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Oxovanadium(IV) Compounds of *cis*-$[VOCl(N\text{-}N)_2]^+$ (N-N = 4,4'- and 5,5'-dimethyl-2,2'-bipyridine); Crystal Structure and Biological Activity

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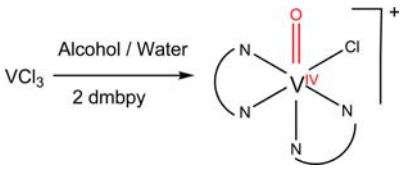
In the last decades, there has been considerable interest in the synthesis, characterization and biological investigation of vanadium compounds as they exhibit interfering physiological processes such as cell proliferation and cellular metabolism as well as insulin-mimetic properties.[1-4] Moreover a number of vanadium complexes with hetero atom ligands display potential antitumor activity.[5-8]

Vanadium(IV) complexes in a general formula of *cis*-$[VOX(N\text{-}N)_2]^{0/+1}$, (X=$Cl^-$, OH^-, SO_4^{-2} and N-N = phen, bipy) have been reported.[5,9-11] We considered the methyl-substituted bipyridine analogues, 4,4'-dimethyl-2,2'-bipyridine (**4dmbpy**) and 5,5'-dimethyl-2,2'-bipyridine (**5dmbpy**) as ligands since our last studies on cytotoxicity of oxovanadium(IV) exhibited that introduction of a methyl group into N-N ligands causes further enhanced cytotoxicity of the related complex.[5,12] Totally, two new oxovanadium(IV), $[VOCl(4dmbpy)_2]\cdot Cl\cdot H_2O\cdot C_2H_5OH$ (**1**) and $[VOCl(5dmbpy)_2]\cdot Cl\cdot H_2O\cdot CH_3OH$ (**2**) were synthesized, proposing anticancer activity of the cationic part of the complexes, as ionic vanadium compounds show to increase membrane permeability with a potential for dose reduction.[4] Both complexes were fully characterized by means of elemental analysis, IR and UV-Vis. spectroscopy as well as the X-ray diffraction method. Furthermore their cytotoxicity were assayed in three cell cultures, colorectal adenocarcinoma (Caco-2), colon carcinoma (HT-29) and breast ductal carcinoma (T47D) by means of MTT assay (MTT=3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide) and the results were compared with cisplatin, an anticancer drugs which shows limited activity on colon and breast cancers.[13]

The titled complexes can be obtained by the reaction of VCl_3 salt with **4dmbpy** and **5dmbpy** ligands respectively, in a metal to ligand ratio of 1:2. Both complexes were synthesized at 40-45 °C in an alcoholic moiety. The consumed alcohol was ethanol for complex **1** but complex **2** shows low solubility in the mentioned solvent. So methanol was used for preparation of complex **2**, where better solubility was achieved. Resulting green crystals of complexes **1** and **2** were prepared from the slow evaporation of solvent after a few days. As seen in Scheme 1, the vanadium(III) metal ion



Scheme 1. Synthesis of titled compounds.

in initial salt changed to vanadium(IV), leading to vanadyl complexes (VO^{2+}) in compounds **1** and **2**. This valence change of the vanadium center during treatment with hetero cycle ligand has been observed in other reports as well.[9,14]

The IR spectra of the titled complexes contain several bands in the region 1000-3100 cm^{-1}, which are related to C-H, C=C, C=N, C-C and C-N.[15,16] These bands show some shifts in comparison with the free ligand that can be explained by changing the geometry of the free ligand from syn to anti orientation upon coordination to metal center.[17] In IR spectra of **1** and **2**, a broad peak in the highest frequency 3470 and 3450 cm^{-1} can be assigned to O-H stretching vibrations for uncoordinated water and alcohol molecules. New similar peaks have been observed in **1** and **2**, related to coordination environment of vanadium center where the peaks at 973 (in **1**) and 979 (in **2**) cm^{-1} can be assigned to vanadyl component ($V=O^{2+}$);[18,19] at 424 cm^{-1} (in **1** and **2**) to V-N bond; and at 349 (in **1**) and 356 cm^{-1} (in **2**) to V-Cl bond.[11]

