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Original article

Synthesis, molecular docking and biological evaluation of Schiff base transition metal complexes as potential urease inhibitors

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ABSTRACT

Six transition metal compounds of Schiff base ligands were evaluated for the inhibitory activity on *jack bean* urease, of which compounds **2**–**6** were determined by single crystal X-ray analysis. It was found that copper(II) complexes **1** and **4** showed strong inhibitory activity against *jack bean* urease ($IC_{50} = 0.52$ and 0.46 μM), compared with acetohydroxamic acid ($IC_{50} = 42.12$ μM) as a positive reference. Cobalt(II), nickel(II) and zinc(II) compounds also exhibited potent inhibitory activity ($IC_{50} = 3.88$–25.20 μM). A docking analysis using the AUTODOCK 4.0 program could explain the inhibitory activities of **1** and **4** against urease.

1. Introduction

Transition metal complexes of Schiff base ligands have been extensively investigated for many years due to their novel structures and potential applications in many fields [1]. Among them, salen-type Schiff base complexes have been becoming the hot topics of contemporary research [2]. Schiff base ligands, derived from the condensation of salicylaldehyde and its derivatives with various primary amines, may act as the bidentate N,O- [3] and tridentate N,O,O-donor ligands [4], and so on [5], to construct mono-, di-mer, and one-dimensional (1D), two-dimensional (2D), three-dimensional (3D) complexes [6]. Currently, the use foreground of such metal complexes is promising such as acting as single-molecule magnets (SMMs) [7], as luminescent probes [8], as catalysts for specific DNA [9] and RNA [10] cleavage reactions. In addition, Schiff base complexes as the inhibitor were also investigated in prostate cancer cells [11] and tumor cells [12]. Recently, some salen-type Schiff base complexes possessing potent inhibitory activities against xanthine oxidase and excellent antibacterial activities have been reported by our group [13]. And some transition metal complexes (M = Cu, Co, Ni, etc.,) of Schiff base ligands with the potent inhibitory activities against urease have also been reported by us [14].

Urease could affect not only human health, for example, causing peptic ulcers, stomach cancer, etc [15], but also the efficiency of soil nitrogen fertilization, and ammonia volatilization and root damage [16]. It's interesting to find the excellent urease inhibitors [17]. As a follow-up to our previous characterization of Schiff base transition metal complexes as the urease inhibitor, urease inhibitory activities of compounds **1**–**6** were investigated and reported in this paper. A docking analysis using the AUTODOCK 4.0 program could explain the inhibitory activities of complexes **1** and **4** against urease and the structure activity relationship was also discussed.

2. Results and discussion

2.1. Synthesis

Schiff base ligands HL^{1-4} were prepared by reaction of 3,5-dibromosalicylaldehyde with 2-chlorobenzylamine (L^1, $C_{14}H_9NOBr_2Cl$), benzylamine (L^2, $C_{14}H_{10}NOBr_2$), cyclohexylamine (L^3, $C_{13}H_{14}NOBr_2$) and N,N′-dimethylethylenediamine (L^4, $C_{11}H_{13}N_2OBr_2$), in nearly 80–90% yield in MeOH, respectively. Complex **1** was obtained from reaction of HL^1 with $Cu(NO_3)_2 \cdot 3H_2O$ in MeOH. Unfortunately, attempts to grow suitable single crystals for its structural determination failed. However, spectroscopic data support the proposed structure as shown in Scheme 1. Similar to analogous copper(II) complexes [14a,18], the central Cu(II) atom of complexes **1** and **4** is four-coordinated by the oxygen and nitrogen donor atoms of two

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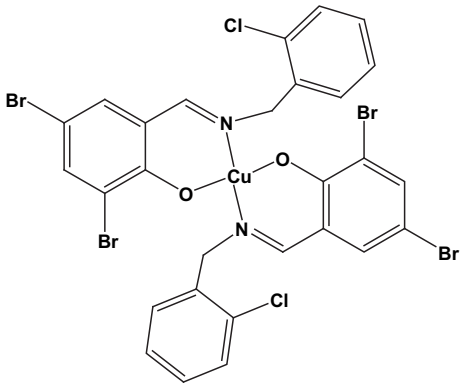

Scheme 1. Proposed structure of Schiff base compound **1**.

Schiff base ligands. The elemental analysis is in good agreement with the chemical formula proposed for compounds **1**–**6**.

