Insulinomimetic Vanadyl–Hydroxythiazolethione Complexes with VO(S_2O_2) Coordination Mode: The Correlation between the Activity and Hammett's Substituent Constant

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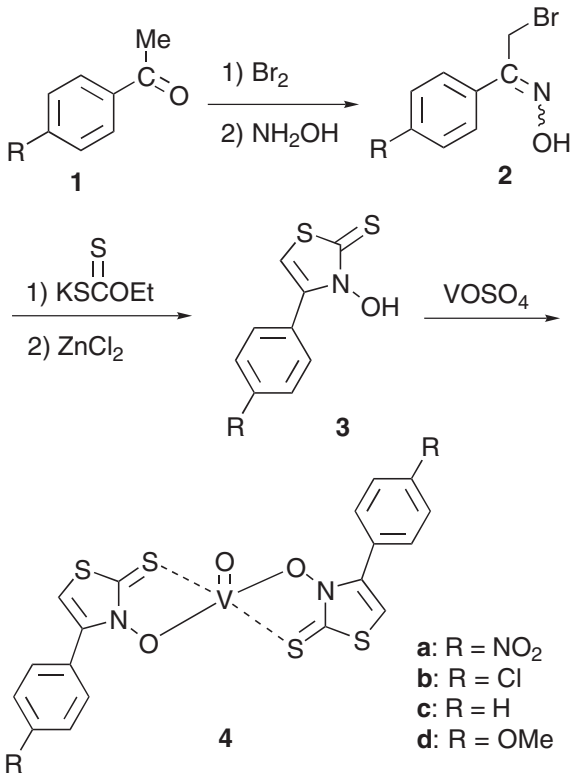
Four kinds of vanadyl–hydroxythiazolethione complexes with VO(S_2O_2) coordination mode were newly synthesized, and demonstrated for the first time that the insulinomimetic activity apparently correlates to the Hammett's substituent constant of the ligands.

The number of people suffering from diabetes mellitus (DM) has been estimated over 16 million in Japan, including potential patients. DM is generally classified into insulin-dependent type 1 DM caused by destruction of pancreatic β cells, and noninsulin-dependent type 2 DM caused by aging, obesity, spiritual stress, or other environmental factors.[1] Orally active chemotherapeutic agents for type 2 DM have already been developed and are currently available.[2] Patients with type 1 DM, however, can be only treated by daily injections of insulin several times in a day, which are painful and elevate the levels of patient stress, especially in young people. From these reasons, the creation and development of new orally active synthetic drugs in place of insulin injections are extremely desirable.[3] The establishment of a clear correlation between the structure and the insulin-mimetic activity is quite important for the strategy in the molecular design and development of new chemotherapeutic agents. Only a few factors, for example, the hydrophilic/lipophilic balance,[4,5] the stability constant,[5,6] the redox potential,[4] and the absolute configuration[4] have been proposed owing to an absolute lack of available data. We considered that the introduction of the *p*-substituted phenyl group results in the change of the electronic structure of a ligand, the stability and the insulinomimetic activity of a vanadyl complex.

As intensive studies on heterocycles[7] for the application to chemotherapeutic agents for DM, we describe here the synthesis of insulinomimetic vanadyl complexes of 4-(*p*-substituted phenyl)-3-hydroxythiazole-2(3*H*)-thiones with VO (S_2O_2) coordination mode, and a proposal of a new factor, the correlation between the Hammett's substituent constant and the insulinomimetic activity, in determining the structure-activity relationship.

4-(*p*-Substituted phenyl)-3-hydroxythiazole-2(3*H*)-thiones (**3**) were synthesized from acetophenones (**1**) via 4 steps by the modified literature method[8] as shown in Scheme 1. The p*K*a values of 3-hydroxythiazole-2(3*H*)-thiones in H_2O were estimated by means of the pH titration method; p*K*a = 3.9 for **3a**, 4.9 for **3b**, 4.0 for **3c**, and 4.6 for **3d**.[9] A typical procedure for synthesis of vanadyl complexes is as follows. A solution of **3a** (303 mg, 1.19 mmol) in H_2O (50 mL) was adjusted to pH 10 with 10 M KOH to dissolve completely. To the solution was added dropwise a solution of $VOSO_4 \cdot 3H_2O$ (140 mg, 0.64 mmol) in H_2O (10 mL). After stirring for 6 h, the pH of the reaction mixture was adjusted to 5 (p*K*a + 1) with 6 M HCl, and stirred for another 10 h at room temperature. The resulting yellow precipitate was collected by suction filtration, washed with small amount of H_2O, and then dried over anhydrous P_2O_5 to give vanadyl complex (**4a**), [2,3-dihydro-2-thioxo-4-(*p*-nitrophenyl)-3-thiazololato]oxovanadium (IV), (240 mg, 71%): mp 225 °C (decomp.); IR(KBr): 1063 ($\nu_{C=S}$), 1513 (ν_{NO_2}), 1348 (ν_{NO_2}), 997 ($\nu_{V=O}$), and 854 cm^{-1} ($\delta_{C\text{-}H}$); Anal Found: C, 37.97; H, 1.60; N, 9.63%. Calcd for $C_{18}H_{10}N_4O_7S_4V$: C, 37.70; H, 1.76; N, 9.77%.[10]



Scheme 1.

