Synthesis of New Vanadyl Complexes of Hydroxyazine-type Heterocycles and Their Insulin-Mimetic Activities

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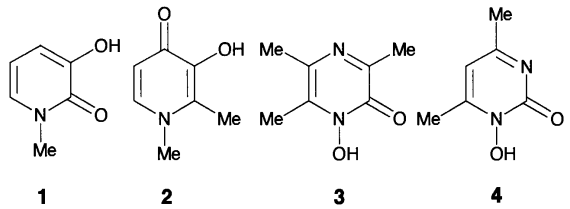
Four kinds of vanadyl complexes of hydroxyazine-type heterocycles were synthesized. Bis(1,2-dihydro-4,6-dimethyl-2-oxo-1-pyrimidinolato)- and bis(1,2-dihydro-2-oxo-3,5,6-trimethyl-1-pyrazinolato)oxovanadium(IV) complexes showed higher insulin-mimetic activity than vanadyl sulfate as a positive control.

Diabetes mellitus (DM), one of the most widespread diseases in the world, is generally classified into insulin-dependent DM (IDDM) and non-insulin dependent DM (NIDDM). Several chemotherapeutic agents for NIDDM have already been developed and clinically used.[1] Patients with IDDM, however, can be only treated by daily hypodemic injections of insulin, because it is a polypeptide with a molecular mass of 5.8 KDa and easily degradated by the ingestive systems. Much effort, therefore, has been devoted to design and synthesize orally active compounds in place of insulin. Under such circumstances, several papers concerning orally active insulin-mimetic vanadyl [oxovanadium(IV)] complexes have been reported,[1] but only two heterocycles, viz., pyridine[2] and 4*H*-pyrone,[3] were used as bidentate ligands, to the best of our knowledge. In addition, the establishment of a clear correlation between structure of the vanadyl complex and insulin-mimetic activity has been yet very difficult owing to absolute lack of available data.

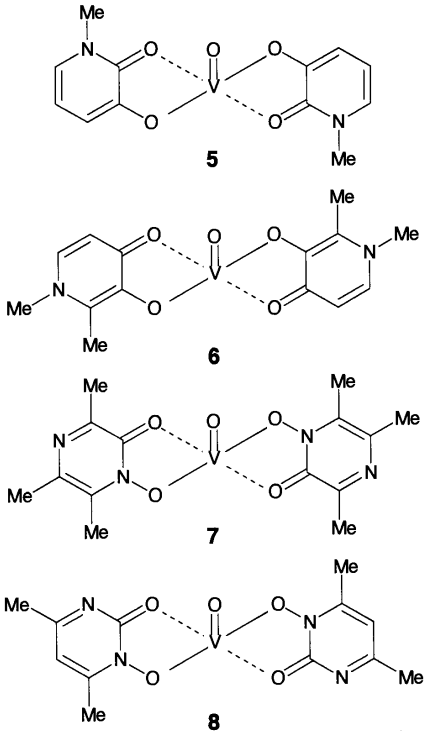
As extensive studies on heterocycles for application to chemotherapeutic agents,[4] we describe here synthesis of new four vanadyl complexes of hydroxyazine-type heterocycles and their insulin-mimetic activities.

3-Hydroxy-1-methyl-2(1*H*)-pyridinone (**1**),[5] 3-hydroxy-1,2-dimethyl-4(1*H*)-pyridinone (**2**),[6] 1-hydroxy-3,5,6-trimethyl-2(1*H*)-pyrazinone (**3**),[7] and 1-hydroxy-4,6-dimethyl-2(1*H*)-pyrimidinone (**4**)[7] were prepared according to literature methods.



