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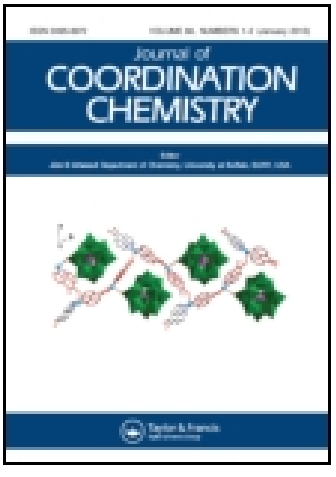

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Syntheses, characterization, and urease inhibition of oxidovanadium(V) complexes with tridentate hydrazone and bidentate benzohydroxamate ligands

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Accepted author version posted online: 11 Jul 2014.Published online: 08 Aug 2014.

To cite this article: Shao-Song Qian, Zhonglu You, Yang Huo, Yu-Ting Ye, Xiao-Shan Cheng & Hai-Liang Zhu (2014) Syntheses, characterization, and urease inhibition of oxidovanadium(V) complexes with tridentate hydrazone and bidentate benzohydroxamate ligands, Journal of Coordination Chemistry, 67:14, 2415-2424, DOI: 10.1080/00958972.2014.944176

To link to this article: http://dx.doi.org/10.1080/00958972.2014.944176

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Journal of Coordination Chemistry, 2014
Vol. 67, No. 14, 2415–2424, http://dx.doi.org/10.1080/00958972.2014.944176

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Syntheses, characterization, and urease inhibition of oxidovanadium(V) complexes with tridentate hydrazone and bidentate benzohydroxamate ligands

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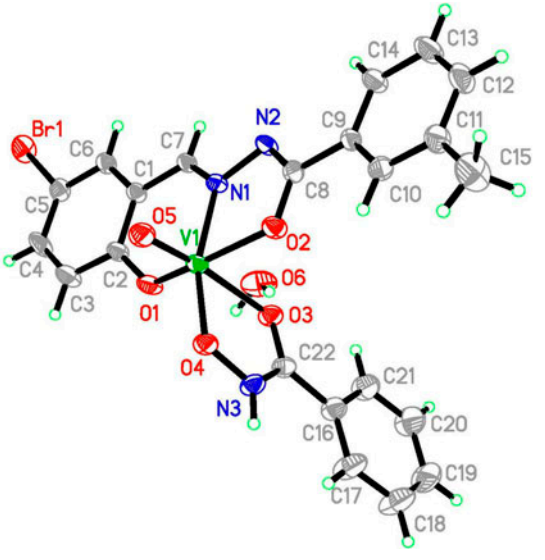
(*Received 23 April 2014; accepted 13 June 2014*)



New oxidovanadium(V) complexes, $[VOL^1(bzh))]\cdot H_2O$ (**1**) and $[VOL^2(bzh))]$ (**2**), were prepared by the reaction of $[VO(acac)_2]$ (where acac = acetylacetonate) and benzohydroxamic acid (Hbzh) with *N*′-(5-bromo-2-hydroxybenzylidene)-3-methylbenzohydrazide (H_2L^1) and *N*′-(5-bromo-2-hydroxybenzylidene)-4-methylbenzohydrazide (H_2L^2), respectively, in methanol. Structures of the complexes were determined by elemental analysis, infrared and UV–vis spectra. Single crystal structures of the complexes were determined by X-ray diffraction. Vanadiums have octahedral coordination. Thermal stability and the inhibition of urease of the complexes were studied.

Keywords: Hydrazone ligand; Oxidovanadium complex; Crystal structure; Thermal property; Urease inhibition

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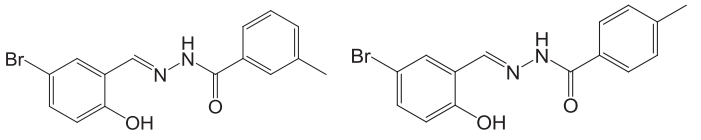
1. Introduction

