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SYNTHESIS OF OXOVANADIUM(IV) AND ZINC(II) COMPLEXES OF 3-HYDROXY-4-(*p*-SUBSTITUTED)PHENYLTHIAZOLE-2(3*H*)-THIONES WITH A S_2O_2 COORDINATION MODE AND THEIR INSULIN-MIMETIC ACTIVITIES†

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Abstract – Oxovanadium(IV) and zinc(II) complexes with five kinds of 3-hydroxy-4-(*p*-substituted)phenylthiazole-2(3*H*)-thiones as bidentate ligands were newly synthesized. Zinc(II) complexes showed approximately 15 times higher *in vitro* insulin-mimetic activities than that of $ZnSO_4$ as a positive control. Oxovanadium(IV) complexes also exhibited *in vitro* insulin-mimetic activities, in which a correlation between the activity and the Hammett's constant of the substituent R was found. Among zinc(II) complexes, bis[2,3-dihydro-2-thioxo-4-(*p*-nitrophenyl)-(**6a**) and bis[2,3-dihydro-2-thioxo-4-(*p*-chlorophenyl)-3-thiazololato]zinc(II) (**6b**) substantially lowered the blood glucose levels in KK-A^y mice. Oral glucose tolerance tests for **6a** and **6b** indicated the improvement of the diabetic states of animals.

INTRODUCTION

Diabetes mellitus (DM) is a medical disorder characterized by persistent hyperglycemia. The number of people suffering from DM has been estimated to be nearly 173 million all over the world.[1] DM develops many secondary complications such as atherosclerosis, microangiopathy, renal function failure, cardiac disease, diabetic retinopathy, and other ocular disorders including blindness.

† Dedicated to Prof. Dr. Ivar Ugi as the memory.

DM is generally classified into two types. Type 1 DM (insulin-dependent DM) is caused by destruction of pancreatic β-cells. On the other hand, type 2 DM (non-insulin-dependent DM) is caused by aging, obesity, spiritual stress, and/or other environmental factors. Although several types of insulin preparations for type 1 DM and orally active medicines for type 2 DM have been developed and clinically used, they still have some problems such as physical and mental pain caused by daily insulin injections and serious side effects such as hypoglycemia and body weight gain. Since the first discovery that vanadium and zinc ions showed insulin-mimetic activities,[2-11] many medicinal chemists have focused their interests on these metals, because they could be potential candidates for safe and orally active medicines. Numerous efforts, therefore, have been dedicated to exploit these metal ions as medicines for DM, and revealed that these metals in the form of inorganic salts could decrease and normalize the blood glucose level in diabetic animals even by the oral dosage.[8-12] However, it has been shown that these elements in the inorganic forms are poorly absorbed, and thus high doses of these elements are required to improve the blood glucose levels, where some undesirable side effects developed. In order to solve the problems with respect to absorption and dosage, it is desirable to administrate these elements in the form of complexes with organic ligands.

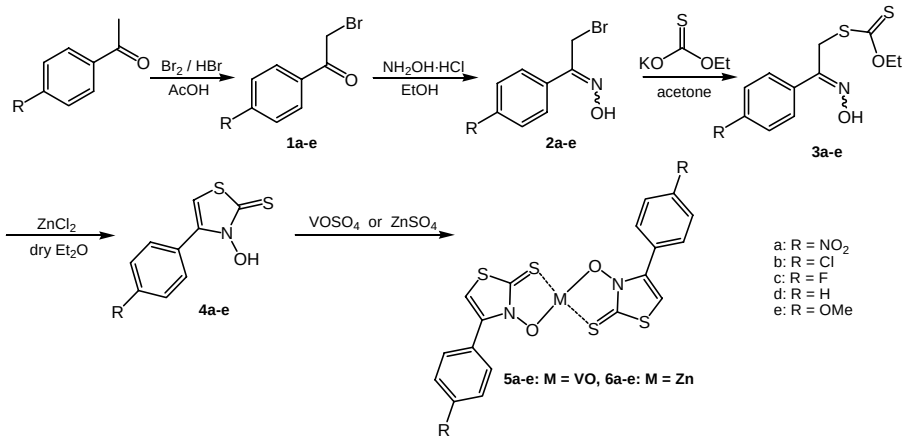
In 1990's, it was demonstrated for the first time that complexes composed of oxovanadium(IV) and low-molecular-weight organic ligands lowered the blood glucose level in type 1 DM rats.[13-15] Following the finding, zinc(II) complexes with small organic ligands were also found to exhibit higher insulin-mimetic activity than inorganic zinc salts both *in vitro* and *in vivo*.[10,16] Since these findings, a number of oxovanadium(IV) and zinc(II) complexes of organic ligands with various coordination modes such as $M(O_4)$, $M(N_2O_2)$, $M(N_2S_2)$, $M(S_2O_2)$ and $M(S_4)$ [M=VO or Zn] have been synthesized, and new candidates showing higher anti-diabetic activities with lower doses both *in vitro* and *in vivo* than the inorganic salts, $VOSO_4$ and $ZnSO_4$ have been proposed.[17-31] During these studies, it has been proposed that the hydrophilic/hydrophobic balance,[27,32] the stability constant[24] and the absolute configuration[32] are important factors for gaining high insulin-mimetic activity. However, establishment of a clear correlation between the chemical structures of oxovanadium(IV) and zinc(II) complexes and their insulin-mimetic activities is still difficult due to lack of available data.

