

席夫碱双氧钒(V)配合物的合成、结构及其抑制 幽门螺旋杆菌脲酶研究

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摘要: 本文合成了 2 个双氧钒(V)配合物, $[\text{VO}_2\text{L}^1]$ (**1**)和 $[\text{VO}_2\text{L}^2]_2$ (**2**)($\text{L}^1=4\text{-氯-2-}[(2\text{-苯胺基乙亚胺基})\text{甲基}]\text{苯酚盐}$; $\text{L}^2=4\text{-}[(2\text{-}[(1\text{-}(5\text{-氯-2-羟基苯基})\text{甲亚胺基})\text{胺基}]\text{乙亚胺基})\text{-2-戊酮})]$, 并通过物理化学方法和单晶 X-射线衍射表征了它们的结构。在单核配合物 **1** 中, V 原子采取畸变的四方锥配位构型, 在双核配合物 **2** 中, V 原子采取八面体配位构型。研究了这 2 个配合物对幽门螺旋杆菌脲酶的抑制活性。在浓度为 $100\ \mu\text{mol}\cdot\text{L}^{-1}$ 时, 配合物 **1** 和 **2** 对脲酶的抑制率分别为 $(52.1\pm 1.8)\%$ 和 $(34.2\pm 3.3)\%$ 。还做了配合物和幽门螺旋杆菌脲酶的分子对接研究。配合物的结构和其抑制脲酶活性的关系表明, 配合物分子的尺寸和形状对脲酶的抑制作用具有重要影响。

关键词: 席夫碱; 钒氧(V)配合物; 晶体结构; 抑制脲酶

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Synthesis and Structures of Dioxovanadium(V) Complexes with Schiff Bases and Their Inhibition Studies on Helicobacter Pylori Urease

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Abstract: Two dioxovanadium(V) complexes, $[\text{VO}_2\text{L}^1]$ (**1**) and $[\text{VO}_2\text{L}^2]_2$ (**2**) ($\text{L}^1=4\text{-chloro-2-}[(2\text{-phenylaminoethylimino})\text{methyl}]\text{phenolate}$; $\text{L}^2=4\text{-}[(2\text{-}[(1\text{-}(5\text{-chloro-2-hydroxyphenyl})\text{methylidene})\text{amino}]\text{ethylamino})\text{ethylimino}]\text{pentan-3-en-2-one}$), have been synthesized and characterized by physico-chemical methods and single-crystal X-ray diffraction. The V atom in the mononuclear complex **1** is in distorted square pyramidal coordination, and those in the dinuclear complex **2** are in octahedral coordination. The complexes were tested for their Helicobacter pylori urease inhibitory activity. The inhibition rate of the complexes **1** and **2** at the concentration of $100\ \mu\text{mol}\cdot\text{L}^{-1}$ against urease are $(52.1\pm 1.8)\%$, and $(34.2\pm 3.3)\%$. The molecular docking study of the complexes with the Helicobacter pylori urease was performed. The relationship between the structures of the complexes and the urease inhibitory activities indicates that the size and shape of the complex molecules play an important role for the inhibition. CCDC: 832899, **1**; 832900, **2**.

Key words: Schiff base; oxovanadium(V) complex; crystal structure; urease inhibition

Urease (urea amidohydrolase; E.C.3.5.1.5) is a nickel-containing metalloenzyme that catalyzes the hydrolysis of urea to form ammonia and carbamate^[1-2]. The resulting carbamate spontaneously decomposes to

yield a second molecule of ammonia and carbon dioxide. High concentration of ammonia arising from these reactions, as well as the accompanying pH elevation, has important negative implications in