The electrochemical behavior of vanadyl complexes in the presence of 0.1 M tetrabutylammonium perchlorate in MeCN: $CHCl_3$ (1:1) mixture was evaluated by means of cyclic voltammetry: scan rate 100 mV s^{-1}; a working electrode Pt; an auxiliary electrode Pt wire; a reference electrode Ag/AgCl. The electron transfer processes of the complexes were irreversible, the i_{pa}/i_{pc} ratio being 1.5 for **4a**, 3.0 for **4b**, and 2.2 for **4d**. $E_{1/2}$ vs Ag/AgCl values (880 for **4a**; 840 for **4b**, and 960 mV for

4d) were higher than that of bis(maltolato)oxovanadium(IV),[11] indicating that these complexes are more stable towards the oxidation. Further, $\Delta\nu_{C=S}$ ($\nu_{C=S}$ for complex–$\nu_{C=S}$ for ligand) was calculated to be +7 for **4a**, +10 for **4b**, +17 for **4c**, and −22 for **4d**, indicating that the single bond character of C=S double bond increases with an increase of the electron-donating character of the substituent R upon complexation, and **4d** is most stable owing to the π-delocalization of the thiazole-2(3*H*)-thione ring.

The insulinomimetic activity of vanadyl complexes was evaluated by *in vitro* experiments, in which the inhibition of the release of free fatty acid (FFA) from isolated rat adipocytes treated with epinephrine was estimated by comparing the activity of vanadyl sulfate (VS).[12] The effect of vanadyl complexes was found to be dose-dependent in the concentration range from 1×10^{-5} to 5×10^{-4} M. The apparent IC_{50} value, which is a 50% inhibition concentration of FFA release in each complex, was estimated, and the results are summarized in Table 1 together with IC_{50} value of VS.

Vanadyl complex (**4a**) with 4-(*p*-nitrophenyl)-3-hydroxythiazole-2(3*H*)-thione showed the highest insulinomimetic activity among the complexes tested. Plots of IC_{50} values vs the Hammett's substituent constants (σ_p) of the ligands are shown in Figure 1. It is noteworthy that the IC_{50} value decreased with increase of the electron-withdrawing property of the substituent R. This is the first example showing the correlation between the insulinomimetic activity and the Hammett's substituent constant.

Further investigation on *in vivo* experiment of vanadyl complex (**4a**) with STZ-rats (streptozotocin-induced diabetic rats) is currently under way. On the basis of *in vitro* results, vanadyl complexes (**4a**–**4c**) are expected to be potent insulinomimetic complexes for treating type 1 DM in animals.

Table 1. Estimated IC_{50} values for the epinephrine-stimulated FFA release from isolated rat adipocytes

Compound	Coordination Mode	IC_{50} Value/mM[a]
4a	S_2O_2	0.08 ± 0.01
4b	S_2O_2	0.15 ± 0.02
4c	S_2O_2	0.35 ± 0.07
4d	S_2O_2	1.49 ± 0.37
$VOSO_4$	ionic	0.67 ± 0.03

[a]Each value is expressed as the mean ± SD for 3 experiments.

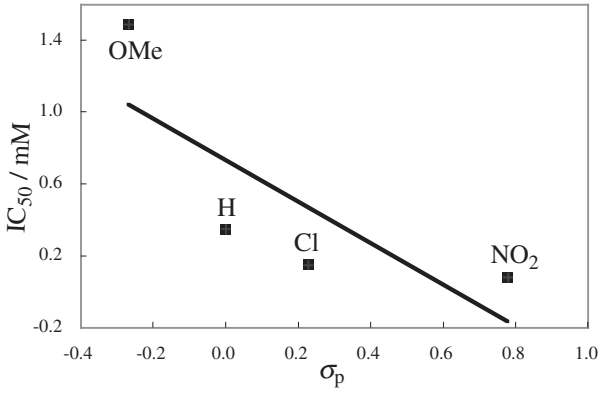


Figure 1. The relationship between IC_{50} values and the Hammett's substituent constants.

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9 Owing to low solubility in H_2O, these p*K*a values were estimated from the extrapolation of p*K*a values in various ratio of THF:H_2O mixture.
10 [2,3-Dihydro-2-thioxo-4-(*p*-chlorophenyl)-3-thiazololato]oxovanadium(IV) (**4b**): IR(KBr): 1061 ($\nu_{C=S}$), 995 ($\nu_{V=O}$), and 829 cm^{-1} (δ_{C-H}); Anal Found: C, 37.96; H, 1.82; N, 4.77%. Calcd for $C_{18}H_{10}Cl_2N_2O_3S_4V \cdot H_2O$: C, 37.90; H, 2.12; N, 4.91%. (2,3-Dihydro-2-thioxo-4-phenyl-3-thiazololato)oxovanadium(IV) (**4c**): IR(KBr): 1069 ($\nu_{C=S}$), 988 ($\nu_{V=O}$), 752, and 700 cm^{-1} (δ_{C-H}); Anal Found: C, 44.76; H, 2.66; N, 5.64%. Calcd for $C_{18}H_{12}N_2O_3S_4V$: C, 44.71; H, 2.50; N, 5.79%. [2,3-Dihydro-2-thioxo-4-(*p*-methoxyphenyl)-3-thiazololato]oxovanadium(IV) (**4d**): IR(KBr): 1030 ($\nu_{C=S}$), 984 ($\nu_{V=O}$), and 832 cm^{-1} (δ_{C-H}); Anal Found: C, 45.66; H, 3.36; N, 5.12%. Calcd for $C_{20}H_{16}N_2O_5S_4V \cdot 0.5THF$: C, 45.59; H, 3.48; N, 4.93%.
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