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Synthesis, Crystal Structures and Insulin-Enhancing Activity of Vanadium(V) Complexes with Hydrazone Ligands[1]

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Abstract—Two new isostructurally mononuclear vanadium(V) complexes, [VO(L^1)(OMe)(MeOH)] (**I**) and [VO(L^2)(OMe)(MeOH)] (**II**), where L^1 and L^2 are the dianionic form of 2-chloro-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide and 2-bromo-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide, respectively, have been prepared. The complexes have been characterized by physico-chemical methods and single crystal X-ray determination (CIF files CCDC nos. 1824513 (**I**) and 1824514 (**II**)). The V atoms in both complexes are coordinated by the three donor atoms of the hydrazone ligands, two O atoms from one deprotonated methanol ligand and one neutral methanol ligand, and one oxo group, forming octahedral coordination. Insulin-mimetic tests on C2C12 muscle cells using biovision glucose assay indicates that the complexes significantly stimulated cell glucose utilization with cytotoxicity at 0.15 g L^{-1}.

Keywords: hydrazone, vanadium complex, crystal structure, X-ray diffraction, insulin mimetic

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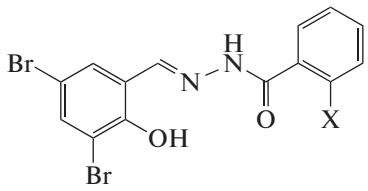
INTRODUCTION

In recent years, inorganic vanadium salts and vanadium complexes with various ligands have been reported to possess potent pharmacological effects of insulin-mimetic activity [1–4]. Studies indicated that vanadium compounds improve not only hyperglycemia in human subjects and animal models of type **I** diabetes but also glucose homeostasis in type **II** diabetes [5, 6]. However, the inorganic vanadium salts are considered as less active and more toxic. In order to reduce the side effects of inorganic vanadium salts, vanadium complexes have received particular attention and demonstrated to be effective [7–9]. Hydrazone compounds have interesting biological activities [10–14]. In view of the increasing importance of vanadium complexes with hydrazone type Schiff bases, we report herein the synthesis, characterization, and insulin-enhancing activities of two new mononuclear vanadium(V) complexes, [VO(L^1)(OMe)(MeOH)] (**I**) and [VO(L^2)(OMe)(MeOH)] (**II**), where L^1 and L^2 are the dianionic form of 2-chloro-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide and 2-bromo-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide, respectively, as shown in Scheme 1:



X = Br for H_2L^1, Cl for H_2L^2.

EXPERIMENTAL

Materials and measurements. Commercially available chemicals were purchased from Lancaster and used without further purification. C, H and N elemental analyses were performed with a Perkin-Elmer 240C elemental analyser. IR spectra were recorded on a Nicolet AVATAR 360 spectrometer as KBr pellets in the 4000–400 cm^{-1} region. UV-Vis spectra were recorded on a Lambda 900 spectrometer. Absorbance was recorded on a Bio-Tek model ELx800 96-well plate reader.

Synthesis of complex I. To a methanolic solution (10 mL) of 3,5-dibromosalicylaldehyde (0.1 mmol, 28.0 mg) and 2-bromobenzohydrazide (0.1 mmol, 21.5 mg) was added a methanolic solution (10 mL) of $VO(Acac)_2$ (0.1 mmol, 26.5 mg) with continuous stirring. The mixture was stirred for 30 min at room tem-

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perature to give a deep brown solution. Upon keeping the solution in air for about a week, brown block-like single crystals suitable for X-ray diffraction were deposited at the bottom of the vessel. The isolated product was washed three times with cold methanol and dried in a vacuum over anhydrous $CaCl_2$. The yield was 23.2 mg (38% on the basis of V).

IR data (KBr; ν, cm^{-1}): 3455 ν(OH), 1607 ν(C=N), 950 ν(V=O). UV-Vis (acetonitrile; $c = 3.2 \times 10^{-5}$ mol L^{-1}; λ_{max}, nm (ε, mol^{-1} L cm^{-1})): 270 (27380), 325 (19750), 400 (15200).

