An Extremely High Insulin-Mimetic Activity of Bis(1,4-dihydro-2-methyl-1-phenyl-4-thioxo-3-pyridinolato)zinc(II) Complex

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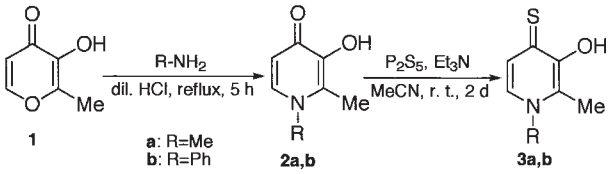
Vanadyl and zinc(II) complexes with $VO(O_2S_2)$ and $Zn(O_2S_2)$ coordination mode, respectively, were synthesized. Among them, bis(1,4-dihydro-2-methyl-1-phenyl-4-thioxo-3-pyridinolato)zinc(II) complex exhibited an extremely high insulin-mimetic activity ($IC_{50} = 0.04$ mM when IC_{50} value of a positive control, $VOSO_4$ was estimated to be 1.0 mM) compared to vanadyl and zinc(II) complexes reported previously.

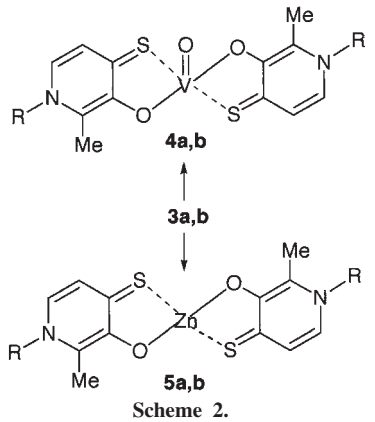
Diabetes mellitus (DM) affects over 140 million people all over the world.[1] DM is generally classified into type I DM (insulin-dependent) and type II DM (non-insulin-dependent). The treatment of type II DM is essentially based on dietary measures, exercise and stimulation of insulin sensitivity by oral chemotherapeutic agents. Such agents have some undesirable side effects. On the other hand, daily injections of insulin are still necessary for the treatment of type I DM.[2] Much effort, therefore, has been devoted to find less toxic and orally active drugs that enhance the insulin sensitivity or insulin-mimetic activity. A number of orally active insulin-mimetic vanadyl complexes have been reported.[3,4] Among them, a vanadyl complex, bis(1-oxy-2-pyridinethiolato)oxovanadium(IV), with $VO(O_2S_2)$ coordination mode has been demonstrated to treat both types of DM in experimental animals.[5] Then, we have focused on developing insulin-mimetic zinc(II) complexes with similar $Zn(O_2S_2)$ coordination mode, because zinc(II) is generally less toxic than vanadyl.[6] Further, the following points were taken into account for the molecular design; 1) Zn(II) complex with a bidentate ligand is superior to inorganic Zn(II) salt from the viewpoint of efficacy of gastrointestinal absorption and 2) a bidentate ligand, which can be easily synthesized and changed the hydrophobicity by introducing a variety of substituents at *N*-1 position, should be selected for the elucidation of the structure-activity relationship. Previously, we proposed the first insulin-mimetic zinc(II) complexes of maltol and 2-hydroxypyridine *N*-oxide with a $Zn(O_4)$ coordination mode.[7] In extending our studies on heterocycles for application to chemotherapeutic agents,[8] we found an extremely high insulin-mimetic bis(1,4-dihydro-2-methyl-1-phenyl-4-thioxo-3-pyrdinolato)zinc(II) complex. This paper reports the first insulin-mimetic Zn(II) complexes with $Zn(O_2S_2)$ coordination mode.

1,2-Dimethyl-3-hydroxy-(**2a**) and 3-hydroxy-2-methyl-1-phenyl-4(1*H*)-pyridinone (**2b**) were prepared from a commercially available maltol (**1**) and the corresponding amines according to literature methods.[9,10] 1,2-Dimethyl-3-hydroxy-(**3a**) and 3-hydroxy-2-methyl-1-phenyl-4(1*H*)-pyridinethione (**3b**) were prepared by treatment of **2a,b** with P_2S_5 in the presence of Et_3N in dry MeCN at room temperature (Scheme 1).



Scheme 1.