The complexes **1** and **2** are green in the solid state and DMSO solution. In Figure 1, UV-Vis spectra of both compounds in DMSO are brought in the range of 350-900 nm. Two d-d bands at around 760 and 640 nm (sh) were observed for the compounds which were assigned to $b_2(d_{xy}) \rightarrow e(d_{xz},d_{yz})$ and $b_2(d_{xy}) \rightarrow b_1(d_{x2-y2})$ transitions, on the assumption of C_{4v} symmetry.[11] The spectra also exhibit a third band in the ultraviolet region at 380 nm for **1** and 384 nm for **2**, which can be attributed to the charge transitions of the ligand to the metal atom (LMCT).[20] Furthermore, the π-π transition of the aromatic ligand disappears at 282 nm for **1** and 292 nm for **2**.

Crystallographic data for **1** and **2** are given in Table 1 and

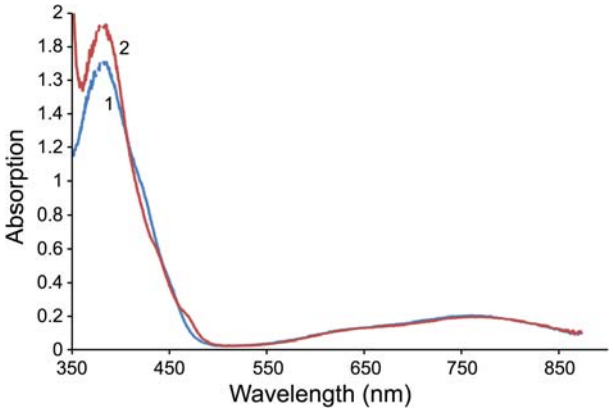


Figure 1. Room temperature absorption spectra for **1** (5.84×10^{-3} M) and **2** (5.99×10^{-3} M), in DMSO solution.

the selected bond lengths and angles are presented in Table 2. As shown in Figures 2 and 3, the asymmetric unit of both complexes contain one vanadyl cation complex *cis*-[VOCl(N-N)$_2$]+ (N-N = **4dmbpy** in **1** and **5dmbpy** in **2**) and three uncoordinated components chloride ion, water and alcohol molecules. In the main cation complex, the coordination environment around the vanadium center is distorted octahedral, involving one oxygen atom, one chloride and four nitrogen atoms from two dimethyl-bipyridine ligands, which has also been observed in other bis(phen) and bis(bpy) oxovanadium complexes.[5,9,11,21]

The V1-O1 bond distances in vanadyl component are 1.642(3) Å (in **1**) and 1.583(4) Å (in **2**), which are typical for

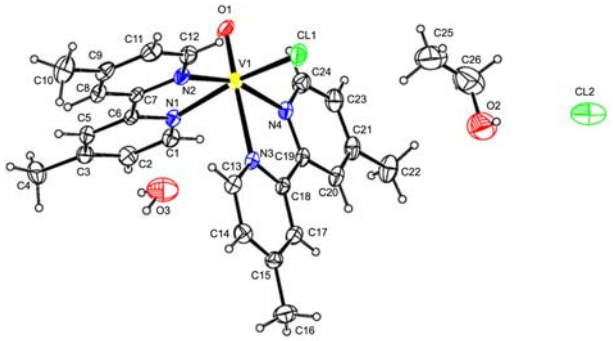


Figure 2. ORTEP drawing of **1**, showing the atom labeling scheme with thermal ellipsoids at 30% probably level.

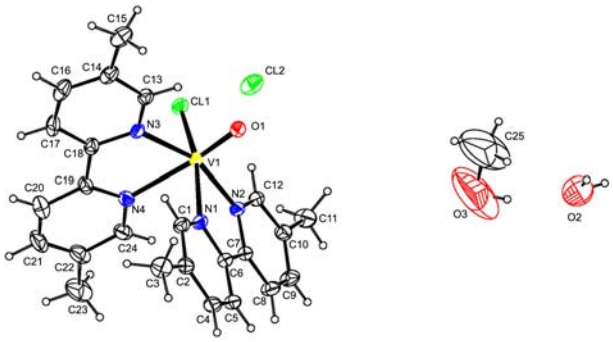


Figure 3. ORTEP drawing of **2**, showing the atom labeling scheme with thermal ellipsoids at 30% probably level.

