Discovery of TAK-875: A Potent, Specific, and Orally Bioavailable GPR40 Agonist

Nobuyuki Negoro, Shinobu Sasaki, Satoshi Mikami, Masahiro Ito, Masami Suzuki, Yoshiyuki Tsujihata, Ryo Ito, Ayako Harada, Koji Takeuchi, Nobuhiro Suzuki, Junichi Miyazaki, Takashi Santou, Tomoyuki Odani, Naoyuki Kanzaki, Miyuki Funami, Toshimasa Tanaka, Akifumi Kogame, Shinichiro Matsunaga, Tsuneo Yasuma,* and Yu Momose

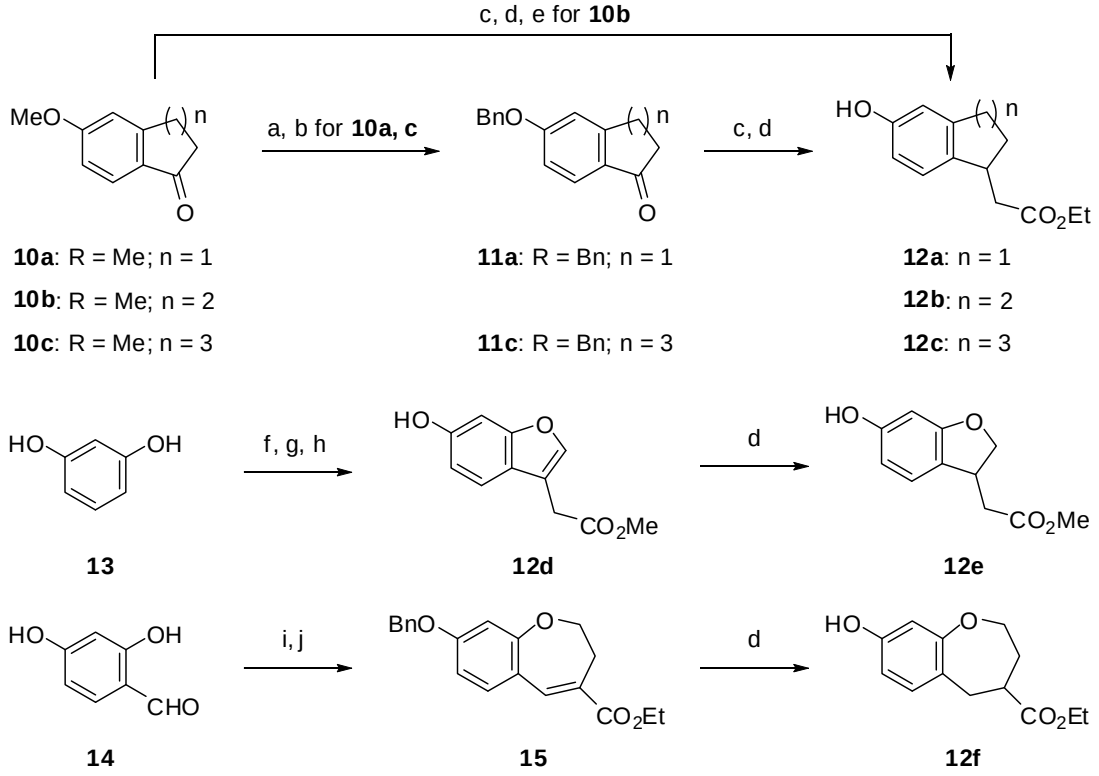
Experimental Section

General. Melting points were determined on a BÜCHI B-545 melting point apparatus and were uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker Ultra Shield-300 (300 MHz) instruments. Chemical shifts are given in parts per million (ppm) with tetramethylsilane as an internal standard. Abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublets of doublet, br = broad. Coupling constants (*J* values) are given in hertz (Hz). Elemental analyses were carried out by Takeda Analytical Laboratories, Ltd., and were within 0.4% of the theoretical values unless otherwise noted. Low-resolution mass spectra (MS) were determined on a Waters Liquid Chromatography–Mass Spectrometer System (MS), using a CAPCELL PAK UG-120 ODS (Shiseido Co., Ltd.) column (2.0 mm i.d. × 50 mm) with aqueous CH_3CN (10–95%) containing 0.05% trifluoroacetic acid (TFA), and an HP-1100 (Agilent Technologies) apparatus for monitoring at 220 nm. All MS experiments were performed using electrospray ionization (ESI) in positive ion mode. Analytical HPLC was performed on a Shimadzu LC-VP instrument, equipped with CAPCELL PAK C18 UG120 S-3 μm, 2.0 × 50 mm column with a 4 min linear gradient from 90/10 to 5/95 and subsequently with a 1.5 min isocratic elution 5/95 A/B, where A = H_2O–0.1%TFA, B = CH_3CN–0.1%TFA, at a flow rate of 0.5 μL/min, with UV detection at 220 and 254 nm, at column temperature of 25 ˚C, or performed on a Waters Quattro micro API (Agilent HP1100, Gilson215) instrument, equipped with CAPCELL PAK C18 UG120 S-3 μm, 1.5 × 35 mm column, by gradient elution: 0.00 min (A/B = 100/0), 2.00 min (A/B = 0/100), 3.00 (A/B = 0/100), 3.01 (A/B = 100/0), 3.30 (A/B = 100/0) where A = 2% CH_3CN/H_2O with 5 mM NH_4OAc; B = 95% CH_3CN/H_2O with 5 mM NH_4OAc, at a flow rate of 0.5 mL/min, with UV detection at 220 nm, at column temperature of 40 ˚C. Optical rotations were determined on a JASCO P-1030 polarimeter. Reagents and solvents were obtained from commercial sources and used without further purification. Reaction progress was determined by thin layer chromatography (TLC) analysis on Merck Kieselgel 60 F254 plates or Fuji Silysia NH plates. Chromatographic purification was carried out on silica gel columns [(Merck Kieselgel 60, 70–230 mesh or 230–400 mesh, Merck) or (Chromatorex NH-DM 1020, 100–200 mesh)] or on

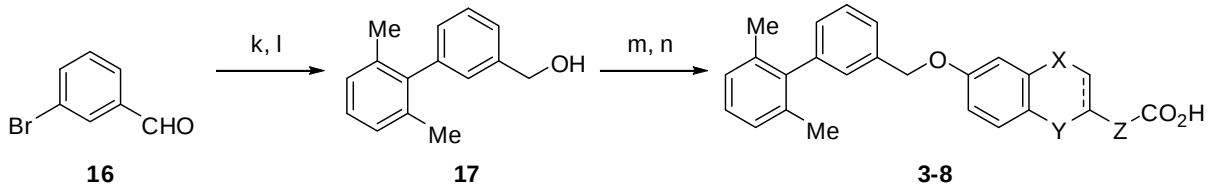
Purif-Pack (SI □ 60 μM or NH □ 60 μM, Fuji Silysia Chemical, Ltd.). Abbreviations of the solvents are used as follows: AcOEt, ethyl acetate; THF, tetrahydrofuran; EtOH, ethanol; DMF, *N*,*N*-dimethylformamide; Et_2O, diethyl ether; MeOH, methanol; CH_3CN, acetonitrile.

Scheme 1. Synthesis of Fused Phenylalkanoic Acids[a]

(A) Synthesis of phenols **12a-f**

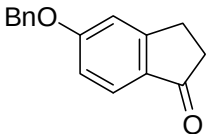


(B) Synthesis of fused phenylalkanoic acids **3-8**



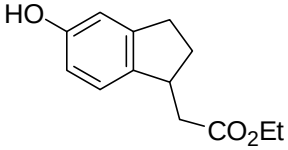
[a] Reagents and conditions: (a) $AlCl_3$, toluene, reflux; (b) benzyl bromide, K_2CO_3, acetone, reflux; 91–94% (2 steps); (c) $(EtO)_2P(O)CH_2CO_2Et$, NaH, toluene, reflux; (d) H_2 (balloon pressure), 10% Pd/C, EtOH or MeOH, rt, 48–89% (2 steps), 76–100% for **12e**, **f**; (e) $AlCl_3$, 1-dodecanethiol, toluene, rt, 98%; (f) ethyl 4-chloroacetoacetate, H_2SO_4, rt, 84%; (g) 1 M NaOH aq., reflux, 83%; (h) H_2SO_4, MeOH, reflux, 70%; (i) benzyl chloride, KF, MeCN, reflux; 43%; (j) ethyl 4-bromobutyrate, Cs_2CO_3, DMF, 80 °C, 41%; (k) 2,6-dimethylphenylboronic acid, $Pd(PPh_3)_4$, 1 M Na_2CO_3 aq., EtOH, toluene, reflux, 97%; (l) $NaBH_4$, 1,2-dimethoxyethane, THF, 0 °C, 83%; (m) **12a**–**f**, ADDP, $P(n\text{-}Bu)_3$, toluene, rt, 23–96%; (n) 2 M NaOH aq., MeOH or EtOH, THF, rt, 53–87%.