We have focused on synthesis of hydroxyazine-type heterocyclic compounds and their utilization for chemotherapeutic agents.[33] In 2000, we demonstrated for the first time the insulin-mimetic activities of four kinds of oxovanadium(IV) complexes of hydroxymonoazine- and hydroxydiazine-type heterocyclic compounds.[34] Since then, we have intensively explored insulin-mimetic oxovanadium(IV) and zinc(II) complexes of various hydroxyazine-type heterocycles.[26,35,36] The hydrophilic/hydrophobic balance[37] and the S_2O_2 coordination mode seem to be significant factors for bringing about high insulin-mimetic activity.

As an important factor for designing effective medicines for DM, we consider that the introduction of the substituted phenyl group on a central heterocyclic nucleus results in change of the electronic structure of the ligand, the stability and the insulin-mimetic activity of its oxovanadium(IV) and zinc(II) complexes. With the objective for providing a new family of insulin-mimetic metal complexes with S_2O_2 coordination mode, we synthesized oxovanadium(IV) and zinc(II) complexes of 3-hydroxy-4-(*p*-substituted)phenylthiazole-2(3*H*)-thiones, and evaluated their *in vitro* and *in vivo* insulin-mimetic activities.

RESULTS AND DISCUSSION

Synthesis and characterization of oxovanadium(IV) and zinc(II) complexes: 3-Hydroxy-4-(*p*-substituted)phenylthiazole-2(3*H*)-thiones **4a-e** were synthesized from acetophenones *via* 4 steps by the modified literature method [38] as shown in Scheme 1. On the bromination of acetophenones, the use of pure AcOH (purity 99.9%) was crucial. The bromoacetophenones **1a-e** were successfully converted into the corresponding oximes **2a-e**, followed by reacting with *O*-ethyl xanthic acid potassium salt to afford *O*-ethyl *S*-[oximino-2-(*p*-substituted phenyl)ethyl]dithiocarbonate **3a-e** in moderate to good yields. The cyclization of the esters **3a-e** was carried out in the presence of $ZnCl_2$ to give thiazole-2(3*H*)-thiones **4a-e** in good yields. It is worthy of note that freshly distilled anhydrous Et_2O and handling of $ZnCl_2$ under nitrogen atmosphere are crucial for effective cyclization of **3a-e**.



Scheme 1 Synthesis of metal complexes with 3-hydroxy-4-(*p*-substituted)phenylthiazole-2(3*H*)-thiones

Table 1 The Estimated p*K*a values from the plots of H_2O/THF ratio vs. apparent p*K*a values

Compound (R)	pKa value
4a (NO_2)	3.92
4b (Cl)	4.91
4c (F)	4.80
4d (H)	4.01
4e (OMe)	4.63

Owing to the poor solubility of compounds **4a-e** in H_2O, apparent p*K*a values were measured at various ratios of H_2O/THF mixture by the pH titration method. The p*K*a values in H_2O were estimated by extrapolating the plots of apparent p*K*a values against the H_2O/THF ratio. The estimated p*K*a values for 3-hydroxythiazole-2(3*H*)-thiones are summarized in Table 1.

Oxovanadium(IV) complexes **5a-e** were synthesized by treatment of **4a-e** with $VOSO_4$. In the reaction, the control of solution pH is important. Prior to treatment with $VOSO_4$, an aqueous solution of **4a-e** was

adjusted to pH 10 to dissolve the ligand completely. During the reaction, pH of the reaction mixture was adjusted to one unit higher value than the estimated p*K*a. On raising the pH of the solution, the solution color turned from yellow into green, and oxovanadium(IV) complexes **5a-e** were obtained as grayish or yellowish precipitates. The structure assignment of oxovanadium(IV) complexes **5a-e** was carried out by IR spectral data and the combustion analysis. In IR spectra, the absorption bands due to C=S stretching vibrations of the complexes shifted to larger or smaller wavenumbers compared to ligands, and the characteristic absorption bands due to V=O stretching vibrations were observed in the range of 980-1000 cm^{-1}. In general, the absorption bands due to V=O stretching vibrations for five-coordinated species, in which two bidentate ligands and oxygen atom are coordinated to central vanadium ion, is observed at 990 cm^{-1} and over, while six-coordinated species with two ligands, oxygen, and other molecules such as solvents[39] exhibit their V=O stretching absorption at 950-960 cm^{-1}. From these results, it was found that these complexes were five-coordinated vanadyl species.