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medicine and agriculture^[3-6]. Control of the activity of urease through the use of inhibitors could counteract these negative effects. In recent years, urease inhibitors play an important role in the treatment of the infections caused by urease producing bacteria^[7]. Inhibitors of urease can be broadly classified into two fields: (1) organic compounds, such as acetohydroxamic acid, humic acid, and 1,4-benzoquinone^[8-10]; (2) heavy metal ions, such as Cu^{2+} , Zn^{2+} , Pd^{2+} , and Cd^{2+} ^[11-12]. The complexes containing metal atoms as urease inhibitors have seldom been reported, which indicates that the organotin(IV), bismuth(III), copper(II), and cadmium(II) complexes bearing interesting urease inhibitory activities^[13-19]. Vanadium complexes have been widely investigated in biological chemistry, especially for their insulin-enhancing activities. After searching the literature, we noticed that the vanadium complexes also possess interesting urease inhibitory activity^[20]. As an extensive study on the oxovanadium complexes and their urease inhibitory activity, in this paper, two new dioxovanadium(V) complexes, $[\text{VO}_2\text{L}^1]$ (**1**) and $[\text{VO}_2\text{L}^2]_2$ (**2**), where L^1 is 4-chloro-2-[(2-phenylaminoethylimino)methyl]phenolate, and L^2 is 4-[2-(2-[[1-(5-chloro-2-hydroxyphenyl)methylidene]amino]ethylamino)ethylimino]pentan-3-en-2-one), have been synthesized and structurally characterized. The urease inhibitory activity of the complexes and the molecular docking analysis of the complexes with *Helicobacter pylori* urease were investigated.

1 Experimental

1.1 General methods and materials

Starting materials, reagents and solvents were purchased from commercial suppliers and purified before use. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer. The IR spectra were recorded on a Jasco FT/IR-4000 spectrometer as KBr pellets in the $4\,000\sim 200\text{ cm}^{-1}$ region. The ^1H NMR spectra were recorded on a Bruker AVANCE 400 MHz spectrometer with tetramethylsilane as the internal reference. Molar conductance was measured with a Shanghai DDS-11A conductometer. X-ray diffraction was carried out on a Bruker SMART 1000 CCD

diffractometer.

1.2 Synthesis of the complexes

$[\text{VO}_2\text{L}^1]$ (**1**): 4-Chlorosalicylaldehyde (0.156 g, 1 mmol) and *N*-phenylethane-1,2-diamine (0.136 g, 1 mmol) were mixed in methanol (30 mL). The mixture was stirred at ambient temperature for 30 min to give yellow solution. To the solution was added with stirring a methanolic solution (15 mL) of $\text{VO}(\text{acac})_2$ (0.27 g, 1 mmol). The final mixture was further stirred for 30 min at ambient temperature to give a deep brown solution. Upon standing at room temperature, brown block-shaped crystals of **1**, suitable for X-ray crystal structural determination were formed. The crystals were isolated, washed three times with cold methanol and dried in air. Yield 53%. IR data (cm^{-1}): 3 221 (sh, w), 1 628 (s), 1 602 (m), 1 541 (m), 1 494 (m), 1 466 (s), 1 385 (w), 1 304 (m), 1 202 (w), 1 183 (m), 1 135 (w), 1 092 (w), 1 044 (w), 928 (s), 825 (m), 755 (m), 710 (m), 688 (w), 660 (w), 543 (m), 467 (w), 405 (w). Anal. Calcd. for $\text{C}_{15}\text{H}_{14}\text{ClN}_2\text{O}_3\text{V}$ (%): C, 50.5; H, 4.0; N, 7.9. Found (%): C, 50.2; H, 4.1; N, 7.8.

$[\text{VO}_2\text{L}^2]_2$ (**2**): 4-Chlorosalicylaldehyde (0.156 g, 1 mmol) dissolved in methanol (20 mL) was added slowly to the methanolic solution (30 mL) of diethylenetriamine (0.136 g, 1 mmol) with continuous stirring for 30 min. To the yellow solution was added with stirring a methanolic solution (15 mL) of $\text{VO}(\text{acac})_2$ (0.27 g, 1 mmol). The final mixture was further stirred for 30 min at ambient temperature to give a deep brown solution. Upon standing at room temperature, brown block-shaped crystals of **2**, suitable for X-ray crystal structural determination were formed. The crystals were isolated, washed three times with cold methanol and dried in air. Yield 63%. IR data (cm^{-1}): 3 225 (sh, w), 1 634 (s), 1 575 (m), 1 539 (m), 1 465 (m), 1 420 (w), 1 383 (m), 1 303 (m), 1 280 (m), 1 185 (w), 1 092 (w), 1 009 (w), 930 (s), 843 (m), 711 (m), 661 (w), 646 (w), 558 (w), 471 (w). Anal. Calcd. for $\text{C}_{32}\text{H}_{42}\text{Cl}_2\text{N}_6\text{O}_8\text{V}_2$ (%): C, 47.4; H, 5.2; N, 10.4. Found(%): C, 47.7; H, 5.3; N, 10.2.