Table 1. Crystal structure data and refinement for complexes **1** and **2**

	1	**2**
Empirical formula	$C_{26}H_{32}Cl_2N_4O_3V_1$	$C_{25}H_{30}C_{12}N_4O_3V_1$
Formula weight	570.40	556.37
Temerature (K)	298 (2)	298 (2)
Wavelenght / λ (Å)	0.71073	0.71073
Crystal system	Triclinic	Triclinic
Space group	$P\bar{1}$	$P\bar{1}$
a (Å)	9.9037 (12)	11.1166 (13)
b (Å)	11.1521 (13)	11.6609 (15)
c (Å)	14.6561 (16)	12.2455 (14)
α (°)	67.924 (8)	61.336(9)
β (°)	71.988 (9)	82.153 (9)
γ (°)	81.678 (9)	87.149 (10)
Volume (Å^3)	1225.8 (3)	1379.6 (3)
Z	2	2
Calculated density (g cm^{-3})	1.329	1.339
Absorbtion coefficient	0.568	0.585
F(000)	594	578
Crystal size (mm)	0.19 × 0.23 × 0.25	0.20 × 0.24 × 0.29
θ Range for data collection	1.97 - 29.27	1.85 - 29.27
Index ranges	$-13 \le h \le 13$	$-15 \le h \le 15$
	$-15 \le k \le 15$	$-13 \le k \le 16$
	$-20 \le l \le 20$	$-16 \le l \le 16$
Data collected	16957	15874
Unique data (R_{int})	7648 (0.037)	7436 (0.0818)
Parameters, restrains	339, 0	329, 0
Final R_1, wR_2[a] (Observed data)	0.0815, 0.2007	0.0758, 0.1496
Final R_1, wR_2[a] (All data)	0.1208, 0.2237	0.1480, 0.1765
Goodness of fit on F^2 (S)	1.034	1.009
Largest diff peak and hole (e Å^{-3})	1.750, -0.601	0.470, -0.334

[a] $R_1 = \Sigma||F_o|-|F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma(w(F_o^2-F_c^2)^2)/\Sigma w(F_o^2)^2]^{1/2}$

double bond V^{IV}=O[5,21-23] and shorter (*ca.* 0.4 Å) than single bond V^{IV}-O bond length.[5,24,25] The V-Cl1 bond lengths are comparable, 2.3174(16) Å in **1** and 2.3382(12) Å in **2** and are in the usual range.[11,21] The V-N bond lengths are in the range of 2.105(3)-2.120(3) Å, except for the nitrogen being trans to oxo ligand that are longer [2.287(3) Å in **1** and 2.312(4) Å in **2**] as a consequence of the strong trans influence of the mentioned oxo ligand.

The bite angles of N1-V-N2 and N3-V-N4 are about 77° and 73° in both complexes which significantly deviate from the ideal 90° and is comparable with the value of 74.9° in [VOCl(bpy)$_2$]+. These small bite angles, accompanied with the different bond distances of V-N, V-O and V-Cl are the main factors accounting for the distortion in octahedral geometry around the vanadium moiety.

To explore the *in vitro* antitumor activity of the titled vanadium complexes, cultures of three cancer cells; Caco-2, HT-29 and T47D were treated with these compounds and cell viability was determined by means of a colometric microculture assay (MTT assay). The results were compared with