2.2. IR and UV analysis

The IR spectra of compounds **1**–**6** exhibit strong absorption at 1609–1649 cm^{-1}, assignable to the ν(C=N) absorption [19,22a]. Due to the impact of metal atoms, the amino-absorption peaks of the Schiff base ligands shift to lower wave number. The strong absorption at 1642, 1633, 1632, 1618, 1612 cm^{-1} are attributed to the stretching vibration of C=N group of compounds **1**–**6**, respectively. For compounds **1**–**6**, the appearances of the broad strong absorption at about 1600, 1585, 1500 and 1450 cm^{-1} could be reasonably attributed to the presence of the benzene ring C=C backbone stretching vibration absorption peak.

The UV–Vis spectra for the compounds **1**–**6** were obtained in assay condition (DMSO:H_2O, 1:1 v/v). The weak but characteristic absorption bands in the 370–396 nm regions are attributed to the L → M charge transfer (CT) in the UV–Vis spectra of **1**–**6** [8b,20]. The middle absorption at around 270 nm may also be associated with charge transfer and/or n → π^* transitions [21]. The strong absorption bands at about 250 nm could be attributed to intra-ligand $\pi \rightarrow \pi^*$ transitions.

2.3. Crystal structure description

Crystal structures of compounds **2**–**6** are shown in Figs. 1–5, respectively. Single crystal X-ray diffraction reveals that these compounds are the mononuclear structures. The Schiff base ligands (HL^1, HL^2, HL^3, HL^4) derived from 3,5-dibromosalicylaldehyde act as a bidentate N,O-donor ligand. Crystal structure of complex **2** contains two independent mononuclear cobalt(II) molecules in asymmetric unit. As shown in Fig. 1, cobalt(II) atom is in a distorted-tetrahedral geometry and is four-coordinated by two N atoms and two O atoms from two Schiff base ligands (L^1). The average bond distances of Co1–O and Co1–N are 1.903(8) and 1.991(8) Å, respectively, while the average bond distances of Co2–O and Co2–N are 1.901(8) and 2.010(8) Å, respectively. The angles subtended at the Co(II) ion of the distorted-tetrahedral geometry (CoN_2O_2) is in the range 95.5(4)–118.9(4)°. The angle between two six-membered chelate planes was 89.1(2)°. In contrast to complex **2**, the Ni(II) atom of complex **3** lies on a crystallographic inversion center (symmetry codes: 1 − x, 1 − y, −z). Nickel atom is four-coordinated by two N atoms and two O atoms from two Schiff base ligands (L^2), which affords a square planar *trans*-[NiN_2O_2] coordination geometry. The bond distances of Ni1–O1 and Ni1–N1 are 1.847(3) and 1.944(4) Å, respectively, which are comparable with the corresponding values reported for analogous square planar Ni(II) species [14a,22].

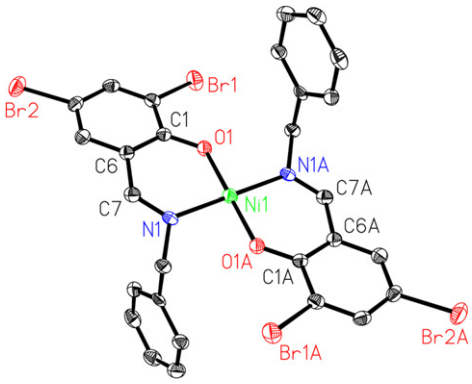

Fig. 2. An ORTEP diagram showing the structure of compound **3** with selected atom-labeling. The thermal ellipsoids plotted at 30% probability and H atoms omitted for the plot clarity. Symmetry codes A: 1 − x, 1 − y, −z.

The molecular structures of **4** and **5** were determined by single crystal X-ray analysis. Their structures are very much alike. Crystal structure of complex **4** is shown in Fig. 3. The copper(II) atom is four-coordinated by two N atoms and two O atoms from two Schiff base ligands (L^3) in the usual *trans* arrangement. Cu(II) atom is in a slightly distorted-tetrahedral geometry, which is intermediate between square planar and tetrahedral. Analogous tetrahedral Cu(II) species were previously reported in the literatures [18,23]. The average bond distances of Cu–O and Cu–N are 1.890(3) and 1.970(4) Å, respectively. The angles subtended at the Cu(II) ion in the distorted-tetrahedral geometry (CuN_2O_2) is in the range 91.28(13)–

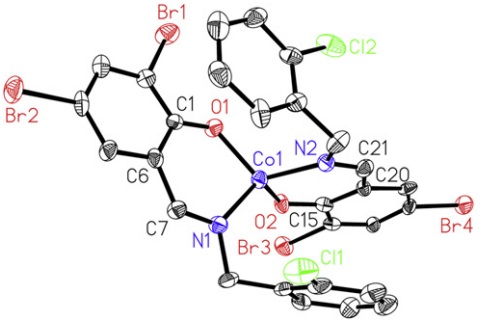

Fig. 1. An ORTEP diagram showing the structure of compound **2** with selected atom-labeling. The thermal ellipsoids plotted at 30% probability and H atoms omitted for the plot clarity.