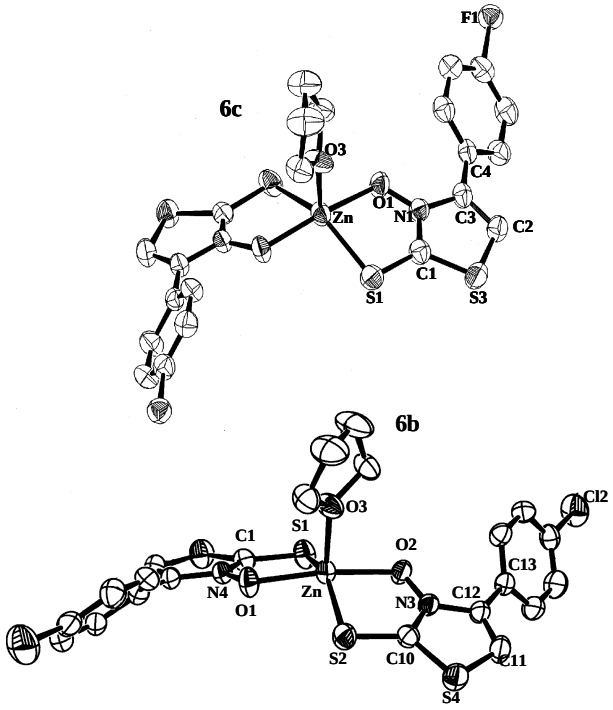


Figure 1. ORTEP diagrams of **6b** and **6c** showing the crystallographic number; *for* **6b** Zn1-S2 2.365(1), Zn1-O2 2.063(4), Zn1-O3 2.089(2), S2-C10 1.696(5)Å, S1-Zn1-S2 146.00(4), O2-Zn1-S2 85.97(8)° ; *for* **6c** Zn1-S1 2.3462(2), Zn1-O1 2.052(5), Zn1-O3 2.055(3), S1-C1 1.676(7)Å, S1-Zn1-S2 145.97(5), O2-Zn1-S2 85.30(1)°

Table 2 Selected crystallographic data

	6b	**6c**
Detector	Rigaku AFC-5 II	Rigaku Saturn
Formula	$C_{26}H_{26}N_2O_4Cl_2S_4Zn$	$C_{26}H_{26}F_2N_2O_4S_4Zn$
*F*w	695.03	662.12
Crystal system	Triclinic	Triclinic
Space group	*P*1 (#2)	*P*1 (#2)
a/Å	11.807(2)	11.122(4)
b/Å	12.678(3)	11.720(4)
c /Å	11.209(2)	12.575(5)
V /Å^3	1455.4(6)	1421.2(9)
Z	2	2
μ(Mokα)	13.50 cm^{-1}	12.07 cm^{-1}
Trans factor	0.7554-0.9997	0.6274-0.9067
$2\theta_{max}$/deg.	55.0 °	55.0 °
No. of variables	355	353
R	0.051	0.081
*R*w	0.135	0.289
GOF	1.00	1.248

$$R = \Sigma||F_0|-|F_c||/\Sigma|F_0|$$
$$Rw = [\Sigma w(|F_0|-|F_c|)^2/\Sigma w\, F_0^2]^{1/2}$$

Compounds **4a-e** were treated with $ZnSO_4$ in the presence of LiOH in H_2O:THF (1:1) mixture. The precipitated zinc(II) complexes **6a-e** were collected by suction filtration.[40] The structure assignment of zinc(II) complexes **6a-e** was carried out by means of ^{1}H-NMR and IR spectroscopies, and the combustion analysis. In ^{1}H-NMR spectra, all OH signals of ligands **4a-e** disappeared upon complexation with zinc(II) ion. In addition, the olefinic C-5 proton on the thiazole-2(3*H*)-thione ring shifted to the lower

magnetic field at the magnitude of about 0.3 ppm. In IR spectra, the absorption bands owing to C=S stretching vibrations shifted to larger or smaller wavenumbers upon complexation. From X-ray crystallographic analysis of compounds **6b** and **6c**, the following characteristics became clear; 1) each complex takes a square pyramidal structure, 2) two 3-hydroxythiazole-2(3*H*)-thiones coordinate to zinc(II) ion in *trans* form, 3) one THF molecule (recrystallization solvent) strongly binds to central zinc(II) ion at the apical position, and 4) the C=S bond length (1.696 Å) of complex **6b** is very close to that (1.689 Å)[41] of **4b** (Figure 1 and Table 2).

