Studies on Non-Thiazolidinedione Antidiabetic Agents. 3.[1,2] Preparation and Biological Activity of the Metabolites of TAK-559

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Preparation and biological activity of the metabolites of the potent antihyperglycemic and antihyperlipidemic agent, (*E*)-4-{4-[(5-methyl-2-phenyl-1,3-oxazol-4-yl)methoxy]benzyloxyimino}-4-phenylbutyric acid (TAK-559) (1), were investigated. Metabolites M-I (2), M-II (3), M-III (4) and M-IV (5) were synthesized and their biological activities were evaluated by *in vitro* and *in vivo* experiments. Compounds 2—4 activate human peroxisome proliferator-activated receptor gamma one (hPPARγ1) and hPPARα, but their activities are weaker than those of TAK-559 (1). Compound 5 only activates hPPARγ1 weakly. TAK-559 (1) showed potent *in vivo* plasma glucose and triglyceride lowering activities in Wistar fatty rats after intraperitoneal administration, while its metabolites (2—5) showed comparatively weak activities.

Key words antidiabetic agent; oxyiminoalkanoic acid; type 2 diabetes; peroxisome proliferator-activated receptor; TAK-559

Peroxisome proliferator-activated receptor gamma (PPARγ) is one of a subfamily of PPARs encoded by independent genes. Three human PPARs, designated PPARα, PPARγ, and PPARδ, have been identified.[3—5] Recent studies suggest that PPARγ ligands have the ability to lower plasma glucose and triglyceride levels in insulin-resistant animal models.[6] Some glucose and lipid lowering agents, which have transcriptional activity at PPARγ, are currently used for clinical treatment of type 2 diabetes.[7—9]

In a previous paper, we showed (*E*)-4-{4-[(5-methyl-2-phenyl-1,3-oxazol-4-yl)methoxy]benzyloxyimino}-4-phenylbutyric acid (TAK-559) (**1**), a novel oxyiminoalkanoic acid derivative, had strong functional activity at PPARγ (Chart 1).[2] TAK-559 also exhibits marked glucose and lipid lowering activities in insulin-resistant animal models, KKA^y mice and Wistar fatty rats,[2,10—13] and is under clinical trials.

Metabolites M-I, M-II, M-III and M-IV were found during preclinical and clinical studies on TAK-559 (**1**), and their proposed structures are shown in Chart 1. To confirm the structures and to study the biological properties of the metabolites, compounds **2**—**5** (Charts 2—4) were synthesized and their biological activities were evaluated by both *in vitro* and *in vivo* experiments. Analytical high-performance liquid chromatography (HPLC) and mass spectroscopy (MS) of isolated metabolites (M-I, M-II, M-III, M-IV) and synthesized compounds (**2**—**5**) were shown to be identical respectively (data not shown). Transactivation activities of compounds **1**—**5** for human PPAR subtypes were examined using *in vitro* experiments, and the glucose and lipid lowering effects of the compounds were evaluated in Wistar fatty rats using *in vivo* experiments. The results are described below.

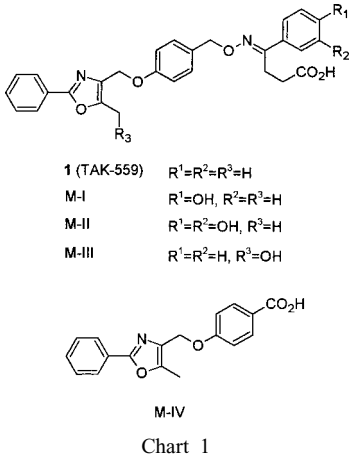

Chart 1

Chemistry

Compounds **2** and **3** were synthesized from commercially available **6a** and **6b**, respectively (Chart 2). Friedel-Crafts acylation of **6a**—**b** gave aryl ketones **7a**—**b**, of which the methoxy groups were converted into hydroxy groups using boron tribromide to yield **8a**—**b**. Reactions of **8a**—**b** with **9**[2] gave aldoximes, which were hydrolyzed to afford **2** and **3**.

Compound **4** was prepared following the procedure described in Chart 3. The hydroxy group of compound **10**[14] was protected using chloromethyl methyl ether to give **11**. Lithiation at the 5-position of the oxazole **11** with *n*-butyllithium, followed by treatment with *N*,*N*-dimethylformamide, yielded aldehyde **12**. Reduction of **12** gave the corresponding alcohol, which was protected using *tert*-butylchlorodiphenylsilane to yield **13**. Selective removal of the methoxymethyl group of **13** was achieved using bromotrimethylsilane to give alcohol **14**. Compound **14** was reacted with methanesulfonyl chloride, then coupled with **15**[2] to provide **16**. Removal of the *tert*-butyldiphenylsilyl group of **16** and subsequent hydrolysis of the ester group gave the desired compound **4**.

