# A Highly Potent Insulin–mimetic Zinc(II) Complex with a $Zn(S_2O_2)$ Coordination Mode: Bis(1,6-dimethyl-3-hydroxy-5-methoxy-2-pentyl-1,4-dihydropyridine-4-thionato)zinc(II)

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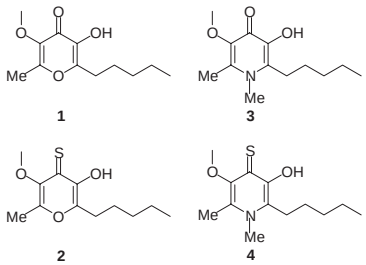
Zn(II) complexes of allixin and its sulfur and *N*-methyl analogs were newly prepared, and their insulin–mimetic activities were evaluated. Among them, a new bis(1,6-dimethyl-3-hydroxy-5-methoxy-2-pentyl-1,4-dihydropyridine-4-thionato)-zinc(II) complex exhibited the most potent insulin–mimetic activity ($IC_{50} = 11\,\mu M$) in terms of inhibition of free fatty acid release in adipocytes.

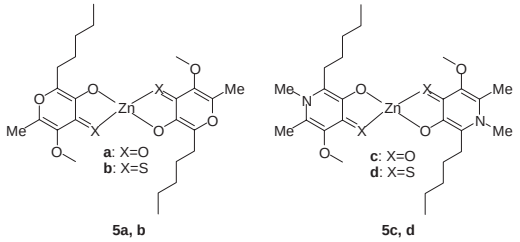
The number of patients suffering from diabetes mellitus (DM) in 2002 was reported to be approximately 173 million people worldwide.[1] DM is generally classified into insulin-dependent type 1 DM and non-insulin-dependent type 2 DM. Although several types of insulin preparations have been developed for patients with type 1 DM and synthetic therapeutics are available for clinical use in patients with type 2 DM, both types of treatments have been associated with problems such as physical and mental pain due to daily insulin injections and certain severe side effects, respectively. Therefore, the development of a new type of anti-diabetic agent is indispensable not only to treat DM but also to improve the quality of life (QOL) in DM patients. Owing to the worldwide necessity for the development of new types of reagents, we have prepared several Zn(II) complexes with various coordination modes,[2] and have found a potent bis(maltolato)zinc(II) complex ($[Zn(ma)_2]$) with a $Zn(O_4)$ coordination mode that exhibits anti-diabetic activity in the form of a blood glucose-lowering effect in the hereditary diabetic mice KK-$A^y$ mice, which is an excellent animal model of human type 2 DM and obesity.[3–5] Following this finding, we examined the in vitro and in vivo structure-activity relationships of $Zn(ma)_2$-related complexes by using this $[Zn(ma)_2]$ as a leading compound.[6] A Zn(II) complex with allixin (3-hydroxy-5-methoxy-6-methyl-2-pentyl-4*H*-pyran-4-one) isolated from garlic was then found to exhibit the highest insulin–mimetic activity among $Zn(ma)_2$-related complexes.[6,7] On the basis of the results, we have attempted to develop more active insulin–mimetic Zn(II) complexes than $[Zn(alx)_2]$ by using the following hypothesis: 1) the concept of equivalent transformation of ligand atoms, from oxygen to sulfur, is useful for altering the activity of the complex;[8,9] and 2) the substitution of ether oxygen at the *O*-1 position of allixin to *N*-$CH_3$ changes the lipophilicity of allixin,[9] and found that the bis(1,6-dimethyl-3-hydroxy-5-methoxy-2-pentyl-1,4-dihydropyridine-4-thionato)zinc(II) complex has extremely high insulin–mimetic activity (**5d** in Scheme 2).

Allixin, 3-hydroxy-5-methoxy-6-methyl-2-pentyl-4*H*-pyrane-4-one (**1**), 3-hydroxy-5-methoxy-6-methyl-2-pentyl-4*H*-pyran-4-thione (**2**), 1,6-dimethyl-3-hydroxy-5-methoxy-2-pentyl-1,4-dihydropyridine-4-one (**3**), and 1,6-dimethyl-3-hydroxy-5-methoxy-2-pentyl-1,4-dihydropyridine-4-thione (**4**), all used in this study (Scheme 1), were synthesized according to previously reported method.[10]




**Scheme 1.**

$[Zn(alx)_2]$ (**5a**) was prepared as previously reported.[6] Other Zn(II) complexes (**5b**–**5d**, Scheme 2) were prepared as follows. To a suspension of ligand (160 μmol) and $ZnSO_4 \cdot 7H_2O$ (80–90 μmol) in $H_2O$ (2 mL) was added a solution of $LiOH \cdot H_2O$ (160 μmol) in $H_2O$ (3 mL), and the reaction mixture was then stirred for 3–7 h at room temperature. The resulting precipitate was collected by filtration, washed well with cold water, and then dried in vacuo.[11] The preparation of **5b** was carried out under nitrogen atmosphere in a gloved box, because the compound **2** was unstable in air. The compositions were determined by elemental analyses, mass spectrometry, and IR spectra, and then compared with the data for the $[Zn(alx)_2]$ complex.[5,11]