For $C_{16}H_{14}N_2O_5Br_3V$			
Anal. calcd., %	C, 31.77	H, 2.33	N, 4.63
Found, %	C, 31.61	H, 2.45	N, 4.72

Synthesis of complex II. To a methanolic solution (10 mL) of 3,5-dibromosalicylaldehyde (0.1 mmol, 28.0 mg) and 2-chlorobenzohydrazide (0.1 mmol, 17.1 mg) was added a methanolic solution (10 mL) of $VO(Acac)_2$ (0.1 mmol, 26.5 mg) with continuous stirring. The mixture was stirred for 30 min at room temperature to give a deep brown solution. Upon keeping the solution in air for about a week, brown block-like single crystals suitable for X-ray diffraction were deposited at the bottom of the vessel. The isolated product was washed three times with cold methanol and dried in a vacuum over anhydrous $CaCl_2$. The yield was 23.2 mg (38% on the basis of V).

IR data (KBr; ν, cm^{-1}): 3455 ν(OH), 1608 ν(C=N), 955 ν(V=O). UV-Vis (acetonitrile; $c = 3.7 \times 10^{-5}$ mol L^{-1}; λ_{max}, nm (ε, mol^{-1} L cm^{-1})): 270 (28550), 325 (19275), 400 (14870).

For $C_{16}H_{14}N_2O_5ClBr_2V$			
Anal. calcd., %	C, 34.29	H, 2.52	N, 5.00
Found, %	C, 34.40	H, 2.61	N, 4.87

X-ray crystal structure determination. Diffraction intensities for complexes **I** and **II** were collected at 298(2) K using a Bruker APEX II area-detector with MoK_α radiation (λ = 0.71073 Å). The collected data were reduced using the SAINT program [15], and multi-scan absorption corrections were performed using the SADABS program [16]. Structures of the complexes were solved by direct methods and refined against F^2 by full-matrix least-squares methods using the SHELXTL program [17]. All of the non-hydrogen atoms were refined anisotropically. The methanol H atoms were located from electronic density maps, and refined isotropically, with U_{iso}(H) restrained to 1.5U_{iso}(O). The remaining hydrogen atoms were located as riding model. Crystallographic data for the complexes are summarized in Table 1. Selected bond lengths and angles are given in Table 2.

Crystallographic data for **I** and **II** have been deposited with the Cambridge Crystallographic Data Centre (CCDC nos. 1824513 and 1824514, respectively; deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

Cell culture and viable cell counts. The biological assay was determined according to the literature method [18]. In general, C2C12 mouse skeletal muscle cells were cultured in Dulbecco modified Eagle's medium with 4 mmol L^{-1} L-glutamine adjusted to contain 1.5 g L^{-1} Na_2CO_3, 4.5 g L^{-1} glucose, and 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. C2C12 cells were sub-cultured in log phase to 70% confluence and seeded at a density of 5000 cells per well into 96-well culture plates. To limit batch-to-batch variation, cell subcultures were limited to 10 passages. After three days culture myotube formation was induced by replacing the fetal bovine serum in the medium with 10% horse serum. All experiments were done in five days when more than 75% of the cells were differentiated morphologically. The cells were suspended in a trypan blue (0.1% w/w) phosphate buffered saline solution and the ratio of stained to nonstained cells was determined after 5 min of incubation time. Viable cell counts were performed using a hemocytometer.

Glucose uptake determination. Three hours prior to glucose uptake, cells were incubated in glucose and serum-free media. On the fifth day, the medium was removed and replaced with 50 mL modified Dulbecco modified Eagle's medium without phenol red, supplemented with 8 mmol L^{-1} glucose and 0.1% bovine serum albumin containing either the vanadium complexes at concentration of 0.10 g L^{-1} or the positive controls, insulin, or metformin, at 1 mmol L^{-1} were added to the 96-well plate. The plate was then incubated for 2 h at 37°C and 5% CO_2. After incubation, 4 mL media was removed from each well and transferred to a new 96-well plate to which 196 mL deionized water was added in each well. A total of 50 mL of this diluted medium was transferred to a new 96-well plate and 50 mL of the prepared glucose assay reagent was added per well and incubated for 30 min at 37°C. Absorbance was taken at 570 nm on a 96-well plate reader. The glucose concentration per well was calculated from a standard curve. Glucose utilization was determined by subtracting the glucose concentration left in the medium of the relevant wells following incubation to media not exposed to cells during incubation. All assays were performed in triplicate to minimize the error.