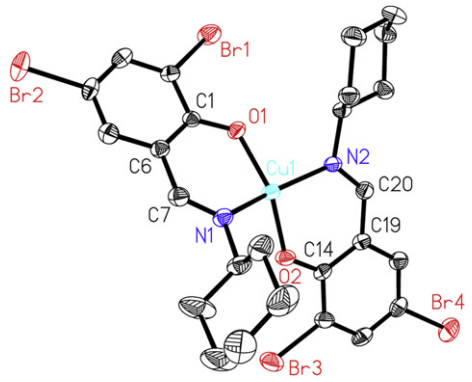

Fig. 3. An ORTEP diagram showing the structure of compound **4** with selected atom-labeling. The thermal ellipsoids plotted at 30% probability and H atoms omitted for the plot clarity.

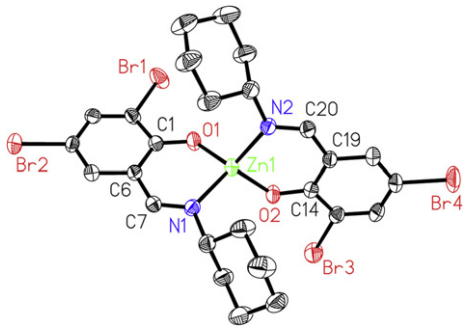

Fig. 4. An ORTEP diagram showing the structure of compound **5** with selected atom-labeling. The thermal ellipsoids plotted at 30% probability and H atoms omitted for the plot clarity.

154.81(15)°. The angle between two six-membered chelate planes was 39.4(2)°. In contrast, Zn(II) atom of complex **5** has a distorted-tetrahedral coordination, which is essentially similar to that of complexes **2** and **4**. The zinc(II) atom is four-coordinated by two N atoms and two O atoms from two Schiff base ligands (L^3), as shown in Fig. 4. Analogous tetrahedral Zn(II) species were previously reported in the literatures [13b,24]. The average bond distances of Zn–O and Zn–N are 1.908(3) and 1.999(3) Å, respectively. The angles subtended at the Zn(II) ion in the distorted-tetrahedral geometry (ZnN_2O_2) is in the range 96.31(11)–126.45(13)°. The angle between two six-membered chelate planes was 100.5(2)°.

Single crystal X-ray diffraction reveals that compound **6** crystallizes in monoclinic space group $P2_1/c$. Crystal structure of the mononuclear unit $[Zn(HL^4)_2]^{2+}$ is shown in Fig. 5. Compound **6** consists of the mononuclear unit $[Zn(HL^4)_2]^{2+}$, two nitrate counter ions and two lattice acetonitrile molecules. There are two independent mononuclear zinc(II) cations $[Zn(HL^4)_2]^{2+}$ in asymmetric unit. Each zinc(II) atom of compound **6**, lying on an inversion center (symmetry codes: $1-x, 2-y, -z$), is six-coordinated by two N atoms and four O atoms from two Schiff base ligands (L^4) and two water molecules. The bond distances of Zn1–O1, Zn1–O2 and Zn1–N1 are 2.014(3), 2.261(3), 2.124(3) Å. The bond distances of Zn2–O3, Zn2–O4 and Zn2–N3 are 1.995(3), 2.296(3), and 2.119(3) Å, respectively.

2.4. Inhibitory activity against jack bean urease

As shown in Table 1, transition metal ions as enzyme inhibitors exhibit different ability to inhibit urease, which follows the order: $Cu^{2+} > Ni^{2+} > Co^{2+} \approx Zn^{2+}$. This is in accords with inhibitory efficiency of metal ions toward urease following the order: $Cu^{2+} > Ni^{2+} > Co^{2+} > Zn^{2+}$, which has been reported in the literature [25,26]. Compared with the standard inhibitor acetohydroxamic acid ($IC_{50} = 42.12$ μM), the Schiff base ligands (HL^{1-4}) have no influence on

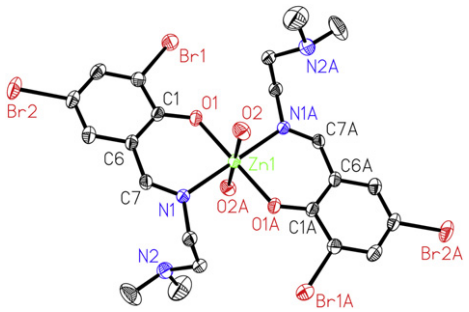

Fig. 5. An ORTEP diagram showing the structure of the cation $[Zn(HL^4)_2 \cdot 2H_2O]^{2+}$ in compound **6** with selected atom-labeling (symmetry codes: $1-x, 2-y, -z$). The thermal ellipsoids plotted at 30% probability and H atoms omitted for the plot clarity.

