New Vanadium(V) Complexes with Salicylaldehyde Semicarbazone Derivatives: Synthesis, Characterization, and in vitro Insulin-Mimetic Activity − Crystal Structure of [$V^{V}O_2$(salicylaldehyde semicarbazone)]

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The new dioxo(semicarbazone)vanadium(V) complexes *cis*-VO_2L, where L = salicylaldehyde semicarbazone (L^1), salicylaldehyde 4-*n*-butylsemicarbazone (L^2), or salicylaldehyde 4-(2-naphthyl)semicarbazone (L^3), have been synthesized, characterized by ^1H and ^{13}C NMR and FTIR spectroscopy and tested for bioactivity as potential insulin-mimetic agents. All dioxovanadium(V) complexes exhibited essentially no in vitro insulin-mimetic activity, but the VO_2L^2 complex developed activity in the presence of ascorbic acid, similar to that of vanadyl sulfate. The molecular structure of the novel complex VO_2L^1 has been solved by X-ray diffraction methods. It crystallizes in the tetragonal space group $P4_2/n$ with a = 12.7674(7), c = 11.5308(5) Å, and Z = 8. The vanadium atom is in a distorted square-pyramidal coordination, with L^1 acting as a tridentate ligand through its azomethyne nitrogen atom, carbonyl oxygen atom and deprotonated phenol oxygen atom. The coordination sphere is completed by two oxo ligands at *cis* positions.

Introduction

The interaction of simple vanadium species with ligand groups bearing pharmacological activity, particularly those with antitumoral and insulin-mimetic properties, is of growing interest. A more detailed physicochemical characterization of vanadium compounds with pharmacologically interesting ligands will help in furthering the inorganic pharmacology of vanadium.[1]

In particular, the discovery of the insulin-like in vitro and in vivo activity of oxovanadates(V) and oxovanadium(IV) compounds such as vanadyl sulfate has stimulated research on vanadium compounds that may have promising application in the treatment of non-insulin-dependent type-2 diabetes mellitus.[2−9] Different approaches have been attempted to develop more potent and orally active vanadium-containing insulin enhancing agents.[10] Some vanadium complexes, such as bis(alkylmaltolato)oxovanadium(IV) ones, are promising candidates as oral complements for insulin in the treatment of diabetes. Although such complexes have shown pharmacological advantages (better gastrointestinal absorption, higher potency and less toxicity) compared to the uncomplexed $VOSO_4$, further improvement in ligand design is needed, focusing on identifying new vanadium compounds with increased potency and decreased toxicity.[11]

Semicarbazones and thiosemicarbazones (Figure 1) show a wide range of biological activities.[12−15] Although some (thiosemicarbazone)vanadium complexes have been synthesized and characterized, there are very few examples of the analogous semicarbazone ligands.[12] To further discern the chelating behaviour of semicarbazones of pharmacological

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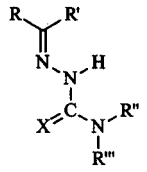


R, R', R'', R''' = H or alkyl or aryl group

Figure 1. General formula of semicarbazones (X = O) and thiosemicarbazones (X = S)

interest,[16−18] a more detailed physicochemical characterization of their vanadium complexes has been made, involving the synthesis, characterization and in vitro biological evaluation as potential insulin-mimetic agents of a series of novel (semicarbazone)VO_2^+ complexes with the general formula *cis*-V^VO_2L. Selected ligands L are salicylaldehyde semicarbazone derivatives bearing moieties with different lipophilicity − a physicochemical property that determines an adequate bioresponse of drugs, and is related to their absorption, distribution, metabolism, elimination and toxicity.[19] The ligands L (Figure 2) are salicylaldehyde semicarbazone (L^1), salicylaldehyde 4-*n*-butylsemicarbazone (L^2) and salicylaldehyde 4-(2-naphthyl)semicarbazone (L^3).

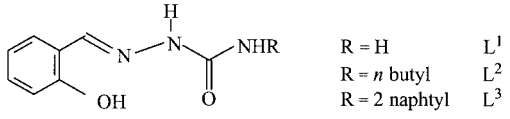


Figure 2. Selected ligands: salicylaldehyde semicarbazone (L^1), salicylaldehyde 4-*n*-butylsemicarbazone (L^2) and salicylaldehyde 4-(2-naphthyl)semicarbazone (L^3)

Although almost no $V^VO_2^+$ complex has shown good insulin-enhancing properties, V^V complexes may be reduced in vivo to form potentially bioactive $V^{IV}O^{2+}$ complexes.[20] In particular, vanadium(v) dipicolinate is an effective oral agent in live animals.[21] Thus, it seemed interesting to test the insulin-mimetic activity of the three novel complexes reported here.