Cytotoxicity assay. MTT (3-(4,5-dimethylthiazo)-2-yl)-2,5-diphenyltetrazolium bromide) was dissolved in phosphate-buffered saline without phenol red at a concentration of 2.0 g L^{-1}. Dulbecco modified Eagle's medium in the 96-well plate was refreshed with 200 mL of fresh media followed by addition of 50 mL of MTT solution to each well. The plate was wrapped

Table 1. Crystallographic data and structure refinement for complexes **I** and **II**

Parameter	Value	
	I	**II**
Fw	604.96	560.50
Crystal shape/colour	Block/brown	Block/brown
Crystal size, mm	0.27 × 0.27 × 0.23	0.19 × 0.19 × 0.16
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/n$
a, Å	7.8739(14)	7.8797(6)
b, Å	23.511(2)	23.2640(17)
c, Å	10.8686(19)	10.8391(9)
β, deg	101.848(3)	101.152(2)
V, Å^3	1969.1(5)	1949.4(3)
Z	4	4
μ(MoK_α), cm^{-1}	6.621	4.775
T_{min}/T_{max}	0.2680/0.3112	0.4640/0.5154
Reflections/parameters	3667/249	3632/249
Restraints	1	1
Goodness of fit on F^2	1.015	1.030
R_1, wR_2 ($I \geq 2\sigma(I)$)*	0.0295, 0.0582	0.0416, 0.0927
R_1, wR_2 (all data)*	0.0461, 0.0635	0.0671, 0.1069
ρ_{max}, ρ_{min}, *e* Å^{-3}	0.646, –0.412	0.628, –0.473

* $R_1 = \Sigma||F_o| - |Fc||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - Fc^2)^2/\Sigma w(F_o^2)^2]^{1/2}$, $w_I = [\sigma^2(F_o)^2 + (0.0239(F_o^2 + 2F_c^2)/3)^2 + 1.5541(F_o^2 + 2F_c^2)/3]^{-1}$, $w_{II} = [\sigma^2(F_o)^2 + (0.0496(F_o^2 + 2F_c^2)/3)^2 + 0.7256(F_o^2 + 2F_c^2)/3]^{-1}$.

in aluminium foil to prevent light and incubated at 37°C for 4 h, after which the media with MTT was removed and replaced with 200 mL DMSO and 25 mL Sorensen's glycine buffer. Absorbance was read at 570 nm in a plate reader.

RESULTS AND DISCUSSION

Facile condensation of 3,5-dibromosalicylaldehyde with 2-bromobenzohydrazide and 2-chlorobenzohydrazide, respectively, in methanol, gave the hydrazone ligands. The ligands were further reacted with $VO(Acac)_2$ to give the vanadium complexes. Crystals of the complexes are stable in open air at room temperature. Elemental analyses are in good agreement with the chemical formulae proposed for the compounds.

Figure 1 gives perspective view of complexes **I** with the atomic labeling system. Structures of both complexes are very similar except for the substitute groups of the hydrazone ligands, viz. Br for **I** and Cl for **II**. The V atoms in the complexes are in octahedral coordination with the phenolate O, imino N, and enolate O atoms of the hydrazone ligands, and the deprotonated methanol O atom defining the equatorial plane, and with one oxo O and the neutral methanol O atom locating at the axial positions. The V atoms deviate from the least-squares planes defined by the equatorial atoms by 0.308(1) Å for **I** and 0.312(1) Å for **II**. The coordinate bond lengths in both complexes are similar to each other, and also comparable to those observed in vanadium complexes with hydrazone ligands [19, 20]. Distortion of the octahedral coordination can be observed from the coordinate bond angles, ranging from 73.74(9)° to 103.97(10)° for **I** and from 73.74(13)° to 103.95(14)° for **II**, for the perpendicular angles, and from 154.44(10)° to 174.43(11)° for **I**, and from 154.20(14)° to 174.13(14)° for **II**, for the diagonal angles. The dihedral angles between the benzene rings of the hydrazone ligands are 11.1(3)° for **I** and 11.6(3)° for **II**. In the crystal structures of the complexes, molecules are linked through O–H···N hydrogen bonds (**I**: O(5)–H(5)···N(2)$^{\#1}$, O(5)–H(5) 0.85(1), H(5)···N(2)$^{\#1}$ 2.012(12), O(5)···N(2)$^{\#1}$ 2.854(3) Å, O(5)–H(5)···N(2)$^{\#1}$ 175(4)°; **II**: O(5)–H(5)···N(2)$^{\#2}$, O(5)–H(5) 0.85(1), H(5)···N(2)$^{\#2}$ 2.029(12), O(5)···N(2)$^{\#2}$ 2.873(5) Å, O(5)–H(5)···N(2)$^{\#2}$