Urease is a nickel-containing enzyme, which can catalyze the hydrolysis of urea. Unfortunately, urease has many negative effects [1–4]. In recent years, great efforts have been made to control the activity of urease by using various inhibitors. Notably, metal complexes have proved to be versatile enzyme inhibitors [5]. Among versatile metal complexes, those derived from hydrazones have received particular attention in biological and medicinal chemistry [6–9]. Vanadium complexes with many types of ligands have been reported to have effective biological effects such as normalizing high blood glucose levels and acting as models of haloperoxidases [10–12]. Ara and co-workers reported that some binuclear vanadium(IV) complexes possess interesting urease inhibitory activities [13]. Aslam and co-workers reported that Schiff bases of hydrazone type possess urease inhibitory activities [14]. Recently, our research group has reported a few vanadium complexes with urease inhibitory activities [15, 16]. In order to explore more potent urease inhibitors, in the present paper, two new oxidovanadium(V) complexes are presented, $[VOL^1(bzh))]\cdot H_2O$ (**1**) and $[VOL^2(bzh))]$ (**2**), where L^1, L^2, and hzh are the deprotonated forms of *N′*-(5-bromo-2-hydroxybenzylidene)-3-methylbenzohydrazide (H_2L^1; scheme 1), *N′*-(5-bromo-2-hydroxybenzylidene)-4-methylbenzohydrazide (H_2L^2; scheme 1), and benzohydroxamic acid, respectively.



Scheme 1. H_2L^1 (left) and H_2L^2 (right).

2. Experimental

2.1. *Materials and measurements*

Commercially available 5-bromosalicylaldehyde, 3-methylbenzohydrazide, 4-methylbenzohydrazide, and benzohydroxamic acid were purchased from Aldrich, and used without purification. Other solvents and reagents were made in China and used as received. C, H, and N elemental analyses were performed with a Perkin-Elmer elemental analyzer. Infrared spectra were recorded on a Nicolet AVATAR 360 spectrometer as KBr pellets from 4000 to 400 cm^{-1}. UV–vis spectra were recorded on a Lambda-900 spectrometer. Thermal stability analysis was performed on a Perkin-Elmer Pyris Diamond TG-DTA thermal analysis system.

2.2. *Synthesis of $[VOL^1(bzh))]\cdot H_2O$ (1)*

5-Bromosalicylaldehyde (1.0 mM, 201 mg), 3-methylbenzohydrazide (1.0 mM, 150 mg), and benzohydroxamic acid (1.0 mM, 137 mg) were dissolved in methanol (20 mL) and stirred at room temperature for 10 min. Then, 20 mL methanolic solution containing $[VO(acac)_2]$ (1.0 mM, 265 mg) was added dropwise to the solution. The mixture was further

stirred at room temperature for 10 min to give a deep brown solution. The solution was filtered to obtain a clear filtrate, which was kept in the dark for slow evaporation. A few days later, well-shaped brown single crystals of the complex were formed and separated by filtration. The product was washed three times with cold methanol and dried in air. Yield: 37%. UV–vis (acetonitrile) λ_{max} (logε) 272 (1.98); 335 (1.72); 440 (1.52) nm. IR (KBr) ν_{max} 3438, 1605, 1540, 1462, 1412, 1338, 1276, 1140, 1084, 1034, 975, 923, 818, 700, 595, 513, 465 cm^{-1}. ^1H NMR (d^6-DMSO, 500 MHz) δ 2.33 (s, 1H), 6.85 (d, 1H), 7.26 (d, 1H), 7.38–7.43 (m, 2H), 7.63–7.82 (m, 6H), 8.01 (d, 2H), 8.37 (s, 1H), 9.26 (s, 1H). Anal. C 48.1%, H 3.6%, N 7.5%, Calcd for $C_{22}H_{19}BrN_3O_6$ V, C 47.8%, H 3.5%, N 7.6%.

