Studies on Non-Thiazolidinedione Antidiabetic Agents. 3.1,2) Preparation and Biological Activity of the Metabolites of TAK-559

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Peroxisome proliferator-activated receptor gamma (PPAR-γ) is one of a subfamily of PPARs encoded by independent genes. Three human PPARs, designated PPARα, PPARγ, and PPARδ, have been identified.1–5) 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.3–9)

In a previous paper, we showed (E)-4-{4-[5-methyl-2-phenyl-1,3-oxazol-4-yl]methoxy}benzoylxyimino]-4-phenylbutyric acid (TAK-559) (1), a novel oximinoalkanoic acid derivative, had strong functional activity at PP AR

and Biological Activity of the Metabolites of TAK-559 (Chart 1). TAK-559 also exhibits marked glucose and lipid lowering activities 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.3–9)

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}benzoylxyimino]-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 hPPARγ2, 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; oximinoalkanoic acid; type 2 diabetes; peroxisome proliferator-activated receptor; TAK-559

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 in insulin-resistant animal models, KKAγ mice and Wistar fatty rats, 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.

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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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 EC50 values for 1, 2, 3, 4 and 5 were 31 nM, 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 EC50 values for 1, 2, 3, and 4 were 67 nM, 130 nM, 210 nM, and 200 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
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 ED25 values of plasma glucose and triglyceride lowering activities in Wistar fatty rats. TAK-559 (1) showed potent 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 ED25 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 lowering 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 hPPARγ 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 5×10^5 cells in a 150 cm² tissue culture flask, and cultured in 5% CO₂ 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-hPPARα or pMCMVneo-hPPARγ), pMCMVneo-hRXRα, 5 μg of reporter plasmid pGL3-PPRE×4-k-luc-neo and 5 μg of plasmid pRl-Tk (Promega, U.S.A.). Cells were incubated in 25 ml of transfection mixture for 3 h in 5% CO₂ at 37°C. After adding 25 ml of Dulbecco’s modified eagle medium (DMEM, Nippon Bio Medical Lab, Japan) containing 0.1% fatty acid-free bovine serum albumin (BSA), the cells were then incubated for 24 h in 5% CO₂ 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 8.8×10^4 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% CO₂ at 37°C for 48 h. After removing the medium, 40 μl of PICAGENE-LET7.5 (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 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 (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 ED25 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. 1H-NMR spectra were recorded on a Gemini-200 spectrometer in CDCl₃ or DMSO-d₆, using tetramethylsilane as an internal standard. Chemical shifts are expressed as δ (ppm) values for protons relative to the internal standard. All compounds exhibited 1H-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, quint = quintet, sext = sextet, sept = septet, br = broad, dca = decomposed.
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1732, 1603, 1225, 1169, 839. Anal. Calcd for C_{12}H_{14}O_{4}: C, 64.85; H, 6.35.

**Ethyl 4-(3,4-Dihydroxy)-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). 1H-NMR (CDCl_3): δ 1.28 (3H, t, J = 7.1 Hz), 2.75 (2H, d, J = 17.2 Hz), 3.45 (2H, s), 3.79—3.83 (4H, m), 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, 1619, 1595, 1292, 1169, 912, 743. Anal. Calcd for C_{10}H_{12}O_{2}: C, 60.50; H, 5.92. Found: C, 60.50; H, 5.81.

(E)-4-[5-(Methyl-2-phenyl-1,3-oxazol-4-yl)methoxy]benzyloximinoo]-4-(4-hydroxyphenyl)butanoic Acid (2) A mixture of 4-[5-(methyl-2-phenyl-1,3-oxazol-4-yl)methoxy]benzyloximino (9, 1.54 g, 4.95 mmol), ethyl 4-(4-hydroxyphenyl)butanoate (10, 0.64 g, 4.50 mmol) in chloroform (17 ml) (5.27; N, 5.53. Found: C, 66.18; H, 5.21; N, 5.32.