5-Benzyloxy-1-indanone (11a).



To a suspension of 5-methoxy-1-indanone (**10a**) (10.3 g, 63.5 mmol) in toluene (150 mL) was added portionwise aluminum chloride (16.9 g, 127 mmol) at 0 °C, and the mixture was stirred at reflux under nitrogen atmosphere for 4 h. The reaction mixture was allowed to cool to room temperature and poured into ice-water. The mixture was extracted with AcOEt–THF. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated to give 5-hydroxy-1-indanone as a yellow solid. This product was suspended in acetone (120 mL). To the suspension were added benzyl bromide (10.9 g, 64.0 mmol) and potassium carbonate (12.3 g, 88.9 mmol), and the mixture was stirred at reflux under nitrogen atmosphere for 1 h. The reaction mixture was concentrated, and to the residue were added AcOEt and water. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated. The resulting solid was washed with AcOEt to give **11a** (10.8 g) as colorless crystals. The second crop and third crop were similarly obtained (3.36 g) (washed with hexane–AcOEt). Total 14.2 g (94%). ^{1}H NMR ($CDCl_3$) δ 2.65–2.69 (m, 2H), 3.09 (t, J = 6.0 Hz, 2H), 5.15 (s, 2H), 6.97–7.00 (m, 2H), 7.32–7.46 (m, 5H), 7.68–7.72 (m, 1H). MS *m/z* 239 $(M + H)^+$.

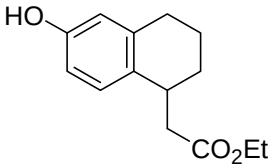
Ethyl (5-Hydroxy-2,3-dihydro-1*H*-inden-1-yl)acetate (12a).



To a solution of triethyl phosphonoacetate (15.7 g, 70.0 mmol) in toluene (50 mL) was added portionwise sodium hydride (60% in mineral oil, 2.25 g, 56.3 mmol) at 0 °C, and the mixture was stirred at 50 °C under nitrogen atmosphere for 1 h. The reaction mixture was added dropwise to a suspension of **11a** (10.7 g, 44.9 mmol) in toluene (50 mL) at 0 °C under nitrogen atmosphere, and the reaction mixture was stirred at reflux for 6 h. The mixture was quenched with diluted hydrochloric acid solution, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–40:60) to give a yellow oil. This oil was dissolved in EtOH (80 mL) and hydrogenated on 10% palladium on carbon (2.0 g, containing 50% water) under hydrogen atmosphere (balloon pressure) at room temperature for 24 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–40:60) to give **12a** (5.27 g, 54% in 2 steps) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J = 7.1 Hz, 3H), 1.69-1.81 (m, 1H), 2.32–2.44 (m,

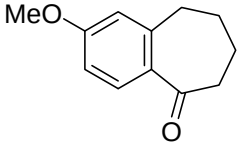
2H), 2.71 (dd, *J* = 15.3, 5.8 Hz,1H), 2.77–2.94 (m, 2H), 3.46-3.56 (m, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.71 (s, 1H), 6.62 (dd, *J* = 8.1, 2.2 Hz, 1H), 6.70 (d, *J* = 2.2 Hz, 1H), 7.02 (d, *J* = 8.1 Hz, 1H). MS *m/z* 221 $(M + H)^+$.

Ethyl (6-Hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)acetate (12b).[1]



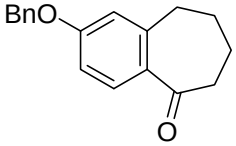
Triethyl phosphonoacetate (15.0 ml, 75.0 mmol) was added dropwise to a suspension of sodium hydride (60% in mineral oil, 2.80 g, 70.0 mmol) in toluene (35 ml) at 0 °C under nitrogen atmosphere, and the mixture was stirred at 50 °C for 1 h. The mixture was cooled to 0 °C and a solution of 6-methoxy-1-tetralone (**10b**) (8.81 g, 50.0 mmol) in toluene (35 ml) was added dropwise. The resulting mixture was stirred at reflux for 6 h. The mixture was quenched with diluted hydrochloric acid solution, and extracted with AcOEt. The extract was washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–25:75) to give a colorless oil. This oil was hydrogenated on 10% palladium on carbon (1.0 g, containing 50% water) in EtOH (100 ml) under hydrogen atmosphere (balloon pressure) at room temperature for 22 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–15:85) to give ethyl (6-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)acetate (6.68 g, 54% in 2 steps) as a colorless oil. To a mixture of this oil (6.68 g, 26.9 mmol) and 1-dodecanethiol (7.73 ml, 32.3 mmol) in toluene (75 ml) was added portionwise aluminum chloride (10.8 g, 81.0 mmol) at 0 °C, and the resulting mixture was stirred at room temperature under nitrogen atmosphere for 18 h. The mixture was quenched with ice-water, and extracted with AcOEt. The extract was washed subsequently with 2 M hydrochloric acid solution and brine, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–40:60) to give **12b** (6.19 g, 98%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.27 (t, *J* = 7.1 Hz, 3H), 1.63–1.95 (m, 4H), 2.48 (dd, *J* = 15.1, 9.6 Hz, 1H), 2.62–2.79 (m, 3H), 3.23–3.32 (m, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 4.63 (s, 1H), 6.54 (d, *J* = 2.5 Hz, 1H), 6.61 (dd, *J* = 8.2, 2.5 Hz, 1H), 7.02 (d, *J* = 8.2 Hz, 1H). MS *m/z* 235 $(M + H)^+$.

2-Methoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (10c).



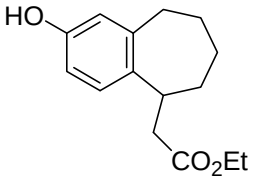
To a solution of triethyl 4-phosphonocrotonate (24.0 g, 95.9 mmol) in THF (100 mL) was added portionwise sodium hydride (60% in mineral oil, 3.84 g, 96.0 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere for 30 min. To the mixture was added dropwise a solution of 3-methoxybenzaldehyde (12.3 g, 90.0 mmol) in THF (100 mL) and the mixture was stirred at room temperature for 2 h. To the mixture was added DMF (50 mL) and the mixture was further stirred at room temperature for 18 h. The reaction mixture was concentrated, and the residue was diluted with AcOEt, washed sequentially with 1 M hydrochloric acid solution and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–30:70) to give ethyl (2*E*,4*E*)-5-(3-methoxyphenyl)penta-2,4-dienoate (7.70 g, yield 37%) as a yellow oil. This oil was hydrogenated on 10% palladium on carbon (1.1 g, containing 50% water) in EtOH (100 ml) under hydrogen atmosphere (balloon pressure) at room temperature. After reaction was completed, the catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–20:80) to give ethyl 5-(3-methoxyphenyl)pentanoate (6.01 g, 77%) as a colorless oil. To a solution of this oil (6.01 g, 25.4 mmol) in EtOH (50 mL) and THF (50 mL) was added 2 M aqueous sodium hydroxide solution (25 mL), and the mixture was stirred at room temperature for 3 h. To the mixture was added 1 M hydrochloric acid solution, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated to give 5-(3-methoxyphenyl)pentanoic acid (5.28 g, 99%) as a red-brown oil. A mixture of phosphorus (V) oxide (10 g) and methanesulfonic acid (70 mL) was stirred at 100 °C for 1 h. The obtained solution and 5-(3-methoxyphenyl)pentanoic acid (5.28 g, 25.4 mmol) were mixed and stirred at 100 °C for 1 h. The reaction mixture was poured into ice-water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–30:70) to give **10c** (4.02 g, 83%) as a red-brown oil. ^{1}H NMR ($CDCl_3$) δ 1.75–1.93 (m, 4H), 2.67–2.74 (m, 2H), 2.89–2.93 (m, 2H), 3.85 (s, 3H), 6.70 (d, *J* = 2.5 Hz, 1H), 6.81 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 1H). MS *m/z* 191 $(M + H)^+$.

2-(Benzyloxy)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (11c).



