urea and 0.002% phenol red, was added and incubated at 37 °C

TABLE 1. Experimental Data for Crystal Structure of HL^1 and $[Cu_2(L^1)_4DMSO_2]\cdot 2DMSO$

Crystallographic parameter	HL^1	$[Cu_2(L^1)_4DMSO_2]\cdot 2DMSO$
Molecular formula	$C_{11}H_{10}O_4$	$C_{48}H_{48}Cu_2O_{18}S_2$, $2(C_2H_6OS)$
Molecular weight	206.19	1260.38
Temperature, K	293	273
Radiation λ, Å	MoK_α (0.7107)	MoK_α (0.7107)
Crystal system	Monoclinic	Triclinic
Space group	*C*2/*c*	*P*-1
a, *b*, *c*, Å	23.2671(17), 11.6009(8), 7.5005(6)	14.3915(11), 14.7299(11), 16.8817(13)
α, β, γ, deg.	β 102.473(3)	108.044(2), 107.509(2), 90.116(2)
V, Å^3	1976.8(3)	3227.1(4)
Z	8	2
D_c, g/cm^3	1.386	1.297
Crystal size, mm	0.20×0.18×0.16	0.30×0.28×0.26
F(000)	864	1308
θ range, deg.	3.2-25.1	2.2-25.2
Reflections collected / unique	9222 / 1763 [R_{int} = 0.045]	31099 / 11536 [R_{int} = 0.047]
Refns obs. $I > 2\sigma(I)$	1088	7264
Goodness of fit on F^2	1.024	1.113
Data / restraints / parameters	1763 / 7 / 182	11536 / 494 / 703
Largest diff. peak and hole, e/Å^3	0.131 and –0.172	1.630 and –0.921
R_1, wR_2 [$I > 2\sigma(I)$][a]	0.0485, 0.1070	0.0789, 0.2294
R_1, wR_2 (all data)[a]	0.0963, 0.1262	0.1298, 0.2552

[35]. The reaction time was measured by microplate reader (570 nm), which was required to produce enough ammonium carbonate to raise the pH of a HEPES buffer from 6.8 to 7.7, the endpoint being determined by the color of phenol red indicator [36].

RESULTS AND DISCUSSION

Crystal structure of HL^1 and complex. The crystal structure of (*E*)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylic acid is shown in Fig. 1, and experimental data for crystal structure of HL^1 and complex are listed in Table 1. The crystal structure of the Cu(II) complex is shown in Fig. 2. Selected bond distances (Å) and angles (deg.) for HL^1 and the complex are collected in Table 2.

Complex $[Cu_2(L^1)_4DMSO_2]\cdot 2DMSO$ crystallizes in a triclinic space group *P*-1. The perspective view of the crystal structures of the complex is shown in Fig. 2. Each molecule consists of two copper ions, four L^1 anions, and four dimethyl sulfoxide molecules. Each copper ion is five-coordinated with four O atoms of L^1 and one O atom of DMSO in a square pyramidal arrangement ($\tau = 0.0002$ for Cu1 and Cu1A), thus forming a $[CuO_5]$ chromophore. Two copper ions are linked together by four bridging bidentate carboxylic groups of four L^1. Four O atoms of four L^1 anions form the basal plane and one O atom of DMSO is in the axial position. The in-plane Cu–O bond distances average 2.05 Å and axial Cu–O bond distances 2.139 Å, which are in the normal range (Fig. 2 and Table 2).

Inhibitory activity against jack bean urease. Inhibition of urease by the tested materials is shown in Table 3.

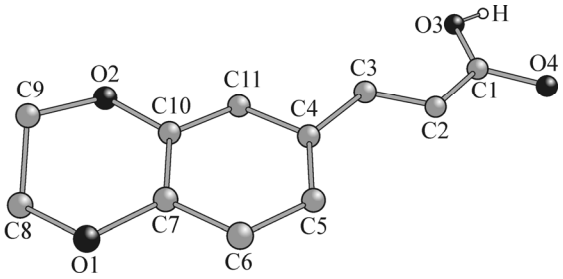


Fig. 1. Crystal structure of (*E*)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylic acid (HL^1).