Results and Discussion

Three novel V^V semicarbazone complexes have been synthesized and characterized. Complexes with the formula V^VO_2L, where L = salicylaldehyde semicarbazone (L^1), salicylaldehyde 4-*n*-butylsemicarbazone (L^2) or salicylaldehyde 4-(2-naphthyl)semicarbazone (L^3), were prepared in good yields and high purity. The analytical data agree with the proposed formulae.

In contrast to $V^{IV}O^{2+}$ complexes, those of VO_2L do not show electronic d−d transitions in the visible region due to the absence of d-electrons. Thus, the yellow color of the V^V complexes arises from intense absorption tailing-in from the UV region, originating in O→V charge transfer transitions.[22]

Selected vibration bands of the free ligands and their vanadium complexes, which are useful for determining the ligand's mode of coordination, are given in Table 1. The shifts in the ν(C=O) and the ν(C=N) bands upon coordination are consistent with bidentate coordination of the semicarbazone portion of the ligand through the carbonyl oxygen atom and azomethine nitrogen atom.[17,18] The third coordination position is the deprotonated phenol oxygen; thus the O−H stretching band is not in the spectrum of the complexes. In addition, the typical strong bands owing to asymmetric and symmetric stretching modes of the *cis*-VO_2 moiety are observed.[22]

Table 1. Selected vibration bands of the ligands L and the V^VO_2L complexes (cm^{-1}); ν: stretching; as: asymmetric, s: symmetric

Compound	ν(C=O)	ν(C=N)	ν(C−O)	ν(O−H)	$\nu_{as}(VO_2^+)$	$\nu_s(VO_2^+)$
VO_2L^1	1661	1606	1557	−	908	933
L^1	1683	1598	1522	3422	−	−
VO_2L^2	1645	1577	1473	−	933	912
L^2	1651	1573	1488	3300	−	−
VO_2L^3	1601	1560	1508	−	907	889
L^3	1647	1566	1508	3307	−	−

NMR Studies

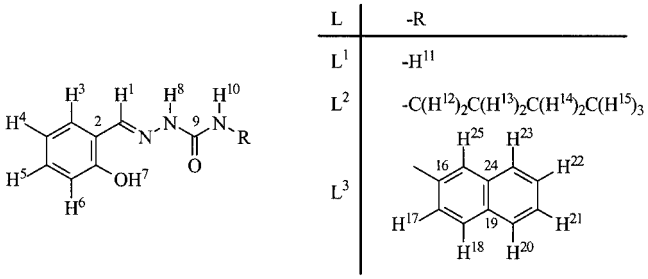
The NMR spectra show narrow signals typical of V^V diamagnetic complexes. HETCOR experiments allowed us to assign all of signals for the studied complexes. The ^1H NMR chemical shifts along with the chemical shift differences between each complex and the corresponding ligand, expressed as Δδ, are listed in Table 2. The formula in the table shows the numbering scheme of the free ligands. The ^1H NMR integrations and signal multiplicities agree with the proposed formula. The three complexes show similar ^1H and ^{13}C chemical shifts for the salicylaldehyde semicarbazone common portion. When the ligand is coordinated, the deshielding effect of the metal atom is apparent in some protons, causing a downfield shift of the corresponding ^1H NMR peaks. Upfield shifts in the ^1H NMR spectra of the signals of 4- and 6-H of the complexes could be the result of ligand deprotonation upon coordination, due to the lack of 7-H (Table 2).[18] The upfield shift of the signal of 3-H could be due to the decreasing azomethine anisotropic effect in the coordinated form of L. When L is coordinated, the azomethine moiety is fixed by the vanadium core in an opposite spatial distribution to 3-H. Thus, after coordination, this proton is less affected by the magnetic anisotropy of the C=N double bond than in the free ligand.[23]

Structural Results

Intramolecular bond lengths and angles around the metal ion in VO_2L^1 (Table 3) and an ORTEP[24] drawing of the molecule (Figure 3) are shown here.

