(*S*)-1,2,3,4-Tetrahydroisoquinoline Derivatives Substituted with an Acidic Group at the 6-Position as a Selective Peroxisome Proliferator-Activated Receptor γ Partial Agonist

Ko Morishita,*a Tomohiro Miike,a Shigemitsu Takeda,a Masaki Fukui,a Yuma Ito,a Tatsuya Kitao,a Shin-ichiro Ozawa,b Shuichi Hironoa and Hiroaki Shirahasea

a Drug Discovery Research Department, Kyoto Pharmaceutical Industries, Ltd.; 38 Nishinokyo Tsukinowa-cho, Nakagyo-ku, Kyoto 604–8444, Japan: and b School of Pharmacy, Kitasato University; 5–9–1 Shirokane, Minato-ku, Tokyo 108–8641, Japan.

Received July 2, 2019; accepted August 26, 2019

A novel series of 2,6,7-substituted 3-unsubstituted 1,2,3,4-tetrahydroisoquinoline derivatives were synthesized to find a peroxisome proliferator-activated receptor γ (PPARγ) partial agonist. Among the derivatives, (E)-7-[2-(cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-2-[3-(2-furyl)acryloyl]-6-(1H-tetrazol-5-yl)-1,2,3,4-tetrahydroisoquinoline (20g) exhibited potent partial agonist activity (EC50 = 13 nM, maximal response 30%) and very weak protein tyrosine phosphatase 1B (PTP1B) inhibition (IC50 = 1100 nM), indicating a selective PPARγ partial agonist. A computational docking calculation revealed that 20g bound to PPARγ in a similar manner to that of known partial agonists. In male and female KK-A' mice with insulin resistance and hyperglycemia, 20g at 30 mg/kg for 7 d significantly reduced plasma glucose levels, but not triglyceride levels. The effects of 20g were similar to those of pioglitazone at 10 mg/kg. In conclusion, the 2,6,7-substituted 1,2,3,4-tetrahydroisoquinoline with an acidic group at the 6-position provides a novel scaffold for selective PPARγ partial agonists and 20g exerted anti-diabetic effects via the partial activation of PPARγ.

Key words peroxisome proliferator-activated receptor γ; partial agonist; diabetes; tetrahydroisoquinoline; insulin resistance; adverse effect

Introduction

Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-dependent transcription factor belonging to the nuclear receptor family. PPARγ agonists, such as pioglitazone (Fig. 1) and rosiglitazone, have long been used as anti-diabetic drugs to reduce blood glucose levels by improving insulin resistance in type 2 diabetic patients.1–5) PPARγ agonists increase insulin sensitivity by promoting adipocyte differentiation, resulting in increases in adiponectin, an anti-insulin-resistance adipokine, and decreases in tumor necrosis factor α (TNFα), an insulin resistance-evoking adipokine.6) PPARγ agonists are also expected for the treatment of various diseases, including Alzheimer’s disease, non-alcoholic steatohepatitis, cardiovascular diseases, and cancer.6–8) However, pioglitazone and rosiglitazone induce edema and increase the risks of weight gain, the liver, heart, and kidneys.9–11) Thus, extensive efforts have been made to develop safer PPARγ agonists, and various PPARα/γ dual agonists and PPAR pan-agonists have been reported. However, their development has been mostly suspended due to the potential risks of cardiovascular events, carcinogenicity, liver injury, and/or renal dysfunction.12–14) The overactivation of PPAR with PPARα/γ dual agonists and PPAR pan-agonists may lead to carcinogenesis and adverse effects in the liver, heart, and kidneys.15–19)

PPARγ partial agonists, such as INT-131 (Fig. 2), have been investigated as PPARγ modulators and examined clinically.20,21) A PPARγ partial agonist is defined to partially activate PPARγ and antagonize the activation of PPARγ by a full agonist, such as farglitazar (Fig. 1). In the concept of PPARγ modulators, the partial activation of PPARγ fully induces insulin sensitization without adverse effects. Although many structurally different PPARγ partial agonists showed higher efficacy with lower toxicity in experimental diabetic animals, none have been successfully developed to date.20,22–23) Therefore, new partial agonists that are structurally different from known agonists are desired and their efficacy and adverse effects need to be compared with those of full agonists.

The majority of PPARγ partial agonists have different structures from those of known full agonists20–23) (Fig. 2). We previously reported various types of PPARγ agonists using the same scaffold, 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid: a selective PPARγ full agonist, PPARγ full agonist with protein tyrosine phosphatase 1B (PTP1B) inhibition, PPARα/γ dual agonist with PTP1B inhibition, and PPARγ partial agonist with PTP1B inhibition.25,26) PTP1B negatively regulates the insulin signal and its overexpression has been implicated in insulin resistance; thus, the inhibition of PTP1B is expected to exert synergistic effects with PPARγ activation on insulin sensitization.22–24) One of the 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acids with PPARα/γ agonist and PTP1B inhibitory activities has been reported to exhibit effective anti-diabetic activities with high safety,25) and this may be due to its weak PPARα activation and PTP1B inhibition. However, it was not a partial PPARγ agonist because it did not antagonize a PPARγ full agonist (unpublished data). A partial agonist based on 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid was also reported; however, its maximal activation level was still high (65%).21) As described above, our previous studies dem-
onstrated that 3-carboxyl 1,2,3,4-tetrahydroisoquinoline derivatives exerted PPARγ full agonist activity or PPARγ partial agonist activity with relatively high maximal activation. In the present study, the 2,7-substituted 3-unsubstituted 1,2,3,4-tetrahydroisoquinoline structure with an acidic group at the 6-position was revealed to be a novel scaffold for a selective PPARγ partial agonist, and compound 20g was found to possess a partial agonist property with high affinity, low maximal activation, and antagonistic activity, and to show anti-diabetic effects in db/db mice.