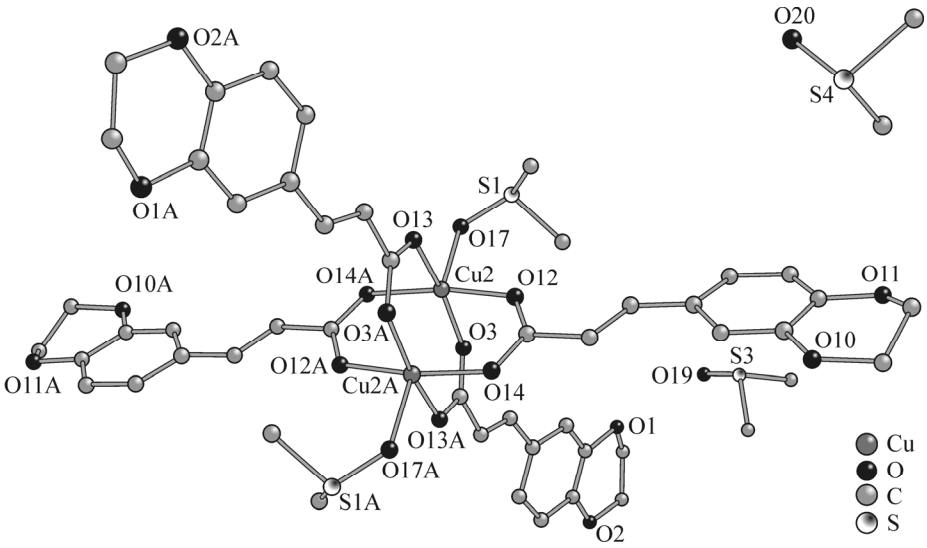


Fig. 2. Crystal structure of $[Cu_2(L^1)_4DMSO_2]\cdot 2DMSO$.

TABLE 2. Selected Bond Distances (Å) and Angles (deg.) for HL^1 and $[Cu_2(L^1)_4DMSO_2]\cdot 2DMSO$

		HL^1	
O(1)–C(7)	1.371(2)	O(1)–C(8)	1.434(2)
C(1)–O(3)	1.274(19)	C(1)–O(4)	1.327(12)
C(1)–C(2)	1.430(15)	O(2)–C(10)	1.366(2)
O(2)–C(9)	1.429(2)	C(2)–C(3)	1.308(13)
C(2)–H(2A)	0.9300	C(3)–C(4)	1.571(8)
C(3)–H(3A)	0.9300	O(3)–H(3B)	0.8200
C(4)–C(11)	1.377(3)	C(4)–C(5)	1.385(3)
C(5)–C(6)	1.368(3)	C(5)–H(5)	0.9300
C(6)–C(7)	1.375(3)	C(6)–H(6)	0.9300
C(7)–C(10)	1.384(3)	C(8)–C(9)	1.474(3)
C(10)–C(11)	1.371(3)	O(3)–C(1)–O(4)	120.4(12)
C(7)–O(1)–C(8)	113.06(16)	O(4)–C(1)–C(2)	115.4(11)
O(3)–C(1)–C(2)	124.2(11)	C(3)–C(2)–C(1)	120.4(7)
C(10)–O(2)–C(9)	112.77(16)	C(11)–C(4)–C(5)	117.6(2)
C(2)–C(3)–C(4)	121.4(7)	C(5)–C(4)–C(3)	135.0(4)
C(11)–C(4)–C(3)	107.4(4)	C(5)–C(6)–C(7)	120.7(2)
C(6)–C(5)–C(4)	121.0(2)	O(1)–C(7)–C(10)	122.41(17)
O(1)–C(7)–C(6)	118.49(19)	O(1)–C(8)–C(9)	110.76(18)
C(6)–C(7)–C(10)	119.10(19)	O(2)–C(10)–C(11)	118.44(18)
O(2)–C(9)–C(8)	110.74(17)	C(11)–C(10)–C(7)	119.47(18)
O(2)–C(10)–C(7)	122.09(17)	C(10)–C(11)–C(4)	122.1(2)
		Complex	
Cu(1)–O(6)	1.956(4)	Cu(1)–O(16)	1.965(4)
Cu(1)–O(9)	1.967(4)	Cu(1)–O(18)	2.152(4)
Cu(2)–O(3)	1.942(4)	Cu(2)–O(13)	1.950(4)
Cu(2)–O(12)	1.972(4)	Cu(2)–O(17)	2.139(4)
Cu(1)–O(15)#1	1.961(4)	Cu(1)–Cu(1)#1	2.6211(12)
O(15)–Cu(1)#1	1.961(4)	Cu(2)–O(14)#2	1.967(4)
Cu(2)–Cu(2)#2	2.6286(13)	O(14)–Cu(2)#2	1.967(4)
O(3)–Cu(2)–O(13)	168.19(19)	O(3)–Cu(2)–O(12)	88.0(2)
O(13)–Cu(2)–O(12)	90.34(19)	O(3)–Cu(2)–O(17)	96.51(19)
O(13)–Cu(2)–O(17)	95.30(19)	O(12)–Cu(2)–O(17)	99.51(16)
O(6)–Cu(1)–O(16)	89.14(16)	O(6)–Cu(1)–O(9)	88.72(17)
O(16)–Cu(1)–O(9)	168.87(16)	O(6)–Cu(1)–O(18)	95.98(16)
O(16)–Cu(1)–O(18)	95.75(17)	O(9)–Cu(1)–O(18)	95.33(17)
O(6)–Cu(1)–O(15)#1	169.04(16)	O(15)#1–Cu(1)–O(16)	89.10(16)
O(15)#1–Cu(1)–O(9)	90.93(16)	O(15)#1–Cu(1)–O(18)	94.96(16)
O(6)–Cu(1)–Cu(1)#1	85.73(12)	O(15)#1–Cu(1)–Cu(1)#1	83.33(11)
O(16)–Cu(1)–Cu(1)#1	84.38(12)	O(9)–Cu(1)–Cu(1)#1	84.57(12)
O(18)–Cu(1)–Cu(1)#1	178.29(12)	O(13)–Cu(2)–O(14)#2	89.0(2)
O(3)–Cu(2)–O(14)#2	90.26(19)	O(17)–Cu(2)–Cu(2)#2	177.48(12)
O(14)#2–Cu(2)–O(12)	168.65(17)	O(14)#2–Cu(2)–O(17)	91.83(16)
O(3)–Cu(2)–Cu(2)#2	83.53(14)	O(13)–Cu(2)–Cu(2)#2	84.67(14)
O(14)#2–Cu(2)–Cu(2)#2	85.65(12)	O(12)–Cu(2)–Cu(2)#2	83.00(12)