2.3. *Synthesis of [VOL^2(bzh))] (2)*

Complex **2** was prepared by the same method as that described for **1** with 3-methylbenzohydrazide replaced by 4-methylbenzohydrazide (1.0 mM, 150 mg). Yield: 46%. UV–vis (acetonitrile) λ_{max} (logε) 227 (1.96), 272 (1.91); 335 (1.70); 440 (1.54) nm. IR (KBr) ν_{max} 1605, 1493, 1411, 1375, 1333, 1276, 1189, 1140, 1034, 973, 910, 818, 737, 694, 650, 588, 496, 453 cm^{-1}. ^1H NMR (d^6-DMSO, 500 MHz) δ 2.32 (s, 1H), 6.85 (d, 1H), 7.31 (d, 2H), 7.39 (d, 1H), 7.60–7.72 (m, 3H), 7.92 (d, 2H), 8.01 (d, 2H), 8.37 (s, 1H), 9.37 (s, 1H). Anal. Calcd 49.3%, H 3.4%, N 7.7%, Calcd for $C_{22}H_{17}BrN_3O_5V$, C 49.5%, H 3.2%, N 7.9%.

Table 1. Crystallographic data and refinement parameters for **1** and **2**.

Complex	**1**	**2**
Chemical formula	$C_{22}H_{19}BrN_3O_6$ V	$C_{22}H_{17}BrN_3O_5$ V
Mr	552.2	534.2
Crystal color, habit	Brown, block	Brown, block
Crystal size (mm^3)	0.17 × 0.15 × 0.15	0.30 × 0.27 × 0.22
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
Unit cell parameters		
a (Å)	7.430(1)	11.1834(6)
b (Å)	14.189(2)	16.6061(9)
c (Å)	27.809(2)	12.2129(6)
β (°)	90.298(1)	102.388(2)
V (Å^3)	2931.6(6)	2215.3(2)
Z	4	4
D_{Calcd} (g cm^{-3})	1.251	1.602
Temperature (K)	298(2)	298(2)
μ (mm^{-1})	1.735	2.290
F (0 0 0)	1112	1072
Number of unique data	5426	4110
Number of observed data [$I > 2\sigma(I)$]	4167	3368
Number of parameters	308	293
Number of restraints	4	1
R_1, wR_2 [$I > 2\sigma(I)$]	0.0737, 0.2440	0.0338, 0.0830
R_1, wR_2 (all data)	0.0950, 0.2589	0.0469, 0.0906
Goodness of fit on F^2	1.128	1.019

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Table 2. Selected bond distances (Å) and angles (°) for **1** and **2**.

1			
V1–O1	1.866(5)	V1–O2	1.947(5)
V1–O3	2.233(4)	V1–O4	1.861(4)
V1–O5	1.588(5)	V1–N1	2.085(5)
O5–V1–O4	96.5(2)	O5–V1–O1	100.5(3)
O4–V1–O1	102.5(2)	O5–V1–O2	99.2(2)
O4–V1–O2	93.2(2)	O1–V1–O2	153.2(2)
O5–V1–N1	98.2(2)	O4–V1–N1	162.7(2)
O1–V1–N1	83.9(2)	O2–V1–N1	75.4(2)
O5–V1–O3	171.2(2)	O4–V1–O3	74.8(2)
O1–V1–O3	82.1(2)	O2–V1–O3	81.1(2)
N1–V1–O3	90.4(2)		
2			
V1–O1	1.866(2)	V1–O2	1.953(2)
V1–O3	2.179(2)	V1–O4	1.858(2)
V1–O5	1.585(2)	V1–N1	2.082(2)
O5–V1–O4	97.7(1)	O5–V1–O1	98.6(1)
O4–V1–O1	103.6(1)	O5–V1–O2	98.6(1)
O4–V1–O2	92.0(1)	O1–V1–O2	154.9(1)
O5–V1–N1	99.6(1)	O4–V1–N1	159.8(1)
O1–V1–N1	84.1(1)	O2–V1–N1	75.1(1)
O5–V1–O3	174.1(1)	O4–V1–O3	76.5(1)
O1–V1–O3	84.1(1)	O2–V1–O3	80.6(1)
N1–V1–O3	85.9(1)		

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2.4. *X-ray crystallography*