Chemistry

(S)-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid derivative 10 was synthesized as shown in Chart 1. Compound 3 was amided with methyl 2-amino-3-oxobutanoate hydrochloride via acyl chloride, which was followed by cyclization with I₂, triphenylphosphine (PPh₃) and Et₃N to give oxazole 5. Compound 5 was reduced with lithium aluminium hydride (LiAlH₄) and then chlorinated with SOCl₂ to give 6. The hydroxyl group of compound 7, which was prepared as described previously,²⁹ was alkylated with compound 6 to afford 8. Deprotection of the tert-butoxycarbonyl (Boc) group with HCl in i-PrOH/HCO₂H, followed by acylation with (E)-3-(2-furyl)acrylic acid and 1-(3-dimethylaminopropyl)-3-ethylcarbodi-imide hydrochloride (EDC·HCl) gave 9. Compound 9 was hydrolyzed with aqueous LiOH, and desired compound 10 was then isolated as a tert-butyl amine salt.

The general approach to the synthesis of 6-substituted-7-(2-cyclopentenyl-5-methyloxazol-4-ylmethoxy)-2-(3-furan-2-yl-propenonyl)tetrahydroisoquinoline derivatives (20a–g) is outlined in Chart 2.

The formylation of the starting material 11¹⁶ afforded a mixture of regioisomers 12, which was used without the isolation of each regioisomer. The mixture was treated with benzyl bromide (BnBr) and K₂CO₃, followed by the oxidation and isolation of a regioisomer to obtain 13. The trifluoroacetyl group of 13 was removed by hydrolysis with aqueous LiOH, and then treated with di-tert-butyl dicarbonate (Boc₂O) to afford 14. Compounds 15a, 15d, and 15h are obtained by esterification, amidation, and reduction, respectively, from compound 14. Deprotection of the benzyl group of compounds 15a, 15d, and 15h gave compounds 17a, 17d, and 17h. Separately, the nitro group of 13 was reduced by zinc and hydrolyzed with aqueous LiOH, and desired compound 10 was obtained by oxidation and cyclization. The Boc groups of 17a, 17d, and 17h to 18a, 18b, 18d, and 18h was accomplished by alkylation with 6. The carbamoyl moiety of 18d was converted to a cyano group (compound 18e), which was treated with n-Bu₃SnN₃ to give tetrazole 18g. Compound 18c was treated with hydroxylamine hydrochloride, which was cyclized to give the 4H-[1,2,4]oxadiazol-5-one-3-yl 18f. The imidazolyl derivative 18e was prepared from 18h by oxidation and cyclization. The Boc groups of 18a–g were removed and acylated with (E)-3-(2-furyl)acrylic acid to give 19a, 20b–g. Compound 19a was hydrolyzed with aqueous LiOH to afford 20a. Compound 20a was isolated as a tert-butylamine salt, and 20b and 20c were isolated as hydrochloride salts.

The synthesis of (E)-2-[3-(2-furyl)acryloyl]-7-[2-(cyclopent-3-enyl)-5-methyloxazol-4-ylmethylsulfanyl]-6-(1H-tetrazol-5-yl)-1,2,3,4-tetrahydroisoquinoline (26) is outlined in Chart 3.

Intermediate 13 was esterified with methyl iodide (MeI) and K₂CO₃, followed by deprotection of the benzyl group to give compound 21. Compound 22 was synthesized via the Newman–Kwart rearrangement of the corresponding O-aryl dimethylthiocarbamate, which was prepared from compound 21. The protection group of 22 was changed from trifluoroacetyl to the Boc group, which was hydrolyzed with MeONa solution to give 23. Compound 23 was alkylated with compound
6, followed by hydrolysis and amidation to give compound 24. Compound 24 was converted to tetrazole 25 by the same procedure from 16d to 16g, and the Boc group of 25 were then deprotected with HCl/HCO$_2$H and acylated with (E)-3-(2-furyl)acrylic acid to give 26.

### Results and Discussion

We previously reported various series of 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid derivatives. Among them, a series of 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid with a 7-bulky substituent exhibited PPAR$\gamma$ partial agonist activity (EC$_{50}$ = 48–193 nM); however, their maximal activation levels were relatively high (50–90%), which are higher than those of reported partial agonists. On the other hand, the derivatives with 3-acetyl and 3-difluoroethyl exhibited potent activities (EC$_{50}$ = 30 and 62 nM) and lower maximal activation (21 and 24%); however, their oral absorption was reduced due to low water solubility. 3-carboxyl ($20a$) showed a PPAR$\gamma$ partial agonist with low maximal activation and high water solubility, novel 3-unsubstituted 7-[2-(cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-2-(3-furan-2-yl-acryloyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid t-Butylamine Salt ($10$)

To gain structural insights into the mechanisms underlying partial agonism by our compounds, we performed computational studies of the compounds synthesized were shown in Table 1. The 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid derivative ($10$) was a PPAR$\gamma$ full agonist (EC$_{50}$ = 528 nM, maximal activation 98.7%). Compound $20a$ with 6-carboxyl exhibited PPAR$\gamma$ partial agonist activity (EC$_{50}$ = 179 nM, maximal activation 28.5%), indicating that 6-carboxyl is more suitable than 3-carboxyl for PPAR$\gamma$ partial agonism. Compound $20c$ with the 6-cyano group had no agonist activities, and compounds with 6-dimethylamino ($20b$), 6-amide ($20d$), and 6-imidazole ($20e$) all showed low activation levels at 1000 nM (21.4–26.9%). However, they caused cytotoxicity at higher concentrations, and thus maximal activation levels and antagonistic activities were unable to be measured. It remains to be determined whether $20b$, $20d$, and $20e$ are PPAR$\gamma$ partial agonists.