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

	1	**2**
Bond length		
V1-Cl1	2.3174(16)	2.3382(12)
V1-O1	1.642(3)	1.583(4)
V1-N1	2.118(3)	2.120(3)
V1-N2	2.106(3)	2.105(3)
V1-N3	2.287(3)	2.120(3)
V1-N4	2.124(3)	2.312(4)
Bond angle		
N1-V1-N2	76.85(12)	77.06(13)
O1-V1-N1	94.77(14)	96.42(16)
N1-V1-Cl1	164.52(11)	162.97(12)
O1-V1-Cl1	98.82(12)	99.81(13)
N1-V1-N3	80.02(12)	90.84(13)
Cl1-V1-N2	93.05(10)	94.55(10)
O1-V1-N2	101.89(14)	101.57(14)
N1-V1-N4	93.83(12)	81.24(14)
N2-V1-N3	89.33(12)	159.38(15)
N2-V1-N4	161.39(13)	88.38(14)
N3-V1-N4	73.06(12)	73.15(13)
O1-V1-N3	166.31(15)	96.28(13)
O1-V1-N4	94.84(14)	169.06(12)
Cl1-V1-N3	88.26(9)	92.49(10)
Cl1-V1-N4	92.37(9)	83.75(9)

Table 3. Cytotoxicity of the complexes against human cell lines

Comp.	IC_{50} (μM)		
	Caco2	HT29	T47D
1	78.14 ± 13.11	20.16 ± 13.67	12.86 ± 1.30
2	> 100	52.30 ± 4.19	3.54 ± 0.49
Cisplatin	12.9 ± 0.8	37.9 ± 1.3	56.3 ± 5.1

the original Pt-based drugs cisplatin, as summarized in Table 3. To note, the difference between complexes **1** and **2** is only the variety of methyl position on bipyridine as ligands (Fig. 2 and 3). However, it leads to changes on antitumor capacity of the complexes. Complex **1** demonstrated more activity than cisplatin for HT29 and especially T47D as its IC_{50} values are lower. It seems, compound **2** has more selective cytotoxicity, where it exhibits no cytotoxic activity on Caco2, less cytoxicity than cisplatin on HT29, and further cytotoxicity related to cisplatin on T47D cell (more than twenty times).

We have shown the synthesis of two vanadium(IV) complexes with dimethyl-bipyridine from vanadium(III) chloride salt. The methyl group position in ligand has been found to be an effective parameter on cytotoxicity of relevant compounds where IC_{50} values of two complexes in different cultures are extensively different.[26] Future studies will be directed toward the toxicity evaluation of the titled compounds on normal cells as we expect that vanadium complexes exhibit less toxicity compared to platinum-based drugs, and consequently in vivo evaluation of them.

Experimental

Reagent grade chemicals were purchased from Merck and Aldrich and used as received without further purification. Infrared spectra (250-4000 cm^{-1}) were recorded on a shimadzu-470 plus spectrophotometer using CsI pellets. UV-Vis spectra were obtained on a Cary 100 BIO-Varian using a 1 cm path length cell. Analyses for C, H and N were carried out by a Heraeus CHN-O Rapid analyzer.

For synthesis of $[VOCl(4dmbpy)_2]\cdot Cl\cdot H_2O\cdot C_2H_5OH$ (**1**), 4,4'-dimethyl-2,2'-bipyridine (0.30 g, 1.62 mmol) in C_2H_5OH (7 mL) was added to a solution of VCl_3 (0.13 g, 0.81 mmol) in C_2H_5OH (4 mL) and H_2O (2 mL) and the resulting violet solution was stirred at 40-45 °C for 30 min. The green block crystals of **1** were obtained after 2 weeks with slow evaporation. (yield 0.36 g, 77.9%). IR (CsI, cm^{-1}): 3470br, 3029w, 2914w, 1620s, 1556w, 1494w, 1445m, 1308w, 1245w, 1032m, 973s, 925w, 840m, 563w, 519m, 424w, 349s, 253m. Anal. Calcd. C, 54.74; H, 5.61; N, 9.82%. Found: C, 54.28; H, 5.57; N, 9.75%.