Chart 4 shows the synthesis of **5**. Chloromethyloxazole **17**[15] was reacted with methyl 4-hydroxybenzoate under basic conditions to yield **18**. Hydrolysis of **18** gave **5** in good yield.

Results and Discussion

To investigate the effect of TAK-559 (**1**) and its metabolites (**2**—**5**) on the full-length PPAR subtypes, COS-1 cells were transiently transfected with PPAR expression plasmid, human retinoid X receptor alpha (hRXRα) expression plasmid and reporter construct containing four copies of the rat acetyl-CoA oxidase peroxisome proliferator response ele-

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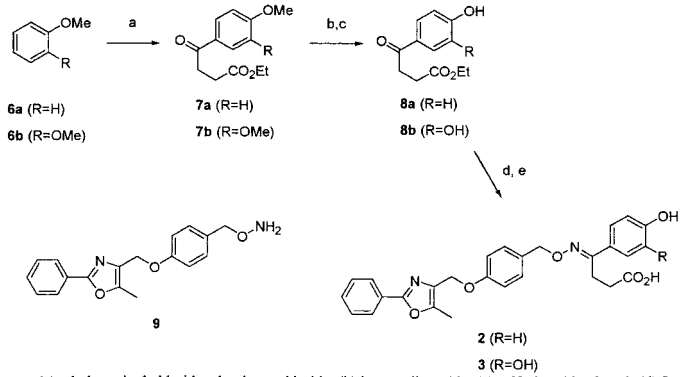
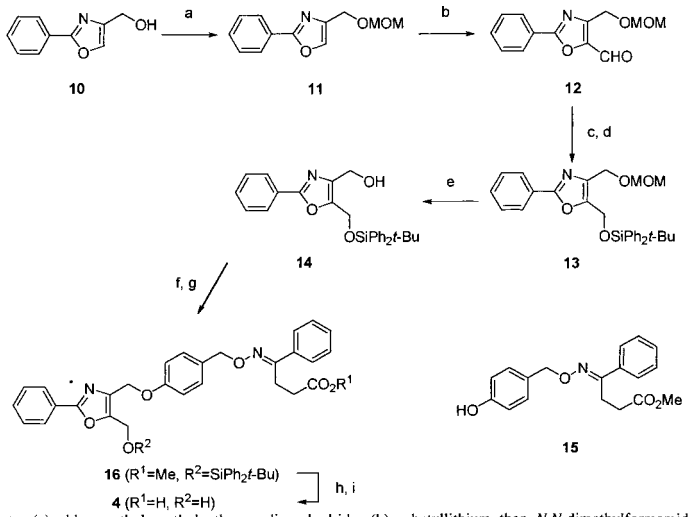


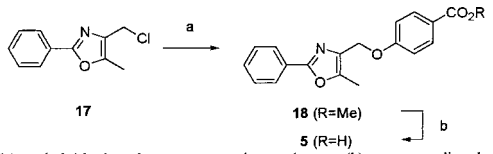
Reagents: (a) ethyl succinyl chloride, aluminum chloride; (b) boron tribromide; (c) sulfuric acid, ethanol; (d) **9**, acetic acid, sodium acetate; (e) aqueous lithium hydroxide.

Chart 2



Reagents: (a) chloromethyl methyl ether, sodium hydride; (b) *n*-butyllithium then *N*,*N*-dimethylformamide; (c) sodium borohydride; (d) *t*-butylchlorodiphenylsilane, imidazole; (e) bromotrimethylsilane; (f) methanesulfonyl chloride, triethylamine; (g) **15**, potassium carbonate; (h) tetrabutylammonium fluoride; (i) aqueous lithium hydroxide.

Chart 3



Reagents: (a) methyl 4-hydroxybenzoate, potassium carbonate; (b) aqueous sodium hydroxide.

Chart 4

ment (PPRE), and then transfected cells were treated with TAK-559 and its metabolites.

TAK-559 (**1**) and its metabolites (**2**—**5**) activated hPPARγ1 in a dose-dependent manner (data not shown). The EC_{50} values for **1**, **2**, **3**, **4** and **5** were 31 nM,[16] 130 nM, 210 nM, 200 nM and 970 nM, respectively (Table 1). Thus, TAK-559 is the most potent activator of hPPARγ1 among these compounds.

In a transient cotransfection assay for hPPARα, TAK-559 (**1**) and its metabolites (**2**—**4**) activated hPPARα in a dose-dependent manner (data not shown), while compound **5** failed to activate it. The EC_{50} values for **1**, **2**, **3**, and **4** were 67 nM,[16] 240 nM, 540 nM and 580 nM, respectively (Table 1), indicating that TAK-559 (**1**) is significantly more potent than its metabolites as an activator of hPPARα.

To examine whether TAK-559 (**1**) and its metabolites (**2**—**5**) activate hPPARδ, we performed a transient cotransfection assay with or without hPPARδ expression plasmid and a high concentration (10 μM) of these compounds. Although the hPPARδ activation by TAK-559 was significantly dependent on the exogenous expression of hPPARδ in COS-1 cells, its metabolites did not activate the exogenous hPPARδ (data not shown).