Table 1
Inhibition of *jack bean* urease by compounds **1**–**6**, Schiff base ligands and metal ions.

Tested materials	IC_{50} (μM)
HL^{1-4}	>100
Cu^{2+}	0.37
Ni^{2+}	2.87
M^{2+} (Co^{2+}, Zn^{2+})	>100
1	0.52
2	25.20
3	3.88
4	0.46
5	19.18
6	9.27
Acetohydroxamic acid	42.12

the activity of *jack bean* urease. Under the same condition, Schiff base Cu(II) complexes **1** and **4** showed better inhibitory activity with IC_{50} values of 0.52 and 0.46 μM, respectively. This is near to Schiff base Cu (II) complexes as the potent urease inhibitor with IC_{50} values of 0.43 μM [14a]. It should be noted that Co(II) complex **2** and Zn(II) complexes **5**, **6** possess the inhibitory activities although the corresponding metal ions had no inhibitory activities against urease. In contrast, both Ni(II) ion and its Schiff base complex **3** showed potent urease inhibitory activities. This indicates that inhibitory activities of Schiff base metal complexes as the urease inhibitor depend on not only the central ions but also the organic ligand.

2.5. Molecular docking study

The binding models of Schiff base copper(II) complexes **1** and **4** in the enzyme active site of urease were depicted in Figs. 6 and 7, respectively [27]. It's interesting that there is one kind of hydrogen

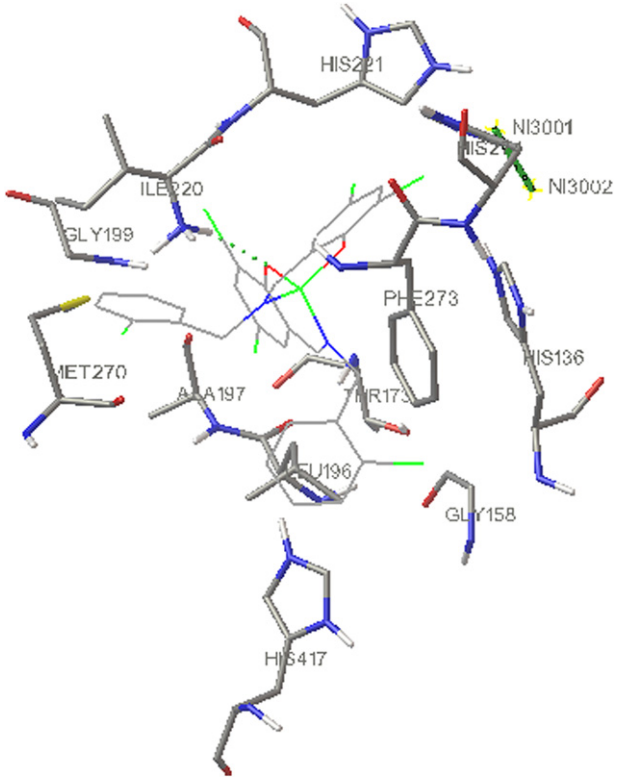

Fig. 6. Compound **1** (colored by atom: carbons–gray; nitrogens–blue; oxygens–red; bromine–green) is bound into urease (entry 1E9Z in the Protein Data Bank). The dotted lines show the hydrogen bond. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