**Scheme 2.**

The insulin–mimetic activity of Zn(II) complexes was evaluated in an in vitro experiment, in which the inhibitory activity of the release of free fatty acids (FFA) from isolated rat adipocytes treated with epinephrine was evaluated according to the previous report.[12] All the Zn(II) complexes examined exhibited complex concentration-dependency in the range of 10–500 μM (Figure 1), and the apparent $IC_{50}$ value, which is a 50% inhibitory concentration of FFA-release in each complex, was calcu-

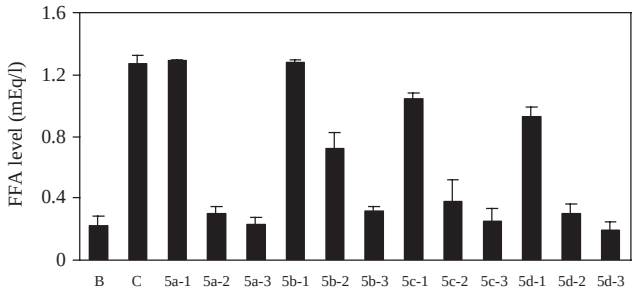



**Figure 1.** Inhibitory effects of Zn(II) complexes on FFA-release from rat adipocytes treated with epinephrine in the presence of 5 mM glucose and 2% DMSO. **B** is blank without epinephrine and complex, and C is control without complex. The concentration of **5a** and **5c**: **1** = 100 μM; **2** = 224 μM; **3** = 500 μM. The concentrations of **5b** and **5d**: **1** = 10 μM; **2** = 50 μM; **3** = 100 μM. In each system, adipocytes were treated with the complexes for 30 min, and then incubated with 10 μM epinephrine for 3 h at 37 °C. Each column is expressed as the mean ± SD for 3 experiments.

lated from these data (Table 1). Zn(II) complexes with a $Zn(S_2O_2)$ coordination mode (**5b** and **5d**) were found to exhibit extremely high insulin–mimetic activities in comparison with Zn(II) complexes (**5a** and **5c**) with a $Zn(O_4)$ coordination mode, indicating that $S_2O_2$ ligation to Zn(II) is superior to $O_4$ ligation in developing in vitro insulin–mimetic activity. Although no difference in the insulin–mimetic activity was observed between new Zn(II) complex (**5c**) with a $Zn(O_4)$ coordination mode and that of $[Zn(alx)_2]$ (**5a**), Zn(II) complex (**5d**) with a $Zn(S_2O_2)$ coordination mode exhibited higher potent insulin–mimetic activity than the complex (**5b**) with the same coordination mode.

**Table 1.** $IC_{50}$ values of Zn(II) complexes and partition coefficients of the corresponding ligands

| Compound | Coordination mode | $IC_{50}$ value (μM) | log *P* of ligand (ligand No.) |
|---|---|---|---|
| $ZnSO_4$ | Ionic | 408 ± 28 | — |
| $[Zn(ma)_2]$ | $O_4$ | 220 ± 28[a] | 0.12 ± 0.06 |
| **5a** | $O_4$ | 151 ± 11[ab] | 1.99 ± 0.06 (**1**) |
| **5b** | $S_2O_2$ | 31 ± 3[abc] | 1.36 ± 0.06 (**2**) |
| **5c** | $O_4$ | 159 ± 26[ab] | 1.73 ± 0.01 (**3**) |
| **5d** | $S_2O_2$ | 11 ± 1[abcd] | 2.04 ± 0.08 (**4**) |

Significance: [a] $p < 0.01$ vs $ZnSO_4$, [b] $p < 0.05$ vs $[Zn(ma)_2]$, [c] $p < 0.01$ vs **5a**, [d] $p < 0.01$ vs **5b**

The partition coefficients ($C_{n\text{-octanol}}/C_{buffer}$) of the ligands were measured by using UV spectrophotometory.[13] We have previously reported that the lipophilicity of the ligand in the Zn(II) complex is an important factor in the development of insulin–mimetic activity because the action sites of Zn(II) are thought to be primarily in the cells.[6,14,15] When a *N*-$CH_3$ group was substituted in place of oxygen at the *O*-1 position of allixin, the lipophilicity of the ligand **3** was decreased (Table 1), giving a comparable $IC_{50}$ value to that of the $[Zn(alx)_2]$ complex. By substitution of sulfur in place of oxygen at the ketone group of allixin, the lipophilicity of the ligand (**2**) was not increased over that of allixin, although *N*-$CH_3$ substitution (**4**) at the *O*-1 position of ligand **2** enhanced the lipophilicity, which in turn produced an extremely high insulin–mimetic activity.

In conclusion, we have developed new potent insulin–mimetic Zn(II) complexes with a $Zn(S_2O_2)$ coordination mode (**5b** and **5d**), with bis(1,6-dimethyl-3-hydroxy-5-methoxy-2-pentyl-1,4-dihydropyridine-4-thionato)zinc(II) (**5d**) showing the most potent insulin–mimetic activity among previously reported Zn(II) complexes.[2,6] On the basis of these results, we propose here the new insulin–mimetic Zn(II) complex (**5d**) with a $Zn(S_2O_2)$ coordination mode. The effectiveness of the complex in diabetic animals will be reported in the near future.

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**References and Notes**

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