Typical procedures for syntheses of vanadyl and zinc(II) complexes are as follows (Scheme 2). To a solution of **3a** (155 mg, 1 mmol) in H_2O (5 mL) was added dropwise a solution of $VOSO_4 \cdot 3H_2O$ (109 mg, 0.5 mmol) in H_2O (5 mL). The pH of the solution was adjusted to 10.5 with 2 M 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 bis(1,4-dihydro-1,2-dimethyl-4-thioxo-3-pyridinolato)oxovanadium(IV) (**4a**; 169 mg, 90%).[11] To a solution of **3b** (869 mg, 4 mmol) and $LiOH \cdot H_2O$ (84 mg, 2 mmol) in H_2O (40 mL) was added a solution of $ZnSO_4 \cdot 7H_2O$ (575 mg, 2 mmol) in H_2O (40 mL), and then the reaction mixture was stirred overnight at room temperature. The resulting white precipitate was collected by filtration, washed well with H_2O, and then dried over P_2O_5 *in vacuo* to give bis(1,4-dihydro-2-methyl-1-phenyl-4-thioxo-3-pyridinolato) zinc (II) (**5b**; 905 mg, 91%).[12]



Scheme 2.

The insulin-mimetic activity of vanadyl and zinc(II) 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 effects of vanadyl and zinc(II) complexes were found to be dose-

dependent in the concentration range from 5×10^{-5} to 1×10^{-3} M (Figure 1), and the apparent IC_{50} value, which is a 50% inhibition concentration of FFA release in each complex, was estimated from these data, in which the IC_{50} values for VS are normalized to 1.0 mM to compare with other complexes. The obtained IC_{50} values for two vanadyl complexes (11.2 mM for **4a** and 2.45 mM for **4b**) were shown to be higher than that of $VOSO_4$ ($IC_{50} = 1.0$ mM) as a positive control. On the other hand, zinc(II) complex (**5b**) ($IC_{50} = 0.04$ mM) exhibited an extremely high insulin-mimetic activity than $VOSO_4$ ($IC_{50} = 1.0$ mM). The partition coefficient ($C_{octanol}/C_{buffer}$) of zinc(II) complex (**5b**) was measured to be 1.62 by means of UV-vis spectroscopy, indicating the importance of some degree of the hydrophobicity.[3,8] Unfortunately, the activity of zinc(II) complex (**5a**) could not be measured accurately owing to its low solubility under the employed condition.

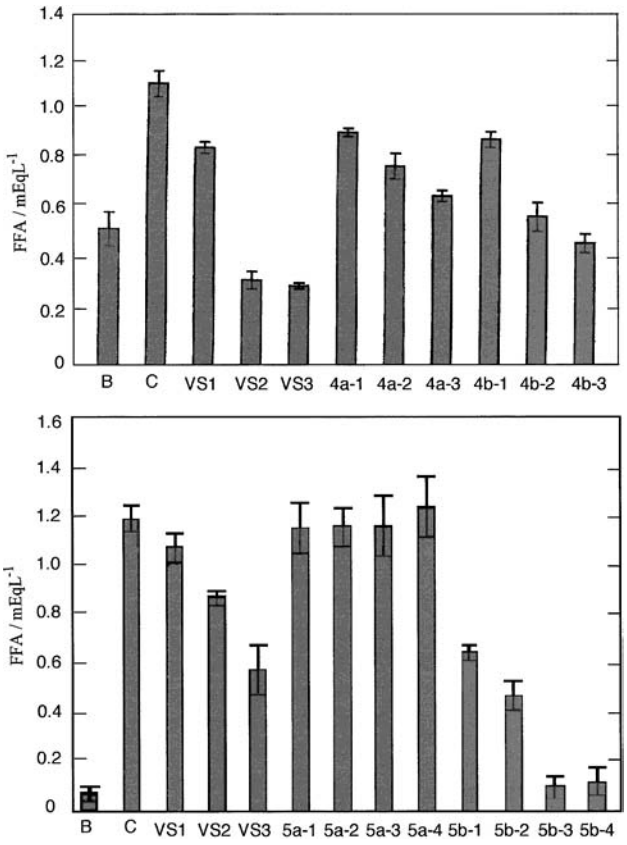


Figure 1. Inhibitory effects of vanadul (upper) and zinc(II) complexes (bottom) on FFA release from rat adipocytes treated with epinephrine in the presence of 0.1% glucose.[13] **B** is blank without epinephrine and complex, and **C** is control without complex. In the case of **VS1-3, 4a-1-3,** and **4b-1-3, 1-3** stand for concentrations of the complexes: **1** $= 1 \times 10^{-4}$; **2** $= 5 \times 10^{-4}$, **3** $= 1 \times 10^{-3}$ M. **5a-1-4** and **5b-1-4** also stand for concentrations: **1** $= 5 \times 10^{-5}$; **2** $= 1 \times 10^{-4}$; **3** $= 5 \times 10^{-4}$; **4** $= 1 \times 10^{-3}$ M. In each system, adipocytes ($2.3 \pm 0.6 \times 10^6$ cells/mL) were treated with complexes for 30 min, and then incubated with epinephrine (1×10^{-5} M) for 3 h at 37 °C. Each column is expressed as the mean ± SD for three repeated experiments.