The *in vitro* results described above indicate that TAK-559

Table 1. Transactivation Activities of TAK-559 and Its Metabolites for hPPARγ1 and hPPARα[a)]

Compounds	EC_{50} (nM)	
	hPPARγ1	hPPARα
1 (TAK-559)	31[b)]	67[b)]
2	130	240
3	210	540
4	200	580
5	970	ND[c)]

a) Full-length hPPARγ1 (or hPPARα)– hRXRα expression plasmids were cotransfected into COS-1 cells with PPAR responsive luciferase reporter plasmid, and cells were cultured in the presence of 1, 3, 10, 30, 100, 300, 1000, 3000, and 10000 nM of each compound or vehicle (0.1% DMSO) alone for 48 h. The cell extracts were then assayed for luciferase activity. Transactivation activity is represented as EC_{50}. Data are the mean (n=5). *b*) From reference 16. *c*) Not determined because of poor activity.

Table 2. Effects of TAK-559 and Its Metabolites on Plasma Glucose and Triglyceride Levels in Wistar Fatty Rats[a)]

Compounds	ED_{25} (mg/kg/d)	
	Plasma glucose	Plasma triglyceride
1 (TAK-559)	0.13 (0.062—0.19)	0.079[b)]
2	>3	>3
3	2.4 (0.94—ND)	>3
4	>3	>3
5	>3	2.6 (1.0—ND)

a) TAK-559 and its metabolites were intraperitoneally injected into male Wistar fatty rats (27-week-old) for 7 d. Values in the parentheses show the 95% confidence interval of the ED_{25} values. The ED_{25} values greater than 3.0 mg/kg/day are shown as ">3". ND: not determined. *b*) This value was obtained by extrapolation below the lowest dose.

plays the key role for *in vivo* glucose and lipid lowering activities, rather than its metabolites (**2**—**5**). However, it is well known that the *in vivo* activities of compounds can be significantly different from their *in vitro* activities. So, to understand the role of metabolites (**2**—**5**) on glucose and lipid lowering activities, the *in vivo* activities of these metabolites were measured in Wistar fatty rats after intraperitoneal administration.

Table 2 shows the effects of TAK-559 (**1**) and its metabolites (**2**—**5**) on the ED_{25} values of plasma glucose and triglyceride lowering activities in Wistar fatty rats. TAK-559 (**1**) showed potent plasma glucose and triglyceride lowering activities, decreasing the plasma triglyceride level by 30% even at the lowest dose (0.1 mg/kg). Hence, it was necessary to estimate the ED_{25} value for the plasma triglyceride lowering effect of TAK-559 (**1**) by extrapolation. Compounds **2** and **4** had less potent effects on plasma glucose and triglyceride levels than TAK-559 (**1**). The plasma glucose levels in **3**-injected rats and the plasma triglyceride levels in **5**-injected rats showed relatively weak effects compared with those in **1**-injected rats.

In conclusion, TAK-559 (**1**) is a more potent activator of both hPPARγ1 and hPPARα than its metabolites (**2**—**5**). TAK-559 (**1**) also showed potent plasma glucose and triglyceride lowering activities, while its metabolites (**2**—**5**) showed comparatively weak activities, in Wistar fatty rats after intraperitoneal administration. These results indicate that TAK-559 itself plays a key role for treatment of diabetes, rather than its metabolites (**2**—**5**).

Experimental

Biological Procedures. (a) *In Vitro* Transient Cotransfection Assay COS-1 cells were seeded at 5×10^6 cells in a 150 cm^2 tissue culture flask, and cultured in 5% CO_2 at 37 °C overnight. Transfections were performed with LipofectAMINE (GIBCO BRL, U.S.A.) according to the instructions of the manufacturer. Briefly, the transfection mixture contained 125 μl of LipofectAMINE, 100 μl of LipofectAMINE Plus, 2.5 μg of each expression plasmid pMCMVneo-hPPARγ (pMCMVneo-hPPARδ or pMCMVneo-hPPARα), pMCMVneo-hRXRα, 5 μg of reporter plasmid pGL3-PPRE×4-tk-luc-neo and 5 μg of pRL-tk (Promega, U.S.A.). Cells were incubated in 25 ml of transfection mixture for 3 h in 5% CO_2 at 37 °C. After adding 25 ml of Dulbecco's modified eagle medium (DMEM, Nikken Bio Medical Lab., Japan) containing 0.1% fatty acid-free bovine serum albumin (BSA), the cells were then incubated for 24 h in 5% CO_2 at 37 °C. After transfection, cells were detached by treating with trypsin-EDTA (GIBCO BRL, U.S.A.) centrifuged and then suspended in DMEM medium containing 0.1% fatty acid free-BSA. The suspended cells were added in an OPAQUE PLATE (white 96 well plate, COSTAR, U.S.A.) at the density of 8.8×10^3 cells/well in 80 μl of DMEM medium containing 0.1% fatty acid free-BSA and 20 μl of test compounds, and then cultured in 5% CO_2 at 37 °C for 48 h. After removing the medium, 40 μl of PICAGENE-LT7.5 (Wako Pure Chemical Ind., Ltd., Japan) was added. After stirring, luciferase activities were determined in a microplate-based luminescence reader (Amersham Pharmacia, U.K.).