The X-ray diffraction study shows that the complex VO_2L^1 consists of a discrete monomeric molecule. The vanadium(v) ion is in a distorted square-pyramidal environ-

Table 2. ^{1}H NMR chemical shifts (δ, ppm) of V^VO_2L, with L = L^1, L^2 and L^3, in [D_6]DMSO/D_2O at 303 K



H	$V^VO_2L^1$		$V^VO_2L^2$		$V^VO_2L^3$		Δδ[a]		
	δ_{Ligand}	$\delta_{Complex}$	δ_{Ligand}	$\delta_{Complex}$	δ_{Ligand}	$\delta_{Complex}$	$\Delta\delta_{L1}$	$\Delta\delta_{L2}$	$\Delta\delta_{L3}$
1	8.14	8.58	8.12	8.63	8.21	8.69	0.44	0.51	0.48
3	7.72	7.48	7.74	7.51	7.74	[b]	−0.24	−0.23	–
4	6.81	6.79	6.85	6.85	6.87	6.85	−0.02	0	−0.02
5	7.16	7.37	7.17	7.39	7.23	[b]	0.21	0.22	–
6	6.87	6.79	6.80	6.80	6.89	6.80	−0.08	0	−0.09
7	9.85	not present	9.97	not present	10.08	not present	–	–	–
8	10.19	12.50	10.17	not observed[c]	10.70	9.93	2.31	–	−0.77
10	6.53	7.96	6.80	8.35	9.07	9.82	1.43	1.55	0.75
11			–	–	–	–		–	–
12	–	–	3.12	3.20	–	–	–	0.08	–
13	–	–	1.45	1.49	–	–	–	0.04	–
14	–	–	1.30	1.33	–	–	–	0.03	–
15	–	–	0.89	0.90	–	–	–	0.01	–
17	–	–	–	–	7.97	8.06	–	–	0.09
18	–	–	–	–	[d]	[b]	–	–	–
20	–	–	–	–	[d]	[b]	–	–	–
21	–	–	–	–	7.37	[b]	–	–	–
22	–	–	–	–	7.46	[b]	–	–	–
23	–	–	–	–	[d]	[b]	–	–	–
25	–	–	–	–	8.31	8.42	–	–	0.11

[a] $\Delta\delta = (\delta_{Complex} - \delta_{Ligand})$. [b] 7.30−7.90 ppm, as multiplet. [c] Owing to exchange with solvent protons. [d] 7.77−7.87 ppm, as multiplet.

Table 3. Bond lengths [Å] and angles [°] around the vanadium atom in [VO_2(salicylaldehyde semicarbazone)], VO_2L^1

V−O(11)	1.622(4)	O(11)−V−O(12)	108.1(2)
V−O(12)	1.641(4)	O(11)−V−O(2)	105.1(2)
V−O(2)	1.894(4)	O(12)−V−O(2)	95.9(2)
V−O(1)	1.998(4)	O(11)−V−O(1)	101.8(2)
V−N(3)	2.154(5)	O(12)−V−O(1)	92.1(2)
		O(2)−V−O(1)	147.9(2)
		O(11)−V−N(3)	102.2(2)
		O(12)−V−N(3)	148.7(2)
		O(2)−V−N(3)	83.2(2)
		O(1)−V−N(3)	74.2(2)

ment, coordinated at the pyramid basis to a salicylaldehyde semicarbazone molecule that acts as a tridentate ligand through its azomethine nitrogen atom [d(V−N) = 2.154(5) Å], and its carbonyl and deprotonated phenol oxygen atoms [V−O distances of 1.998(4) and 1.894(4) Å, respectively], and to an oxo ligand [d(V−O) = 1.641(4) Å]. The fivefold coordination is completed by another oxo ligand at the pyramid apex [d(V−O) = 1.622(4) Å]. Both oxo ligands are in *cis* positions. The average V−O bond length and O−V−O angle are quite similar to those previously reported for the *cis*-VO_2 moiety in other complexes.[25] The *trans*-ligand−V−ligand angles are O1−V−O2 147.9(2)° and N3−V−O12 148.7(2)° and the *cis*-ligand−V−ligand angles vary from 74.2(2) to 108.1(2)°.

The nitrogen atom and the three coordinated oxygen atoms at the pyramid base are nearly in a plane (rms deviation of NO_3 atoms from the least-squares plane is 0.017 Å) with the metal ion 0.401 Å apart from this plane, towards the axial oxo ligand.