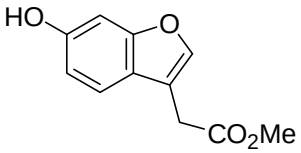
The title compound was prepared from **10c** by a similar to that described for **11a** in 91% yield as colorless prisms (hexane–AcOEt). ^{1}H NMR ($CDCl_3$) δ 1.76–1.93 (m, 4H), 2.71 (t, *J* = 6.0 Hz, 2H), 2.91 (t, *J* = 6.0 Hz, 2H), 5.11 (s, 2H), 6.79 (d, *J* = 2.5 Hz, 1H), 6.88 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.31–7.45 (m, 5H), 7.78 (d, *J* = 8.7 Hz, 1H). MS *m/z* 267 $(M + H)^+$.

Ethyl (2-Hydroxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-yl)acetate (12c).



The title compound was prepared from **11c** by a similar to that described for **12a** in 89% yield as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.22 (t, *J* = 7.2 Hz, 3H), 1.44–1.92 (m, 6H), 2.61–2.86 (m, 4H), 3.36–3.44 (m, 1H), 4.12 (q, *J* = 7.2 Hz, 2H), 4.66 (s, 1H), 6.54–6.59 (m, 2H), 6.95 (d, *J* = 7.9 Hz, 1H). MS *m/z* 249 $(M + H)^+$.

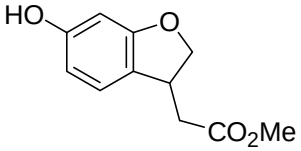
Methyl (6-Hydroxy-1-benzofuran-3-yl)acetate (12d).[2,3]



Ethyl 4-chloroacetoacetate (14.0 g, 85.0 mmol) was dissolved in concentrated sulfuric acid (30 mL) at 0 °C, and resorcinol (**13**) (8.81 g, 80.0 mmol) was added portionwise. The mixture was stirred at room temperature for 2 h. The reaction mixture was poured into ice-water, and the resulting solid was collected by filtration, washed with water, and dried to give 4-(chloromethyl)-7-hydroxy-2H-chromen-2-one (14.1 g, 84%) as a beige solid. A mixture of the obtained solid (10.9 g, 51.8 mmol) and 1 M aqueous sodium hydroxide solution (500 mL) was stirred at reflux for 2 h. The reaction mixture was acidified with concentrated sulfuric acid and extracted with AcOEt. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated to give (6-hydroxy-1-benzofuran-3-yl)acetic acid (8.27 g, 83%) as brown crystals. The obtained crystals (9.85 g, 51.3 mmol) were suspended in methanol (45 mL), and to the suspension was added concentrated sulfuric acid (5 mL), and the mixture was stirred at reflux for 4 h. After evaporation of the solvent, the residue was diluted with Et_2O, washed sequentially

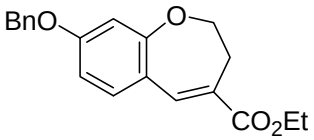
with water, saturated sodium hydrogen carbonate solution, and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–50:50) to give a solid, which was washed with hexane–AcOEt to give **12d** (7.38 g, 70%) as pale-yellow crystals. ^{1}H NMR ($CDCl_3$) δ 3.67 (d, *J* = 0.9 Hz, 2H), 3.73 (s, 3H), 4.91 (s, 1H), 6.79 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.95 (d, *J* = 2.2 Hz, 1H), 7.38 (d, *J* = 8.3 Hz, 1H), 7.52 (s, 1H). MS *m/z* 207 $(M + H)^+$.

Methyl (6-Hydroxy-2,3-dihydro-1-benzofuran-3-yl)acetate (12e).



Compound **12d** (11.4 g, 55.3 mmol) was hydrogenated on 10% palladium on carbon (2 g, containing 50% water) in MeOH (100 mL) under hydrogen atmosphere (balloon pressure) at room temperature for 18 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 20:80–50:50) to give a solid. Recrystallization from hexane–AcOEt gave **12e** (8.74 g, 76%) as colorless prisms. mp 108–109 °C. ^{1}H NMR ($CDCl_3$) δ 2.55 (dd, *J* = 16.4, 9.1 Hz, 1H), 2.74 (dd, *J* = 16.4, 5.7 Hz, 1H), 3.72 (s, 3H), 3.74–3.84 (m, 1H), 4.26 (dd, *J* = 9.1, 5.7 Hz, 1H), 4.75 (t, *J* = 9.1 Hz, 1H), 4.82 (s, 1H), 6.31–6.34 (m, 2H), 6.97 (d, *J* = 8.7 Hz, 1H). MS *m/z* 209 $(M + H)^+$.

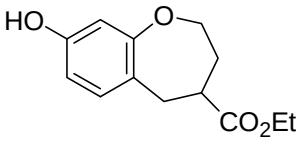
Ethyl 8-(Benzyloxy)-2,3-dihydro-1-benzoxepine-4-carboxylate (15).



A mixture of 2,4-dihydroxybenzaldehyde (**14**) (13.8 g, 100 mmol), benzyl chloride (20.1 ml, 175 mmol), and potassium fluoride (11.6 g, 200 mmol) in CH_3CN (100 ml) was stirred at reflux for 20 h. The mixture was concentrated, diluted with water, and extracted with AcOEt. The extract was washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–40:60) to give 4-(benzyloxy)-2-hydroxybenzaldehyde (9.76 g, 43%) as colorless crystals. A mixture of this product (9.76 g, 42.8 mmol), ethyl 4-bromobutyrate (7.34 ml, 51.3 mmol), and Cs_2CO_3 (20.9 g, 64.1 mmol) in DMF (100 ml) was stirred at 80 °C for 4 days. After evaporation of the solvent, the residue was diluted with water, and extracted with AcOEt. The extract was washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–40:60) to give a solid. Recrystallization from heptane–AcOEt gave **15** (5.74 g, 41%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ 1.34 (t, *J* = 7.2

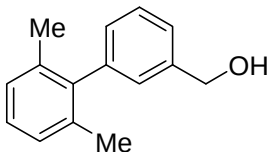
Hz, 3H), 2.92–2.98 (m, 2H), 4.21–4.30 (m, 4H), 5.06 (s, 2H), 6.59 (d, *J* = 2.4 Hz, 1H), 6.66 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.24 (d, *J* = 8.6 Hz, 1H), 7.30–7.45 (m, 5H), 7.54 (s, 1H). MS *m*/*z* 325 $(M + H)^+$.

Ethyl 8-Hydroxy-2,3,4,5-tetrahydro-1-benzoxepine-4-carboxylate (12f).



Compound **15** (3.76 g, 11.6 mmol) was hydrogenated on 10% palladium on carbon (0.6 g, containing 50% water) in EtOH (50 ml) under hydrogen atmosphere (balloon pressure) at room temperature for 24 h. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 5:95–40:60) to give **12f** (2.74 g, 100 %) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.1 Hz, 3H), 2.10–2.29 (m, 2H), 2.56–2.67 (m, 1H), 2.90–3.10 (m, 2H), 3.77–3.87 (m, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 4.23–4.33 (m, 1H), 4.85 (s, 1H), 6.43–6.51 (m, 2H), 7.00 (d, *J* = 8.0 Hz, 1H). MS *m*/*z* 237 $(M + H)^+$.

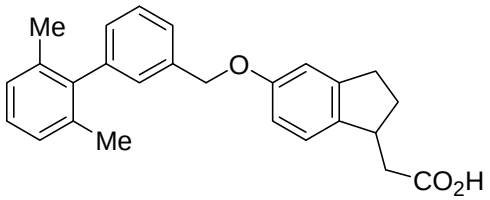
(2',6'-Dimethylbiphenyl-3-yl)methanol (17).



3-Bromobenzaldehyde (**16**) (18.5 g, 100 mmol) and (2,6-dimethylphenyl)boronic acid (21.0 g, 140 mmol) were dissolved in a mixture of 1 M aqueous sodium carbonate solution (200 mL), EtOH (100 mL), and toluene (200 mL). After argon substitution, tetrakis(triphenylphosphine)palladium(0) (5.78 g, 5.00 mmol) was added. The reaction mixture was stirred under argon atmosphere at 80 °C for 20 h. The reaction mixture was cooled, and water was added to the reaction mixture. The mixture was diluted with AcOEt, and the insoluble material was filtered through Celite. The organic layer of the filtrate was washed with brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–10:90) to give 2',6'-dimethylbiphenyl-3-carbaldehyde (20.4 g, 97%) as a colorless oil. This product (18.5 g, 88.0 mmol) was dissolved in a mixture of DME (100 mL) and THF (100 mL), and sodium borohydride (1.66 g, 44.0 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 3 h, further stirred at room temperature for 3 h. The reaction mixture was quenched with diluted hydrochloric acid, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column

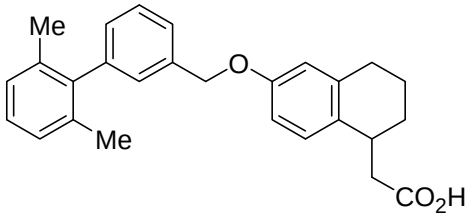
chromatography (AcOEt:hexane = 10:90–50:50) to give **17** (15.6 g, 83%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.66 (t, *J* = 5.9 Hz, 1H), 2.03 (s, 6H), 4.74 (d, *J* = 5.9 Hz, 2H), 7.07–7.19 (m, 5H), 7.35 (d, *J* = 7.5 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 1H). MS *m/z* 195 $(M + H - 18)^+$.