The compound with 6-oxadiazolone ($20f$) showed approximately 4-fold higher activity (EC$_{50}$ = 44 nM, maximal activation 27.6%), while that with 6-tetrazole ($20g$) showed approximately 14-fold higher activity (EC$_{50}$ = 13 nM, maximal activation 30.0%) than that of 6-carboxyl ($20a$). The order of acidity was $20a > 20g > 20f$, and the order of lipophilicity was $20f > 20a > 20g$. These compounds are considered to bind to the PPAR$\gamma$ protein via ionic and/or hydrophobic interactions. The above-mentioned 3-carboxyl ($10$) showed lower activity for PPAR$\gamma$ than its corresponding ether derivative $20g$ (discussed below). Three derivatives substituted with an acidic group at the 6-position ($20a$, $20f$, and $20g$) showed very weak PTP1B inhibitory activities (IC$_{50}$ = 8200, 3500, and 1100 nM, respectively), which were approximately 50–90-fold lower than their PPAR$\gamma$ partial agonist activities, suggesting selective PPAR$\gamma$ agonists.

To gain structural insights into the mechanisms underlying partial agonism by our compounds, we performed computa-
ntional docking calculations on 20a, 20f, 20g, and 26 into the human PPARγ proteins. Full agonists (compounds 1, 2, and 10) were also docked in the same manner for comparisons. The human PPARγ protein structures were derived from the Protein Data Bank (PDB; www.rcsb.org), 38) and 5Y2O (complexed with a full agonist) and 4A4V chain A (complexed with a partial agonist) were selected as the docking templates. Computational docking calculations were performed for both structures, followed by energy minimization to refine the docking pose. As a result, full agonists 1, 2, and 10 were more stably docked into the template 5Y2O 39) than 4A4V, and 20a, 20f, and 20g into the template 4A4V than 5Y2O.

The molecular orientation of the compounds was similar in each series (Figs. 3, 4). The docking scores (kcal/mol) of the compounds 1, 2, 10, 20a, 20f, and 20g were −8.6, −8.4, −8.0, −9.1, −10.1, and −10.4 for 4A4V and −10.3, −10.6, −8.7, −7.8, −8.6, and −8.6 for 5Y2O, respectively. In our study, the docking score and experimental negative logarithm of the half maximal effective concentration (pEC50) value were not correlated well, but an order of magnitude difference in the agonist activity could be verified by the docking score. By contrast, the orientation of 26 in its top-ranked pose was different from that of other partial agonists, and the pose with an orientation similar to them showed lower docking score (−7.6 kcal/mol).

The bulky substitution from ether to thioether might disturb the correct positioning of the compound deep inside the binding pocket, leading the lower agonist activity.

PPARγ full agonists, such as thiazolidinediones (TDZ), are known to interact with Helix 12 in the activation function 2 (AF-2) pocket of PPARγ and stabilize the active conformation via direct hydrogen bonding to Tyr473 (Helix 12), which allowed Helix 12 to dock to Helix 3 and Helix 11. 40) In 5Y2O, pioglitazone also showed that the nitrogen atom of the TZD ring interacts with Tyr473 (Helix 12) via a direct hydrogen bond (Fig. 3a). Furthermore, two carbonyl groups of the TZD ring formed hydrogen bonds with His323 (Helix 4/5), Ser289 (Helix 3), and His449 (Helix 10/11). The phenyl and ethyl-pyridyl groups formed hydrophobic interactions with Cys285.

Reagents and conditions: (i) HMTA, TFA; (ii) BnBr, K2CO3, DMF; (iii) 2-methyl-2-butene, NaClO2, NaH2PO4, t-BuOH, H2O, THF; (iv) LiOH aq., 1,4-dioxane then Boc2O; (v) MeI, K2CO3, DMF; (vi) ClCO2i-Bu, N-methylmorpholine, NH4Cl aq.; (vii) BH3·THF, THF; (viii) H2, Pd·C, MeOH; (ix) Zn, AcOH; (x) 37% formalin, NaBH(OAc)3, THF; (xi) 6, K2CO3, DMF; (xii) POCl3, Et3N, CH2Cl2; (xiii) NH2OH·HCl, Et3N, DMSO; (xiv) isobutyl chloroformate, pyridine, DMF then xylene reflux; (xv) n-Bu3SnN3, toluene; (xvi) SO3·Py, DMSO, i-Pr2NEt, CH2Cl2; (xvii) NH3 aq., glyoxal aq.; (xviii) HCl in i-PrOH, HCO2H; (xix) (E)-3-(2-furyl)acrylic acid, EDC·HCl, DMF, Et3N, CH2Cl2; (xx) LiOH aq., MeOH, THF then t-BuNH2, AcOEt.

Chart 2. Synthesis of 6-Substituted-7-(2-cyclopentenyl-5-methyloxazol-4-ylmethoxy)-2-(3-furan-2-yl-propenonyl)tetrahydroisoquinoline Derivatives
These interactions were considered to stabilize Helix 12 into an active conformation.