The salicylaldehyde semicarbazone molecule is also nearly planar (rms deviation of ligand atoms from the least-squares plane is 0.065 Å) and subtends an angle of 5.8(1)° with the NO_3 plane. Of the possible isomeric forms for the ligand, the (*E*) isomer is preferred. The crystal is further stabilized by a net of medium-to-strong intermolecular N−H···O bonds, involving the terminal NH_2 group of a given ligand molecule and the phenol oxygen atom [d(N1···O2′) = 2.972 Å, N1−H1A···O2 = 174.9°] and the equatorial oxo ligand [d(N1···O12″) = 2.870 Å, N1−H1B···O12″ = 171.4°] of neighboring ligand molecules, and also the N−H group in an N−H···O bond with the axial oxo ligand [d(N2···O11″) = 2.723 Å, N2−H2···O11″ = 153.2°].

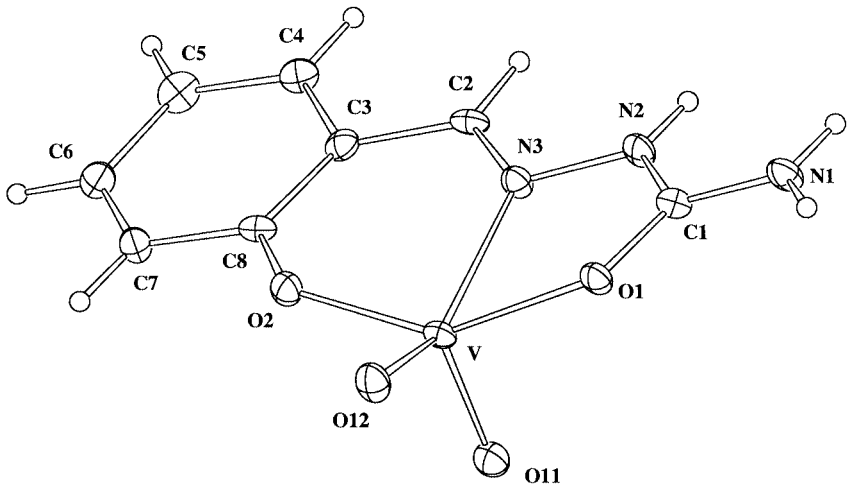
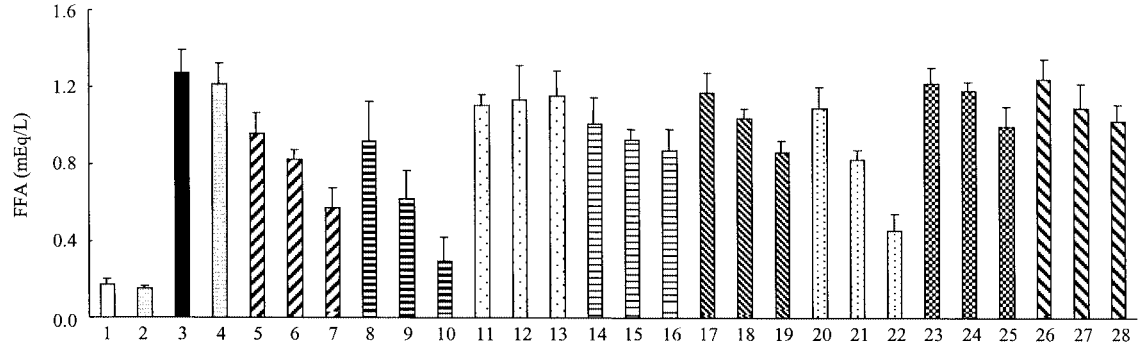


Figure 3. [VO_2(salicylaldehyde semicarbazone)] complex showing the labeling scheme of the non-H atoms and their displacement ellipsoids at the 30% probability level



1	Blank		11	VO_2L^1	10^{-4} M	17	VO_2L^2	10^{-4} M	23	VO_2L^3	10^{-4} M
2	Blank + ascorbic acid		12		5×10^{-4} M	18		5×10^{-4} M	24		5×10^{-4} M
3	Control		13		10^{-3} M	19		10^{-3} M	25		10^{-3} M
4	Control + ascorbic acid										
5	$VOSO_4$	10^{-4} M									
6		5×10^{-4} M	14	VO_2L^1 + ascorbic acid	10^{-4} M	20	VO_2L^2 + ascorbic acid	10^{-4} M	26	VO_2L^3+ ascorbic acid	10^{-4} M
7		10^{-3} M	15		5×10^{-4} M	21		5×10^{-4} M	27		5×10^{-4} M
8	$VOSO_4$ + ascorbic acid	10^{-4} M	16		10^{-3} M	22		10^{-3} M	28		10^{-3} M
9		5×10^{-4} M									
10		10^{-3} M									