1.3 X-ray crystallography

Diffraction intensities for the complexes were collected at 298(2) K using a Bruker SMART 1000 CCD area-detector diffractometer with $\text{Mo K}\alpha$ radiation

($\lambda = 0.071\ 073\ \text{nm}$). The collected data were reduced with the SAINT program^[21], and multi-scan absorption correction was performed using the SADABS program^[22]. The structures were solved by direct method. The complexes were refined against F^2 by full-matrix least-squares method using the SHELXTL package^[23]. All of the non-hydrogen atoms were refined anisotropically. The amino H atoms in the complexes were located from difference Fourier maps and refined isotropically,

with N-H distances restrained to $0.090(1)\ \text{nm}$. The remaining hydrogen atoms were placed in calculated 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. Crystallographic data for the complexes have been deposited with the Cambridge Crystallographic Data Centre.

CCDC: 832899, **1**; 832900, **2**.

Table 1 Crystal data for the complexes

Complex	1	2
Formula	$\text{C}_{15}\text{H}_{14}\text{ClN}_2\text{O}_3\text{V}$	$\text{C}_{32}\text{H}_{42}\text{Cl}_2\text{N}_6\text{O}_8\text{V}_2$
M_r	356.7	811.5
T / K	298(2)	298(2)
Crystal shape / color	Block / brown	Block / brown
Crystal size / mm	0.32×0.30×0.30	0.23×0.20×0.20
Crystal system	Monoclinic	Monoclinic
Space group	Pc	$P2_1/c$
a / nm	1.264 5(3)	1.361 3(3)
b / nm	0.509 4(1)	0.878 8(2)
c / nm	1.209 4(2)	3.136 1(3)
$\beta / (^\circ)$	92.97(3)	96.002(3)
V / nm^3	0.778 0(3)	3.731 2(12)
Z	2	4
$D_c / (\text{g} \cdot \text{cm}^{-3})$	1.523	1.445
$\mu (\text{Mo } K\alpha) / \text{mm}^{-1}$	0.822	0.700
$F(000)$	364	1 680
Reflections collected	5 592	29 296
Independent reflections (R_{int})	3 137 (0.041 2)	6 559 (0.090 2)
Observed reflections ($I \geq 2\sigma(I)$)	2096	3560
Min. and max. transmission	0.779 and 0.791	0.856 and 0.873
Parameters	203	467
Restraints	3	4
Goodness-of-fit on F^2	1.042	1.007
$R_1, wR_2 (I \geq 2\sigma(I))$	0.054 5, 0.096 0	0.070 1, 0.135 3
R_1, wR_2 (all data)	0.088 8, 0.110 1	0.138 5, 0.166 4

Table 2 Selected bond lengths (nm) and angles ($^\circ$) for the complexes

1					
V1-O1	0.188 4(4)	V1-O2	0.160 4(3)	V1-O3	0.161 2(3)
V1-N1	0.213 3(5)	V1-N2	0.217 3(4)		
O2-V1-O3	110.1(2)	O2-V1-O1	98.5(2)	O3-V1-O1	102.5(2)
O2-V1-N1	133.5(2)	O3-V1-N1	114.8(2)	O1-V1-N1	83.2(2)
O2-V1-N2	90.4(2)	O3-V1-N2	93.2(2)	O1-V1-N2	157.8(2)
N1-V1-N2	76.0(2)				

Continued Table 2

2					
V1-O1	0.189 5(4)	V1-N1	0.215 4(4)	V1-N2	0.215 2(5)
V1-O5	0.234 9(3)	V1-O6	0.166 2(3)	V1-O7	0.160 3(4)
V2-O3	0.190 2(4)	V2-N4	0.216 0(4)	V2-N5	0.215 8(5)
V2-O5	0.166 9(3)	V2-O6	0.231 2(3)	V2-O8	0.160 5(4)
O7-V1-O6	106.5(2)	O7-V1-O1	102.4(2)	O6-V1-O1	98.8(2)
O7-V1-N2	93.0(2)	O6-V1-N2	93.4(2)	O1-V1-N2	156.6(2)
O7-V1-N1	96.1(2)	O6-V1-N1	155.8(2)	O1-V1-N1	84.2(2)
N2-V1-N1	76.6(2)	O7-V1-O5	171.1(2)	O6-V1-O5	78.1(2)
O1-V1-O5	84.1(2)	N2-V1-O5	79.0(2)	N1-V1-O5	78.3(2)
O8-V2-O5	107.1(2)	O8-V2-O3	101.6(2)	O5-V2-O3	99.0(2)
O8-V2-N5	92.8(2)	O5-V2-N5	93.9(2)	O3-V2-N5	156.8(2)
O8-V2-N4	96.2(2)	O5-V2-N4	155.4(2)	O3-V2-N4	83.8(2)
N5-V2-N4	76.7(2)	O8-V2-O6	169.2(2)	O5-V2-O6	79.1(2)
O3-V2-O6	85.9(2)	N5-V2-O6	77.7(2)	N4-V2-O6	76.7(2)