Diffraction intensities for the complex were collected at 298(2) K using a Bruker D8 VENTURE PHOTON diffractometer with MoKα radiation (λ = 0.71073 Å). The collected data were reduced using the SAINT program [17], and multi-scan absorption corrections were performed using SADABS [18]. The structure was solved by direct methods and refined against F^2 by full-matrix least-squares, using SHELXTL [19]. All non-hydrogen atoms were refined anisotropically. The amino hydrogens of both complexes, and the water hydrogens in **1** were located from difference Fourier maps and refined isotropically with N–H, O–H, and H···H distances restrained to 0.90(1), 0.85(1), and 1.37(2) Å, respectively. The remaining hydrogens were placed in idealized 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. *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 conditions (5% O_2, 10% CO_2, and 85% N_2). The method of preparation of *H. pylori* urease by Mao was followed [20]. Briefly, broth cultures (50 mL, 2.0×10^8 CFU mL^{-1}) were centrifuged (5000 g, 4 °C) to collect the bacteria, and after washing twice with phosphate-buffered saline (pH 7.4), the *H. pylori* precipitate was stored at −80 °C. When the *H. 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

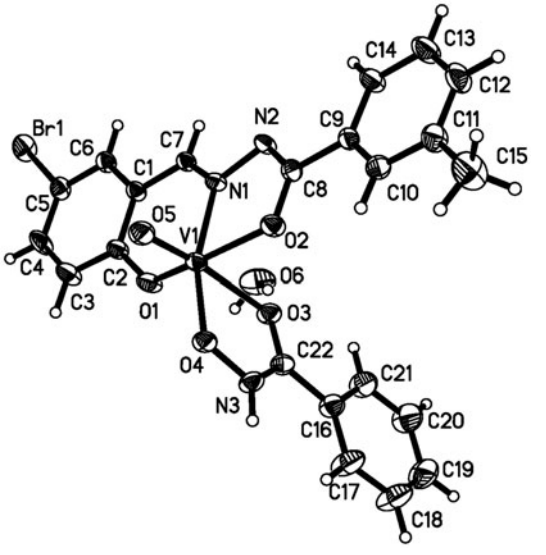

Figure 1. ORTEP plot of the crystal structure of **1**. Displacement ellipsoids of non-hydrogen atoms are drawn at the 30% probability level.

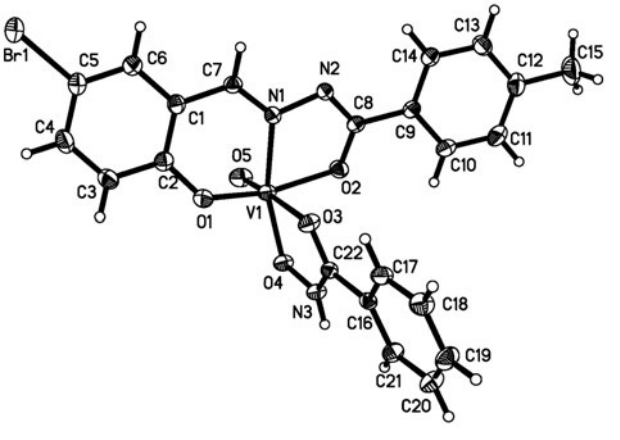

Figure 2. ORTEP plot of the crystal structure of **2**. Displacement ellipsoids of non-hydrogen atoms are drawn at the 30% probability level.

urease solution was added to an equal volume of glycerol and stored at 4 °C, until used in the experiment. The mixture, containing 25 µL (4U) of *H. pylori* urease and 25 µL of the test compound of various concentrations (dissolved in solution of DMSO : H_2O = 1 : 1 (v/v)), was pre-incubated for 3 h at room temperature in a 96-well assay plate. Urease activity was determined by measuring ammonia production by the indophenol method.

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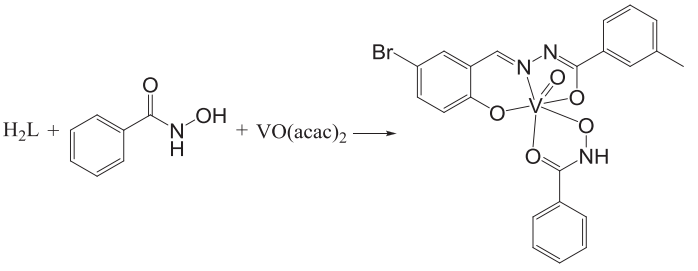
3. Results and discussion

3.1. *General*

Replacement of two acetylacetonate ligands in $[VO(acac)_2]$ by hydrazone and benzohydroxamate ligands in methanol resulted in formation of the complexes, with similar structures (scheme 2). The complexes are soluble in DMF, DMSO, methanol, ethanol, and acetonitrile. Molar conductance of the complexes at 10^{-4} M is about 20–30 Ω^{-1} cm^2 M^{-1}, indicating they are non-electrolytes [21].