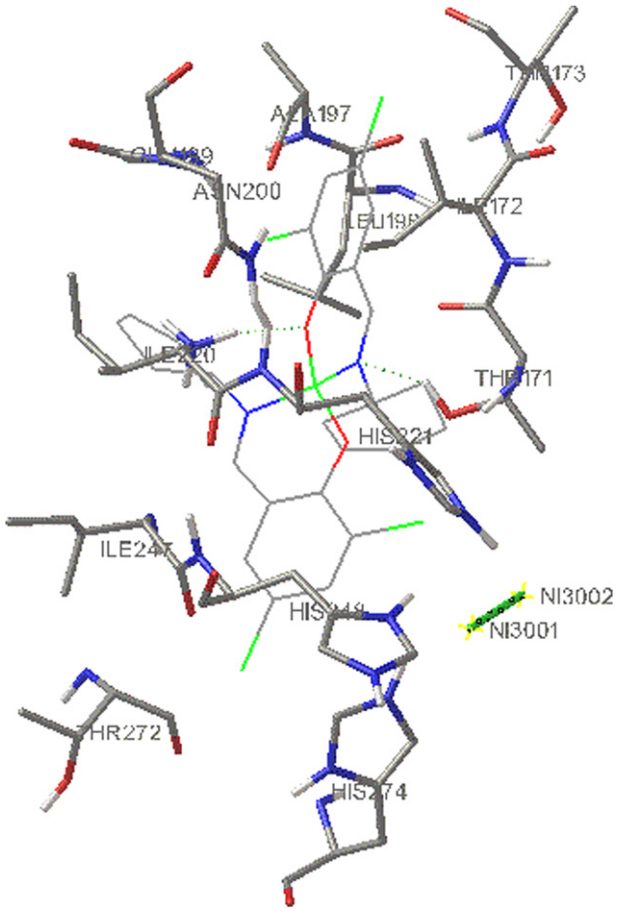

Fig. 7. Compound **4** (colored by atom: carbons–gray; nitrogens–blue; oxygens–red; bromine–green) is bound into urease (entry 1E9Z in the Protein Data Bank). The dotted lines show the hydrogen bond. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bond that is formed by the phenolic oxygen atom of complex **1** and the amino hydrogen of Ile220 (length of hydrogen bond: Ile220 N–H···$O_{complex\text{-}1}$ = 1.908 Å ; angle of hydrogen bond: Ile220 N–H···$O_{complex\text{-}1}$ = 152.941°). In contrast, there are two kinds of hydrogen bonds in the binding model of complex **4** (Fig. 7). One kind was formed by the phenolic oxygen atom of complex **4** and hydroxy of THR171 (length of hydrogen bond: THR171O–H···$N_{complex\text{-}4}$ = 1.753 Å ; angle of hydrogen bond: THR171O–H···$N_{complex\text{-}4}$ = 141.5°). And the other was composed of amino hydrogen of ILE220 and the nitrogen atom of complex **4** (length of hydrogen bond: ILE220N–H···$O_{complex\text{-}4}$ = 2.159 Å ; angle of the hydrogen bond: ILE220N–H···$O_{complex\text{-}4}$ = 147.7°). In addition, complex **1** may form hydrophobic interactions with Ala197, Leu196 and Phe273 of urease. In comparison to **1**–urease interactions, complex **4** may also form hydrophobic interactions with Ile172, Leu196, Ala197, Ile220 and Ile247. The result of molecular docking study could explain the better inhibitory activity of **1** and **4** against *jack bean* urease.

3. Experimental section

3.1. Materials and measurements

3,5-Dibromosalicyladehyde, cyclohexylamine, 2-chlorobenzylamine, benzylamine, were purchased from Aldrich and used without further purification. Elemental analyses for C, H and N were carried out on a Perkin–Elmer 2400 analyzer. IR spectra were recorded using KBr pellets (4000–400 cm^{-1}) on a Nexus 870 FT-IR spectrophotometer. Electronic spectra in the 200–800 nm range were measured using DMSO–H_2O (1:1 v/v) solution on a Shimadzu UV-160 Aspectrophotometer.

3.2. Compounds synthesis

General procedure for the synthesis of compounds **1**–**6**: Primary amine (0.2 mmol) was added to the solution of 3,5-dibromosalicyladehyde (56 mg, 0.2 mmol) in an aqueous acetonitrile solution (5 mL). The mixture was stirred for 5 min to give an orange solution, which was added to a methanol solution (1 mL) of $M(NO_3)_2 \times H_2O$ (0.1 mmol) (M = Cu, $x = 3$; M = Co, Ni, Zn, $x = 6$). The mixture was stirred for another 5 min at room temperature to give a clear solution and then filtered. The filtrate was kept in air for about a week, forming block crystals. The crystals were isolated, washed three times with distilled water and dried in a vacuum desiccator containing anhydrous $CaCl_2$.

3.2.1. $[Cu(L^1)_2]$ (**1**)

Black solid, yield: 40.8 mg (47%). IR (KBr, cm^{-1}): 3020, 2966, 2947, 1612, 1514, 1450, 1163, 864, 756, 717, 592, 522, 476. UV–Vis (DMSO–H_2O, k/nm): 243, 257, 378. Anal. Calcd. for $C_{28}H_{18}Br_4Cl_2CuN_2O_2$: C, 38.72; H, 2.09; N, 3.23. Found: C, 38.56; H, 2.25; N, 3.11%.