Among previously reported 2,7-substituted 1,2,3,4-tetrahydroisoquinoline 3-carboxyl acids, compound 1 exhibited PPARγ full agonist activity (EC50 = 22 nM, maximal activation 104%) and weak PPARα agonist activity (EC50 = 142 nM) and compound 2 exhibited PPARγ full agonist activity (EC50 = 70 nM, maximal activation 102%) both were revealed here to have similar binding modes to that of pioglitazone. In compound 1, the phenyl ring in the 7-substituent was attached to the side of Helix 11 and Helix 12, forming interactions with Tyr473 (Helix 12) and His449 (Helix 10/11) by aromatic and/or hydrophobic interactions (Fig. 3b). The 7-substituent in compound 2 was also close to the side of Helix 11 and Helix 12 and may form van der Waals interactions with Met348 (Helix 12) and partially recruit a coactivator, resulting in PPARγ partial agonism. In 4A4V, the carboxyl group of amorfrutin 2 interacted with Ser342 (β-strand 3) by a direct hydrogen bond (Fig. 4a). In addition, the n-pentyl and isoprenyl groups, which are compact side chains of amorfrutin 2, formed weakly hydrophobic interactions with Met348 (β-strand 4), Ile341 (β-strand 3), Leu330 (Helix 4/5), and Leu353 (Helix 6). Compound 20a substituted with carboxyl at the 6-position showed a different binding mode from that of 10 substituted with carboxyl at the 3-position: changes in the molecular orientation and the disappearance of interactions with Helix 11 and Helix 12, which may be due to the introduction of the 6-substituent, at which the carboxyl oxygen forms a hydrogen bond with Glu343 (loop) and oxazole with Cys285 (Helix 3) (Figs. 4b, c). The present results demonstrated that the 3-carboxyl substituent was preferable for interactions with the PPARγ protein as a full agonist, and the 6-carboxyl substituent as a partial agonist. In addition to the interactions shown in compound 10, a nitrogen atom of 6-oxadiazolone in 20f interacted with Glu291 (Helix 3) via a hydrogen bond, and 6-tetrazole in 20g forms a salt bridge with Arg288 (Helix 3) and a hydrogen bond with Ser342 (β-strand 3) (Figs. 4d, e). Amorfrutin 2 does not form the salt bridge with Arg288 (Helix 3), and, thus, may exhibit weaker activity (EC50 = 1200 nM) than 20g. A similar binding mode to β-sheet and Helix 3 was shown in other reported partial agonists, such as nTZDpa (Fig. 2, PDB: 2Q4S). As described above, the order of the partial agonist activity 20g > 20f > 20a may be explained by the interaction with the PPARγ protein. Among the derivatives, compound 20g with the lowest lipophilicity (c log D14 = 0.95) showed the most potent PPARγ par-
tial agonist properties in the transactivation assay. 20g showed no PPARα or PPARδ agonist activity (EC₅₀ > 10000 nM). The plasma Cₘₐₓ of 20g at 10 mg/kg after oral administration reached 7.0 ± 2.1 µg/mL in Sprague-Dawley (SD) rats and 19.8 ± 0.6 µg/mL in KK-A₁y mice, indicating good oral absorption. Compound 20g and pioglitazone were administered as a food admixture for 7 d and their anti-diabetic effects were compared. Despite very low maximal activation (30%), compound 20g at 30 mg/kg significantly lowered blood glucose, which was similar to pioglitazone, a full agonist at 10 mg/kg (Table 2). In the present experiments, neither 20g nor pioglitazone exerted severe adverse effects. Repeated dosing for a longer period may be required to clarify the less adverse effects of 20g than those of pioglitazone.

In summary, the present study found that 7-[2-(cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-2-(3-furyl)-acryloyl]-1,2,3,4-tetrahydroisoquinoline derivatives substituted with an acidic group at the 6-position are selective PPARγ partial agonists. Computational docking calculations revealed the interaction mode of these partial agonists as well as 1,2,3,4-tetrahydroisoquinoline-based full agonists. Among the three partial agonists, 20g with 6-tetrazole had the most potent activity and lowest lipophilicity, and showed good oral absorption and experimental anti-diabetic activity.

Experimental

General Melting points were measured on a melting point apparatus (Yamato MP-21; Yamato Scientific Co., Ltd., Tokyo, Japan) and were uncorrected. ¹H-NMR spectra were obtained on a NMR spectrometer at 400 MHz (JNM-AL400; JEOL Ltd., Tokyo, Japan) using tetramethylsilane (TMS) as an internal standard. IR spectra were recorded with a HORIBA FT-720 spectrometer (HORIBA, Kyoto, Japan). Mass spectra were obtained on an Expression CMS-L (Advion, Ithaca, U.S.A.). Column chromatography was performed on a silica gel (Daisogel No.1001W; Daiso Co., Ltd., Osaka, Japan). Reactions were monitored by TLC (TLC silica gel 60F254, Merck KGaA, Darmstadt, Germany).

Methyl 2-[(Cyclopent-3-ene-carbonyl)aminol]-3-oxobutyrate (3) Cyclopent-3-enecarboxylic acid (2) (5.00 g, 44.6 mmol) was dissolved in SOCl₂ (9.80 mL, 134 mmol) and stirred at room temperature for 1 h. The reaction mixture was concentrated in a vacuum and the residue was dissolved in toluene and concentrated.

Et₃N (18.7 mL, 134 mmol) was added dropwise to a suspension of the obtained residue and methyl 2-amino-3-oxobutanoate hydrochloride (8.20 g, 48.9 mmol) in CH₂Cl₂ (80 mL) under ice-cooling, which was then stirred at the same temperature for 0.5 h. After the addition of a 10% aqueous citric acid solution, the reaction mixture was extracted with AcOEt, washed...
with brine, and dried over Na₂SO₄. The solvent was evaporated under reduced pressure, and the obtained residue was purified by column chromatography to give 3 (6.93 g, 69% yield for 2 steps) as an oil. \(^{1}\)H-NMR (CDCl₃) δ: 2.39 (3H, s), 2.60–2.70 (4H, m), 3.08 (1H, quintet, \(J = 7.8\) Hz), 3.82 (3H, s), 5.27 (1H, d, \(J = 6.6\) Hz), 5.68 (2H, s), 6.58–6.73 (1H, br).