Table 2. Selected bond lengths (Å) and bond angles (deg) for **I** and **II**

Bond	*d*, Å	Bond	*d*, Å
		I	
V(1)–O(2)	1.950(2)	V(1)–O(3)	1.747(2)
V(1)–O(4)	1.579(2)	V(1)–O(5)	2.356(3)
V(1)–N(1)	2.129(3)	V(1)–O(1)	1.856(2)
		II	
V(1)–O(1)	1.856(3)	V(1)–O(2)	1.952(3)
V(1)–O(3)	1.581(3)	V(1)–O(4)	1.750(3)
V(1)–N(1)	2.134(3)	V(1)–O(5)	2.362(3)
Angle	ω, deg	Angle	ω, deg
		I	
O(4)V(1)O(3)	103.09(12)	O(4)V(1)O(1)	99.52(13)
O(3)V(1)O(1)	103.97(10)	O(4)V(1)O(2)	95.83(12)
O(3)V(1)O(2)	92.19(10)	O(1)V(1)O(2)	154.44(10)
O(4)V(1)N(1)	97.64(11)	O(3)V(1)N(1)	156.06(11)
O(1)V(1)N(1)	84.02(10)	O(2)V(1)N(1)	73.74(9)
O(4)V(1)O(5)	174.43(11)	O(3)V(1)O(5)	81.65(10)
O(1)V(1)O(5)	81.97(10)	O(2)V(1)O(5)	80.94(10)
N(1)V(1)O(5)	77.13(9)		
		II	
O(3)V(1)O(4)	103.24(16)	O(3)V(1)O(1)	99.80(17)
O(4)V(1)O(1)	103.95(14)	O(3)V(1)O(2)	95.86(15)
O(4)V(1)O(2)	92.14(14)	O(1)V(1)O(2)	154.20(14)
O(3)V(1)N(1)	97.67(15)	O(4)V(1)N(1)	155.90(13)
O(1)V(1)N(1)	83.90(14)	O(2)V(1)N(1)	73.74(13)
O(3)V(1)O(5)	174.13(14)	O(4)V(1)O(5)	81.75(13)
O(1)V(1)O(5)	81.77(13)	O(2)V(1)O(5)	80.74(13)
N(1)V(1)O(5)	76.82(12)		

175(6)°; symmetry codes: $^{\#1}$ $1-x, 2-y, 1-z$; $^{\#2}$ $1-x, -y, 1-z$), to form dimers (Fig. 2).

The typical strong ν(C=N) absorption bands are located at 1607 cm^{-1} for **I** and 1608 cm^{-1} for **II**. The bands indicative of the V=O vibrations are located at 950 cm^{-1} for **I** and 955 cm^{-1} for **II**. Electronic spectra of the complexes were recorded in 10^{-5} M in acetonitrile in the range 200–800 nm. In the UV-Vis region the complexes show bands at 325 nm and 400 nm, which can be attributed to intramolecular charge transfer transitions from the p_π orbital on the nitrogen and oxygen to the empty *d* orbitals of the metal [21]. The intense bands observed at 270 nm are assigned to intraligand π–π* transitions [21].

Thermal gravimetric (TG) analyses were conducted to examine the stability of the complexes. For **I** (Fig. 3a) and **II** (Fig. 3b), the first step started at about 100°C and completed at 130°C, corresponding to the loss of the methanol ligand. Then, the complex decomposed gradually until 475°C, corresponds to the loss of the remaining ligands, and the formation of the final product (V_2O_5).