Figure 4. Inhibitory effects of $VOSO_4$ and dioxovanadium(v) complexes with or without 1 mM ascorbic acid on FFA release from isolated rat adipocytes treated with epinephrine

Biological Activity of the Complexes

In vitro insulin-mimetic activities of the three dioxovanadium(v) complexes were examined, with or without 1 mM ascorbic acid, with regard to inhibition of FFA release from isolated rat adipocytes treated with epinephrine (Figure 4). Ascorbic acid exhibited essentially no inhibitory effect on FFA release under the experimental conditions. The inhibitory effects of dioxovanadium(v) complexes were compared with those of $VOSO_4$ as a positive control (IC_{50} = 1.00 ± 0.27 mM). In the absence of ascorbic acid, all dioxovanadium(v) complexes exhibited scarcely any inhibitory effects at concentrations of 10^{-4}, 5×10^{-4} and 10^{-3} M. The in vitro insulin-mimetic activities of the three dioxovanadium(v) complexes were lower than that of $VOSO_4$. However, the addition of 1 mM ascorbic acid to $VOSO_4$ or VO_2L^2 complex ($VOSO_4$-asc and VO_2L^2-asc) significantly enhanced the inhibitory effect of FFA release (Table 4, $VOSO_4$-asc: 0.34 ± 0.15 mM, VO_2L^2-asc: 0.73 ± 0.27 mM). Thus, the in vitro insulin-mimetic activity of VO_2L^2 was improved by the addition of the reducing agent ascorbic acid, even though it remained lower than that of pure $VOSO_4$ under the same conditions.

Upon addition of $VOSO_4$ to the adipocytes under air, the vanadyl state was found to be oxidized during the 3 h of incubation (unpublished results). Therefore, the addition of ascorbic acid was thought to maintain the vanadyl state during incubation, which in turn enhances the insulin-mimetic activity of $VOSO_4$. Interestingly, of the tested dioxovanadium(v) complexes only VO_2L^2 exhibited insulin-mi-

Table 4. Estimated IC_{50} values of the dioxovanadium(v) complexes

Compound	IC_{50} [mM]
$VOSO_4$	1.00 ± 0.27
$VOSO_4$ + ascorbic acid	0.34 ± 0.15[a]
VO_2L^1	none
VO_2L^1 + ascorbic acid	none
VO_2L^2	none
VO_2L^2 + ascorbic acid	0.73 ± 0.22[b][c]
VO_2L^3	none
VO_2L^3 + ascorbic acid	none

[a] Significance at $P < 0.05$ vs. $VOSO_4$. [b] Significance at $P < 0.05$ vs. $VOSO_4$ + ascorbic acid. [c] Significance at $P < 0.05$ vs. VO_2L^2.

metic activity in the presence of ascorbic acid, although spectroscopic experiments showed that all three complexes were reduced by the addition of ascorbic acid, as described below. These results suggest the importance of the interaction between the reduced forms of the complexes and the adipocytes, and that this interaction strongly depends on the chemical nature of the ligands.

Reaction of V^VO_2L with Ascorbic Acid

The reaction of $V^VO_2L^2$ with ascorbic acid was monitored by ^1H NMR spectroscopy at 303 K in [D_6]DMSO/D_2O. Immediately after the addition of ascorbic acid, the solution changed from yellow to dark-green and the narrow signals characteristic of the V^V diamagnetic complex were converted into broad ones. This strongly suggests that during the reaction the $V^VO_2L^2$ complex is reduced by the ascorbic acid to a paramagnetic V^{IV} species. Signals of free L^2 were not detected, indicating that the ligand remained coordinated to the central atom in the V^{IV} species.

Similar conclusions arise from the spectrophotometric study of the reduction with ascorbic acid of $V^VO_2L^2$ and $V^VO_2L^3$, as previously reported for (maltol)- and (ethylmaltol)vanadium(v) complexes.[25] In each case, after addition of the reducing agent a new band in the visible region near 670 nm was detected, which is attributable to a d−d transition of the resulting V^{IV} species.[22] After keeping the solution under air for 8 h, the new band disappeared gradually and finally the initial spectrum was obtained (Figure 5).