Scheme 2. Synthesis of the complexes. $H_2L = H_2L^1$ for **1**, H_2L^2 for **2**.

3.2. *Crystal structure description of the complexes*

The molecular structures and atom numbering schemes of **1** and **2** are shown in figures 1 and 2, respectively. The vanadiums in the complexes are octahedral, with the three donors of the hydrazone ligands with hydroxy of the benzohydroxamate, defining the equatorial plane, and with one oxo and the carbonyl O occupying axial positions. The axial bond lengths of V1–O5 in both **1** and **2** are 1.59 Å, indicating they are typical V=O double

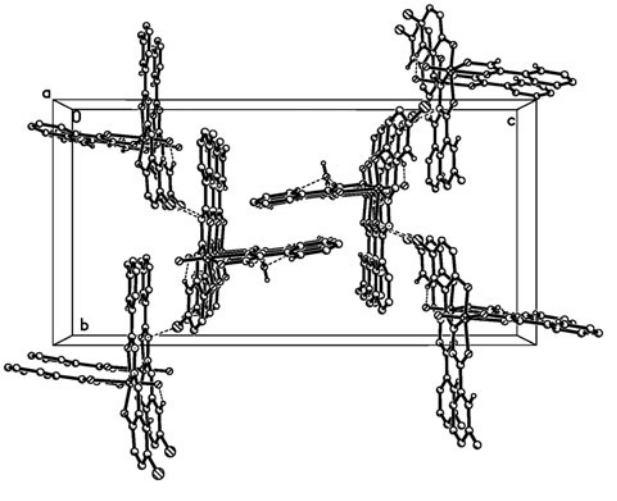


Figure 3. Packing diagram of **1**. Hydrogen bonds are shown as dashed lines.

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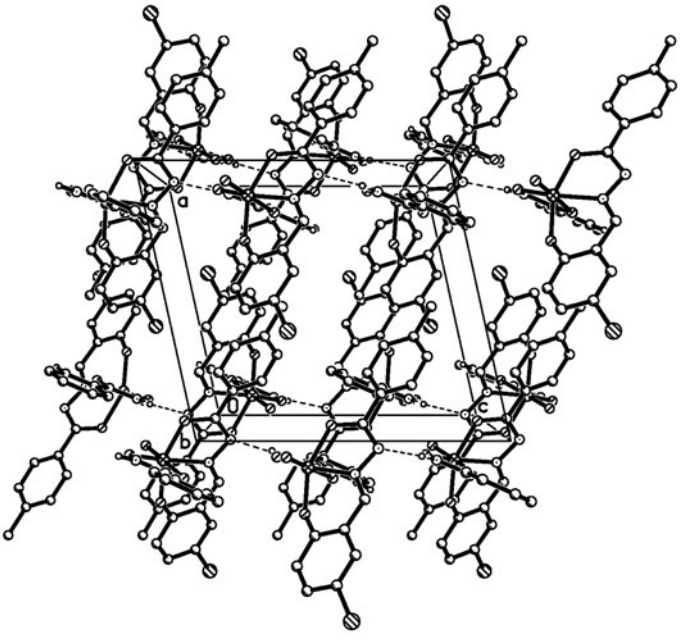

Figure 4. Packing diagram of **2**. Hydrogen bonds are shown as dashed lines.