1.4 Urease inhibitory activity assay

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 condition (5% O₂, 10% CO₂, and 85% N₂). The method of preparation of *Helicobacter pylori* urease by Mao^[24] was followed. Briefly, broth cultures (50 mL, 2.0×10⁸ CFU·mL⁻¹) were centrifuged (5 000 g, 4 °C) to collect the bacteria, and after washing twice with phosphate-buffered saline (pH 7.4), the *Helicobacter pylori* precipitation was stored at -80 °C. While the *Helicobacter pylori* was returned to room temperature, and mixed with 3 mL of distilled water and protease inhibitors, sonication was performed for 60 s. Following centrifugation (15 000 g, 4 °C), the supernatant was desalted through SephadexG-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 (4U) of *Helicobacter pylori* urease and 25 μL of the test compound, was pre-incubated 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^[25].

1.5 Molecular docking study

Molecular docking of the complexes into the 3D X-ray structures of *Helicobacter pylori* urease structure (entry 1E9Y in the Protein Data Bank) was carried out by using the AutoDock 4.2 software as implemented through the graphical user interface AutoDockTools (ADT 1.5.4).

The graphical user interface Auto Dock Tools was employed to setup the enzymes: all hydrogens were added, Gasteiger charges were calculated and non-polar hydrogens were merged to carbon atoms. The Ni initial parameters are set as $r=0.117$ nm, $q=+2.0$, and van der Waals well depth of 0.418 kJ·mol⁻¹^[26]. The Auto Dock Tools was used to generate the docking input files. In the docking grid box size of 6.6 nm×5.0 nm×5.0 nm for **1** and 11.0 nm×8.0 nm×7.0 nm for **2** points in x , y , and z directions were built, the maps were centered on the original ligand molecule in the catalytic site of the protein. A grid spacing of 0.037 5 nm and a distances-dependent function of the dielectric constant were used for the calculation of the energetic map. 100 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 2.5×10⁶ energy evaluations, and a maximum number of 2.7×10⁴ generations. A mutation rate of 0.02 and a crossover rate of 0.8 were chosen.

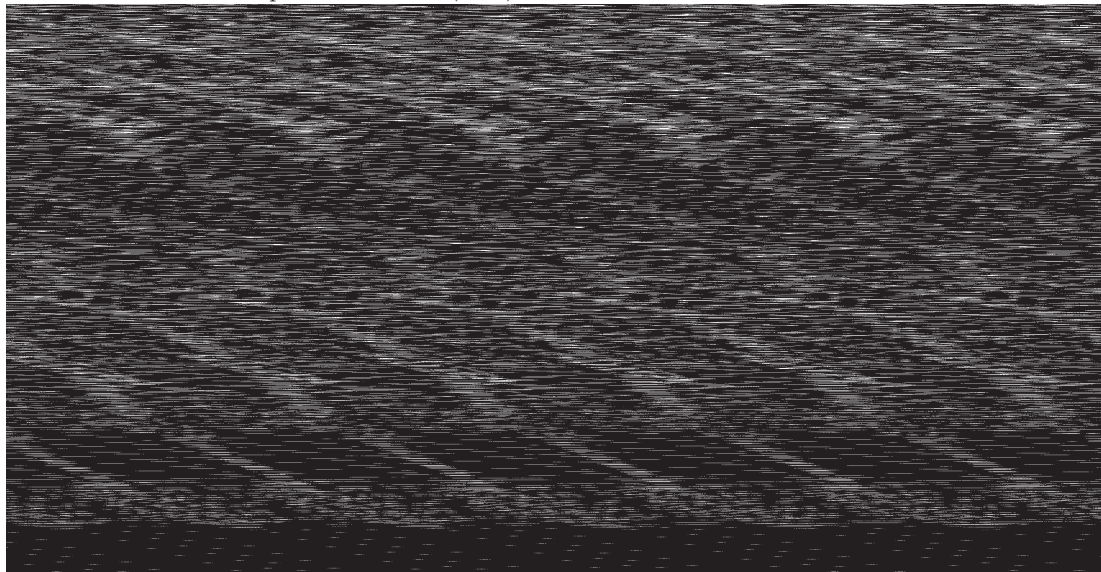
The results of the most favorable free energy of binding were selected as the resultant complex structures.