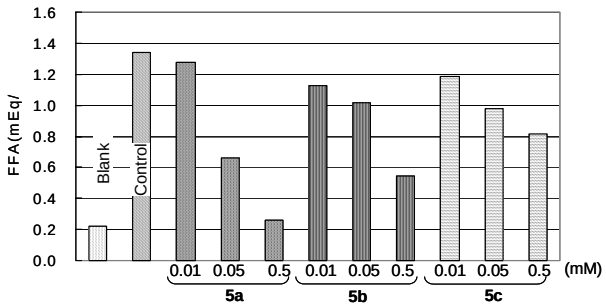


Figure 2. The selected inhibitory effects of vanadyl complexes (**5a**, **5b**, and **5c**) on epinephrine-stimulated FFA release from isolated rat adipocytes

Table 3 *In vitro* insulin-mimetic activities of vanadium(IV) complexes **5a-e**

Compound	(R)	σ_p	IC_{50} value (mM)
5a	(NO_2)	0.78	0.08±0.01#
5b	(Cl)	0.23	0.15±0.02*
5c	(F)	0.06	0.55±0.09
5d	(H)	0.00	0.35±0.07
5e	(MeO)	-0.27	1.49±0.19#

Each value is expressed as the means ±SD for 3 experiments. #Significance at $p<0.01$ vs. **6d,** *Significance at $p<0.05$ vs. **6e**

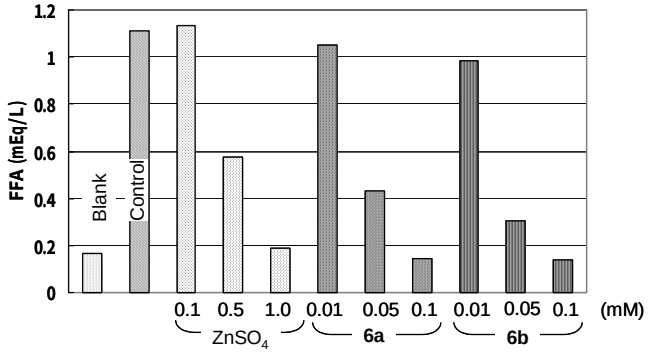


Figure 3. The selected inhibitory effects of zinc(II) complexes (**6a** and **6b**) on epinephrine-stimulated FFA release from isolated rat adipocytes

Table 4 *In vitro* insulin-mimetic activities of zinc(II) complexes (**6a-e**)

Compound	IC_{50} value (mM)
$ZnSO_4$	0.47±0.03
6a	0.036±0.006*
6b	0.030±0.003*
6c	0.044±0.004
6d	0.037±0.003*
6e	0.037±0.006*

Each value is expressed as the means±SD for 3 experiments.
*Significance at $p<0.05$ vs. $ZnSO_4$

***In vitro* insulin-mimetic activity**: Previously it was demonstrated that the amount of glucose in blood is mutually related to the amount of free fatty acid (FFA) in the blood.[12] To evaluate the *in vitro* insulin-mimetic activity of oxovanadium(IV) and zinc(II) complexes, we examined the effects of the complexes on FFA release from isolated rat adipocytes, this being proposed to be simple and convenient method free from any radioactive materials.[12] The activity of complexes was estimated in terms of IC_{50} value, which stands for a 50% inhibitory concentration of the FFA release in each complex from isolated

rat adipocytes. The results for oxovanadium(IV) and zinc(II) complexes are summarized in Figure 2 and Table 3, and in Figure 3 and Table 4, respectively. As seen from Table 3, all oxovanadium(IV) complexes showed substituent-dependent insulin-mimetic activities *in vitro*.

Among these complexes, **5a**, which has a strong electron-withdrawing nitro group, showed the highest insulin-mimetic activity. When IC_{50} values were plotted against the Hammett's substituent constants (σ_p) of R, as shown in Figure 4, it was indicated that IC_{50} value decreases with an increase of the electron-withdrawing property of the substituent R. This is the first example showing the correlation between the *in vitro* insulin-mimetic activity of oxovanadium(IV) complexes and the Hammett's substituent constants.[36] In contrast to the oxovanadium(IV) complexes, all zinc(II) complexes showed almost the same insulin-mimetic activity regardless of the substituents R *in vitro*. It is noteworthy that these IC_{50} values are much smaller than that of $ZnSO_4$ as a positive control and even zinc(II) complexes reported previously,[24,30] indicating these complexes are potent candidates for anti-diabetic agents. The difference in the bioactivity between zinc(II) and oxovanadium(IV) complexes may attributable to the different mode of action of these metal complexes toward rat adipocytes. It has already reported that oxovanadium(IV) complex with maltol exhibited higher glucose-uptake-enhancing activity and lower inhibitory activity of FFA release than the corresponding zinc(II) complex.[42]

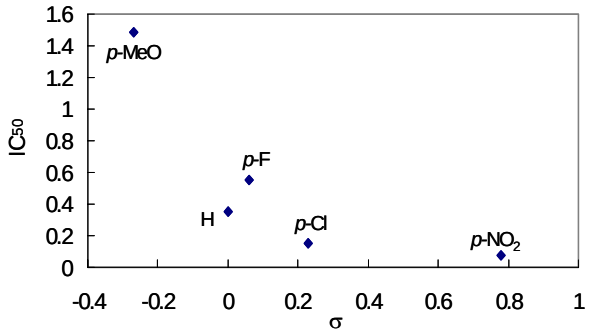


Figure 4. Plots of IC_{50} values, which are index of *in vitro* insulin-mimetic activity, of oxovanadium(IV) complexes **5a-e** against the Hammett's substituent constants.