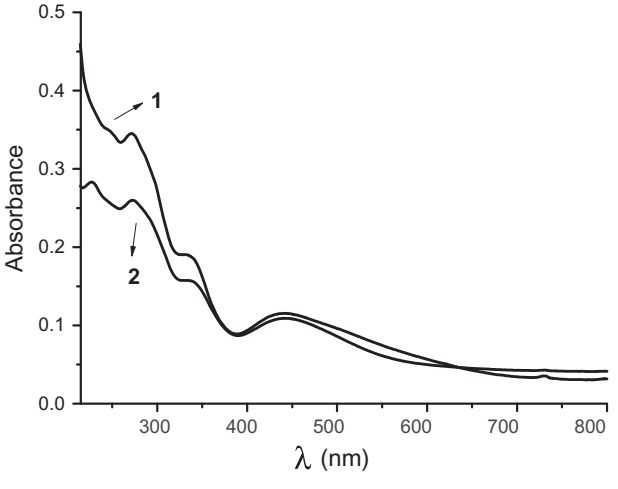

Figure 5. UV–vis spectra of the complexes.

bonds. The other axial bond lengths in the complexes are significantly longer than the remaining coordinate bonds, which might be due to the *trans* effect of the V=O bonds. This is not uncommon for oxidovanadium complexes [22, 23]. In both complexes, the coordinate bond lengths are comparable and also similar to those observed in mononuclear oxidovanadium(V) complexes with octahedral coordination [22, 23]. The angular distortion in the

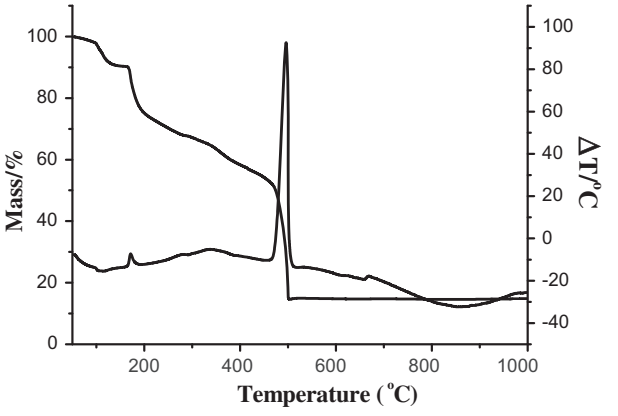

Figure 6. DT-TGA curve of **1**.

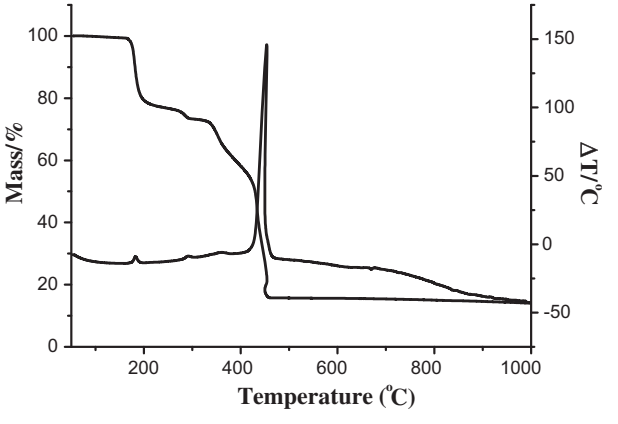

Figure 7. DT-TGA curve of **2**.

octahedral environment around V comes from the five- and six-membered chelate rings taken by the hydrazone ligands. For the same reason, the *trans* angles significantly deviate from the ideal values of 180°. Distortion of the octahedral coordination can be observed from the coordinate bond angles, ranging from 74.8(2)° to 102.5(2)° for perpendicular angles, from 153.2(2)° to 171.2(2)° for *trans* angles for **1**, from 75.1(1)° to 103.6(1)° for the perpendicular angles, and from 154.9(1)° to 174.1(1)° for *trans* angles for **2**. The displacement values of V from the equatorial planes are 0.285(1) Å for **1** and 0.286(1) Å for **2**. The dihedral angles between the benzene rings of the hydrazone ligands are 10.3(3)° for **1** and 4.3(3)° for **2**.