The insulin-like capacity of vanadium compounds is usually related to their ability to lower the blood glucose level by activating the glucose transport into the cell of the peripheral tissues. In this study, we have investigated the in vitro glucose uptake by C2C12 muscle cells following exposure to the vanadium complexes:

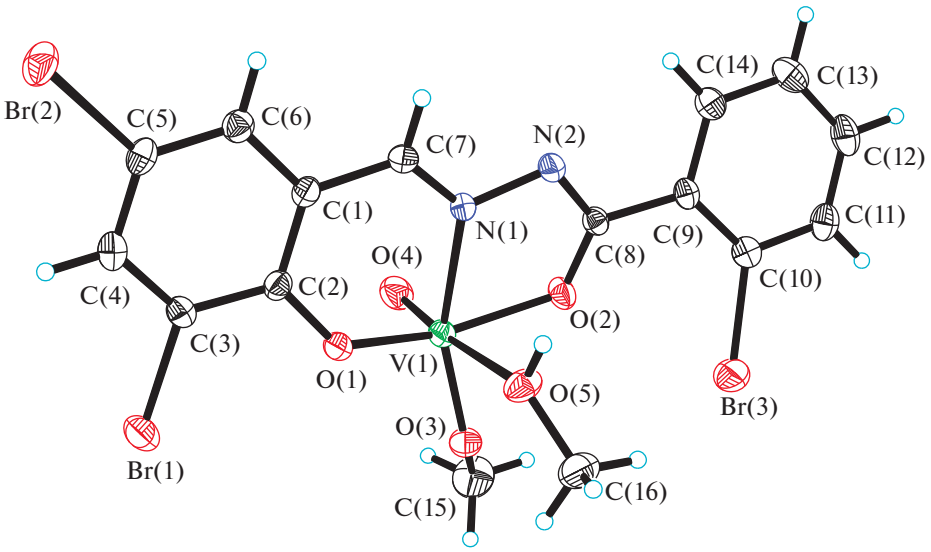

Fig. 1. Molecular structures of complex **I**, showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radii.

Compound	Percentage in glucose utilization
I	157 ± 15
II	163 ± 18
Insulin	151 ± 13
Metformin	145 ± 17

Insulin-mimetic test on C2C12 muscle cells indicates that the complexes significantly stimulated cell glucose utilization with cytotoxicity at 0.15 g L^{-1}. Complex **II** has stronger activity than complex **I**, indicating that the *o*-chloro group is better than the *o*-bromo group of the hydrazone ligands

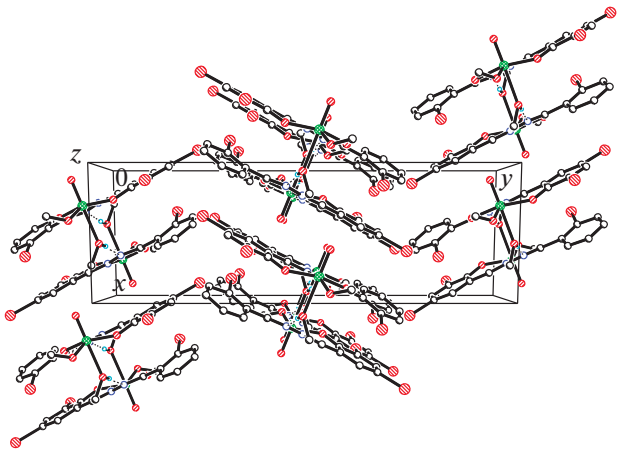

Fig. 2. Molecular packing diagrams of complex **I**.