1 **2** **3** **4**

A typical procedure for synthesis of vanadyl complexes is as follows. To a solution of **4** (700 mg, 5 mmol) in H_2O (8 mL) was added dropwise a solution of $VOSO_4 \cdot 3.6H_2O$ (570 mg, 2.5 mmol) in H_2O (5 mL). The pH of the solution was adjusted to 7 with 2M KOH, and the mixture was stirred overnight. The resulting precipitate was collected by filtration, washed several times with H_2O, and then dried over anhydrous P_2O_5 *in vacuo* to give the product, bis(1,2-dihydro-4,6-dimethyl-2-oxo-1-pyrimidinolato)oxovanadium (IV) (**8**) as gray powders (587 mg, 68%): mp 160 °C (decomp.); IR(KBr): 1618 ($\nu_{C=O}$) and 979 cm^{-1} ($\nu_{V=O}$); ESR parameters in DMSO at 77 K: g_0 = 1.977, A_0= 87.9 × 10^{-4} cm^{-1}, $g_{//}$ = 1.939, $A_{//}$ = 172.8 × 10^{-4} cm^{-1}, $g_{\perp}$ = 1.996, $A_{\perp}$ = 45.6 × 10^{-4} cm^{-1}; UV–vis: λ_{max} (c = 5 × 10^{-3} M in DMSO)/nm 817(ε/dm^3 mol^{-1} cm^{-1} 33), 610 (31) and 411 (297); magnetic susceptibility of 3.56 × 10^{-6} cgs unit and m_{eff} 1.80 (n = 1.06); FAB-MS: *m/z* 346 $[M+1]^+$. Anal Found: C, 39.35; H, 4.65; N, 15.2%. Calcd for $C_{12}H_{14}N_4O_5V \cdot 1.2H_2O$: C, 39.3; H, 4.5; N, 15.25%. Similarly other vanadyl complexes were synthesized, in which the solution pH was adjusted to 9.5 for **5**,[8] 10 for **6**,[9] and 5.5 for **7**.[10] These four vanadyl complexes showed typical isotopic eight line ESR spectra at room and liquid nitrogen temperature, indicating that they exist in a single isomer. Further, some neutral vanadyl complexes of bidentate ligands have been proven to be the *trans* isomer by X-ray crystallographic analysis.[1,11,12] From these data, the obtained vanadyl complexes probably exist in the *trans* form.



5 **6** **7** **8**

The insulin-mimetic activity of four 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) as a positive control.[13]

The *in vitro* experiments were carried out according to the method described in references.[2,13] The effects of vanadyl complexes and VS were found to be dose-dependent over the concentration range from 10^{-4} to 10^{-3} M as shown in Figure 1. The apparent IC_{50} value, which is a 50% inhibition concentration of FFA release in each compound, was estimated from these results. The obtained IC_{50} values for vanadyl complexes as follows when IC_{50} of VS was calibrated to be 1.0 mM: 2.13 M for **5**, 0.61 mM for **7**, and 0.39 mM for **8**. Complex (**6**) did not show measurable activity (not shown here). It is noteworthy that vanadyl complex (**8**) showed the highest activity amomg four heterocycle–vanadyl complexes, followed by complex (**7**), and also showed higher activity than bis(picolinato)oxovanadium(IV) complex (0.64 mM),[14] which has been already proven to be a potent therapeutic agent for DM in the *in vivo* experiment with STZ-rats (rats with streptozotocin-induced diabetes).[2] On the basis of the *in vitro* results, compound (**8**) may be a potent insulin-mimetic complex on the treatment of IDDM in animals.

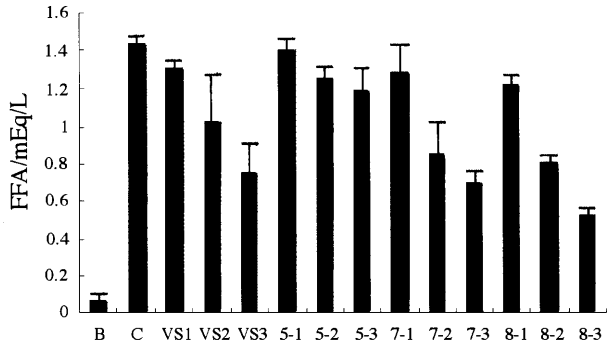


Figure 1. Inhibitory effects of vanadyl complexes on FFA release from rat adipocytes treated with epinephrine in the presence of 0.1% glucose. **B** and **C** are blank without epinephrine and complex, and control without complex, respectively. **VS** 1-3, **5**-1-3, **7**-1-3, and **8**-1-3 are $VOSO_4$, **5**, **7**, and **8**, respectively. In each system, adipocytes ($2.3\pm0.6\times10^6$ cells/mL) were treated with 10^{-4}, 5×10^{-4}, and 10^{-3} M of **5**, **7**, and **8**, respectively, for 30 min and then incubated with 10^{-5} M of epinephrine for 3 h at 37 ℃. Each column is expressed as the mean ± SD for three repeated experiments.