2 Results and discussion

2.1 Chemistry

The Schiff bases 4-chloro-2-[(2-phenylaminoethylimino)methyl]phenol and 2-[[2-(2-aminoethylamino)ethylimino)methyl]-4-chlorophenol were prepared by the reaction of 5-chlorosalicylaldehyde with *N*-phenylethane-1,2-diamine and diethylenetriamine, respectively, in methanol. The Schiff bases were not isolated and purified, which were further used to prepare the dioxovanadium complexes with VO(acac)₂

(Scheme 1). It is interesting that the amino group of 2-[[2-(2-aminoethylamino)ethylimino)methyl]-4-chlorophenol was condensed with the carbonyl group of VO(acac)₂, generating a new ligand 4-[2-(2-[[1-(5-chloro-2-hydroxyphenyl)methylidene]amino)ethylamino)ethylimino] pentan-3-en-2-one. The single crystals were obtained by slow evaporation of the methanolic solution of the complexes. Both complexes are stable in air at room temperature. The molar conductivity of the complexes measured in methanol at concentration of approximately 10⁻³ mol·L⁻¹ are 18 Ω⁻¹·cm²·mol⁻¹ for **1**, and 30 Ω⁻¹·cm²·mol⁻¹ for **2**, indicating the non-electrolytic nature of the complexes in solution^[27].



Scheme 1 Preparation of the complexes

2.2 Structure description of the complexes

The molecular structure of the complex **1** is shown in Fig.1. X-ray crystallography reveals that the complex is a mononuclear dioxovanadium(V) compound. The V atom is five-coordinated through two bonds to oxo groups and through three bonds to the Schiff base ligand L¹. The Schiff base serves as a tridentate ligand to form a five- and a six-membered chelate rings with the V atom in the complex. The coordination geometry around the metal center in each of the complexes can be best described as a distorted square pyramid, with τ parameter of 0.40 ($\tau=0$ for an ideal square pyramid; $\tau=1$ for an ideal trigonal bipyramid). The three donor atoms of the Schiff base ligand and one oxo O atom define the basal plane of the square pyramidal

geometry, and the other oxo O atom occupies the apical position. The V atom lies 0.047 9(2) nm from the least-squares plane of the basal donor atoms, in the direction of the axial oxo ligand. The bond distances between the V atom and the oxo O atoms indicate that they are

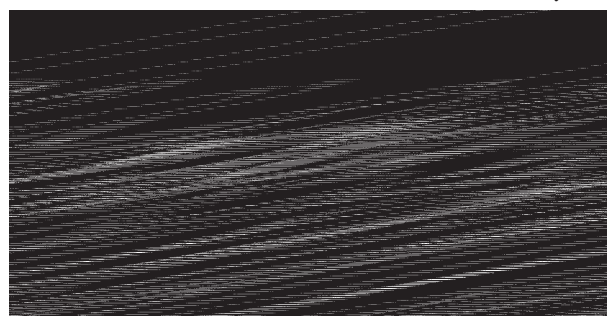
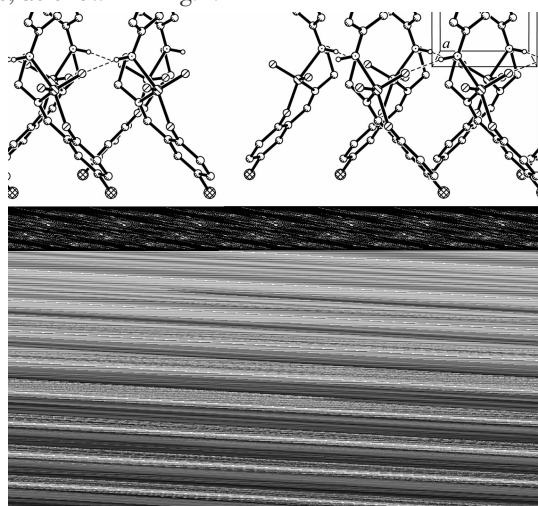