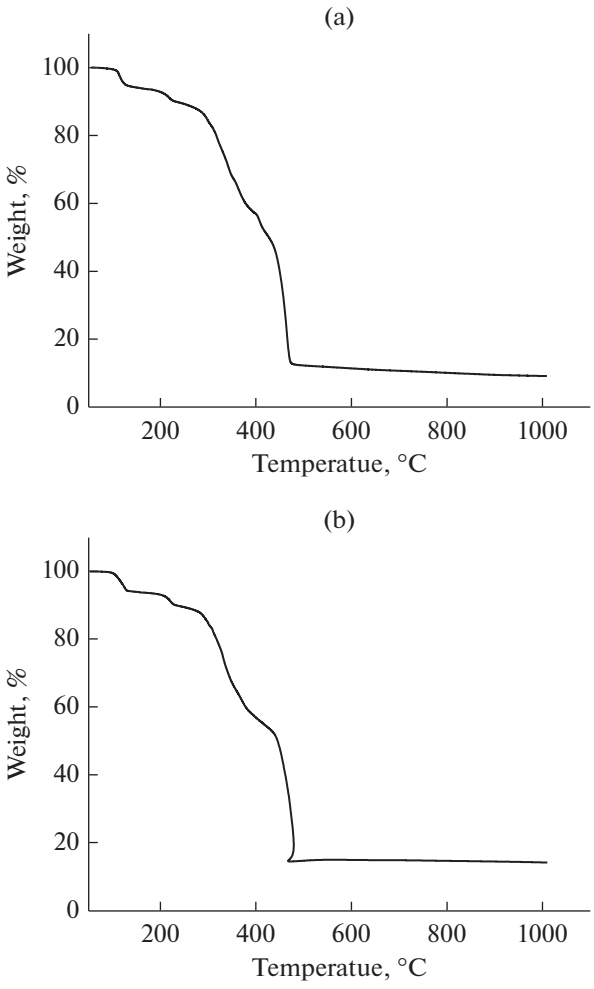

Fig. 3. TG curves of complex **I** (a) and **II** (b).

during the biological processes. In general, the insulin enhancing activities of the two vanadium complexes are similar to the reference drugs insulin and metformin. So, they are promising vanadium-based insulin-like materials.

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REFERENCES

1. Pillai, S.I., Subramanian, S.P., and Kandaswamy, M., *Eur. J. Med. Chem*., 2013, vol. 63, p. 109.
2. Smee, J.J., Epps, J.A., Ooms, K., et al., *J. Inorg. Biochem*., 2009, vol. 103, no. 4, p. 575.
3. Sanna, D., Micera, G., and Garribba, E., *Inorg. Chem.*, 2013, vol. 52, no. 20, p. 11975.
4. He, L., Wang, X.S., Zhao, C., et al., *Metallomics*, 2014, vol. 6, no. 5, p. 1087.
5. Crans, D.C., Trujillo, A.M., Pharazyn, P.S., et al., *Coord. Chem. Rev.*, 2011, vol. 255, nos. 19–20, p. 2178.
6. Sheela, A., Roopan, S.M., and Vijayaraghavan, R., *Eur. J. Med. Chem.*, 2008, vol. 43, no. 10, p. 2206.
7. Zhang, Y., Yang, X.D., Wang, K., et al., *J. Inorg. Biochem*., 2006, vol. 100, no. 1, p. 80.
8. Dornyei, A., Marcao, S., Pessoa, J.C., et al., *Eur. J. Inorg. Chem.*, 2006, vol. 18, no. 18, p. 3614.
9. Haratake, M., Fukunaga, M., Ono, M., et al., *J. Bio. Inorg. Chem.*, 2005, vol. 10, no. 3, p. 250.
10. Amir, M., Ali, I., Hassan, M.Z., et al., *Arch. Pharm.*, 2014, vol. 347, no. 12, p. 958.
11. Rajitha, G., Prasad, K.V.S.R.G., Umamaheswari, A., et al., *Med. Chem. Res.*, 2014, vol. 23, no. 12, p. 5204.
12. El-Sayed, M.A.A., Abdel-Aziz, N.I., Abdel-Aziz, A.A.M., et al., *Bioorg. Med. Chem.*, 2011, vol. 19, no. 11, p. 3416.
13. Horiuchi, T., Chiba, J., Uoto, K., et al., *Bioorg. Med. Chem. Lett*., 2009, vol. 19, no. 2, p. 305.
14. Zhang, M., Xian, D.-M., Li, H.-H., et al., *Aust. J. Chem.*, 2012, vol. 65, no. 4, p. 343.
15. *SMART and SAINT*, Madison: Bruker AXS Inc., 2002.
16. Sheldrick, G.M., *SADABS, Program for Empirical Absorption Correction of Area Detector*, Göttingen: Univ. of Göttingen, 1996.
17. Sheldrick, G.M., *SHELXTL, Version 5.1, Software Reference Manual*, Madison: Bruker AXS Inc., 1997.
18. Nejo, A.A., Kolawole, G.A., Opoku, A.R., et al., *J. Coord. Chem.*, 2009, vol. 62, no. 21, p. 3411.
19. Zhao, Y., Han, X., Zhou, X.-X., et al., *Chin. J. Inorg. Chem.*, 2013, vol. 29, no. 4, p. 867.
20. Huo, Y., Ye, Y.-T., Cheng, X.-S., et al., *Inorg. Chem. Commun.*, 2014, vol. 45, p. 131.
21. Asgedom, G., Sreedhara, A., Kivikoski, J., et al., *J. Chem. Soc., Dalton Trans.*, 1996, no. 1, p. 93.