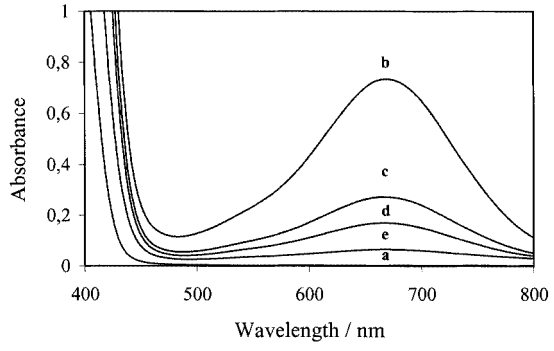


Figure 5. Electronic spectra (400−800 nm) of a 9.5×10^{-4} M DMSO solution of $V^VO_2L^2$ (a) before and (b) immediately after the addition of ascorbic acid, and (c) 20, (d) 60 and (e) 300 min after the addition

With $V^VO_2L^1$ re-oxidation of the V^{IV} species occurred almost immediately under the experimental conditions tested.

Thus, NMR and spectrophotometric experiments strongly indicate that ascorbic acid reduces V^VO_2L to a $V^{IV}O^{2+}L$ complex.

In particular, the reduction of the $V^VO_2L^2$ complex could explain the enhancement in insulin-mimetic activity of this complex in the presence of ascorbic acid.

These results, together with those of the biological tests in the presence of ascorbic acid, encouraged us to try to synthesize and isolate the $V^{IV}L^2$ complex, which could show the desirable in vitro and/or in vivo insulin-mimetic activity. We are thus extending our research to examine the structure-activity relationship of these complexes − involving redox potentials, partition coefficients, stability constants, molecular sizes and the elucidation of the chemical structure of the reduced forms. The emerging results will be the subject of a future publication.

Experimental Section

Materials: All common laboratory chemicals were purchased from commercial sources and used without further purification. 4-*n*-Butylsemicarbazide, 4-(2-naphthyl)semicarbazide and $V^{IV}O(acac)_2$, where acac = acetylacetonate, were prepared according to well-established literature procedures.[27−29] Vanadyl sulfate ($VOSO_4 \cdot 2.8H_2O$) and (+)-L-ascorbic acid were purchased from Wako Pure Chemical (Osaka, Japan). The purity of $VOSO_4 \cdot 2.8H_2O$ was determined by chelatometry using Cu-Pan [Cu-1-(2-pyridylazo-2-naphthol)]. Collagenase (Type II), bovine serum albumin (BSA; fraction V) and (±)-epinephrine hydrochloride (adrenaline) were obtained from Sigma Chemical (St. Louis, MO, USA). (+)-D-Glucose was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Other reagents were of the highest purity commercially available.

Physicochemical Characterization: C, H, N analyses were performed with a Carlo Erba Model EA1108 elemental analyzer. FTIR spectra (4000−400 cm^{-1}) were measured as KBr pellets with a Bomen M102 instrument. Electronic spectra were recorded with a Spectronic 3000 spectrophotometer. ^1H and ^{13}C NMR spectra of the free ligands and of the complexes were recorded with a Bruker DPX-400 instrument (at 400 and 100 MHz, respectively). Experiments were performed at 303 K in [D_6]DMSO/D_2O (8:2) (stability of the complexes in this medium had been tested previously). Heteronuclear correlation experiments (2D-HETCOR), HMQC (multiple quantum) and HMBC (multiple bond) were performed with the same instrument. MS experiments of the free ligands were obtained in the mass range 40−500 amu using a Shimadzu MSQP 1100 EX spectrometer with EI at 70 eV and a direct insertion probe.

Syntheses of the Ligands: All reactions were carried out under nitrogen. An equimolar mixture of 2-hydroxybenzaldehyde (salicylaldehyde) and the corresponding semicarbazide was stirred with *p*-TsOH (catalytic amounts) in dry toluene as solvent at room temperature until no more carbonyl compound was detected (SiO_2, 1% MeOH in CH_2Cl_2). For L^1 and L^2 the resulting solid was collected by filtration. For L^3 the solution was concentrated in vacuo and the solid was filtered off. The ligands were characterized by ^1H and ^{13}C NMR, IR and MS.

Syntheses of the Complexes: The $V^{V}O_2L$ complexes were prepared by boiling under reflux $V^{IV}O(acac)_2$ (100 mg, 0.375 mmol, acac = acetylacetonate) with L (0.375 mmol) in ethanol (10 mL) for 10 h.