{5-[(2',6'-Dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1*H*-inden-1-yl}acetic Acid (3).



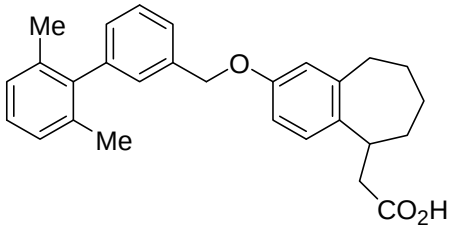
To a mixture of **12a** (0.529 g, 2.40 mmol), **17** (0.637 g, 3.00 mmol), and tributylphosphine (1.20 mL, 4.80 mmol) in toluene (40 mL) was added 1,1'-(azodicarbonyl)dipiperidine (1.21 g, 4.80 mmol) at 0 °C, and the mixture was stirred at room temperature under nitrogen atmosphere for 6 h. To the mixture were added 1,1'-(azodicarbonyl)dipiperidine (0.606 g, 2.40 mmol) and tributylphosphine (0.606 mL, 2.40 mmol). After stirred at room temperature for15 h, 1,1'-(azodicarbonyl)dipiperidine (0.606 g, 2.40 mmol) and tributylphosphine (0.606 mL, 2.40 mmol) were added, and the mixture was stirred at room temperature for 6 h. Hexane (20 mL) was added, and the precipitate was removed by filtration. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–30:70) to give ethyl {5-[(2',6'-dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1*H*-inden-1-yl}acetate (0.239 g, 24%) as a colorless oil. To a solution of this product (0.238 g, 0.574 mmol) in EtOH (2 mL) and THF (2 mL) was added 2 M aqueous sodium hydroxide solution (1.00 mL, 2.00 mmol), and the mixture was stirred at room temperature for 7 h. The mixture was acidified with 1 M hydrochloric acid solution, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated to give a solid. Recrystallization from hexane–AcOEt gave **3** (0.118 g, 53%) as colorless prisms. mp 114 °C. ^{1}H NMR ($CDCl_3$) δ 1.71–1.83 (m, 1H), 2.01 (s, 6H), 2.36–2.51 (m, 2H), 2.76–2.96 (m, 3H), 3.49–3.58 (m, 1H), 5.09 (s, 2H), 6.79 (dd, *J* = 8.3, 2.5Hz, 1H), 6.85 (s, 1H), 7.08–7.17 (m, 5H), 7.20 (s, 1H), 7.38–7.47 (m, 2H). MS *m/z* 387 $(M + H)^+$. HPLC (220nm) 99.8%. Anal. Calcd for $C_{26}H_{26}O_3$: C, 80.80; H, 6.78. Found: C, 80.63; H, 6.97.

{6-[(2',6'-Dimethylbiphenyl-3-yl)methoxy]-1,2,3,4-tetrahydronaphthalen-1-yl}acetic Acid (4).



To a mixture of **12b** (0.351 g, 1.50 mmol), **17** (0.372 g, 1.75 mmol), and tributylphosphine (0.561 mL, 2.25 mmol) in toluene (15 mL) was added 1,1'-(azodicarbonyl)dipiperidine (0.568 g, 2.25 mmol), and the mixture was stirred at room temperature under nitrogen atmosphere for 64 h. Hexane (7.5 mL) was added, and the precipitate was removed by filtration. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (AcOEt:hexane = 0:100–20:80) to give ethyl {6-[(2',6'-dimethylbiphenyl-3-yl)methoxy]-1,2,3,4-tetrahydronaphthalen-1-yl}acetate (0.150 g, 23%) as a colorless oil. To a solution of this product (0.150 g, 0.350 mmol) in EtOH (1 mL) and THF (1 mL) was added 2 M aqueous sodium hydroxide solution (0.500 mL, 1.00 mmol), and the mixture was stirred at room temperature for 18 h. The mixture was acidified with 1 M hydrochloric acid solution, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated to give a solid. Recrystallization from hexane–AcOEt gave **4** (0.080 g, 57%) as colorless prisms. mp 120 °C. ^{1}H NMR ($CDCl_3$) δ 1.67–1.98 (m, 4H), 2.01 (s, 6H), 2.55 (dd, *J* = 15.5, 9.9 Hz, 1H), 2.70–2.77 (m, 3H), 3.26–3.34 (m, 1H), 5.08 (s, 2H), 6.68 (d, *J* = 2.6 Hz, 1H), 6.78 (dd, *J* = 8.5, 2.6 Hz, 1H), 7.07–7.19 (6H, m), 7.37–7.46 (m, 2H). MS *m/z* 401 $(M + H)^+$. HPLC (220nm) 100%. Anal. Calcd for $C_{27}H_{28}O_3$: C, 80.97; H, 7.05. Found: C, 80.89; H, 7.27.

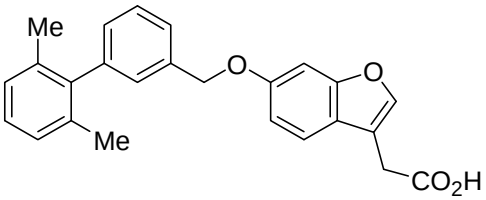
{2-[(2',6'-Dimethylbiphenyl-3-yl)methoxy]-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-yl}acetic Acid (5).



The title compound was prepared from **12c** by a similar to that described for **4** in 43% yield (2 steps) as a colorless crystalline powder (hexane–AcOEt). mp 86–88 °C. ^{1}H NMR ($CDCl_3$) δ 1.44–1.92 (m, 6H), 2.01 (s, 6H), 2.68–2.89 (m, 4H), 3.36–3.44 (m, 1H), 5.08 (s, 2H), 6.70–6.75 (m, 2H), 7.00 (d, *J* = 8.3 Hz, 1H), 7.06–7.19 (m, 5H), 7.37–7.46 (m, 2H). MS *m/z* 415 $(M + H)^+$.

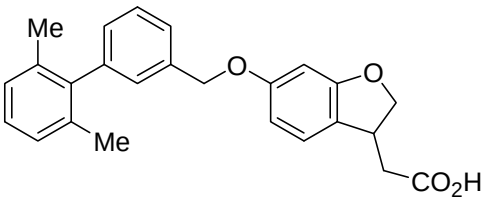
HPLC (220nm) 99.5%. Anal. Calcd for $C_{28}H_{30}O_3$: C, 81.13; H, 7.29. Found: C, 81.03; H, 7.53.

{6-[(2',6'-Dimethylbiphenyl-3-yl)methoxy]-1-benzofuran-3-yl}acetic Acid (6).



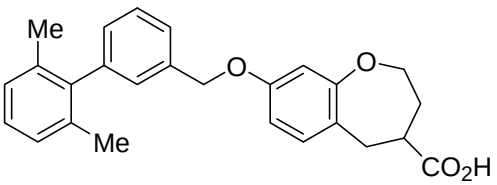
The title compound was prepared from **12d** by a similar to that described for **4** in 77% yield (2 steps) as colorless plates (hexane–AcOEt). mp 128–129 °C. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 3.71 (d, *J* = 0.8 Hz, 2H), 5.15 (s, 2H), 6.97 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.07–7.19 (m, 5H), 7.23 (s, 1H), 7.40–7.48 (m, 3H), 7.54 (s, 1H). MS *m/z* 387 $(M + H)^+$. HPLC (220nm) 99.4%. Anal. Calcd for $C_{25}H_{22}O_4$: C, 77.70; H, 5.74. Found: C, 77.52; H, 5.49.

{6-[(2',6'-Dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetic Acid (7).