Hypoglycemic effect of oxovanadium(IV) and zinc(II) complexes *in vivo*: As seen in Table 3, oxovanadium(IV) complexes **5a** and **5b** exhibited good insulin-mimetic activities. Thus, the effect of these two complexes on lowering the blood glucose level was examined using streptozotocin-induced diabetic rats (STZ-rats), which is a model animal for type 1 DM. Oxovanadium(IV) complexes **5a** and **5b** were injected daily at a dose of 3 mg V/kg of body weight to type 1 STZ-rats for 14 days, and the body weight and blood glucose level were monitored. No significant loss of body weight was observed during the experiments (data not shown), indicating that the complexes do not have side effects such as diarrhea. In the first week from the initial dose of the complexes, remarkable change of the blood glucose levels was not observed, and thereafter, they slightly decreased (data note shown). These modest effects of **5a** and **5b** may be responsible for their fast metabolic speed *in vivo*, despite of their high

activities *in vitro*. An exceedingly fast decomposition of $VO(S_2O_2)$ complexes with pyranthione and pyridinethione and a fast excretion of vanadium in the urine have already reported by Orvig and co-workers.[43]

As seen in Table 4, zinc(II) complexes **6a** and **6b** exhibited good insulin-mimetic activities. The effect of two complexes on lowing the blood glucose level was tested using KK-A^y mice, which are model animals for type 2 DM. Zinc(II) complexes **6a** and **6b** were administrated by daily intraperitoneal (*i.p.*) injections at a dose of 3.0 mg Zn/kg of body weight for the first day and then adjusted to approximately 0.1-0.3 mg Zn/kg of body weight depending on the blood glucose levels for 13 days, and the body weight and blood glucose level were monitored. The mean doses of **6a** and **6b** were 2.7 and 0.7 mg Zn/kg of body weight of mice for total 14 days, respectively, and the results are shown in Figure 5. The blood glucose levels of KK-A^y mice lowered on administration of both **6a** and **6b**. Especially, the blood glucose level of the group treated with **6b** was maintained in almost normal range (100-200 mg/dL) for 14 days.

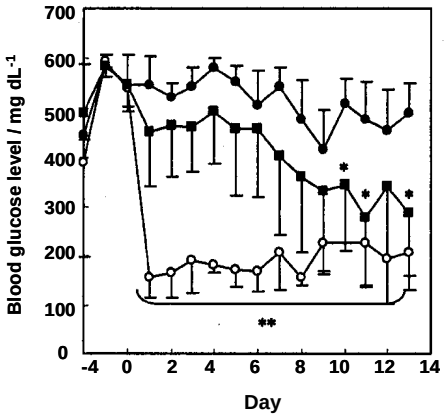


Figure. 5 Serum glucose level in KK-A^y diabetic mice (●), KK-A^y mice given **6a** (■) and KK-A^y mice given **6b** (○) by daily i.p. injections for 14 days. Each complex was administrated at the mean dose of 2.7 mg Zn/kg body weight and 0.7 mg Zn/kg body weight. **p*<0.05 vs. control, ***p*<0.01 vs. control

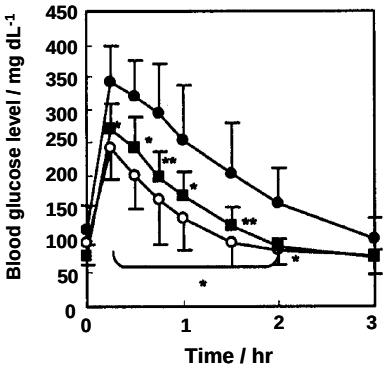


Figure. 6 Glucose tolerance tests in KK-A^y diabetic mice (●), KK-A^y mice given **6a** (■) and KK-A^y mice given **6b** (○). These tests were performed on mice that had fasted for 16 h, and then they were given an oral glucose solution at a dose of 1 g/kg of body weight. **p*<0.05 vs. control, #*p*<0.005 vs. control, ##*p*<0.0005 vs. control

In order to investigate whether zinc(II) complexes **6a** and **6b** improved glucose tolerance in type 2 KK-A^y mice, an oral glucose tolerance test (OGTT) was performed after treating the mice with the complexes, and the results are shown in Figure 6. In control group, the blood glucose level of KK-A^y mice was elevated to a maximal concentration of 350 mg/dL at 15 min after the administration of glucose, after which the level gradually decreased. In contrast, the rise in the blood glucose levels of KK-A^y mice treated with **6a** and **6b** was substantially lowered compared to the control animal, suggesting that **6a** and

6b improved the diabetic states. No change in the body weight was observed during the experiments (data not shown).