(b) *In Vivo* Male Wistar fatty rats[11)] were bred in Takeda Chemical Industries, Ltd. and used at the age of 27-weeks. Throughout the study, they were housed in metal mesh cages and fed a commercial diet CE-2 (Clea, Japan) and water *ad libitum*. They were divided into 16 groups (5 rats in each group) based on plasma glucose and triglyceride, and they were intraperitoneally injected with TAK-559 (**1**) and its metabolites (**2**—**5**) for 7 d. Compounds (**1**—**5**) were suspended in 0.5% methylcellulose saline solution. TAK-559 was injected into 3 groups of rats at the doses of 0.1, 0.3 and 1.0 mg/kg/d, and each metabolite was injected into 3 groups of rats at the doses of 0.3, 1.0 and 3.0 mg/kg/d. Before and after the 7-d treatment, blood was withdrawn from the tail vein, and the plasma glucose and triglyceride levels were enzymatically measured using an Autoanalyzer 7070 (Hitachi, Japan). The change (%) from the initial level of plasma glucose and triglyceride after the 7-d treatment was calculated in each rat. The degrees of variation (% control value) in the plasma parameters after treatment were also estimated from the relative ratio of the change (%) in each rat to the average of the change (%) in the control group, and the ED_{25} values for the glucose and triglyceride lowering activities of TAK-559 and its metabolites were calculated by least-squares linear regression analysis using % control values.

Chemical Methods Melting points were recorded on a Yanagimoto micro melting point apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out in the Takeda Analytical Research Laboratories, and all values are within ±0.4% of calculated values, unless otherwise noted. IR spectra were recorded on a JASCO IR-810. ^{1}H-NMR spectra were recorded on a Varian Gemini-200 spectrometer in $CDCl_3$ or DMSO-d_6 using tetramethylsilane as an internal standard. Chemical shifts are expressed as δ (ppm) values for protons relative to the internal standard. All compounds exhibited ^{1}H-NMR spectra and analytical data consistent with their proposed structures. Column chromatography was performed using Merck Silica Gel 60 (0.063—0.200 mm). The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, quin=quintet, sext=sextet, sept=septet, m=multiplet, br=broad, dec.=decomposed.

Ethyl 4-(4-Methoxyphenyl)-4-oxobutanoate (7a) Ethyl succinyl chloride (48.8 g, 297 mmol) was added dropwise to a suspension of aluminum chloride (43.6 g, 327 mmol) in dichloromethane (300 ml) at 0 °C, and the mixture was stirred at 0 °C for 15 min. To this was added anisole (32.2 ml, 297 mmol) dropwise at 0 °C. The reaction mixture was stirred at room temperature for 15 h, then poured onto ice (500 g). After stirring at room temperature for 1 h, the organic layer was separated, washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was chromatographed on silica gel with ethyl acetate–hexane (1 : 5, v/v) and recrystallized from ethyl acetate–hexane to give **7a** (47.1 g, 67%) as colorless crystals. mp 49—50 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J=7.1 Hz), 2.74 (2H, t, J=6.8 Hz), 3.27 (2H, t, J=6.8 Hz), 3.87 (3H, s), 4.16 (2H, q, J=7.1 Hz), 6.94 (2H, d, J=8.8 Hz), 7.97 (2H, d, J=8.8 Hz). IR (KBr) cm^{-1}: 2980, 1732, 1678, 1601, 1260, 1171, 1030, 833. *Anal.* Calcd for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83. Found: C, 66.08; H, 6.58.

Ethyl 4-(3,4-Dimethoxyphenyl)-4-oxobutanoate (7b) Using the procedure for preparation of **7a**, **7b** (81% yield) was prepared from veratrole (**6b**) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J=7.1 Hz), 2.75 (2H, t, J=6.8 Hz), 3.29 (2H, t, J=6.8 Hz), 3.93 (3H, s), 3.95 (3H, s), 4.17 (2H, q, J=7.1 Hz), 6.90 (1H, d J=8.4 Hz), 7.54 (1H, d, J=2.0 Hz), 7.64 (1H, dd, J=2.0, 8.4 Hz). IR (KBr) cm^{-1}: 2980, 1732, 1678, 1516, 1267, 1157, 1024, 858, 766.