The partition coefficient of the complex is thought to be one of important factors for predicting the insulin-mimetic activity.[1] The partition coefficient ($C_{octanol}/C_{buffer}$) of complexes between octanol and 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) was measured by means of UV–vis spectroscopy.[5,15] The partition coefficient for **8** was estimated to be 0.03, while those for **5**, **6**, and **7** could not determined due to their high water solubility, indicating the importance of some degree of the hydrophobicity. Further, p*K*a values of hydroxyl groups of **5** and **6** were 9.1 and 9.64,[15] while those of **7** and **8** were reported to be 4.7 and 6.1,[7] respectively. The p*K*a values of the latter compounds were close to that of picolinic acid (pKa 5.4). It seems likely that the pK_a of ligand is one of important factors for the occurrence of the insulin-mimetic activity.

In conclusion, new two vanadyl complexes (**7** and **8**) with *N*-hydroxydiazine-type heterocycles (**3** and **4**) were found for the first time to show higher insulin-mimetic activities than vanadyl sulfate as a positive control, especially complex (**8**) being higher than bis(picolinato)vanadium(IV).

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8 Bis(1,2-dihydro-1-methyl-2-oxo-3-pyridinolato)oxovanadium (IV) (**5**): yield: 84%; IR(KBr): 1622 ($\nu_{C=O}$) and 965 cm^{-1} ($\nu_{V=O}$); ESR parameters in KRB buffer at 77K: g_0=1.966, A_0=89.8 × 10^{-4} cm^{-1}, $g_{//}$ = 1.940, $A_{//}$ = 176.3 × 10^{-4} cm^{-1}, $g_{\perp}$ = 1.980, $A_{\perp}$ =44.7 × 10^{-4} cm^{-1}; UV–vis: λ_{max} (c = 2 × 10^{-3} M in H_2O)/nm 540 (ε/dm^3 mol^{-1} cm^{-1} 156). Anal Found: C, 43.75; H, 4.15; N, 8.5%. Calcd for $C_{12}H_{12}N_2O_5V\cdot0.8H_2O$: C, 43.85; H, 4.15; N, 8.45%.

9 Bis(1,4-dihydro-1,2-dimethyl-4-oxo-3-pyridinolato)oxovanadium(IV) (**6**): yield: 84 %; IR(KBr): 1606 ($\nu_{C=O}$) and 967 cm^{-1}($\nu_{V=O}$); ESR parameters in DMSO at 77K: g_0 = 1.979, A_0 = 79.4 × 10^{-4} cm^{-1}, $g_{//}$ = 1.947, $A_{//}$ = 153.9 × 10^{-4} cm^{-1}, $g_{\perp}$ = 1.995, $A_{\perp}$ = 42.1 × 10^{-4} cm^{-1}; UV–vis: λ_{max} (c = 5 × 10^{-3} M in DMSO)/nm 613 (ε/dm^3 mol^{-1} cm^{-1} 96), 555 (86) and 403 (162); magnetic susceptibility of 5.53x10^{-6} cgs unit and m_{eff} 2.21 (n=1.42). Anal Found: C, 48.7; H, 4.55; N 7.8%. Calcd for $C_{14}H_{16}N_2O_5V$: C, 49.0; H, 4.7; N, 8.15%.

10 Bis(1,2-dihydro-2-oxo-3,5,6-trimethyl-1-pyrazinolato)oxovanadium (IV) (**7**): yield: 56%; mp 180 °C (decomp.); IR(KBr): 1590($\nu_{C=O}$) and 982 cm^{-1} ($\nu_{V=O}$); ESR parameters in DMSO at 77K: $g_{//}$ = 1.940, $A_{//}$ = 169.9 × 10^{-4} cm^{-1}; UV–vis: λ_{max} (c = 5 × 10^{-3} M in DMSO)/nm 802 (ε/dm^3 mol^{-1} cm^{-1} 30), 657(23) and 456 (102); magnetic susceptibility of 3.68 × 10^{-6} cgs unit and m_{eff} 1.91 (n = 1.15); FAB-MS: *m/z* 374 $[M+1]^+$. Anal Found: C, 43.6; H, 5.1; N 14.45%. Calcd for $C_{14}H_{18}N_4O_5V\cdot0.7H_2O$: C, 43.55; H, 5.1; N, 14.5%.

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14 The IC_{50} value has benn already reported,[1] but the experiment condition was different from the present one. The IC_{50} value, therefore, was reexamined under almost the same condition as the present study.

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