$V^{V}O_2L^1$: $V^{V}O_2L^1$ was filtered off from the hot solution as a yellow-greenish solid. Yield 69 mg, 70%. Single yellow-greenish crystals, suitable for X ray diffraction, were obtained by slow concentration of an ethanolic solution at room temperature. $C_8H_8N_3O_4V$ (261.1): calcd. C 36.8, H 3.09, N 16.1; found C 37.1, H 3.41, N 15.8. UV/Vis (MeOH): λ_{max} = 237, 278, 315 nm. ^{1}H NMR: δ = 6.79 (m, 2 H), 7.37 (t, 1 H), 7.48 (d, 1 H), 7.96 (br. s, 2 H), 8.58 (br. s, 1 H), 12.50 (br. s, 2 H) ppm. ^{13}C NMR: δ = 118.20, 119.30, 120.00, 133.00, 134.30, 150.0, 161.4, 164.20 ppm.

$V^{V}O_2L^2$: $V^{V}O_2L^2$ was obtained as a microcrystalline powder by slow concentration of the reaction mixture at room temperature. Yield 47 mg, 40%. $C_{12}H_{16}N_3O_4V$ (317.2): calcd. C 45.4, H 5.08, N 13.2; found C 45.1, H 5.31, N 13.1. UV/Vis (MeOH): λ_{max} = 244, 287, 359 nm. ^{1}H NMR: δ = 0.90 (t, 3 H), 1.33 (m, 2 H), 1.49 (m, 2 H), 3.20 (m, 2 H), 6.80 (d, 1 H), 6.85 (t, 1 H), 7.39 (t, 1 H), 7.51 (d, 1 H), 8.35 (br. s, 1 H), 8.63 (br. s, 1 H) ppm. ^{13}C NMR: δ = 14.40, 20.18, 31.86, 41.03, 118.66, 119.77, 120.31, 133.39, 134.65, 150.22, 160.59, 164.58 ppm.

$V^{V}O_2L^3$: $V^{V}O_2L^3$ was obtained as a yellow solid by slow concentration of the reaction mixture at room temperature, after filtering off the unreacted ligand from the hot solution. Yield 73 mg, 50%. $C_{18}H_{14}N_3O_4V$ (387.3): calcd. C 55.8, H 3.64, N 10.8; found C 55.5, H 3.90, N 10.6. UV/Vis (MeOH): λ_{max} = 242, 283, 330 nm. ^{1}H NMR: δ = 6.80 (br. t, 1 H), 6.85 (br. d, 1 H), 7.30−7.90 (m, 7 H), 8.06 (m, 1 H), 8.42 (br. s, 1 H), 8.69 (br. s, 1 H), 9.82 (br. s, 1 H), 9.93 (br. s, 1 H) ppm. ^{13}C NMR: δ = 116.90, 119.00, 120.10, 121.00, 121.75, 125.17, 127.29, 127.88, 128.34, 128.82, 129.26, 131.48, 134.28, 134.69, 137.59, 150.00, 159.66, 163.79 ppm.

X-Ray Diffraction Data and Crystal Structure Determination and Refinement: Crystal data, the data collection procedure, structure determination methods and refinement results for $V^{V}O_2L^1$ are summarized in Table 5. The hydrogen atoms were included in the molecular model at stereochemical positions and refined with the riding model. CCDC-213179 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Animals: Male Wistar rats (7 weeks old) weighing 200−250 g were obtained from the Shimizu Experimental Material Co. (Kyoto, Japan). Animals were maintained on a 12 h light/dark cycle in a central animal facility. All animals were allowed free access to solid food (MF, Oriental Yeast Co. Tokyo, Japan) and tap water. All animal experiments were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University (KPU) and were performed according to the Guidelines for Animal Experimentation of KPU.

Inhibitory Effects of $VOSO_4$ and Dioxovanadium(v) Complexes on Free Fatty Acid (FFA) Release from Isolated Rat Adipocytes Treated with Epinephrine:[4,7] Epididymal fat pads, excised from male Wistar rats (7−8 weeks) anesthetized with ether, were cut into appropriate pieces and incubated in type-II collagenase digestion in a Krebs−Ringer hydrogen carbonate (KRB) buffer (120 mM NaCl, 1.27 mM $CaCl_2$, 1.2 mM $MgSO_4$, 4.75 mM KCl, 1.2 mM KH_2PO_4 and 24 mM $NaHCO_3$; pH = 7.4) containing 2% BSA and 5 mM glucose at 37 °C with gentle shaking at 100 cycles/min for 1 h. The prepared cells were filtered through sterilized cotton gauze and were then washed with KRB buffer. Isolated rat adipocytes cells ($1.0-2.0 \times 10^6$ cells/mL) were preincubated at 37 °C for 30 min with various concentrations ($10^{-4}-10^{-3}$ M) of $VOSO_4$ or dioxovanadate complexes in KRB buffer containing 2% dimethyl sulfoxide (DMSO) with or without 1 mM (+)-L-ascorbic acid. The reaction mixtures were then incubated at 37 °C for 3 h after the addition of epinephrine (10^{-5} M, adrenaline). The reactions were stopped by soaking in ice/water and the resultant mixtures were centrifuged at 3000 rpm at 4 °C for 10 min. For the outer solution of the cells, the FFA levels were determined with an FFA kit (NEFA C-test Wako, Wako Pure Chemicals). In vitro insulin-mimetic effects of the dioxovanadate complexes were evaluated by the apparent IC_{50} value (50% inhibitory concentration of the FFA released in each system).