It was concluded that 1) bis(1,4-dihydro-2-methyl-1-phenyl-4-thioxo-3-pyridinolato)zinc(II) (**5b**) exhibited the highest insulin-mimetic activity among vanadyl and zinc(II) complexes reported previously in terms of IC_{50} value, 2) zinc(II) complex (**5b**) showed activity about 10 times higher than the first insulin-mimetic zinc(II) complexes, bis(maltolato)zinc(II) and bis(2-hydroxypyridine-*N*-oxide)zinc(II),[7] and 3) zinc(II) complex (**5b**) exhibited insulin-mimetic activity higher than the corresponding vanadyl complex (**4b**). The result 3) is the first report concerning the direct comparison of the activities between vanadyl and zinc(II) complexes. On the basis of the results, we propose here that zinc(II) complex (**5b**) is a potent insulin-mimetic complex. Further investigation on *in vivo* activity as well as action mechanism of the complex in relation to the insulin receptor and glucose transporter is under way.

References and Notes

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11 Bis(1,4-dihydro-1,2-dimethyl-4-thioxo-3-pyridinolato)oxovanadium-(IV) (**4a**): IR(KBr): 962 cm^{-1} ($\nu_{V=O}$); UV-vis: λ_{max} ($c = 5 \times 10^{-3}$ M in DMSO)/nm 649 (ε/dm^3 mol^{-1} cm^{-1} 92) and 541 (146). Anal. Found: C, 44.70; H, 4.11; N, 7.34%. Calcd for $C_{14}H_{16}N_2O_3S_2V$: C, 44.80; H, 4.30; N, 7.46%. Bis(1,4-dihydro-2-methyl-1-phenyl-4-thioxo-3-pyridinolato)-oxovanadium(IV) (**4b**): IR(KBr): 972 cm^{-1} ($\nu_{V=O}$); UV-vis: λ_{max} ($c = 5 \times 10^{-3}$ M in DMSO)/nm 716 (ε/dm^3 mol^{-1} cm^{-1} 38) and 551 (160). Anal. Found: C, 57.33; H, 3.95; N, 5.39%. Calcd for $C_{24}H_{20}N_2O_3S_2V$: C, 57.71; H, 4.04; N, 5.61%.
12 Bis(1,4-dihydro-1,2-dimethyl-4-thioxo-3-pyridinolato)zinc(II) (**5a**): IR(KBr): 1585, 1454, 1313, and 1199 cm^{-1}; UV-vis: λ_{max} ($c = 2 \times 10^{-5}$ M in DMSO)/nm 357 (ε/dm^3 mol^{-1} cm^{-1} 35800), 290 (8400), and 263 (38500); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.47 (3H, s), 3.91 (3H, s), 7.42 (1H,d, $J = 6.2$ Hz), and 7.53 ppm (1H, d, $J = 6.2$ Hz); Anal. Found: C, 45.15; H, 4.27; N, 7.27%. Calcd for $C_{14}H_{16}N_2O_2S_2Zn$: C, 44.99; H, 4.31; N, 7.49%. Bis(1,4-dihydro-2-methyl-1-phenyl-4-thioxo-3-pyridinolato)zinc(II) (**5b**): IR(KBr): 1610, 1571, 1490, 1459, 1321, 1222, and 1170 cm^{-1}; UV-vis: λ_{max} ($c = 2 \times 10^{-5}$ M in DMSO)/nm 363 (ε/dm^3 mol^{-1} cm^{-1} 48500), 289 (9400), and 269 (37700); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.17 (3H, s), 7.51 (1H,d, $J = 6.3$ Hz), 7.56 (1H, d, $J = 6.3$ Hz), and 7.50-7.65 (5H, m); Anal. Found: C, 57.89; H, 3.88; N, 5.51%. Calcd for $C_{24}H_{20}N_2O_2S_2Zn$: C, 57.89; H, 4.05; N, 5.63%.
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