In order to examine whether the diabetic state was improved by the complexes, the several serum parameters were measured. HbA_{1c}, blood pressure, and serum leptin and insulin levels in KK-A^y mice were measured after 14-days treatment, and the results are summarized in Table 5. HbA_{1c} level (7.9% for **6a** and 6.4% for **6b**), which is a parameter to show the number of glucose molecules attached to the hemoglobin in the erythrocytes over a long period, decreased significantly compared to the control animals (10.2%), suggesting that zinc(II) complexes **6a** and **6b** sustained the longitudinal blood glucose-controlling effect. Furthermore, serum leptin and insulin levels were significantly decreased compared to those of the control animals, indicating the improvement in both insulin and leptin resistance that are symptoms of diabetes. In addition, high blood pressure, that is a cause of complication such as arteriosclerosis, was improved compared to the control animals, suggesting that the diabetic state was improved by dosage of **6a** and **6b**.

Table 5 Various parameters of KK-A^y mice receiving daily i.p. treatment for 14 days

	HbA1c (%)	Leptin level (ng/mL)	Insulin level (μU/mL)	Blood pressure (mmHg)
Control	10.2(0.8)	27.2(10.7)	19.5(5.8)	118(8)
6a	7.9(1.5)*	11.6(2.9)$^{\#\#}$	12.2(5.1)$^{\#}$	107(8)$^{\#}$
6b	6.4(0.7)$^{\$}$	13.8(9.8)	9.0(2.0)**	104(8)$^{\#}$

$^{\$}p<0.0001$ vs. control, $^{**}p<0.001$ vs. control, $^{*}p<0.005$ vs. control, $^{\#\#}p<0.01$ vs. control, $^{\#}p<0.05$ vs. control

In conclusion, we demonstrated the insulin-mimetic activity of novel oxovanadium(IV) and zinc(II) complexes with 3-hydroxy-4-(*p*-substituted)phenylthiazole-2(3*H*)-thiones both *in vitro* and *in vivo*. The correlation between the insulin-mimetic activity of oxovanadium(IV) complexes and the Hammett's substituent constants of the ligands were found. Moreover, zinc(II) complexes **6a** and **6b** exhibited a great potential as possible medicines for treating type 2 DM. From ^{1}H-NMR analysis of zinc(II) complex with 4-methyl-3-hydroxythiazole-2(3*H*)-thione in DMSO-d_6 solution under various apparent pD conditions it was suggested that the zinc(II) complex decomposes below pD 3. (data not shown) Therefore, an enteric-coated capsulation of zinc(II) complex should be taken into account for an oral administration.[44]

EXPERIMENTAL

General: Melting points were measured on a Melting Point Apparatus SMP3 in open capillaries and are

uncorrected. IR and UV-vis spectra were recorded on a Jasco FT/IR-470 infrared and a Jasco Ubest V-550 spectrophotometers, respectively. ^{1}H-NMR spectra were measured on a JEOL JNM-LA400D NMR spectrometers, and chemical shifts were expressed in ppm (δ) downfield from the internal TMS. X-ray crystallographic analysis was performed on a Rigaku AFC-5 II and Rigaku Saturn. Combustion analysis was performed on a Perkin Elmer 2400II CHNS/O analyzer.

Compounds **1a-e**, **2a-e**, **3a-e**, and **4a-e** were synthesized from acetophenone and its derivative *via* 4 steps by the modified literature method.[38]

General procedure for oxovanadium(IV) complexes; a typical example: bis[2,3-dihydro-2-thioxo-4-(*p*-nitrophenyl)-3-thiazololato]oxovanadium(IV) (5a): A solution of **4a** (303 mg, 1.19 mmol) in H_2O (50 mL) was adjusted to pH 10 with 10 M KOH. To the mixture was added dropwise a solution of $VOSO_4 \cdot 3H_2O$ (140 mg, 0.64 mmol) in H_2O (10 mL). The pH of the reaction mixture was adjusted to 6 with 5.5 M HCl, and then the resulting mixture was stirred for 10 h at rt. The resulting yellow precipitate was collected by suction filtration, washed with hot H_2O, and THF to give oxovanadium(IV) complex **5a**. Yield: 240 mg (71%), mp: 225 ºC (decomp.), IR (KBr): 3116 (ν_{C-H}), 1513 (ν_{N-O}), 1348 (ν_{N-O}), 1063 ($\nu_{C=S}$), 997 ($\nu_{V=O}$), and 854 cm^{-1} (γ_{C-H}). Anal. Calcd for $C_{18}H_{10}N_4O_7S_4V$: C, 37.70; H, 1.76; N, 9.77%. Found: C, 37.97; H, 1.60; N, 9.63%.