The title compound was prepared from **12e** by a similar to that described for **4** in 53% yield (2 steps) as colorless needles (hexane–AcOEt). mp 147–148 °C. ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 2.61 (dd, *J* = 16.8, 9.2 Hz, 1H), 2.81 (dd, *J* = 16.8, 5.7 Hz, 1H), 3.76–3.86 (m, 1H), 4.29 (dd, *J* = 9.2, 5.7 Hz, 1H), 4.76 (t, *J* = 9.2 Hz, 1H), 5.07 (s, 2H), 6.46–6.51 (m, 2H), 7.04–7.19 (m, 6H), 7.37–7.46 (m, 2H). MS *m/z* 389 $(M + H)^+$. HPLC (220nm) 99.4%. Anal. Calcd for $C_{25}H_{24}O_4$: C, 77.30; H, 6.23. Found: C, 77.08; H, 6.25.

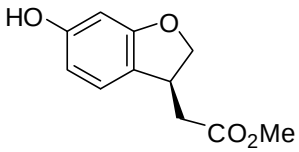
8-[(2',6'-Dimethylbiphenyl-3-yl)methoxy]-2,3,4,5-tetrahydro-1-benzoxepine-4-carboxylic Acid (8).



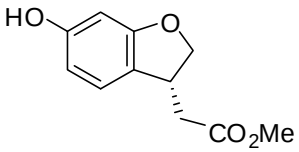
The title compound was prepared from **12f** by a similar to that described for **4** in 68% yield (2 steps) as colorless crystals (hexane–AcOEt). ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 6H), 2.17–2.29 (m, 2H), 2.63–2.72 (m, 1H), 2.96–3.11 (m, 2H), 3.79–3.85 (m, 1H), 4.25–4.32 (m, 1H), 5.07 (s, 2H), 6.60–6.64 (m, 2H), 7.04–7.19 (m, 6H), 7.37–7.46 (m, 2H). MS *m/z* 403 $(M + H)^+$. HPLC (220nm) 96.9%.

[(3*S*)-6-({2',6'-Dimethyl-4'-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid Hemi-hydrate (9a).

a) Methyl [(3*S*)-6-Hydroxy-2,3-dihydro-1-benzofuran-3-yl]acetate (12g) and Methyl [(3*R*)-6-Hydroxy-2,3-dihydro-1-benzofuran-3-yl]acetate (12h).



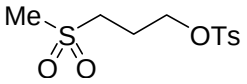
12g



12h

Methyl (6-hydroxy-2,3-dihydro-1-benzo-furan-3-yl)acetate (**12e**) (2.44 g) was resolved using normal phase preparative HPLC [CHIRALPAK AD column, 50 mmID 500 mmL, mobile phase hexane/EtOH (88/12); flow rate 60 mL/min; UV 220 nm; temperature 30 ˚C]. [Analysis: CHIRALPAK AD-H column, 4.6 mmID 250 mmL, mobile phase hexane/2-propanol (80/20); flow rate 0.5 mL/min; UV 220 nm; temperature 30 ˚C]. The (*S*)-isomer **12g** (retention time 16.3 min) was thus obtained (1.20 g, 98% recovered) with a 99.7% ee. The (*R*)-isomer **12h** (retention time 18.7 min was thus obtained (1.19 g, 97% recovered) with a 99.1% ee.

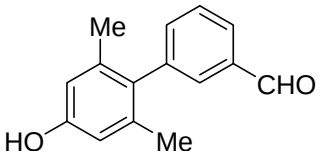
b) 3-(Methylsulfonyl)propyl 4-Methylbenzenesulfonate (18).[4]



To a solution of 3-methylthio-1-propanol (5.30 g, 50.0 mmol), triethylamine (10.5 mL, 75.0 mmol), and *N*,*N*,*N*',*N*'-tetramethyl-1,6-hexanediamine (0.861 g, 5.00 mmol) in toluene (50 mL) was added dropwise *p*-toluenesulfonyl chloride (14.3 g, 75.0 mmol) in toluene (50 mL) at 0 °C, and the mixture was stirred at 0 °C under nitrogen atmosphere for 3 h. The mixture was quenched with water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–40:60) to give 3-(methylthio)propyl 4-methylbenzenesulfonate (12.2 g, 94%) as a colorless oil. To a solution of this product (12.2 g, 46.9 mmol) in MeOH (250 mL) was added dropwise a solution of Oxone (57.7 g, 93.8 mmol) in water (250 mL) at 0 °C, and the mixture was stirred at 0 °C to room temperature for 20 h. MeOH was evaporated. The residue was diluted with water, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous sodium sulfate, and concentrated. The resulting crystals were washed with heptane–AcOEt to give **18** (13.1 g, 96%) as colorless crystals. mp 88–89 °C. ^{1}H NMR ($CDCl_3$) δ 2.17–2.28 (m, 2H), 2.46 (s, 3H), 2.92 (s, 3H), 3.07–3.15 (m, 2H), 4.18 (t, *J* = 5.9 Hz, 2H), 7.37 (d, *J* = 8.3 Hz, 2H), 7.79 (d, *J* = 8.3 Hz, 2H). MS *m/z* 293 (M

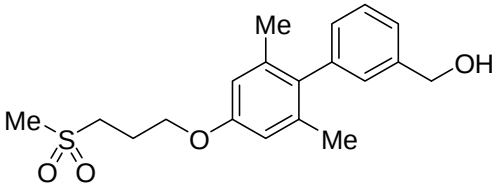
+ H)$^+$. Anal. Calcd for $C_{11}H_{16}O_5S_2$: C, 45.19; H, 5.52. Found: C, 44.96; H, 5.53.

c) 4'-Hydroxy-2',6'-dimethyl-3-biphenylcarbaldehyde (19).



4-Bromo-3,5-dimethylphenol (10.3 g, 51.0 mmol) and (3-formylphenyl)boronic acid (7 .67 g, 51.2 mmol) were dissolved in a mixture of 1 M sodium carbonate aq. (150 mL), EtOH (50 mL) and toluene (150 mL). After argon substitution, tetrakis(triphenylphosphine)palladium(0) (2.95 g, 2.55 mmol) was added. The reaction mixture was stirred at 80 °C under argon atmosphere for 24 h. The reaction mixture was cooled, and water was added. The mixture was diluted with AcOEt, and the insoluble material was filtered off through Celite. The organic layer of the filtrate was washed with brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 10:90–40:60) to give a solid, which was triturated with hexane–AcOEt to give **19** (9.53 g, 83%) as pale yellow crystals. ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 6H), 4.69 (s, 1H), 6.62 (s, 2H), 7.42 (dt, *J* = 7.7, 1.5 Hz, 1H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.66 (t, *J* = 1.5 Hz, 1H), 7.86 (dt, *J* = 7.7, 1.5 Hz, 1H), 10.05 (s, 1H). MS *m/z* 227 (M + H)$^+$.

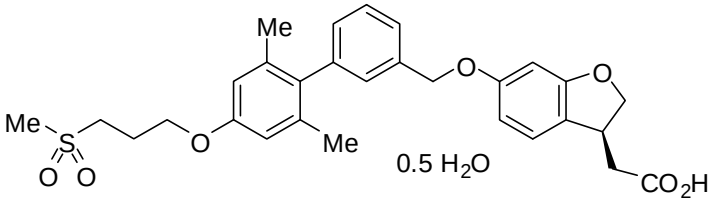
d) {2',6'-Dimethyl-4'-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methanol (20).



A mixture of **19** (2.26 g, 10.0 mmol), **18** (3.51 g, 12.0 mmol), and potassium carbonate (1.80 g, 13.0 mmol) in DMF (20 mL) was stirred at 90 °C under nitrogen atmosphere for 24 h. The mixture was diluted with water and extracted with AcOEt. The organic layer was washed sequentially with 1 M aqueous sodium hydroxide solution and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt:hexane = 40:60–80:20) to give a solid. Recrystallization from heptane–AcOEt gave 2',6'-dimethyl-4'-[3-(methylsulfonyl)propoxy]biphenyl-3-carbaldehyde (2.68 g, 77%) as colorless crystals. To a solution of this product (2.66 g, 7.68 mmol) in MeOH (10 mL) and THF (20 mL) was added portionwise sodium borohydride (0.323 g, 7.68 mmol) at 0 °C, and the mixture was stirred at 0 °C under nitrogen atmosphere for 6 h. The mixture was concentrated, quenched with water and 1 M hydrochloric acid solution, and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and

concentrated to give a solid. Recrystallization from heptane–AcOEt gave **20** (2.60 g, 97%) as colorless crystals. mp 96–98 °C. ^{1}H NMR ($CDCl_3$) δ 1.68 (t, *J* = 5.9 Hz, 1H), 2.00 (s, 6H), 2.30–2.40 (m, 2H), 2.97 (s, 3H), 3.24–3.31 (m, 2H), 4.13 (t, *J* = 5.7 Hz, 2H), 4.73 (d, *J* = 5.9 Hz, 2H), 6.64 (s, 2H), 7.03–7.08 (m, 1H), 7.12 (s, 1H), 7.31–7.37 (m, 1H), 7.41 (t, *J* = 7.5 Hz, 1H). MS *m/z* 331 $(M - 18 + H)^+$. Anal. Calcd for $C_{19}H_{24}O_4S$: C, 65.49; H, 6.94. Found: C, 65.25; H, 7.19.

e) [(3*S*)-6-({2',6'-Dimethyl-4'-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid Hemi-hydrate (9a).