Fig.1 A perspective view of the molecular structure of **1** with the thermal ellipsoids drawn at 30% probability level

typical double bonds. The coordinate bond distances and angles in the complex are comparable to those observed in other similar oxovanadium(V) complexes with Schiff bases^[28-29]. In the crystal structure of **1**, molecules are linked through intermolecular N-H...O hydrogen bonds, to form chains running along the *b* axis, as shown in Fig.2.



Hydrogen bonds are shown as dashed lines; H atoms not involved in hydrogen bonding are omitted for clarity; Symmetry code: i x , $1+y$, z

Fig.2 Molecular packing of **1** viewed along the *c* axis

The molecular structure of the complex **2** is shown in Fig.3. X-ray crystallography reveals that the complex is a dinuclear dioxovanadium(V) compound, with the V...V distance of 0.3122 (1) nm. There are two N-H...O hydrogen bonds between the two Schiff base ligands. Each V atom in the complex is six-coordinated through three bonds to oxo groups and through three bonds to the Schiff base ligand L², forming an octahedral geometry. The distances between atoms V1 and O6, V1 and O7, V2 and O5, and V2 and O8 are in the range 0.160 3 (4)~0.166 9 (3) nm, indicating they are typical V=O bonds. The oxo O5 and O6 atoms act as bridging groups. The coordinate bond lengths in the complex are comparable to those observed in other similar oxovanadium(V) complexes with Schiff bases^[30-31]. The distortion of the octahedral coordination can be observed by the coordinate bond angles, ranging from 76.7(2)° to 106.5(2)° for V1, and from 76.7(1)° to 107.0 (2)° for V2, for the perpendicular angles, and from 155.8 (2)° to 171.1 (2)° for V1, and from 155.4 (2)° to

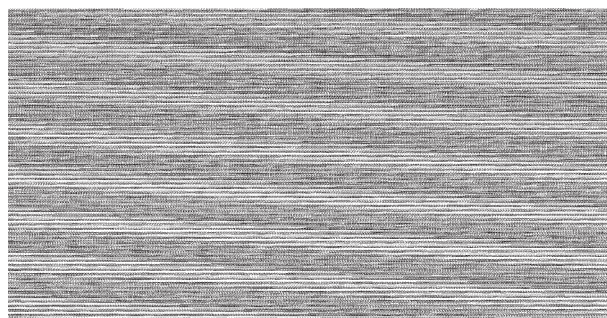


Fig.3 A perspective view of the molecular structure of **2** with the thermal ellipsoids are drawn at the 30% probability level

169.2(2)° for V2, for the diagonal angles.

2.3 Pharmacology

The results of the urease inhibition are summarized in Table 3. The acetohydroxamic acid (AHA) was used as a reference. Complex **1** show effective urease inhibitory activity, while that of the complex **2** is very weak. The IC₅₀ value ((63.3±3.8) μmol·L⁻¹) for **1** was determined since it has effective activity. The results indicate that the inhibitory activity of **1** is much better than the vanadyl sulfate with an IC₅₀ value of (207.13±3.10) μmol·L⁻¹. However, when compared with the acetohydroxamic acid, the activity of **1** is relatively weak.

Table 3 Inhibition of urease by the tested materials

Tested materials	Inhibition ^a / %	IC ₅₀ / (μmol·L ⁻¹)
1	72.1±1.8	63.3±3.8
2	29.6±2.5	—
Vanadyl sulfate	22.3±2.7	207.1±3.1
Acetohydroxamic acid	90.2±3.7	43.1±2.7