TABLE 3. Inhibition of Urease by the Tested Materials

Tested materials	IC_{50}, μmol/l	Tested materials	IC_{50}, μmol/l
DMSO	>100	$[Cu_2(L^1)_4DMSO_2]\cdot 2DMSO$	0.56
$CuSO_4\cdot 6H_2O$	1.99	AHA	10.95
HL^1	>100		

The HL^1, AHA, $CuSO_4\cdot 6H_2O$ and the complex were evaluated for inhibitory activity against *jack bean* urease by the phenol red method. The results are summarized in Table 3. The IC_{50} value of HL^1 was >100 μM. The IC_{50} value of the $CuSO_4\cdot 6H_2O$ was 1.99 μM. Under the same conditions, the IC_{50} of the complex was 0.56 μM, which is better than positive control AHA 10.95 μM and $CuSO_4\cdot 6H_2O$ 1.99 μM. The results of the urease test showed that the complex had obvious inhibitory activity against urease. This offers some hope for the complex to be developed as a drug to treat diseases caused by *H.pylori.*

UV-Vis and IR spectra. The UV-Vis spectra for the complex and HL^1 on TU-1801 were obtained in assay conditions (DMSO). The electronic spectra of the complex and HL^1 show intense UV band near 300 nm. In the visible region, one low-intensity band is observed for the complex in the region 650-800 nm assigned to *d–d* transitions.

The IR spectra of the complex on Nicolet 5700 are compared with the HL^1 in order to determine the coordination sites that may be involved in coordination complex. The important IR bands with their tentative assignments are depicted as follow. Some important calculated and experimental stretching vibrational frequencies of the HL^1 and complex are listed in supplementary material. The medium band at 2986.5 cm^{-1} is attributed to the $\nu_{(OH)}$ vibration of the ligands. In the complex the $\nu_{(OH)}$ vibration disappears. The characteristic bands of the carboxylate anions have typical $\nu(OCO)_{asym}$ {1582.2 cm^{-1} in complex} and $\nu(OCO)_{sym}$ {1399.7 cm^{-1} in complex} values. The calculated Δ(OCO) values {$\nu(OCO)_{asym} - \nu(OCO)_{sym}$} are 182.5 cm^{-1} as typical of bidentate/bridging carboxylate.

CONCLUSIONS

A new Cu(II) complex was synthesized with the (*E*)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylic as first ligand, DMSO as the second ligand. The complex exhibit urease inhibitory activity higher than that shown by the parent ligand and Cu^{2+} ion separately. The activity is probably due to synergy between strong Lewis acid properties of Cu^{2+} and the ligands.

Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre (CCDC-1446504 for (*E*)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylic acid and CCDC-1415174 for 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) 1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www:http://www.ccdc.cam.ac.uk).

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