Bis[2,3-dihydro-2-thioxo-4-(*p*-chlorophenyl)-3-thiazololato]oxovanadium(IV) (5b): Yield: 257 mg (76%), : 91 ºC (decomp.), IR (KBr): 3105 (ν_{C-H}), 1483 (δ_{C-H}), 1061 ($\nu_{C=S}$), 995 ($\nu_{V=O}$), and 829 cm^{-1} (δ_{C-H}). Anal. Calcd for $C_{18}H_{10}Cl_2N_2O_3S_4V \cdot H_2O$: C, 37.90; H, 2.12; N, 4.91%. Found: C, 37.96; H, 1.82; N, 4.77%.

Bis[2,3-dihydro-2-thioxo-4-(*p*-fluorophenyl)-3-thiazololato]oxovanadium(IV) (5c): Yield: 134 mg (96%), IR (KBr): 3100 (ν_{C-H}), 1484 (δ_{C-H}), 1065 ($\nu_{C=S}$), 984 ($\nu_{V=O}$), and 829 cm^{-1} (γ_{C-H}); UV-Vis (DMSO): 782 nm (ε=16). Anal. Calcd for $C_{18}H_{10}F_2N_2O_3S_4V \cdot H_2O$: C, 40.22; H, 2.25; N, 5.21%. Found: C, 39.99; H, 1.98; N, 4.95%.

Bis(2,3-dihydro-2-thioxo-4-phenyl-3-thiazololato)oxovanadium(IV) (5d): Yield: 181 mg (80%), mp: 109 ºC (decomp.), IR (KBr): 3100 (ν_{C-H}), 1069 ($\nu_{C=S}$), 986 ($\nu_{V=O}$), and 700 cm^{-1} (δ_{C-H}); UV-Vis (DMSO): 769 nm (ε=28). Anal. Calcd for $C_{18}H_{12}N_2O_3S_4V$: C, 44.71; H, 2.50; N, 5.79%. Found: C, 44.76; H, 2.66; N, 5.64%**.**

Bis[2,3-dihydro-2-thioxo-4-(*p*-methoxyphenyl)-3-thiazololato]oxovanadium(IV) (5e): Yield: 131 mg (77%), mp: 92 ºC (decomp.), IR (KBr): 3109 (ν_{C-H}), 1030 ($\nu_{C=S}$), 984 ($\nu_{V=O}$), and 832 cm^{-1} (γ_{C-H}); UV-Vis (DMSO): 775 nm (ε=31). Anal. Calcd for $C_{20}H_{16}N_2O_5S_4V \cdot 0.5THF$: C, 45.59; H, 3.48; N, 4.83%. Found: C, 45.66; H, 3.36; N, 5.12%**.**

General procedure for zinc(II) complexes; a typical example: bis[2,3-dihydro2-thioxo-4-(*p*-nitro-phenyl)-3-thiazololato]zinc(II) (6a): To a solution of **4a** (310 mg, 1.2 mmol) and $LiOH \cdot H_2O$ (63 mg,

1.5 mmol) in H_2O:THF (1:1) mixture (20 mL) was added a solution of $ZnSO_4 \cdot 7H_2O$ (200 mg, 0.7 mmol) in H_2O:THF(1:1) mixture (10 mL). The reaction mixture was stirred for 6 h at rt. The resulting yellow precipitate was collected by suction filtration, washed with H_2O, and THF to afford zinc(II) complex **6a**. Yield: 286 mg (82%), ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 7.15 (1H, s, CH), 8.08 (2H, d, *J*=8.7 Hz, 3,5-CH), and 8.30 ppm (2H, d, *J*=8.7 Hz, 2,6-CH), IR (KBr): 3119 (ν_{C-H}), 1513 (ν_{N-O}), 1347 (ν_{N-O}), 1002 ($\nu_{C=S}$), and 857 cm^{-1} (δ_{C-H}). Anal. Calcd for $C_{18}H_{10}N_4O_6S_4Zn$: C, 37.79; H, 1.76; N, 9.80%. Found: C, 37.78; H, 2.09; N, 9.79%.

Bis[2,3-dihydro-2-thioxo-4-(*p*-chlorophenyl)-3-thiazololato]zinc(II) (6b): Yield: 376 mg (67%), ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 6.95 (1H, s, CH), 7.41 (2H, d, *J*=8.8 Hz, 3,5-CH), and 7.78 ppm (2H, d, *J*=8.8 Hz, 2,6-CH), IR (KBr): 3073 (ν_{C-H}), 1480 (δ_{C-H}), 1056 ($\nu_{C=S}$), and 831 cm^{-1} (δ_{C-H}). Anal. Calcd for $C_{18}H_{10}Cl_2N_2O_2S_4Zn \cdot 2THF$: C, 44.93; H, 3.77; N, 4.03%. Found: C, 44.87; H, 3.45; N, 3.98%.