Reaction of $V^{V}O_2L$ with Ascorbic Acid: The reduction of $V^{V}O_2L$ by ascorbic acid was studied by electronic spectroscopy in DMSO solution. Ascorbic acid (0.047 mmol, 8.3 mg) was added to a $V^{V}O_2L$ solution (9.5×10^{-4} M, 5.0 mL) ($V^{V}O_2L$/ascorbic acid, molar ratio 1:10). The spectrum of the resultant solution was measured in the range 400−800 nm both before and after the addition and monitored with time until no bands were detected. The reaction of $V^{V}O_2L^2$ with ascorbic acid was also monitored by ^{1}H NMR at 303 K, by first dissolving $V^{V}O_2L^2$ (0.032 mmol) in [D_6]DMSO/D_2O (8:2) in an NMR tube. After purging the solution with nitrogen for 5 min, the ^{1}H NMR spectrum was measured. The ascorbic acid (0.032 mmol) was then added in one portion, and the ^{1}H NMR spectrum measured again after keeping the mixture under nitrogen for 30 min.

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Table 5. Crystal data and structure solution methods and refinement results for [(salicylaldehyde semicarbazone)VO_2], VO_2L[1]

Empirical formula	$C_8H_8N_3O_4V$
Formula mass	261.11
Temperature [K]	120(2)
Low-temperature device	Oxford Cryosystems
Cooling rate [K/h]	200
Crystal system	tetragonal
Space group	$P4_2/n$
Unit cell dimensions [Å][a]	$a = b = 12.7674(7)$, $c = 11.5308(5)$
Volume [Å^3]	1879.6(2)
Z, calculated density [Mg/m^3]	8, 1.845
Absorption coefficient (μ) [mm^{-1}]	1.058
F(000)	1056
Crystal size [mm]	0.190 × 0.055 × 0.037
Crystal color/shape	greenish-yellow/prism
Diffractometer/scan type	KappaCCD/φ and ω
Radiation [Å], graphite monochromator	Mo-K_α, $\lambda = 0.71073$
ϑ range for data collection [°]	2.26 to 24.99
Index ranges	$-15 \leq h \leq 13$, $-14 \leq k \leq 15$, $-12 \leq l \leq 13$
Reflections collected/unique	6324/1663 [R(int) = 0.102]
Completeness [%]	94.7 (to ϑ = 24.99°)
Reflections observed [$I > 2\sigma(I)$]	1329
Absorption correction	PLATON[29]
Max./min transm.	0.964/0.884
Data collection	COLLECT[30]
Data reduction and correction,[b] structure solution,[c] and refinement[d] programs	DENZO and SCALEPACK,[31] SHELXS-97,[32] SHELXL-97[33]
Refinement method	Full-matrix least-squares on F^2
Weights w	$[\sigma^2(F_o^2) + (0.05P)^2 + 9.68P]^{-1}$; $P = [\max(F_o^2, 0) + 2F_c^2]/3$
Data/restraints/parameters	1663/0/145
Goodness-of-fit on F^2	1.177
Final R(ind.) [$I > 2\sigma(I)$][e]	$R1 = 0.0623$, $wR2 = 0.1674$
R indices (all data)	$R1 = 0.0810$, $wR2 = 0.1766$
Largest peak/hole [e·Å^{-3}]	0.438/−0.668

[a] Least-squares refinement of the angular settings for 6324 reflections in the $2.26° < \vartheta < 24.99°$ range. [b] Corrections: Lorentz, polarization, and absorption. [c] Neutral scattering factors and anomalous dispersion corrections. [d] Structure solved by Patterson and Fourier methods. Final molecular model obtained by anisotropic full-matrix least-squares refinement of the non-hydrogen atoms. [e] R indices defined as: $R1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$.

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