Table 2
Crystal data for compounds **2**–**6**.

Compounds	**2**	**3**	**4**	**5**·CH_3CN	**6**·$2CH_3CN$
Empirical formula	$C_{28}H_{18}Br_4Cl_2CoN_2O_2$	$C_{28}H_{20}Br_4NiN_2O_2$	$C_{26}H_{27}Br_4CuN_2O_2$	$C_{28}H_{31}Br_4ZnN_3O_2$	$C_{26}H_{38}Br_4ZnN_8O_{10}$
Molecular weight	863.92	794.81	782.68	826.57	1007.66
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic	Triclinic
Space group	$P2_1/c$	$P2_1/c$	$P2_1/c$	$P2_1/n$	P-1
a (Å)	16.4449(7)	10.6062(6)	15.309(3)	9.4743(3)	7.5691(3)
b (Å)	12.5497(5)	6.1056(4)	12.858(3)	16.3900(5)	14.8691(5)
c (Å)	31.6709(12)	20.7125(15)	14.161(3)	20.4619(6)	17.6475(6)
α (°)	90	90	90.00	90	78.688(2)
β (°)	116.179(2)	102.431(3)	93.24(3)	91.8410(10)	84.203(2)
γ (°)	90	90	90.00	90	80.706(2)
T (K)	291(2)	291(2)	293(2)	291(2)	291(2)
V (Å^3)	5865.7(4)	1309.84(15)	2783.0(10)	3175.76(17)	1917.06(12)
Z	4	2	4	4	1
$\rho_{calc.}$ (g cm^{-3})	1.957	2.015	1.868	1.729	1.746
F(000)	3336	772	1528	1624	1000
μ(Mo-Kα) (mm^{-1})	6.247	6.872	6.554	5.835	4.868
Data/restraint/parameters	11565/31/703	2712/0/169	6906/0/316	6556/0/350	7409/0/452
Goodness-of-fit on F^2	1.445	1.049	1.010	1.021	1.007
Final R_1, wR_2 [$I > 2\sigma(I)$]	0.0689, 0.1322	0.0432, 0.1081	0.0467, 0.0909	0.0402, 0.1049	0.0436, 0.1020

Table 3
Selected bond lengths [Å] and angles [°] in compounds **2**–**6**.