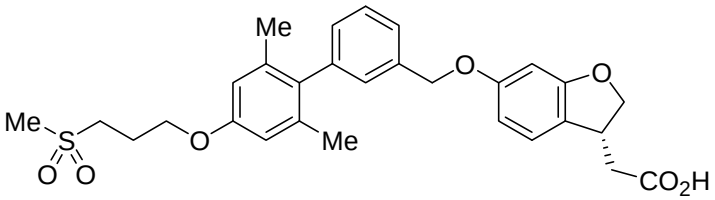


To a mixture of **12g** (0.208 g, 1.00 mmol), **20** (0.348 g, 1.00 mmol), and tributylphosphine (0.324 g, 1.60 mmol) in toluene (15 mL) was added portionwise 1,1'-(azodicarbonyl)dipiperidine (0.404 g, 1.60 mmol), and the mixture was stirred at room temperature under nitrogen atmosphere for 1.5 h. Hexane (8 mL) was added, and the insoluble material was removed by filtration. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (AcOEt:hexane = 40:60–80:20) to give methyl [(3*S*)-6-({2',6'-dimethyl-4'-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.442 g, 82%) as a colorless oil.

To a solution of methyl [(3*S*)-6-({2',6'-dimethyl-4'-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (11.2 g, 20.8 mmol) in MeOH (40 ml) and THF (80 ml) was added 2 M aqueous sodium hydroxide solution (20.0 ml, 40.0 mmol), and the mixture was stirred at 50 °C for 2 h. The mixture was concentrated, diluted with water, acidified with 1 M hydrochloric acid solution, and extracted with AcOEt. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated to give a solid, which was washed with heptane–AcOEt. Recrystallization from EtOH–H_2O gave **9a** (9.31 g, 85%) as colorless crystals. mp 127–129 °C. $[\alpha]_D$ +5.3° (c 0.3085, CH_3CN). 99.6% ee [Analysis: CHRALPAK AD-3 (NC002) column 4.6 mmID × 250 mmL column by isocratic elution: hexane/2-propanol/TFA = 500/500/1 (v/v/v) at a flow rate of 0.5 μL/min, with UV detection at 220 nm, at column temperature of 30 °C]. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.29–2.41 (m, 2H), 2.61 (dd, *J* = 16.9, 9.2 Hz, 1H), 2.81 (dd, *J* = 16.9, 5.5 Hz, 1H), 2.97 (s, 3H), 3.23–3.31 (m, 2H), 3.75–3.87 (m, 1H), 4.13 (t, *J* = 5.8 Hz, 2H), 4.28 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.76 (t, *J* = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.52 (m, 2H), 6.64 (s, 2H), 7.02–7.10 (m, 2H), 7.16 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z*

525 $(M + H)^+$. HPLC (220 nm) 100.0%. Anal. Calcd for $C_{29}H_{32}O_7S \cdot 0.5\ H_2O$: C, 65.27; H, 6.23. Found: C, 65.23; H, 6.15.

[(3*R*)-6-({2',6'-Dimethyl-4'-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (9b).



To a mixture of **12h** (0.208 g, 1.00 mmol), **20** (0.348 g, 1.00 mmol), and tributylphosphine (0.399 ml, 1.60 mmol) in toluene (15 ml) was added portionwise 1,1'-(azodicarbonyl)dipiperidine (0.404 g, 1.60 mmol), and the mixture was stirred at room temperature under nitrogen atmosphere for 2 h. Hexane (7 ml) was added, and the insoluble material was removed by filtration. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (AcOEt:hexane = 40:60–80:20) to give [(3*R*)-6-({2',6'-dimethyl-4'-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.376 g, 87%) as a colorless oil. To a solution of this product (0.370 g, 0.687 mmol) in MeOH (1.5 ml) and THF (3 ml) was added 2 M aqueous sodium hydroxide solution (1.00 ml, 2.00 mmol), and the mixture was stirred at 50 °C for 4 h. The mixture was diluted with water, acidified with 1 M hydrochloric acid solution, and extracted with AcOEt. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated to give a solid. Recrystallization from heptane–AcOEt gave **9b** (0.324 g, 90%) as colorless crystals. mp 127–128 °C. $[\alpha]_D$ −6.5° (c 0.3060, CH_3CN). 99.1% ee [Analysis: CHRALPAK AD-3 (NC002) column 4.6 mmID × 250 mmL column by isocratic elution: hexane/2-propanol/TFA = 500/500/1 (v/v/v) at a flow rate of 0.5 μL/min, with UV detection at 220 nm, at column temperature of 30 °C]. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.29–2.41 (m, 2H), 2.55–2.67 (m, 1H), 2.75–2.86 (m, 1H), 2.97 (s, 3H), 3.23–3.31 (m, 2H), 3.75–3.87 (m, 1H), 4.13 (t, *J*=5.7 Hz, 2H), 4.29 (dd, *J*=9.1, 6.0 Hz, 1H), 4.76 (t, *J*=9.1 Hz, 1H), 5.06 (s, 2H), 6.44–6.52 (m, 2H), 6.64 (s, 2H), 7.02–7.10 (m, 2H), 7.16 (s, 1H), 7.35–7.46 (m, 2H). MS *m/z* 525 $(M + H)^+$. HPLC (220 nm) 99.8%. Anal. Calcd for $C_{29}H_{32}O_7S$: C, 66.39; H, 6.15. Found: C, 66.35; H, 6.19.

References

(1) Node, M.; Kumar, K.; Nishide, K.; Ohsugi, S.; Miyamoto, T. Odorless substitutes for foul-smelling thiols: syntheses and applications. *Tetrahedron Lett.* **2001**, *42*, 9207-9210.

(2) Conconi, M. T.; Guiotto, A.; Manzini, P.; Chilin, A.; Bassani, V.; Parnigotto, P. P.

4-Hydroxymethyltetrahydro- and 4-Hydroxymethyl- Benzopsoralen: Synthesis and Biological Activity. *Farmaco* **1995**, *50*, 125-130.
(3) Fall, Y.; Santana, L.; Teijeira, M.; Uriarte, E. A convenient synthesis of benzofuran-3-acetic acids. *Heterocycles* **1995**, *41*, 647–650.
(4) Yoshida, Y.; Shimonishi, K.; Sakakura, Y.; Okada, S.; Naoya, A.; Tanabe, Y. Facile and practical methods for the sulfonylation of alcohols using Ts(Ms)Cl and $Me_2N(CH_2)_nNMe_2$ as a key base. *Synthesis* **1999**, 1633–1636.

Ca influx activity of CHO cells expressing human GPR40 (FLIPR assay).

CHO dhfr cells stably expressing human GPR40 (accession no. NM_005303) were plated and incubated overnight in 5% CO_2 at 37 °C. Then, cells were incubated in loading buffer (recording medium containing 2.5 μg/mL fluorescent calcium indicator Fluo 4-AM (Molecular Devices), 2.5 mmol/L probenecid (Dojindo) and 0.1% fatty acid-free BSA (Sigma)) for 60 min at 37 ºC. Various concentrations of test compounds or γ-linolenic acid (Sigma) were added into the cells and increase of the intracellular Ca^{2+} concentration after addition were monitored by FLIPR Tetra system (Molecular Devices) for 90 seconds. The agonistic activities of test compounds and γ-linolenic acid on human GPR40 were expressed as [(A-B)/(C-B)] X 100 (increase of the intracellular Ca^{2+} concentration (A) in test compounds-treated cells, (B) in vehicle-treated cells and (C) in 10 μM γ-linolenic acid-treated cells). EC_{50} value of each compound was obtained with Prism 5 software (GraphPad).

Preparation of CHO Membrane for GPR40 Receptor Binding Assay.