For synthesis of $[VOCl\ (5dmbpy)_2]\cdot Cl\cdot H_2O\cdot CH_3OH$ (**2**), 5,5'-dimethyl-2,2'-bipyridine (0.13 g, 1.62 mmol) in CH_3OH (5 mL) was added to a solution of VCl_3 (0.13 mg, 0.81 mmol) in CH_3OH (5 mL) and H_2O (5 mL) and the resulting solution was stirred at 40-45 °C for 30 min. During the treatment, the brown solution first turned to violet and then to green. After 10 days, the green block crystal of complex **2** was obtained (yield 0.33 g, 73.2%). IR (CsI, cm^{-1}): 3451br, 3037w, 2924w, 2695br, 1608m, 1479s, 1390m, 1320w, 1243m, 1161w, 1054s, 979s, 835s, 732w, 658w, 493w, 424m, 356s, 264w. Anal. Calcd. C, 53.97; H, 5.39; N, 10.06%. Found: C, 53.51; H, 5.35; N, 9.97%.

The crystals of **1** and **2** were mounted on glass fibers using X-Ray diffraction data (Mo-Kα, graphite monochromator, λ = 0.71073 Å) and were collected at a temperature of 298(2) K using a Bruker APEX II CCD area detector diffractometer. The single crystals with dimensions 0.19 × 0.23 × 0.25 mm^3 for **1** and 0.20 × 0.24 × 0.29 mm^3 for **2** were selected for structure determination. The structures of **1** and **2** were solved by SHELX-97 and SHELXTL ver. 5.1 and absorption correction were done using the SADABS, X-Area 1.31 and APEX2 programs for **1** and **2** respectively.[27-29] The numerical absorption coefficient, μ, is 0.568 mm^{-1} for **1** and 0.585 mm^{-1} for **2**. Data collection, cell refinement and data reduction were done by X-Area 1.31 and APEX2, SAINT, SHELXTL ver. 5.1, PLATON and MERCURY.[27-32]

For determining the cytotoxicity of the complexes, three carcinoma cell lines have been considered, including Caco-2, HT-29 and T47D obtained from Pasteur Institute (Tehran, Iran). Cell incubation was kept at 37 ºC under an atmosphere of 95% air: 5% carbon dioxide. For MTT assay, the cells were plated in 96-well microtiter plates and incubated for 24 h at the density of 1×10^4 cells/well. Cells were then exposed to 62.5, 125, 250, 500, 750 μg/mL concentrations of the gold drugs. Cells were treated with cell lines for 48 h and incubated with 100 μL of 5 mg/ml MTT solution for 3-4 h at 37 ºC.[33] Cell survival was evaluated by measuring the

absorbance at 690 nm, using a micro plated reader (Anthos 2020, Cambride, UK). The values of 50% inhibition of cell proliferation (IC_{50}) were evaluated from the dose response curves by plotting cell survival versus compound concentration.

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Supplementary. CCDC 842896 and 842897 contain the supplementary crystallographic data for **1** and **2**, respectively. 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 336 033; or e-mail: deposit@ ccdc.cam.ac.uk.