Ethyl 4-(4-Hydroxyphenyl)-4-oxobutanoate (8a) Boron tribromide (2.40 ml, 25.4 mmol) was added to a stirred mixture of ethyl 4-(4-methoxyphenyl)-4-oxobutanoate (**7a**, 2.00 g, 8.46 mmol) in dichloromethane (15 ml) at 0 °C, and the mixture was stirred at 0 °C for 1 h and at room temperature for an additional 15 h. The reaction was quenched by addition of water and extracted with ethyl acetate. The extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo* to leave an oil. The oil was dissolved in ethanol (20 ml), then sulfuric acid (0.2 ml) was added. The mixture was refluxed for 2.5 h. After cooling to room temperature, it was diluted with ethyl acetate, washed with water, brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was chromatographed on silica gel with ethyl acetate–hexane (1 : 2, v/v) to give **8a** (1.16 g, 62%) as crystals. Recrystallization of **8a** from ethyl acetate–hexane gave colorless crystals. mp 111—112 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.1 Hz), 2.76 (2H, t, J=6.6 Hz), 3.25 (2H, t, J=6.6 Hz), 4.18 (2H, q, J=7.1 Hz), 6.06 (1H, br s), 6.85 (2H, d, J=8.8 Hz), 7.86 (2H, d, J=8.8 Hz). IR (KBr) cm^{-1}: 3335,

1732, 1603, 1225, 1169, 839. *Anal.* Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.96; H, 6.59.

Ethyl 4-(3,4-Dihydroxyphenyl)-4-oxobutanoate (8b) Using the procedure for preparation of **8a**, **8b** (45% yield) was prepared from ethyl 4-(3,4-dimethoxyphenyl)-4-oxobutanoate (**7b**) as colorless crystals. mp 118—119 °C (ethyl acetate–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.1 Hz), 2.75 (2H, t, J=6.6 Hz), 3.26 (2H, t, J=6.6 Hz), 4.17 (2H, q, J=7.1 Hz), 5.95—6.40 (2H, br), 6.90 (1H, d, J=8.0 Hz), 7.51 (1H, dd, J=2.2, 8.0 Hz), 7.59 (1H, d, J=2.2 Hz). IR (KBr) cm^{-1}: 3250, 1715, 1669, 1595, 1292, 1169, 912, 743. *Anal.* Calcd for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92. Found: C, 60.50; H, 5.81.

(*E*)-4-[4-(5-Methyl-2-phenyl-1,3-oxazol-4-ylmethoxy)benzyloxyimino]-4-(4-hydroxyphenyl)butanoic Acid (2) A mixture of 4-[(5-methyl-2-phenyl-1,3-oxazol-4-yl)methoxy]benzyloxyamine[2] (**9**, 1.54 g, 4.95 mmol), ethyl 4-(4-hydroxyphenyl)-4-oxobutanoate (**8a**, 1.00 g, 4.50 mmol), acetic acid (0.773 ml, 13.5 mmol), sodium acetate (738 mg, 9.00 mmol), and ethanol (30 ml) was refluxed for 48 h, then diluted with ethyl acetate, washed with water, brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was chromatographed on silica gel with ethyl acetate–hexane (1 : 2, v/v) to leave an oil. The oil was dissolved in tetrahydrofuran (20 ml), water (10 ml) and ethanol (10 ml), then lithium hydroxide monohydrate (755 mg, 18.0 mmol) was added. The reaction mixture was stirred at room temperature for 4 h, then made acidic by addition of 1 M hydrochloric acid (18.5 ml) and extracted with ethyl acetate. The extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was recrystallized from ethyl acetate–hexane to give **2** (1.82 g, 83%) as colorless crystals. mp 178—179 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.30—2.40 (2H, m), 2.45 (3H, s), 2.82—2.92 (2H, m), 5.00 (2H, s), 5.08 (2H, s), 6.77 (2H, d, J=8.8 Hz), 7.04 (2H, d, J=8.8 Hz), 7.35 (2H, d, J=8.8 Hz), 7.44—7.54 (5H, m), 7.90—7.98 (2H, m), 9.72 (1H, s). IR (KBr) cm^{-1}: 3397, 1690, 1514, 1285, 1240, 1019, 943, 912, 808. *Anal.* Calcd for $C_{28}H_{26}N_2O_6$: C, 69.12; H, 5.39; N, 5.76. Found: C, 68.96; H, 5.47; N, 5.64.