A mixture of 4-chloromethyl-5-methyl-2-phenyl-1,3-oxazole (11) (61.4 g, 280 mmol) in diethyl ether (1000 ml), and the mixture was stirred at room temperature for 1 h. The mixture was washed with 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 18 (61.4 g, 98%) as a pale yellow oil.

5-{[5-(tert-Butyldiphenylsilyloxy)methyl]-2-phenyl-1,3-oxazol-4-yl}-methylbenzoate (4) Tetrabutylammonium fluoride (21.5 ml, 0.16 mmol) was added dropwise to a cold (−40°C) stirred solution of 5-{[5-(tert-butyldiphenylsilyloxy)methyl] methoxymethyl}2)-phenyl-1,3-oxazole (11) (20.0 g, 41.0 mmol) in chloroform (250 ml). The mixture was stirred at −40°C for 2 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. 1H-NMR (CDCl_3): δ 1.60 (9H, s), 3.34 (3H, s), 4.64 (2H, s), 4.80 (2H, s), 7.34—7.49 (9H, m), 7.62—7.75 (4H, m), 8.43—8.78 (2H, m). IR (KBr) cm⁻¹: 2932, 2935, 1547, 1411, 1111, 1042, 704.

Methyl (E)-4-[4-[[5-(tert-butyl diphenylsilyloxy)methyl]-2-phenyl-1,3-oxazol-4-yl]-methyl]benzoate (5) Methyl (E)-4-[4-[[5-(tert-butyl diphenylsilyloxy)methyl]-2-phenyl-1,3-oxazol-4-yl]-methyl]benzoate (5) (0.55 ml, 3.43 mmol) was added dropwise at 0°C to a solution of 5-{[5-(tert-Butyldiphenylsilyloxy)methyl]-2-phenyl-1,3-oxazol-4-yl}-methylbenzoate (4) (1.65 g, 3.59 mmol) in dichloromethane (25 ml). The mixture was stirred at 0°C for 3 h, then washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The crude mixture was chromatographed on silica gel with ethyl acetate–hexane (1: 2, v/v) to give 15 (2.84 g, 76.1% as a colorless solid. IR (KBr) cm⁻¹: 3245, 2930, 1696, 1514, 1427, 1238, 1113, 912, 743, 702. Anal. Calcd for C_{28}H_{26}N_{2}O_{7} · 1/4H_{2}O: C, 66.33; H, 5.69; N, 5.16. Found: C, 66.86; H, 5.74; N, 5.64.

(E)-4-[5-(Methyl-2-phenyl-1,3-oxazol-4-yl)methoxy]benzyloximinoo]-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). 1H-NMR (CDCl_3): δ 2.50—2.39 (2H, m), 2.45 (3H, s), 2.79—2.88 (2H, m), 3.70 (2H, s), 7.30—7.48 (9H, m), 7.70—7.82 (2H, m). IR (KBr) cm⁻¹: 3245, 2930, 1696, 1514, 1238, 1026, 781, 693. Anal. Calcd for C_{12}H_{14}O_{4}: C, 66.33; H, 5.76; N, 5.3. Found: C, 66.18; H, 5.72; N, 5.32.

4-(Methoxymethyl)phenyl-1,3-oxazole (11) Sodium hydroxide (0.34 g, 0.014 mmol) was added to a cold (0°C) stirred solution of 2-(phenyl-1,3-oxazol-4-yl)methanol (10, 0.50 g, 2.85 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 30 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: 2, v/v) to give 11 (61.4 g, 98%) as a pale yellow oil. 1H-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-(Methoxymethyl)phenyl-1,3-oxazole-3-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-(methoxymethyl)phenyl-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. 1H-NMR (CDCl_3): δ 3.45—2.83 (2H, m), 4.70 (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-{[5-(tert-Butyldiphenylsilyloxy)methyl]-2-phenyl-1,3-oxazole (13) Sodium borohydride (3.90 g, 103 mmol) was added to a cold (0°C) stirred solution of 4-(methoxymethyl)phenyl-2-
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. 1H-NMR (CDCl3) 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 C19H17NO4: 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 1M 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. 1H-NMR (CDCl3) 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 C18H15NO4: 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