**Methyl 2-Cyclopent-3-enyl-5-methyloxazole-4-carboxylate (4)** PPh₃ (30.7 g, 117 mmol) and Et₃N (33.1 mL, 237 mmol) were added to a solution of I₂ (29.7 g, 117 mmol) in CH₂Cl₂ (460 mL) under ice-cooling, which was then stirred at the same temperature for 10 min. Compound 3 (6.93 g, 30.8 mmol) in CH₂Cl₂ (60 mL) was added dropwise to the reaction mixture under ice-cooling and stirred at the same condition for 0.5 h. The reaction mixture was washed with water and saturated brine, dried over Na₂SO₄, and then evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give 4 (4.76 g, 75% yield) as an oil. \(^{1}\)H-NMR (CDCl₃) δ: 2.60 (3H, s), 2.70–2.87 (4H, m), 3.58 (1H, quintet, \(J = 7.6\) Hz), 3.89 (3H, s), 5.72 (2H, s).

**4-Chloromethyl-2-cyclopent-3-enyl-5-methyloxazole (6)** Lithium aluminum hydride (0.61 g, 16 mmol) was added portionwise to a solution of 5 (4.76 g, 23.0 mmol) in tetrahydrofuran (THF) (95 mL) under ice-cooling and stirred at the same condition for 10 min. Water was added to the reaction mixture.

### Table 2. Effects of 20g Administered Orally for 1 Week to KK-A⁺ Mice

|                | Control                  | 20g 30mg/kg/d             | Pioglitazone 10mg/kg/d |
|----------------|--------------------------|---------------------------|------------------------|
| **Male**       |                          |                           |                        |
| Glucose (mg/dL)| 584.5 ± 28.8             | 474.2 ± 33.4*             | 450.4 ± 35.2*          |
| TG (mg/dL)     | 646.2 ± 72.6             | 661.6 ± 75.2              | 611.6 ± 63.6           |
| Body weight (g)| 49.1 ± 0.9               | 51.7 ± 1.1                | 51.8 ± 1.3             |
| **Female**     |                          |                           |                        |
| Glucose (mg/dL)| 448.0 ± 19.6             | 290.5 ± 43.0**            | 241.2 ± 30.7**         |
| TG (mg/dL)     | 583.0 ± 48.6             | 595.5 ± 82.9              | 663.9 ± 85.2           |
| Body weight (g)| 48.8 ± 1.0               | 50.3 ± 0.8                | 51.9 ± 1.3             |

Mean ± S.E. (n = 7). * \(p < 0.05\), ** \(p < 0.01\) vs. Control, the Student’s t-test.
The precipitate was removed by filtration and washed with AcOEt. The filtrate was separated into two layers and the organic layer was washed with water and brine, dried over Na₂SO₄, and then evaporated under reduced pressure.

The residue was dissolved in SOCl₂ (9.10 mL, 120 mmol) and stirred at room temperature for 30 min. The reaction mixture was concentrated in a vacuum and the residue was dissolved in toluene and concentrated to give 6 (4.12 g, 91% yield) as an oil. ¹H-NMR (CDCl₃) δ: 2.31 (3H, s), 2.68–2.84 (4H, m), 3.50–3.61 (1H, m), 4.46 (2H, s), 5.73 (2H, s).

**Methyl (S)-2-tert-Butyoxycarbonyl-7-(2-cyclopent-3-yl-5-methyloxazol-4-ylmethoxy)-3,4-dihydro-1H-isooquinoline-3-carboxylate (8)** K₂CO₃ (1.35 g, 9.75 mmol) was added to a solution of 7⁹ (1.00 g, 3.25 mmol) and 6 (722 mg, 3.90 mmol) in N,N-dimethylformamide (DMF) (10 mL), and the mixture was stirred at 60°C for 2.5 h. AcOEt was added to the reaction mixture, which was washed with water and saturated brine, and dried over Na₂SO₄. The solvent was evaporated under reduced pressure. The obtained residue was purified by column chromatography to give 8 (722 mg, 3.90 mmol) as a solid. ¹H-NMR (CDCl₃) δ: 1.38 (3H, s), 1.52 (4.5H, s), 2.31 (3H, s), 2.70–2.85 (4H, m), 3.05–3.22 (2H, m), 3.53–3.65 (4H, m), 4.40–4.52 (1H, m), 4.62–4.72 (1H, m), 4.73–4.79 (0.5H, m), 4.84 (2H, s), 5.08–5.16 (0.5H, m), 5.73 (2H, s), 6.70–6.85 (2H, m), 7.00–7.09 (1H, m).

**Methyl (7-2-Cyclopent-3-yl-5-methyloxazol-4-ylmethoxy)-2-(3-furan-2-ylacryloyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (9)** A solution of 8 (450 mg, 0.775 mol) and K₂CO₃ (160 g, 1.16 mol), and the mixture was stirred at room temperature for 0.5 h. The reaction mixture was neutralized with saturated aqueous NaHCO₃ solution and extracted with CHCl₃. The organic layer was dried over Na₂SO₄ and evaporated under reduced pressure. The obtained residue was dissolved in CH₂Cl₂ (3.0 mL), (E)-3-(2-furyl)acrylic acid (160 mg, 1.16 mmol) and EDC·HCl (92.0 mL, 0.775 mol) were added, and the mixture was stirred at room temperature for 2 h. After the addition of a 10% aqueous acetic acid solution, the reaction mixture was extracted with CHCl₃ and washed with saturated aqueous NaHCO₃ solution and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure, and the obtained residue was purified by column chromatography to give 9 (86.7 g, 0.618 mol) as a foam (420 mg). ¹H-NMR (CDCl₃): 3.80–3.95 (2H, m), 4.25–4.35 (1H, m), 4.40–4.52 (1H, m), 6.54–6.65 (1H, m), 6.70–6.90 (4H, m), 6.95–7.06 (1H, m), 7.24–7.37 (1H, m), 7.70–8.40 (3H, m). 1R (ATR) cm⁻¹: 1646, 1604. MS m/z: 473 [M+H], 475 [M+H⁺].