References

1. Stein, G. S.; Lian, J. B. *Endocr. Rev.* **1993**, *14*, 424.
2. Barrio, D. A.; Williams, P. A.; Cortizo, A. M.; Etcheverry, S. B. *J. Biol. Inorg. Chem*. **2003**, *8*, 459.
3. Thompson, K. H.; Orvig, C. *Met. Ions Biol. Syst*. **2004**, *41*, 221.
4. Zhang, Y.; Yang, X. D.; Wang, K.; Crans, D. C. *J. Inorg. Biochem.* **2006**, *100*, 80.
5. Dong, Y.; Narla, R. K.; Sudbeck, E.; Uckun, F. M. *J. Inorg. Biochem*. **2000**, *78*, 321.
6. Noblía, P.; Vieites, M.; Parajón-Costa, B. S.; Baran, E. J.; Cerecetto, H.; Draper, P.; González, M.; Piro, O. E.; Castellano, E. E.; Azqueta, A.; López de Ceráin, A.; Monge-Vega, A.; Gambino, D. *J. Inorg. Biochem.* **2005**, *99*, 443.
7. Rivadeneira, J.; Barrio, D. A.; Etcheverry, S. B.; Baran, E. J. *Biol. Trace Elem. Res.* **2007**, *118*, 159.
8. Rivadeneira, J.; Barrio, D. A.; Arrambide, G.; Gambino, D.; Bruzzone, L.; Etcheverry, S. B. *J. Inorg. Biochem*. **2009**, *103*, 633.
9. Brand, S. G.; Edelstein, N.; Hawkins, C. J.; Shalimoff, G.; Snow, M. R.; Tiekink, E. R. T. *Inorg. Chem*. **1990**, *29*, 434.
10. Tolis, E. J.; Manos, M. J.; Tasiopoulos, A. J.; Raptopoulou, C. P.; Terzis, A.; Sigalas, M. P.; Deligiannakis, Y.; Kabanos, T. A. *Angew. Chem. Int. Ed*. **2002**, *41*, 2797.
11. Triantafillou, G. D.; Tolis, E. I.; Terzis, A.; Deligiannakis, Y.; Raptopoulou, C. P.; Sigalas, M. P.; Kabanos, T. A. *Inorg. Chem*. **2004**, *43*, 79.
12. Frank, P.; Carlson, R. M.; Carlson, E. J.; Hodgson, K. O. *J. Inorg. Biochem.* **2008**, *102*, 809.
13. Wong, E.; Giandomenico, C. M. *Chem. Rev.* **1999**, *99*, 2451.
14. Egdal, R. K.; Larsen, F. B.; Bond, A. D.; McKenzie, C. J. *Inorg. Chim. Acta* **2005**, *358*, 376.
15. Morsali, A.; Payheghader, M.; Poorheravi, M. R.; Jamali, F. *Z. Anorg. Allg. Chem*. **2003**, *29*, 1627.
16. Abedi, A.; Safari, N.; Amani, V.; Khavasi, H. R. *Dalton Trans*. **2011**, *40*, 6877.
17. Khavasi, H. R.; Abedi, A.; Amani, V.; Notash, B.; Safari, N. *Polyhedron* **2008**, *27*, 1848.
18. Yue, H.; Zhang, D.; Chen, Y.; Shi, Z.; Feng, S. *Inorg. Chem. Commun*. **2006**, *9*, 959.
19. Rubcic, M.; Milic, D.; Kamenar, B.; Cindric, M. *Acta Chim. Solv.* **2008**, *55*, 810.
20. Tasiopoulos, A. J.; Troganis, A. N.; Evangelou, A.; Raptopoulou, C. P.; Terzis, A.; Deligiannakis, Y.; Kabanos, T. A. *Chem. Eur. J.* **1999**, *5*, 910.
21. Otieno, T.; Bond, M. R.; Mokry, L. M.; Walter, R. B.; Carrano, C. J. *J. Chem. Soc., Chem. Commun.* **1996**, 37.
22. Qia, Y.; Yang, Y.; Cao, M.; Hu, C.; Wang, E.; Hu, N.; Jia, H. *J. Mol. Struct*. **2003**, *648*, 191.
23. Pacigová, S.; Gyepes, R.; Tatiersky, J.; Sivák, M. *Dalton Trans*. **2008**, 121.
24. Lorber, C.; Choukroun, R.; Donnadieu, B. *Inorg. Chem*. **2003**, *42*, 673.
25. Khan, M. I.; Cevik, S.; Doedens, R. J. *Chem. Commun*. **2001**, 1930.
26. Abedi, A.; Safari, N.; Amani, V.; Tavajohi, S.; Ostad, S. N. *Inorg. Chim. Acta* **2011**, *376*, 679.
27. Sheldrick, G. M. *Programs SHELXS97* (crystal structure solution) and *SHELXL97* (crystal structure refinement), University of Gottingen, Germany 1997.
28. Sheldrick, G. M. *SHELXTL V. 5.10*, Structure Determination Software Suite, Bruker AXS, Madison, Wisconsin, USA 1998.
29. Stoe & Cie, *X-AREA, V. 1.30*: Program for the acquisition and analysis of data; Stoe & Cie GmbH: Darmatadt, Germany, 2005.
30. Bruker, *APEX2 Software Package*, Bruker AXS Inc., 5465, East Cheryl Parkway, Madison, WI 5317, 2005.
31. Spek, A. L. *J. Appl. Cryst*. **2003**, *36*, 7.
32. Mercury 1.4.1, Copyright Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 1EZ, UK, 2001-2005.
33. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.