Cell lines stably expressing human GPR40 and rat GPR40 were used for the experiments. Each cell was cultured in Minimum Essential Medium Alpha (MEM-Alpha, Invitrogen) supplemented with 10% dialyzed Fetal-Bovine-Serum (dialyzed FBS, Thermo Trace Ltd.), 100 unit/mL penicillin and 100 unit/mL streptomycin in 5% CO_2/95% air atmosphere at 37 °C. Cells were harvested at confluence in Dulbecco's Phosphate-Buffered-Saline (D-PBS, Invitrogen) containing 1 mM EDTA and centrifuged. Cells were homogenized in ice-cold membrane preparation buffer (50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.5 mM PMSF (Wako), 20 μg/mL leupeptin, 0.1 μg/mL pepstatin A, 100 μg/mL Phosphoramidon, Peptide Institute, Inc.) and centrifuged (700 x g, 10 min, 4 °C). The supernatant was filtered through 40 μm Cell Strainer (BD Falcon) and ultracentrifuged (100,000 x g, 1 h, 4 °C) with Optima™ L-100 XP Ultracentrifuge (Beckman Coulter). The precipitation was suspended in the same buffer, and the protein concentration was determined with the BCA Protein assay reagent (Pierce) following membrane solubilization with 0.1% SDS and 0.1 M NaOH aqueous solution. The membrane suspension was stored at −80 °C

until receptor binding assay.

GPR40 Receptor Binding Assay.

The frozen cell membranes were resuspended in ice-cold assay buffer (25 mmol/L Tris-HCl (pH7.5), 5 mmol/L EDTA, 0.5 mmol/L PMSF, 20 μg/mL leupeptin, 0.1 μg/mL pepstatin A, 0.05% CHAPS (Wako), 0.2% fatty-acid-free BSA (Sigma)), and used for receptor binding assay. To determine the *Kd* values of 3-[4-({2',6'-dimethyl-6-[(4-[^{3}H])phenylmethoxy]biphenyl-3-yl}methoxy)phenyl] propanoic acid (Amersham Biosciences) for human and rat GPR40, binding assays were performed in the presence of various concentrations of the labeled ligand. After incubation at room temperature for 90 min, the membranes were harvested GF/C filter plates (MILLIPORE), and washed with ice-cold 50 mmol/L Tris-HCl (pH7.5) using FilterMate Harvester (PerkinElmer). The membrane-associated radioactivities were counted using TopCount liquid scintillation counter (PerkinElmer). Non-specific binding was defined as binding in the presence of 10 μmol/L of the unlabeled ligand. To determine the binding affinities of test compounds to human and rat GPR40, binding assays were performed in the presence of both various concentrations of test compounds and 2 nmol/L or 6 nmol/L of the labeled ligand. The 50% inhibitory concentrations (IC_{50} values) of test compounds for the labeled ligand were calculated using non-linear regression analysis in GraphPad Prism 3.0 (GraphPad Software). *Ki* values were converted as $Ki = IC_{50} / \{1 + (\text{the concentration of the labeled ligand}) / Kd\}$.

Figure 1 and 2 show the saturation curves of 3-[4-({2',6'-dimethyl-6-[(4-[^{3}H])phenylmethoxy]biphenyl-3-yl}methoxy)phenyl]propanoic acid for human and rat GPR40. The *Kd* values of the labeled ligand for human and rat GPR40 were 4.8 and 6.3 nmol/L by Scatchard analysis, respectively.

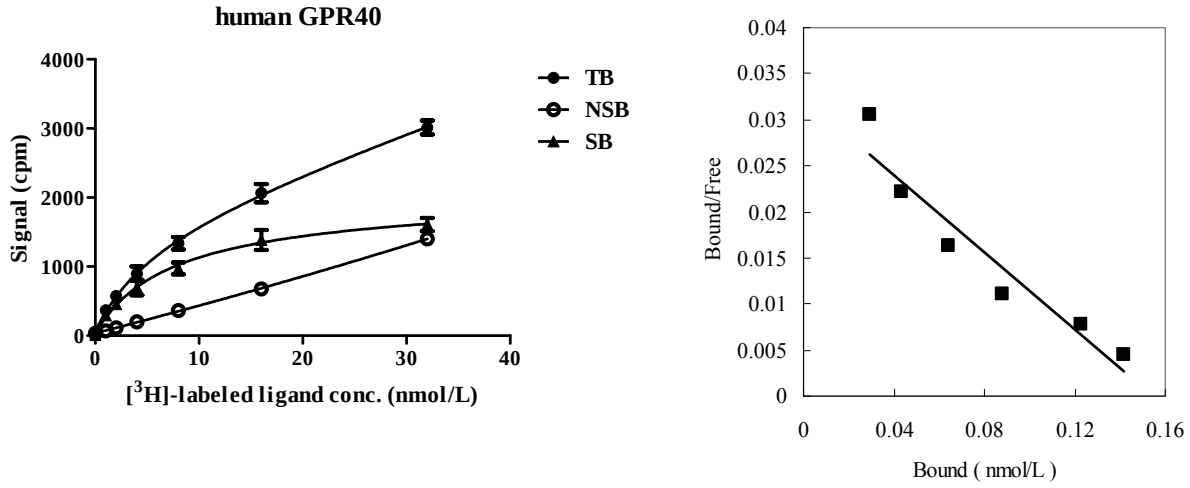


Figure 1. Saturation isotherm and Scatchard plot of 3-[4-({2',6'-dimethyl-6-[(4-[^{3}H])phenylmethoxy]biphenyl-3-yl}methoxy)phenyl]propanoic acid binding to membranes from CHO-hGPR40.

The bound radioactivities to the membranes from CHO-hGPR40 were measured. In left panel, total binding (closed circle), non-specific binding (open circle), and specific binding (closed triangle) are presented. Right panel represent Scatchard analysis of 3-[4-({2',6'-dimethyl-6-[(4-[^{3}H])phenylmethoxy]biphenyl-3-yl}methoxy)phenyl]propanoic acid binding to human GPR40. Data represent means obtained from measurements in triplicate wells. (n = 3, mean ± SDs)

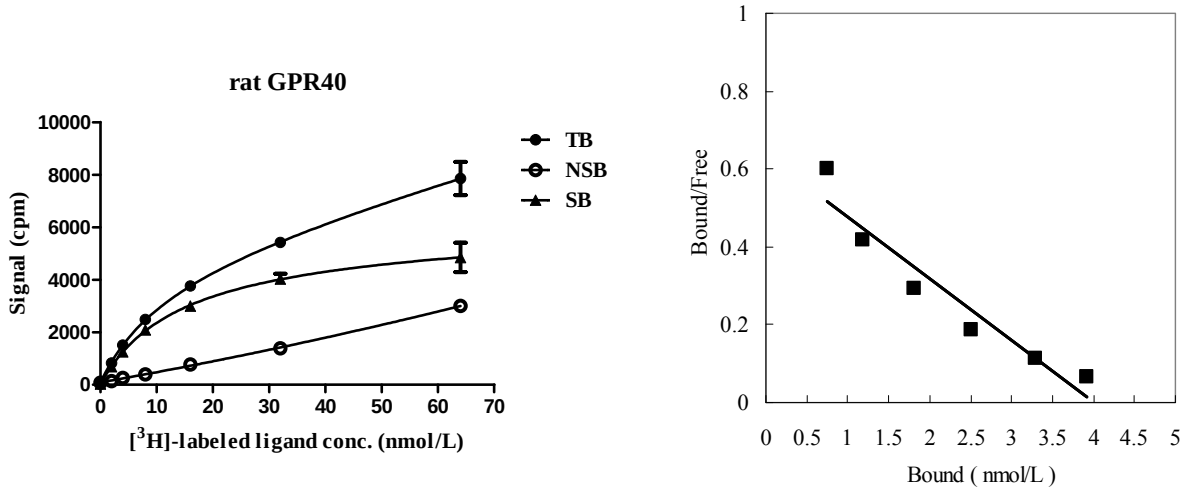


Figure 2. Saturation isotherm and Scatchard plot of 3-[4-({2',6'-dimethyl-6-[(4-[^{3}H])phenylmethoxy]biphenyl-3-yl}methoxy)phenyl]propanoic acid

binding to membranes from CHO-rGPR40.

The bound radioactivities to the membranes from CHO-rGPR40 were measured. In left panel, total binding (closed circle), non-specific binding (open circle), and specific binding (closed triangle) are presented. Right panel represent Scatchard analysis of 3-[4-({2',6'-dimethyl-6-[(4-[^{3}H])phenylmethoxy]biphenyl-3-yl}methoxy)phenyl]propanoic acid binding to rat GPR40. Data represent means obtained from measurements in triplicate wells. (n = 3, mean ± SDs).