(E)-4-[4-(5-Methyl-2-phenyl-1,3-oxazol-4-ylmethoxy)benzyloxyimino]-4-(3,4-dihydroxyphenyl)butanoic Acid (3) Using the procedure for preparation of **2**, **3** (77% yield) was prepared from ethyl 4-(3,4-dihydroxyphenyl)-4-oxobutanoate (**8b**) as pale brown crystals. mp 168—169 °C (ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ: 2.30—2.39 (2H, m), 2.45 (3H, s), 2.79—2.88 (2H, m), 5.00 (2H, s), 5.07 (2H, s), 6.73 (1H, d, J=8.0 Hz), 6.92 (1H, dd, J=2.0, 8.0 Hz), 7.01—7.10 (3H, m), 7.35 (2H, d, J=8.4 Hz), 7.47—7.54 (3H, m), 7.91—7.98 (2H, m), 9.08 (1H, d, J=2.0 Hz), 9.23 (1H, d, J=2.0 Hz), 12.23 (1H, s). IR (KBr) cm^{-1}: 3245, 2930, 1696, 1514, 1238, 1026, 781, 720, 691. *Anal.* Calcd for $C_{28}H_{26}N_2O_7 \cdot 1/4H_2O$: C, 66.33; H, 5.27; N, 5.53. Found: C, 66.18; H, 5.21; N, 5.32.

4-(Methoxymethoxymethyl)-2-phenyl-1,3-oxazole (11) Sodium hydride (60% in oil, 12.5 g, 314 mmol) was added to a cold (0 °C) stirred solution of (2-phenyl-1,3-oxazol-4-yl)methanol[14] (**10**, 50.0 g, 285 mmol) in tetrahydrofuran (700 ml), and the mixture was stirred at 0 °C for 10 min and at room temperature for an additional 1 h. The mixture was cooled to 0 °C, then chloromethyl methyl ether (26.0 ml, 342 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 10 min and at room temperature for an additional 3 h. The mixture was poured into water and extracted with ethyl acetate. The extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was chromatographed on silica gel with diethyl ether–hexane (1 : 1, v/v) to give **11** (61.4 g, 98%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 3.44 (3H, s), 4.61 (2H, s), 4.77 (2H, s), 7.42—7.49 (3H, m), 7.68 (1H, s), 8.03—8.09 (2H, m). IR (KBr) cm^{-1}: 2934, 1555, 1449, 1150, 1055, 716.

4-(Methoxymethoxymethyl)-2-phenyl-1,3-oxazole-5-carbaldehyde (12) *n*-Butyllithium (1.6 M in hexane, 193 ml, 308 mmol) was added dropwise to a cold (−78 °C) stirred solution of 4-(methoxymethoxymethyl)-2-phenyl-1,3-oxazole (**11**, 61.4 g, 280 mmol) in diethyl ether (1000 ml), and the mixture was stirred at −78 °C for 1 h. *N*,*N*-Dimethylformamide (65.0 ml, 840 mmol) was added dropwise to the mixture, then the resultant mixture was allowed to warm to room temperature and stirred at that temperature for 1 h. The mixture was poured into water and extracted with diethyl ether. The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was chromatographed on silica gel with diethyl ether–hexane (1 : 1, v/v) to give **12** (55.9 g, 81%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 3.45 (3H, s), 4.82 (2H, s), 4.90 (2H, s), 7.45—7.58 (3H, m), 8.15—8.21 (2H, m), 10.03 (1H, s). IR (KBr) cm^{-1}: 2944, 1682, 1541, 1451, 1152, 1046, 914, 743.

5-(*tert*-Butyldiphenylsilyloxymethyl)-4-(methoxymethoxymethyl)-2-phenyl-1,3-oxazole (13) Sodium borohydride (3.90 g, 103 mmol) was added to a cold (0 °C) stirred solution of 4-(methoxymethoxymethyl)-2-phenyl-1,3-oxazole-5-carbaldehyde (**12**, 50.9 g, 206 mmol) in tetrahydrofuran (300 ml) and methanol (30 ml). The mixture was stirred at 0 °C for 0.5 h, then poured into water and extracted with ethyl acetate. The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo* to leave an oil. The oil was dissolved in *N*,*N*-dimethylformamide (500 ml). The solution was cooled to 0 °C, then imidazole (29.5 g, 433 mmol) was added, followed by addition of *tert*-butylchlorodiphenylsilane (67.9 g, 247 mmol) dropwise. The reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated *in vacuo*. The residue was diluted with ethyl acetate, washed with 1 M hydrochloric acid, water, brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was chromatographed on silica gel with ethyl acetate–hexane (1 : 8, v/v) to give **13** (80.1 g, 66%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.06 (9H, s), 3.34 (3H, s), 4.37 (2H, s), 4.64 (2H, s), 4.80 (2H, s), 7.34—7.49 (9H, m), 7.69—7.75 (4H, m), 7.97—8.03 (2H, m). IR (KBr) cm^{-1}: 2932, 1555, 1427, 1150, 1111, 1042, 704.