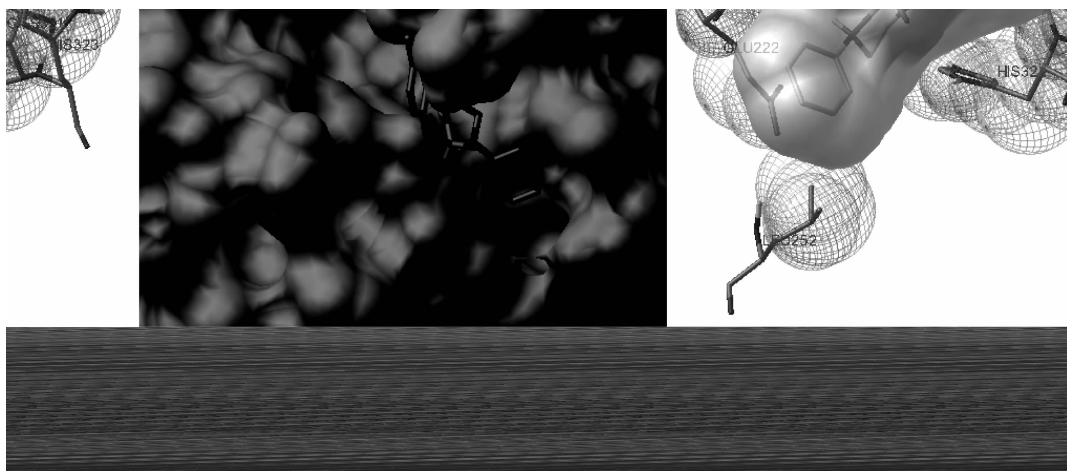
^a Concentration of the tested material is 100 μmol·L⁻¹.

2.4 Molecular docking study

The molecular docking study was performed to investigate the binding effects between the complexes and the active sites of the *Helicobacter pylori* urease. In the X-ray structure available for the native *Helicobacter pylori* urease, the two nickel atoms were coordinated by His136, His138, Kcx219, His248, His274, Asp362 and water molecules, while in the AHA-inhibited urease, these water molecules were replaced by AHA^[32]. In order to give an explanation and understanding of the inhibitory activity of the complexes, molecular docking of the complexes **1** and **2** into the AHA binding site of

the urease was performed on the binding model based on the *Helicobacter pylori* urease complex structure (1E9Y.pdb). The binding models of the complexes **1** and **2** with the urease were depicted in Figs.4 and 5. It can be seen that the complex molecule of **1** is well filled in the active pocket of the urease, while that of **2** is located at the surface of the active pocket. The binding energy of the complexes with the urease are $-1.325 \text{ kJ} \cdot \text{mol}^{-1}$ for **1** and $-0.222 \text{ kJ} \cdot \text{mol}^{-1}$ for **2**, which are higher

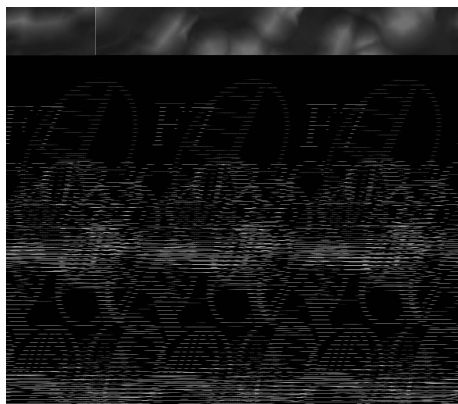
than that of the AHA ($-2.094 \text{ kJ} \cdot \text{mol}^{-1}$). The possible explanation is that the size of complex **2** is too large, and can not enter the pocket of the active center of urease, while that of **1** is relatively small, and forms several short contacts with the residues of the active sites. The results of the molecular docking study agree well with the inhibitory test, and could explain the activity of the complexes against *Helicobacter pylori* urease.



Left: Only the interacting residues are displayed; Interactions are shown as dashed spheres;

Right: Enzyme is shown as Surface; Complex is shown as sticks

Fig.4 Binding mode of **1** with *Helicobacter pylori* urease



Enzyme is shown as surface; Complex is shown as sticks

Fig.5 Binding mode of **2** with *Helicobacter pylori* urease

3 Conclusions

The present study reports the synthesis and structures of two new dioxovanadium(V) complexes with Schiff bases. The structure-activity relationship indicates that the size of the molecules of the complexes play an important role in the urease inhibition. Considering that the oxovanadium complexes have

interesting biological activities and have been widely used in medicine, further work need to be carried out to investigate the inhibitory mechanism of the complex **1**.