Confirmation of selectivities against human GPR41, GPR43 and GPR120.

Human GPR41 (accession No. NM_005304) and GPR43 (NM_005306) cDNAs were amplified by PCR, and ligated into pcDNA3.1 vector (Invitrogen). FreeStyle 293 cells (Invitrogen) were transiently transfected with each vector using the 293 fectin transfection reagent (Invitrogen). Two days after transient transfection, cells were suspended in assay buffer (HBSS (Invitrogen), 0.5% fatty acid-free BSA (Sigma), 100 μmol/L IBMX (Wako), 100 μmol/L Ro20-1724 (BIOMOL), 5 mmol/L HEPES (Invitrogen)). The cells were then incubated with **9a** or sodium propionate (1.5×10^{-9}, 1.5×10^{-8}, 1.5×10^{-7}, 1.5×10^{-6} and 1.5×10^{-5} mol/L as final concentrations) in assay buffer containing 1 μmol/L forskolin and 3 μmol/L propionate for 30 minutes at 37°C. The reaction was terminated by adding 0.1 units/μL Acceptor beads and the Biotin-cAMP/Donor beads mixture, which consists of 0.1 units/μL Biotin-cAMP, 0.1 units/μL Donor beads and 0.3% Tween 20 (AlphaScreen cAMP Assay Kit, PerkinElmer). Emission signals were measured with the Envision™ multilabel plate reader (PerkinElmer), and intracellular cAMP concentrations were determined using a cAMP standard curve. The agonistic activities of **9a** and sodium propionate on human GPR41 and GPR43 were expressed as [(A-B)/(A-C)] x 100 (a concentration of cAMP (A) in 3 μmol/L sodium propionate-treated cells, (B) in test compounds-treated cells, and (C) in 1 mmol/L sodium propionate-treated cells). EC_{50} values of each compound were obtained with Prism 5 software (GraphPad).

CHO (dhfr-) cells stably expressing human GPR120 (accession No. BC101175) were plated at 5,000 cells/well in 384-well black with clear bottom plate (BD), and incubated overnight in 5% CO_2 at 37°C. Cells were then incubated in loading buffer (Fluo 4-AM Calcium Kit, Dojindo) containing 0.1% fatty acid-free BSA (Sigma) for 60 minutes at 37ºC. Compound **9a** or γ-linolenic acid (1×10^{-11}, 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6} and 1×10^{-5} mol/L as final concentrations) were added into the cells, and increase of the intracellular Ca^{2+} concentration after addition were monitored by FLIPR Tetra system (Molecular Devices) for 90 seconds. The agonistic activities of **9a** and γ-linolenic acid on human GPR120 were expressed as [(A-B)/(C-B)] x 100 (increase of the intracellular Ca^{2+} concentration (A) in test compounds-treated cells, (B) in

vehicle-treated cells and (C) in 10 μmol/L γ-linolenic acid-treated cells). EC_{50} values of each compound were obtained with Prism 5 software.

Homology Modeling and Ligand Docking.

A homology model of GPR40 was constructed using the crystal structure of bovine rhodopsin (PDB code 1GZM),[5] which obtained from the RCSB Protein Data Bank, as a structural template. An alignment of the amino acid sequences between GPR40 and rhodopsin was created using ClustalX (version 2.0.11)[6] and manually revised. Procedures of homology modeling were performed in MOE (version 2008.10).[7] The CL2 loop on the extra cellular domain was excluded except Cys170 forming disulfide bond due to the difficulty of estimation. In the previous step, compound **7** was docked into the obtained receptor model using the program GOLD (version 4.1).[8] Then, the resultant docking modes with receptor models, replacing compound **7** with **9a**, were subjected energy minimization with MOE after connecting each residual substituent. In the energy minimization process, the MMFF94s force field was used and the dielectric constant was set to 2*r, where r is the distance between two interacting atoms.

References

(5) Crystal structure of rhodopsin (1GZM): Li, J. *et al*., *J. Mol. Biol.* **343**, 1409-1438 (2004)
(6) *ClustalX, version 2.0.11*: Larkin,M.A. *et al.*, *Bioinformatics*, **23,** 2947-2948 (2007)
(7) *MOE, version 2008.10*: Chemical Computing Group Inc., 1010 Sherbrooke St. W, Suite 910 Montreal, Quebec, Canada H3A 2R7. www.chemcomp.com.
(8) *GOLD, version 4.1*: CCDC, Business & Administration, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K. http://www.ccdc.cam.ac.uk.

Pharmacokinetic Analysis in Rat Cassette Dosing.

Test compounds were administered as a cassette dosing to non-fasted rats. After oral and intravenous administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant was diluted and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

Pharmacokinetic Analysis in Rats.

Compound **9a** was administered to fasted Crl:CD(SD) rats (male, 8 weeks old, n = 3) intravenously (1 mg/kg as **9a** (anhydrous), DMAA/PEG400) and orally (3 mg/kg as **9a**

(anhydrous), 0.5% methylcellulose suspension). At 5, 10, 15, and 30 min and 1, 2, 4, 8, 24, 32 h after intravenous administration or at 5, 15 and 30 min and 1, 2, 4, 8, 24, 32 h after oral administration, blood samples were collected from tail vein. The blood samples were centrifuged to obtain the plasma fraction. The concentrations of **9a** (anhydrous) were determined after extraction from rat plasma by deproteinization with acetonitrile. After centrifugation, the supernatants were evaporated to dryness under a nitrogen gas stream and then the residues were dissolved in acetonitrile/0.01 mol/L ammonium formate (pH 3.0) (11:9, v/v). The solutions were centrifuged and the supernatants were injected into a high-performance liquid chromatograph, which was equipped with an XTerra RP_{18} column and a CAPCELL PAK C_{18} AQ S3 column. Compound **9a** (anhydrous), eluted from the first column, was applied to the second column with a column switching technique. The eluted **9a** (anhydrous) was monitored with absorbance at 235 nm.

Pharmacokinetic Analysis in Dogs.

Compound **9a** was administered to fasted dogs (male, n = 4) intravenously (0.5 mg/kg as **9a** (anhydrous), DMAA/PEG400) and orally (1 mg/kg as **9a** (anhydrous), 0.5% methylcellulose suspension). At 5, 10, 15, and 30 min and 1, 2, 4, 8, 24 h after intravenous administration or at 5, 15 and 30 min and 1, 2, 4, 8, 24 h after oral administration, blood samples were collected from cephalic vein. The blood samples were centrifuged to obtain the plasma fraction. The concentrations of **9a** (anhydrous) were determined as above.

Oral Glucose Tolerance Test (OGTT).

The care and use of the animals and the experimental protocols used in this research were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company Limited. Female Wistar fatty rats were obtained from Takeda Rabics, Ltd. They were fed a commercial diet CE-2 and tap water ad libitum. All blood samples were collected from tail vein. At age of 24 weeks, female Wistar fatty rats were fasted overnight and divided into four groups (n = 6), based on plasma glucose (PG) and triglyceride levels, and body weight. Each group was orally given vehicle (0.5% methylcellulose) or compound **9a** at 0.3, 1, or 3 mg/kg as free acid form of **9a**, one hour prior to oral glucose load (1g/kg). The PG and the plasma insulin levels measured for the grouping of the animals were used as the data before administration of **9a** (pre). Blood samples were collected just before glucose load (time 0), and 10, 30, 60 and 120 min after glucose load. PG levels were enzymatically measured by Autoanalyzer 7080 (Hitachi, Japan). Plasma insulin levels were measured by T.N. TECHNOS., LTD. using radioimmunoassay (RIA) (LINCO Research, USA). For evaluation of the effect of **9a**,

incremental area under the curve $(AUC)_{0\text{-}120\ min}$ of PG levels and incremental $AUC_{pre\text{-}30\ min}$ of plasma insulin levels were calculated using the following formula: [{(0 min PG)+(10 min PG)}×10/2+{(10 min PG)+(30 min PG)}×20/2+{(30 min PG)+(60 min PG)}×30/2+{(60 min PG)+(120 min PG)}×60/2-{(0 min PG)×120}] and [{(pre insulin)+(0 min insulin)}×60/2+{(0 min insulin)+(10 min insulin)}×10/2+{(10 min insulin)+(30 min insulin)}×20/2-{(pre insulin)×90}], respectively. By using these AUC, statistical differences compared with control were analyzed with one-tailed Williams' test.