[5-(*tert*-Butyldiphenylsilyloxymethyl)-2-phenyl-1,3-oxazol-4-yl]-methanol (14) Bromotrimethylsilane (21.5 ml, 163 mmol) was added dropwise to a cold (−40 °C) stirred solution of 5-(*tert*-butyldiphenylsilyloxymethyl)-4-(methoxymethoxymethyl)-2-phenyl-1,3-oxazole (**13**, 20.0 g, 41.0 mmol) in chloroform (250 ml). The mixture was stirred at −40 °C for 0.5 h and at 0 °C for an additional 1 h. The reaction mixture was poured into saturated aqueous sodium hydrogen carbonate and extracted with chloroform. The extract was dried over magnesium sulfate, and concentrated *in vacuo*. The residue was chromatographed on silica gel with ethyl acetate–hexane (1 : 2, v/v) and crystallized from ethyl acetate–hexane to give **14** (10.2 g, 56%) as colorless crystals. mp 95—96 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.06 (9H, s), 2.15 (1H, t, J=6.2 Hz), 4.44 (2H, d, J=6.2 Hz), 4.80 (2H, s), 7.34—7.49 (9H, m), 7.67—7.74 (4H, m), 7.95—8.01 (2H, m). IR (KBr) cm^{-1}: 2932, 1553, 1427, 1113, 912, 743, 702. *Anal.* Calcd for $C_{27}H_{29}NO_3Si$: C, 73.10; H, 6.59; N, 3.16. Found: C, 72.82; H, 6.87; N, 3.08.

Methyl (*E*)-4-(4-{[5-(*tert*-Butyldiphenylsilyloxymethyl)-2-phenyl-1,3-oxazol-4-yl]methoxy}benzyloxyimino)-4-phenylbutanoate (16) Methanesulfonyl chloride (0.617 ml, 7.98 mmol) was added dropwise to a cold (0 °C) stirred solution of [5-(*tert*-butyldiphenylsilyloxymethyl)-2-phenyl-1,3-oxazol-4-yl]methanol (**14**, 3.00 g, 6.70 mmol) in ethyl acetate (30 ml). The mixture was stirred at 0 °C for 2 h, then poured into water and extracted with ethyl acetate. The extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo* to leave an oil. The oil was dissolved in *N*,*N*-dimethylformamide (15 ml). Methyl (*E*)-4-(4-hydroxybenzyloxyimino)-4-phenylbutanoate[2] (**15**, 2.00 g, 6.38 mmol) was added to the solution, followed by addition of potassium carbonate (1.76 g, 12.8 mmol). The reaction mixture was stirred at 50 °C for 1 h, then poured into water and extracted with ethyl acetate. The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was chromatographed on silica gel with ethyl acetate–hexane (1 : 6, v/v) to give **16** (3.43 g, 73%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.05 (9H, s), 2.50—2.59 (2H, m), 3.02—3.11 (2H, m), 3.61 (3H, s), 4.78 (2H, s), 4.84 (2H, s), 5.15 (2H, s), 6.87 (2H, d, J=8.4 Hz), 7.25—7.48 (14H, m), 7.60—7.72 (6H, m), 7.98—8.04 (2H, m). IR (KBr) cm^{-1}: 2932, 1736, 1512, 1238, 1113, 1071, 1024, 824, 704.

(*E*)-4-(4-{[5-(Hydroxymethyl)-2-phenyl-1,3-oxazol-4-yl]methoxy}benzyloxyimino)-4-phenylbutanoic Acid (4) Tetrabutylammonium fluoride trihydrate (2.93 g, 9.28 mmol) was added to a stirred solution of methyl (*E*)-4-(4-{[5-(*tert*-butyldiphenylsilyloxymethyl)-2-phenyl-1,3-oxazol-4-yl]methoxy}benzyloxyimino)-4-phenylbutanoate (**16**, 3.43 g, 4.64 mmol) in tetrahydrofuran (30 ml). The mixture was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo* to leave an oil. The oil was dissolved in tetrahydrofuran (20 ml), water (10 ml) and methanol (10 ml), then lithium hydroxide monohydrate (584 mg, 13.9 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, then made acidic by addition of 1 M hydrochloric acid (14.0 ml) and extracted with ethyl acetate. The extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was crystallized from ethyl acetate–hexane to give **4** (1.89 g, 84%) as colorless crystals. mp 139—141 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.53—2.62 (2H, m), 3.01—3.10 (2H, m), 4.77 (2H, s), 5.11 (2H, s), 5.16 (2H, s), 6.99 (2H, d, J=8.8 Hz), 7.30—7.50 (8H, m), 7.58—7.65 (2H, m), 8.00—8.06 (2H, m). IR (KBr) cm^{-1}: 2938, 1717, 1512, 1236, 1020, 912, 739, 693. *Anal.* Calcd for $C_{28}H_{26}N_2O_6$: C, 69.12; H, 5.39; N, 5.76. Found: C, 69.10; H, 5.24; N, 5.63.