**7-Hydroxy-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline-6-carbaldehyde (12)** Hexamethylenetetramine (HMTA) (86.7 g, 0.618 mol) was added to a solution of 11⁹ (102 g, 0.416 mol) in trifluoroacetic acid (TFA) (1 L), and the mixture was refluxed for 3 h. After cooling to room temperature, TFA was evaporated under reduced pressure. The obtained residue was poured into cold water (1 L), neutralized with NaHCO₃, and extracted with AcOEt. The organic layer was washed with saturated brine, dried over Na₂SO₄, and then evaporated under reduced pressure. The obtained residue was purified by column chromatography to give 12 (74.0 g, 65% yield) as a solid. ¹H-NMR (CDCl₃) δ: 2.83–3.02 (2H, m), 3.80–3.95 (2H, m), 4.70–4.85 (0.9H, m), 5.10–5.25 (1.1H, m), 6.75–6.90 (1H, m), 7.20–7.45 (1H, m), 9.82–9.86 (0.45H, m), 10.23–10.30 (0.55H, m), 11.93–11.98 (0.55H, m).

**7-Benzoyloxy-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline-6-carboxylic Acid (13)** A solution of 12 (210 g, 0.769 mol) in DMF (1000 mL) was added to benzyl bromide (92.0 mL, 0.775 mol) and K₂CO₃ (160 g, 1.16 mol), and the mixture was stirred at room temperature for 2 h. AcOEt was added to the reaction mixture, which was washed with water and saturated brine, and dried over Na₂SO₄. The solvent was evaporated under reduced pressure. The obtained residue was added to n-hexane (500 mL), which was stirred at room temperature for 40 min, and the precipitate that formed was filtered (281 g). NaClO₃ (124 g, 1.37 mol) was added to a solution of NaH₂PO₄ (131 g, 1.09 mol) in water (1200 mL), and the mixture was stirred at room temperature for 0.5 h. The reaction mixture was added to a solution of the obtained precipitate (133 g, 0.366 mol) in i-PrOH–THF (2 : 1) (1800 mL) and 2-methyl-2-butane (275 mL, 2.59 mol) at the same temperature, and then was stirred at room temperature for 1.5 h. The reaction mixture was cooled with an ice bath and stirred for 15 min. The precipitate was filtered to give 13 (49.0 g, 35% yield) as a solid. ¹H-NMR (CDCl₃) δ: 2.80–2.90 (2H, m), 3.79 (2H, t, J = 5.9 Hz), 4.75 (0.8H, s), 4.78 (1.2H, s), 5.15 (2H, s), 7.15 (0.6H, s), 7.20 (0.4H, s), 7.28–7.50 (6H, m), 12.62 (1H, s).

**7-Benzoyloxy-2-tert-butyloxycarbonyl-1,2,3,4-tetrahydroisoquinoline-6-carboxylic Acid (14)** A 1.0 M aqueous LiOH solution (640 mL, 640 mmol) was added to a solution of 13 (92.6 g, 0.244 mol) in 1,4-dioxane (640 mL), and the mixture was stirred at room temperature for 0.5 h. Boc₂O (69.2 g, 366 mmol) was added to the obtained mixture, which was then stirred at the same temperature for 1 h. AcOEt was added to the reaction mixture, which was washed with 10% aqueous acetic acid solution, water, and saturated brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure. The obtained residue was added to n-hexane (100 mL), which was stirred at room temperature for 1 h. The precipitate was added to the solution of carboxylic acid in AcOEt (2.0 mL) and the reaction mixture was stirred at room temperature for 10 min. After the addition of i-PrOMe, the precipitate was filtered to give 10 (178 mg, 71% yield) as a solid, mp 111–114°C. ¹H-NMR (dimethyl sulfoxide (DMSO)-d₆) δ: 1.08–1.17 (9H, m), 2.30 (3H, s), 2.57–2.83 (4H, m), 2.88–2.97 (1H, m), 3.18–3.61 (2H, m), 4.36–4.45 (0.7H, m), 4.53–4.60 (0.7H, m), 4.76–4.95 (3.3H, m), 5.08–5.14 (0.3H, m), 5.73 (2H, s), 6.54–6.65 (1H, m), 6.70–6.90 (4H, m), 6.95–7.06 (1H, m), 7.24–7.37 (1H, m), 7.70–8.40 (3H, m). 1R (ATR) cm⁻¹: 1646, 1604. MS m/z: 473 [M+H]⁻, 475 [M+H⁺].
was filtered to give 14 (88.0 g, 94% yield) as a solid. ¹H-NMR (CDCl₃) δ: 1.50 (9H, s), 2.78–2.88 (2H, m), 3.58–3.68 (2H, m), 4.59 (2H, s), 5.26 (2H, s), 6.85 (1H, s), 7.37–7.48 (5H, m), 7.98 (1H, s), 10.76 (1H, s).

tert-Butyl 7-Benzoxly-6-carbamoyl-3,4-dihydro-1H-isoquinoline-2-carboxylate (15d) N-Methylmorpholine (27.1 g, 0.268 mol) and isobutyl chloroformate (34.8 mL, 0.268 mol) were added dropwise to a suspension of 14 (97.4 g, 0.254 mol) in THF (2L) under ice-cooling, which was stirred at the same temperature for 20 min. A 28% aqueous NH₃ solution (97 mL, 0.268 mol) was added dropwise to the obtained mixture, and the mixture was stirred further at the same temperature for 2h. The solvent was evaporated under reduced pressure to give 15d (117 g, quant.) as a solid. ¹H-NMR (CDCl₃) δ: 1.49 (9H, s), 2.82 (2H, t, J = 5.4 Hz), 3.64 (2H, t, J = 5.4 Hz), 4.59 (2H, s), 5.26 (2H, s), 6.85 (1H, s), 7.39–7.46 (5H, m), 7.98 (1H, s), 9.80–11.20 (2H, br).