2			
Co(1)–O(1)	1.897(8)	Co(2)–O(4)	1.899(8)
Co(1)–O(2)	1.908(8)	Co(2)–O(3)	1.903(9)
Co(1)–N(2)	1.976(11)	Co(2)–N(4)	2.002(11)
Co(1)–N(1)	2.005(10)	Co(2)–N(3)	2.018(10)
O(1)–Co(1)–O(2)	118.9(4)	O(4)–Co(2)–O(3)	124.4(4)
O(1)–Co(1)–N(2)	115.5(4)	O(4)–Co(2)–N(4)	93.6(4)
O(2)–Co(1)–N(2)	95.8(4)	O(3)–Co(2)–N(4)	108.7(4)
O(1)–Co(1)–N(1)	95.5(4)	O(4)–Co(2)–N(3)	119.9(4)
O(2)–Co(1)–N(1)	118.2(4)	O(3)–Co(2)–N(3)	95.0(4)
N(2)–Co(1)–N(1)	114.4(4)	N(4)–Co(2)–N(3)	116.3(4)
3 (a: 1 − *x*, 1 − *y*, −*z*)			
Ni(1)–O(1)	1.847(3)	Ni(1)–O(1)^a	1.847(3)
Ni(1)–N(1)	1.944(4)	Ni(1)–N(1)^a	1.944(4)
O(1)–Ni(1)–O(1)^a	180.00(18)	O(1)–Ni(1)–N(1)^a	88.03(15)
O(1)–Ni(1)–N(1)	91.97(15)	O(1)^a–Ni(1)–N(1)^a	91.97(15)
O(1)^a–Ni(1)–N(1)	88.03(15)	N(1)–Ni(1)–N(1)^a	180.0(2)
4			
Cu(1)–O(1)	1.898(3)	Cu(1)–N(1)	1.975(4)
Cu(1)–O(2)	1.882(3)	Cu(1)–N(2)	1.964(4)
O(1)–Cu(1)–O(2)	151.85(14)	O(1)–Cu(1)–N(1)	92.94(13)
O(1)–Cu(1)–N(2)	93.30(13)	O(2)–Cu(1)–N(1)	91.28(13)
O(2)–Cu(1)–N(2)	94.62(13)	N(2)–Cu(1)–N(1)	154.81(15)
5			
Zn(1)–O(1)	1.905(3)	Zn(1)–N(1)	1.999(3)
Zn(1)–O(2)	1.911(3)	Zn(1)–N(2)	1.998(3)
O(1)–Zn(1)–O(2)	115.97(12)	O(1)–Zn(1)–N(1)	96.31(11)
O(1)–Zn(1)–N(2)	111.50(12)	O(2)–Zn(1)–N(1)	111.31(12)
O(2)–Zn(1)–N(2)	96.63(11)	N(2)–Zn(1)–N(1)	126.45(13)
6 (a: 1 − *x*, 2 − *y*, −z; b: 2 − *x*, 1 − *y*, 1 − *z*)			
Zn(1)–O(1)^a	2.014(3)	Zn(2)–O(3)	1.995(3)
Zn(1)–O(1)	2.014(3)	Zn(2)–O(3)^b	1.995(3)
Zn(1)–N(1)^a	2.124(3)	Zn(2)–N(3)	2.119(3)
Zn(1)–N(1)	2.124(3)	Zn(2)–N(3)^b	2.119(3)
Zn(1)–O(2)	2.261(3)	Zn(2)–O(4b)	2.296(3)
Zn(1)–O(2)	2.261(3)	Zn(2)–O(4)	2.296(3)
O(1)^a–Zn(1)–O(1)	180.00(1)	O(3)–Zn(2)–O(3)^b	180.00(1)
O(1)^a–Zn(1)–N(1)^a	88.37(11)	O(3)–Zn(2)–N(3)	88.92(11)
O(1)–Zn(1)–N(1)^a	91.63(11)	O(3)^b–Zn(2)–N(3)	91.08(11)
O(1)^a–Zn(1)–N(1)	91.63(11)	O(3)–Zn(2)–N(3)^b	91.08(11)
O(1)–Zn(1)–N(1)	88.37(11)	O(3)^b–Zn(2)–N(3)^b	88.92(11)
N(1)^a–Zn(1)–N(1)	180.00(19)	N(3)–Zn(2)–N(3)^b	180.00(1)
O(1)^a–Zn(1)–O(2)^a	90.77(12)	O(3)–Zn(2)–O(4)^b	89.11(12)
O(1)–Zn(1)–O(2)^a	89.23(12)	O(3)^b–Zn(2)–O(4)^b	90.89(12)
N(1)^a–Zn(1)–O(2)^a	89.65(11)	N(3)–Zn(2)–O(4)^b	92.58(11)
N(1)–Zn(1)–O(2)^a	90.35(11)	N(3)^b–Zn(2)–O(4)^b	87.42(11)
O(1)^a–Zn(1)–O(2)	89.23(12)	O(3)–Zn(2)–O(4)	90.89(12)
O(1)–Zn(1)–O(2)	90.77(12)	O(3)^b–Zn(2)–O(4)	89.11(12)
N(1)^a–Zn(1)–O(2)	90.35(11)	N(3)–Zn(2)–O(4)	87.42(11)
N(1)–Zn(1)–O(2)	89.65(11)	N(3)^b–Zn(2)–O(4)	92.58(11)
O(2)^a–Zn(1)–O(2)	180.00(13)	O(4b)–Zn(2)–O(4)	180.00(13)

a and b are for the symmetry related position.

3.2.2. $[Co(L^1)_2]$ (**2**)

Red solid, yield: 60.4 mg (70%). IR (KBr, cm^{-1}): 3065, 2960, 2779, 1642, 1620, 1580, 1450, 1222, 1153, 865, 710, 580, 561, 450. UV–Vis (DMSO–H_2O, k/nm): 246, 272, 370. Anal. Calcd. for $C_{28}H_{18}Br_4Cl_2CoN_2O_2$: C, 38.93; H, 2.10; N, 3.24. Found: C, 39.82; H, 2.30; N, 3.41%.

3.2.3. $[Ni(L^2)_2]$ (**3**)

Brown solid, yield: 23.8 mg (30%). IR (KBr, cm^{-1}): 3028, 2926, 2868, 1618, 1517, 1496, 1444, 1168, 871, 746, 717, 607, 555, 457. UV–Vis (DMSO–H_2O, k/nm): 256, 267, 396. Anal. Calcd. for $C_{28}H_{20}Br_4N_2NiO_2$: C, 42.31; H, 2.54; N, 3.52. Found: C, 42.20; H, 2.99; N, 3.41%.