References:

- [1] Karplus P A, Pearson M A, Hausinger R P. *Acc. Chem. Res.*, **1997**,**30**:330-337
- [2] Sumner J B. *J. Biol. Chem.*, **1926**,**69**:435-441
- [3] Zonia L E, Stebbins N E, Polacco J C. *Plant Physiol.*, **1995**, **107**:1097-1103
- [4] Collins C M, Dórazio S E F. *Mol. Microbiol.*, **1993**,**9**:907-913
- [5] Montecucco C, Rappuoli R. *Nat. Rev. Mol. Cell Biol.*, **2001**,**2**: 457-466
- [6] Zhengping W, Van Cleemput O, Demeyer P, et al. *Biol. Fertil. Soils*, **1991**,**11**:43-47
- [7] Krajewska B. *J. Mol. Catal. B. Enzym.*, **2009**,**59**:9-21
- [8] Amtul Z, Atta-ur-Rahman, Siddiqui R A, et al. *Curr. Med. Chem.*, **2002**,**9**:1323-1348
- [9] Zaborska W, Kot M, Superata K. *J. Enzym. Inhib. Med. Chem.*, **2002**,**17**:247-253

- [10] Pearson M A, Michel L O, Hausinger R P, et al. *Biochemistry*, **1997**, **36**:8164-8172
- [11] Zaborska W, Krajewska B, Olech Z. *J. Enzym. Inhib. Med. Chem.*, **2004**, **19**:65-69
- [12] Zaborska W, Krajewska B, Leszko M, et al. *J. Mol. Catal. B: Enzym.*, **2001**, **13**:103-108
- [13] Asato E, Kamamuta K, Akamine Y, et al. *Bull. Chem. Soc. Jpn.*, **1997**, **70**:639-648
- [14] Khan M I, Baloch M K, Ashfaq M. *J. Enzym. Inhib. Med. Chem.*, **2007**, **22**:343-350
- [15] Hou P, You Z L, Zhang L, et al. *Transition Met. Chem.*, **2008**, **33**:1013-1017
- [16] You Z L, Han X, Zhang G N. *Z. Anorg. Allg. Chem.*, **2008**, **634**:142-146
- [17] Tanaka T, Kawase M, Tani S. *Life Sci.*, **2003**, **73**:2985-2990
- [18] Cheng K, You Z L, Zhu H L. *Aust. J. Chem.*, **2007**, **60**:375-379
- [19] You Z L, Ni L L, Shi D H, et al. *Eur. J. Med. Chem.*, **2010**, **45**:3196-3199
- [20] Ara R, Ashiq U, Mahroof-Tahir M, et al. *Chem. Biodivers.*, **2007**, **4**:58-71
- [21] Bruker, *SMART and SAINT*. Bruker AXS Inc, Madison, **2002**.
- [22] Sheldrick G M. *SADABS*, University of Göttingen, Germany, **1996**.
- [23] Sheldrick G M. *SHELXTL V5.1, Software Reference Manual*, Bruker AXS Inc, Madison, **1997**.
- [24] Mao W J, Lv P C, Shi L, et al. *Bioorg. Med. Chem.*, **2009**, **17**:7531-7536
- [25] Weatherburn M W. *Anal. Chem.*, **1967**, **39**:971-974
- [26] Krajewska B, Zaborska W. *Bioorg. Chem.*, **2007**, **35**:355-365
- [27] Geary W J. *Coord. Chem. Rev.*, **1971**, **7**:81-122
- [28] Kwiatkowski E, Romanowski G, Nowicki W, et al. *Polyhedron*, **2003**, **22**:1009-1018
- [29] Asgedom G, Sreedhara A, Kivikoski J, et al. *Dalton Trans.*, **1996**:93-97
- [30] LI Lian-Zhi(李连之), XU Tao(许涛), WANG Da-Qi(王大奇), et al. *Chinese J. Inorg. Chem.(Wuji Huaxue Xuebao)*, **2004**, **20**(2):236-240
- [31] ZHANG Xiao-Mei(张小梅), ZHOU Yin-Zhuang(周荫庄), TU Shu-Jie(屠淑洁), et al. *Chinese J. Inorg. Chem.(Wuji Huaxue Xuebao)*, **2007**, **23**(10):1700-1704
- [32] Xiao Z P, Ma T W, Fu W C, et al. *Eur. J. Med. Chem.*, **2010**, **45**:5064-5070