The crystal structures of the complexes (figures 3 and 4) are stabilized by intermolecular hydrogen bonds [**1**: N3–H3A = 0.90(1) Å, H3A···O6^{i} = 1.89(2) Å, N3···O6^{i} = 2.776(7) Å, N3–H3A···O6^{i} = 172(9)°; **2**: N3–H3A = 0.90(1) Å, H3A···N2^{ii} = 2.11(1) Å, N3···N2^{ii} = 2.991(3) Å, N3–H3A···N2^{ii} = 167(4)°; symmetry codes: (i) $-1+x$, y, z; (ii) x, $3/2-y$, $-1/2+z$] and $\pi\cdots\pi$ interactions.

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3.3. *Infrared and UV–vis spectra*

Both complexes exhibit similar absorptions. The typical absorption bands at 975 cm^{-1} can be assigned to V=O vibrations [24]. The intense $\nu_{C=N}$ absorptions are observed at 1605 cm^{-1} for both complexes. Weak peaks in the low wavenumber region (450–650 cm^{-1}) may be attributed to vibrations of the V–O and V–N bonds of the complexes.

Acetonitrile solutions of the complexes with concentration of 10^{-5} M L^{-1} have been used to record electronic spectra. The main features of all the spectra are quite similar (figure 5). There are absorptions at 550–390 nm in the complexes, attributed to ligand-to-metal charge transfer transitions. The high energy absorptions at 275 and 337 nm are most likely due to transitions involving ligand orbitals only.

3.4. *Thermal property*

Differential thermal and thermal gravimetric analyses were conducted to examine the stability of the complexes under air and with standard corundum crucible sample holder (figure 6 for **1** and figure 7 for **2**). The rate of the gas flow is 20.0 cm^3 min^{-1}. The heating rate is 10 °C min^{-1}. For **1**, the complex decomposed from 80 to 140 °C, which might be due to the loss of lattice water molecules. Then, the complex decomposed gradually from 170 to 500 °C, corresponding to the loss of the hydrazone and benzohydroxamate ligands, and the formation of V_2O_5. The total observed weight loss of 85.1% is close to the calculated value of 83.5%. For **2**, the complex decomposed from 170 to 460 °C, corresponding to the loss of the hydrazone and benzohydroxamate ligands and the formation of V_2O_5. The total observed weight loss of 84.5% is close to the calculated value of 83.0%.

3.5. *Pharmacology*

The results of urease inhibition are summarized in table 3. Compared with the reference inhibitor acetohydroxamic acid (AHA), the free hydrazone compounds and the vanadyl sulfate have very weak interactions against urease. The complexes at a concentration of 100 μM L^{-1} have the same urease inhibitory activities within the deviations with percent inhibition of about 65%, and with IC_{50} values of *ca.* 10 μM L^{-1}. In general, both complexes have effective urease inhibitory activities, and even close to that of AHA. The position of the methyl substituent seems not important for the influence of urease inhibition. The urease inhibition of the present vanadium complexes is similar to the cobalt complex reported by Qiu and co-workers, but less active than the nickel and copper complexes [25, 26]. When compared with the nickel(II), zinc(II), and cobalt(II) complexes with hydroxyl-rich Schiff base ligands, the present vanadium complexes are more active [27]. The complexes have

Table 3. Inhibition of urease by the compounds.

	Percent inhibition	IC_{50} (μM L^{-1})
1	64 ± 3	10.5
2	67 ± 38	9.8
H_2L^1	14 ± 2	–
H_2L^2	13 ± 2	–
Vanadyl sulfate	28 ± 3	215
AHA	87 ± 4	35.1

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stronger activity against urease than the free hydrazone ligands, which might be due to supramolecular interactions between the compounds and the 3-D cavity of the active site of the urease. Supported by octahedral coordination of vanadium, the complexes may have more interactions with urease than the ligands.

4. Conclusion

Two new oxidovanadium(V) complexes with the tridentate hydrazone ligands and bidentate benzohydroxamate ligands have been prepared and structurally characterized. The complexes may be used as potential urease inhibitors with the IC_{50} value of *ca.* 10 μM L^{-1}.

Supplementary material

CCDC-994819 for **1** and 994820 for **2** contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at http://www.ccdc.cam.ac.uk/const/retrieving.html, or from the Cambridge Crystallographic Data Center (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44(0)1223 336033 or Email: deposit@ccdc.cam.ac.uk.

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