tert-Butyl 7-Benzoxly-6-hydroxymethyl-1,2,3,4-tetrahydroisoquinolin-2-carboxylate (15h) A total of 1.0 M BH₃·THF (25.1 mL, 25.1 mmol) was added dropwise to a solution of (3.20 g, 8.35 mmol) in THF (25 mL) under ice-cooling, and the mixture was stirred at room temperature for 0.5 h. The reaction mixture was added to water and the solvent was evaporated by filtration. The filtrate was basified with saturated aqueous NaHCO₃ solution, which was extracted with AcOEt. The organic layer was washed with water and saturated brine, and then dried over Na₂SO₄.

Compound 6 was synthesized from 15h according to the procedure for the synthesis of 17d. Yield 66%. A pale yellow oil. ¹H-NMR (CDCl₃) δ: 1.48 (9H, s), 2.16–2.33 (1H, br), 2.67–2.75 (2H, m), 3.56–3.65 (2H, m), 4.49 (2H, s), 4.81 (2H, s), 6.64 (1H, s), 6.81 (1H, s), 7.14–7.19 (1H, br).

tert-Butyl 6-Amino-7-hydroxy-1,2,3,4-tetrahydroisoquinolin-2-carboxylate (27) An 85% Zn powder (1.15 g, 15 mmol) was added to a solution of 16 (0.88 g, 3.0 mmol) in acetic acid (5mL), and the mixture was stirred at same temperature for 0.5h. The reaction mixture was added to water and the Zn powder was removed by filtration. The filtrate was basified with saturated aqueous NaHCO₃ solution, which was extracted with AcOEt. The organic layer was washed with water and saturated brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give 27 (597 mg, 76% yield) as a solid.

Methyl 2-tert-Butoxycarbonyl-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-6-carboxylate (17a) Mel (0.325 mL, 5.22 mmol) and K₂CO₃ (721 mg, 5.22 mmol) were added to a solution of 14 (1.00 g, 2.61 mmol) in DMF (10 mL), which was then stirred at room temperature for 16h. AcOEt was added to the reaction mixture, washed with water and saturated brine, then stirred at room temperature for 1 h. The reaction mixture was basified with saturated aqueous NaHCO₃ solution, which was extracted with AcOEt. The organic layer was washed with water and saturated brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give 17b (661 mg, quant.) as an oil.

Methyl 2-tert-Butoxycarbonyl-7-[2-(cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-1,2,3,4-tetrahydroisoquinoline-6-carboxylate (18a) Compound 18a was synthesized from 17a and 6 according to the procedure for the synthesis of 8. Yield 84%. A white solid. ¹H-NMR (CDCl₃) δ: 1.49 (9H, s), 2.34 (3H, s), 2.69–2.85 (6H, m), 3.50–3.67 (3H, m), 3.85 (3H, s), 4.55 (2H, s), 4.97 (2H, s), 5.74 (2H, s), 6.86 (1H, s), 7.58 (1H, s).

17b: 20%.
yield) as a solid. 1H-NMR (CDCl₃) δ: 1.50 (9H, s), 2.32 (3H, s), 2.68–2.86 (6H, m), 3.53–3.68 (3H, m), 4.59 (2H, s), 4.60 (2H, s), 4.91 (2H, s), 5.72 (2H, s), 6.74 (1H, s), 7.02 (1H, s).

tert-Butyl 7-[2-(Cyclopent-3-enyl)-5-methoxyazol-4-ylmethoxy]-6-hydroxyethyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (18b) Compound 18b was synthesized from 17h and 6 according to the procedure for the synthesis of 8. Yield 91%. A white solid. 1H-NMR (CDCl₃) δ: 1.49 (9H, s), 2.31 (3H, s), 2.68–2.85 (7H, m), 3.48–3.67 (3H, m), 4.53 (2H, s), 4.60 (2H, s), 4.91 (2H, s), 5.72 (2H, s), 6.74 (1H, s), 7.02 (1H, s).

tert-Butyl 7-[2-(Cyclopent-3-enyl)-5-methoxyazol-4-ylmethoxy]-6-hydroxyethyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (18h) Compound 18h was synthesized from 17h and 6 according to the procedure for the synthesis of 9. Yield 85%. A yellow oil. 1H-NMR (CDCl₃) δ: 1.50 (9H, s), 2.32 (3H, s), 2.68–2.86 (6H, m), 3.53–3.68 (3H, m), 4.59 (2H, s), 4.97 (2H, s), 5.74 (2H, s), 6.81 (1H, s), 7.32 (1H, s).

tert-Butyl 7-[2-(Cyclopent-3-enyl)-5-methoxyazol-4-ylmethoxy]-6-((1H-imidazol-2-yl)-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (18e) A 40% aqueous glyoxal solution (63 µL, 0.55 mmol) and 28% aqueous NH₄Cl solution (0.15 mL, 2.3 mmol) were added to a solution of 28 (200 mg, 0.466 mmol) in MeOH (1 mL), which was then stirred at room temperature for 14 h. After the addition of saturated aqueous NaOH solution, the mixture was extracted twice with CHCl₃. The organic layer was dried over Na₂SO₄ and then evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give 18e (167 mg, 77% yield) as a solid. 1H-NMR (CDCl₃) δ: 1.50 (9H, s), 2.31 (3H, s), 2.75–2.93 (6H, m), 3.53–3.69 (3H, m), 4.59 (2H, s), 5.00 (2H, s), 5.79 (2H, s), 6.81 (1H, s), 7.00–7.20 (2H, m), 8.10 (1H, s), 11.50–11.80 (1H, br).