Bis[2,3-dihydro-2-thioxo-4-(*p*-fluorophenyl)-3-thiazololato]zinc(II) (6c): Yield: 150 mg (quant.), ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 6.95 (1H, s, CH), 7.49 (2H, d, *J*=8.4 Hz, 2,6-CH), and 7.89 ppm (2H, d, *J*=8.4 Hz, 3,5-CH), IR (KBr): 3073 (ν_{C-H}), 1482 (δ_{C-H}), 1059 ($\nu_{C=S}$), and 773 cm^{-1} (δ_{C-H}). Anal. Calcd for $C_{18}H_{10}F_2N_2O_2S_4Zn \cdot H_2O$: C, 40.34; H, 2.26; N, 5.23%. Found: C, 40.05; H, 2.00; N, 4.97%.

Bis(2,3-dihydro-2-thioxo-4-phenyl-3-thiazololato)zinc(II) (6d): Yield: 114 mg (49%), mp: 191 ºC (decomp.), ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 6.93 (1H, s, CH), 7.42-7.47 (2H, m, 3,4,5-CH), and 7.81-7.84 ppm (2H, m, 2,6-CH), IR (KBr): 3117 (ν_{C-H}), 1485 (δ_{C-H}), 1058 ($\nu_{C=S}$), and 699 cm^{-1} (δ_{C-H}). Anal. Calcd for $C_{18}H_{12}N_2O_2S_4Zn$: C, 44.86; H, 2.51; N, 5.81%. Found: C, 45.08; H, 2.66; N, 5.64%.

Bis[2,3-dihydro-2-thioxo-4-(*p*-methoxyphenyl)-3-thiazololato]zinc(II) (6e): Yield: 120 mg (70%), mp: 64 ºC (decomp.), ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 3.83 (3H, s, OCH_3), 6.84 (1H, s, CH), 6.95 (2H, d, *J*=8.8 Hz, 3,5-CH), and 7.77 ppm (2H, d, *J*=8.8 Hz, 2,6-CH), IR (KBr): 1179 ($\nu_{C=S}$) and 832 cm^{-1} (δ_{C-H}). Anal. Calcd for $C_{20}H_{16}N_2O_4S_4Zn \cdot 2THF$: C, 49.01; H, 4.70; N, 4.08%. Found: C, 48.75; H, 4.54; N, 4.27%.

Evaluation of the inhibitory effect of metal complexes on FFA release from isolated rat adipocytes: Male Wister rats, whose age was 7 weeks, were killed by decapitation under anesthesia with ether, and the adipocytes were isolated from the epididymal fat pads. Fat tissues were incubated for 1 h at 37 ºC in KRB buffer containing 2% bovine serum albumin (BSA). Adipocytes were then separated by filtration through mesh, washed three times with the above buffer, and prepared for 240 μL. Isolated adipocyte solutions were preincubated at 37 ºC for 0.5 h with various concentrations (10^{-4}-10^{-3} M) of oxovanadium(IV) and zinc(II) complexes. Then 10^{-5} M epinephrine was added to the reaction mixtures and the resulting solutions were incubated at 37 ºC for 3 h. The reactions were stopped by soaking in ice water and the mixtures were centrifuged at 3000 rpm at 4 ºC for 10 min. For the outer solution of the cells, FFA levels were determined with an NEFA C-test Wako.

Evaluation of *in vivo* antidiabetic activity: Diabetes was induced in male Wister rats weighting

190-210 g, by a single intravenous (*i.v.*) injection of freshly prepared STZ (40 mg/kg) body in 0.1 M citrate buffer (pH 5.0). Blood samples for analysis of serum glucose were obtained from the tail vein of the rats, and serum glucose levels were measured using the glucose oxidase method (glucose C-II test; Wako Pure Chemicals). STZ rats with a blood glucose level of 14.3-26.9 mM (257-484 mg/dL) at 1 week after STZ administration were used for the experiments. STZ rats with type 1 DM were given oxovanadium(IV) complexes **5a** and **5c** suspended in 5% acacia daily at a dose of 3.0 mg V/kg body weight for 14 days by *i.p.* injections.

KK-A^y mice with type 2 DM were treated by daily *i.p.* injections of zinc(II) complexes **6a** and **6c** at the dose of 3.0 mg Zn/kg body weight for the first day and then adjusted to approximately 0.1-3 mg Zn/kg body weight according to the blood glucose level for 13 days. After administration of the complexes for 14 days, the blood samples were obtained from the orbit of mice with a capillary under anesthesia with ether. The serum concentration of glycated hemoglobin (HbA_{1c}) was measured by an immunoassay method (DCA 2000 System, Bayer-Sankyo Co. Ltd. Tokyo, Japan)

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