Methyl 4-[(5-Methyl-2-phenyl-1,3-oxazol-4-yl)methoxy]benzoate (18) A mixture of 4-chloromethyl-5-methyl-2-phenyl-1,3-oxazole[15] (**17**, 6.00 g,

28.9 mmol), methyl 4-hydroxybenzoate (4.84 g, 31.8 mmol), potassium carbonate (4.80 g, 34.7 mmol) and *N*,*N*-dimethylformamide (50 ml) was stirred at 70 °C for 3 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic extract was washed with water, brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was chromatographed on silica gel with ethyl acetate–hexane (1 : 3, v/v) to give **18** (8.09 g, 87%) as crystals. Recrystallization of **18** from ethyl acetate–diethyl ether gave colorless needles. mp 104—105 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.45 (3H, s), 3.89 (3H, s), 5.05 (2H, s), 7.05 (2H, d, J=9.0 Hz), 7.40—7.53 (3H, m), 7.95—8.08 (4H, m). IR (KBr) cm^{-1}: 1708, 1600, 1275, 1240, 1167, 1100, 993, 765. *Anal.* Calcd for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33. Found: C, 70.50; H, 5.22; N, 4.30.

4-[(5-Methyl-2-phenyl-1,3-oxazol-4-yl)methoxy]benzoic Acid (5) A mixture of methyl 4-[(5-methyl-2-phenyl-1,3-oxazol-4-yl)methoxy]benzoate (**18**, 2.00 g, 6.19 mmol), 1 M sodium hydroxide solution (18.6 ml), methanol (20 ml) and tetrahydrofuran (30 ml) was stirred at 60 °C for 1 h. The reaction mixture was made acidic by addition of 1 M hydrochloric acid and extracted with ethyl acetate. The organic extract was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo* to give **5** (1.73 g, 90%) as crystals. Recrystallization of **5** from ethyl acetate–diisopropyl ether gave colorless needles. mp 185—186 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.46 (3H, s), 5.07 (2H, s), 7.06 (2H, d, J=9.0 Hz), 7.39—7.50 (3H, m), 7.96—8.11 (4H, m). IR (KBr) cm^{-1}: 1670, 1600, 1302, 1252, 1215. *Anal.* Calcd for $C_{18}H_{15}NO_4$: C, 69.89; H, 4.89; N, 4.53. Found: C, 69.60; H, 5.01; N, 4.20.

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References and Notes

1) Part 1: Imoto H., Imamiya E., Momose Y., Sugiyama Y., Kimura H., Sohda T., *Chem. Pharm. Bull.*, **50**, 1349—1357 (2002).
2) Part 2: Imoto H., Sugiyama Y., Kimura H., Momose Y., *Chem. Pharm. Bull.*, **51**, 138—151 (2003).
3) Isseman I., Green S., *Nature* (London), **347**, 645—650 (1990).
4) Schmidt A., Endo N., Rutledge S. J., Vogel R., Shinar D., Rodan G. A., *Mol. Endocrinol.*, **6**, 1634—1641 (1992).
5) Kliewer S. A., Forman B. M., Blumberg B., Ong E. S., Borgmeyer U., Manglesdorf D. J., Umesono K., Evans R. M., *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 7355—7359 (1994).
6) Lehmann J. M., Moore L. B., Smith-Oliver T. A., Wilkison W. O., Willson T. M., Kliewer S. A., *J. Biol. Chem.*, **270**, 12953—12956 (1995).
7) Troglitazone: Yoshioka T., Fujita T., Kanai T., Aizawa Y., Kurumada T., Hasegawa K., Horikoshi H., *J. Med. Chem.*, **32**, 421—428 (1989). Although launched first in the market, troglitazone had been withdrawn because of liver toxicity and related deaths associated with the drug.
8) Pioglitazone: Sohda T., Momose Y., Meguro K., Kawamatsu Y., Sugiyama Y., Ikeda H., *Arzneim.-Forsch.*, **40**, 37—42 (1990).
9) Rosiglitazone: Cantello B. C., Cawthorne M. A., Cottam G. P., Duff P. T., Haigh D., Hindley R. M., Lister C. A., Smith S. A., Thurlby P. L., *J. Med. Chem.*, **37**, 3977—3985 (1994).
10) Iwatsuka H., Shino A., Suzuoki Z., *Endocrinol. Jpn.*, **17**, 23—35 (1970).
11) Ikeda H., Shino A., Matsuo T., Iwatsuka H., Suzuoki Z., *Diabetes*, **30**, 1045—1050 (1981).
12) Suzuki M., Odaka H., Matsumoto M., Suzuki N., Imoto H., Momose Y., Kimura H., Sugiyama Y., *Diabetologia*, submitted for publication.
13) Tsuji Y., Odaka H., Suzuki M., Itasaka M., Sugiyama Y., *Diabetologia*, submitted for publication.
14) Yoo S. K., *Tetrahedron Lett.*, **33**, 2159—2162 (1992).
15) Goto Y., Yamazaki M., Hamana M., *Chem. Pharm. Bull.*, **19**, 2050—2057 (1971).
16) Sakamoto J., Kimura H., Moriyama S., Imoto H, Momose Y., Odaka H., Sawada H., *Eur. J. Pharmacol.*, submitted for publication.