3.2.4. $[Cu(L^3)_2]$ (**4**)

Black solid, yield: 41.5 mg (53%). IR (KBr, cm^{-1}): 2939, 2858, 1642, 1624, 1514, 1448, 1210, 1155, 864, 559, 561, 478. UV–Vis (DMSO–H_2O, k/nm): 243, 276, 384. Anal. Calcd. for $C_{26}H_{28}Br_4CuN_2O_2$: C, 39.85; H, 3.60; N, 3.57. Found: C, 39.70; H, 4.21; N, 3.65%.

3.2.5. $[Zn(L^3)_2]$ (**5**)

Yellow solid, yield: 23.5 mg (30%). IR (KBr, cm^{-1}): 3064, 3030, 2927, 2873, 1635, 1633, 1508, 1446, 1398, 1020, 1153, 866, 597, 547, 501. UV–Vis (DMSO–H_2O, k/nm): 243, 274, 382. Anal. Calcd. for **5**·CH_3CN, $C_{28}H_{31}Br_4ZnN_3O_2$: C, 40.69; H, 3.78; N, 5.08. Found: C, 40.23; H, 3.99; N, 5.00%.

3.2.6. $[Zn(HL^4)_2{\cdot}2H_2O]{\cdot}2NO_3$ (**6**)

Yellow solid, yield: 63.8 mg (69%). IR (KBr, cm^{-1}): 3456, 3062, 2960, 2789, 1633, 1456, 1219, 1143, 875, 704, 567, 460. UV–Vis (DMSO–H_2O, k/nm): 251, 284, 386. Anal. Calcd. for **6**·$2CH_3CN$, $C_{26}H_{38}Br_4ZnN_8O_{10}$: C, 30.99; H, 3.80; N, 11.12. Found: C, 30.55; H, 3.52; N, 11.00%.

3.3. 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 was carried out according to the literature reported by Tanaka et al. [28]. Generally, the assay mixture, containing 25 μL of *jack bean* urease (10 kU/L) and 25 μL of the tested complexes of various concentrations (dissolved in the solution of DMSO:H_2O = 1:1 (v/v)), was preincubated for 1 h at 37 °C in a 96-well assay plate. After preincubation, 0.2 mL of 100 mM HEPES (N-[2-hydroxy-ethyl] piperazine-N′-[2-ethanesulfonic acid]) buffer [29] pH = 6.8 containing 500 mM urea and 0.002% phenol red were added and incubated at 37 °C. The reaction time was measured by micro plate 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 end-point being determined by the color of phenol red indicator [30].

The abilities of the Schiff base ligands $HL^{1–4}$, metal ions (M = Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}) and compounds **1**–**6** as the inhibitors were studied by the IC_{50} values of the material (25 μL, 100 μg) tested against *jack bean* urease (25 μL, 10 kU/L) using urea (500 mM) in HEPES buffer (0.2 mL, 100 mM; pH = 6.8) (Table 1).

3.4. Crystal structure determinations

Diffraction intensities for compounds **2**–**6** were collected at 291 (2) and 293(2) K using a Bruker SMART CCD area detector with Mo-Kα radiation (λ = 0.71073 Å). The collected data were reduced using the SAINT program [31], and empirical absorption corrections were performed using the SADABS program [32]. The structures were solved by direct methods and refined against F^2 by full-matrix least-squares methods using the SHELXTL version 5.1 [33]. All of the non-hydrogen atoms were refined anisotropically. All other hydrogen atoms were placed in geometrically ideal positions and constrained to ride on their parent atoms. The crystallographic data for compounds **2**–**6** are summarized in Table 2. Selected bond lengths and angles are given in Table 3.

4. Conclusions

Six transition metal compounds of Schiff base ligands were evaluated for the inhibitory activity on *jack bean* urease. It was found that most of them have inhibitory activity against *jack bean* urease. It is noteworthy that copper(II) complexes **1** and **4** exhibit stronger activity to inhibit *jack bean* urease (IC_{50} = 0.52 and 0.46 μM) than those of the nickel(II), cobalt(II) and zinc(II) compounds (IC_{50} = 3.88–25.20 μM). A docking analysis using the AUTODOCK 4.0 program could explain the inhibitory activities of compounds **1** and

4 against urease. The trend in this work is in accord with the studies reported earlier. Detailed investigations are continuing to study the mechanisms of the inhibitory activity reported here.

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Supplementary data

Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre Nos. 754514 (**2**), 754513 (**3**), 759980 (**4**), 754515 (**5**) and 754516 (**6**). 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; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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