tert-Butyl 7-[2-(Cyclopent-3-enyl)-5-methoxyazol-4-ylmethoxy]-6-((2-furan-2-yl-acryloyl)-1,2,3,4-tetrahydroisoquinoline-6-carboxylate (20a) A 1.0 M aqueous LiOH solution (2.58 mL, 2.58 mmol) was added to a solution of 19a (420 mg, 0.860 mmol) in MeOH (2.5 mL) and THF (7.5 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was acidified with a 1.0 M aqueous HCl solution, which was extracted with CHCl₃. The organic layer was washed with saturated brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure. tert-Butylamine (90 µmol, 0.88 mmol) was added to a solution of the obtained residue in AcOEt (2.0 mL), and the precipitate was then filtered to give 20a (125 mg, 27% yield) as a solid. 1H-NMR (CDCl₃) δ: 1.28 (3H, s), 2.28 (3H, s), 2.56–2.96 (2H, m), 3.45–3.55 (1H, m), 3.78–3.90 (2H, m), 4.76–4.86 (2H, m), 4.98 (2H, s), 6.44–6.49 (1H, m), 6.57 (1H, d, J = 3.2 Hz), 6.73 (1H, d, J = 15.2 Hz), 6.93 (1H, s), 7.46 (1H, s), 7.50 (1H, d, J = 15.2 Hz), 7.60 (1H, s).

(E)-7-[2-(Cyclopent-3-enyl)-5-methoxyazol-4-ylmethoxy]-6-(3-furan-2-yl-acryloyl)-1,2,3,4-tetrahydroisoquinoline-6-carboxylic Acid t-Butylamine Salt (20a) A 1.0 M aqueous LiOH solution (2.58 mL, 2.58 mmol) was added to a solution of 19a (420 mg, 0.860 mmol) in MeOH (2.5 mL) and THF (7.5 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was acidified with a 1.0 M aqueous HCl solution, which was extracted with CHCl₃. The organic layer was washed with saturated brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure. tert-Butylamine (90 µmol, 0.88 mmol) was added to a solution of the obtained residue in AcOEt (2.0 mL), and the precipitate was then filtered to give 20a (125 mg, 27% yield) as a solid. 1H-NMR (CDCl₃) δ: 1.28 (3H, s), 2.28 (3H, s), 2.56–2.96 (2H, m), 3.45–3.55 (1H, m), 3.78–3.90 (2H, m), 4.76–4.86 (2H, m), 4.98 (2H, s), 6.44–6.50 (1H, m), 6.56 (1H, d, J = 3.2 Hz), 6.77 (1H, d, J = 15.2 Hz), 6.86 (1H, s), 7.44–7.47 (1H, m), 7.49 (1H, d, J = 15.2 Hz), 7.60 (1H, s). IR (ATR) cm⁻¹: 1646, 1604. MS m/z: 473 [M⁺].

(E)-7-[2-(Cyclopent-3-enyl)-5-methoxyazol-4-ylmethoxy]-6-dimethylamino-2-[3-(2-furyl)acryloyl]-1,2,3,4-tetrahydroisoquinoline Hydrochloride (20b) A total of 8.6 M HCl in i-PrOH (0.29 mL, 2.5 mmol) was added to a solution of 18b (380 mg, 0.838 mmol) in HCO₂H (2 mL), and the mixture was stirred at room temperature for 15 min. The reaction mixture was basified with saturated aqueous NaHCO₃ solution, which was extracted with AcOEt. The organic layer was washed with water and saturated brine, and
dried over Na₂SO₄. The solvent was evaporated under reduced pressure.

The obtained residue was dissolved in CH₂Cl₂ (5.0mL) to which was added (E)-3-(2-furyl)acrylic acid (120mg, 0.869mmol) and EDC·HCl (210mg, 1.10mmol), and the mixture was stirred at room temperature for 2h. After the addition of a 10% aqueous citric acid solution, the reaction mixture was extracted with CH₂Cl₂, washed with saturated aqueous NaHCO₃ solution and brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure, and the obtained residue was purified by column chromatography. A total of 7.3M HCl in i-PrOH (0.13mL, 0.95mmol) and i-Pr₂O (2.0mL) was added to the obtained residue in AcOEt (2.0mL), and the precipitate was then filtered to give 20b (281mg, 75% yield for 3 steps) as a white solid, mp 179–181°C. 1H-NMR (CDCl₃) δ: 2.33 (3H, s), 2.68–2.78 (2H, m), 2.82–2.98 (4H, m), 3.68–3.76 (1H, m), 5.14–5.21 (2H, s), 5.27 (2H, s), 5.72 (2H, s), 6.60–6.65 (1H, m), 6.81 (1H, d, J = 6.0Hz), 7.01 (1H, d, J = 15.1Hz), 7.39 (1H, d, J = 15.1Hz), 7.42–7.50 (1H, m), 7.74 (2H, s), 7.82 (1H, s), 7.90 (1H, s), 14.30–14.45 (1H, br). IR (ATR) cm⁻¹: 1645, 1601. MS m/z: 495 [M − H]⁻, 497 [M + H]⁺.

(E)-7-[2-(Cyclopent-3-enyl)-5-methylxazol-4-ylmethoxy]-2-[3-(2-furyl)acryloyl]-6-(5-oxo-[1,2,4]oxadiazolidin-3-yl)-1,2,3,4-tetrahydroquinoline (20f) Compound 20f was synthesized from 18f according to the procedure for the synthesis of 9. Yield 47% for 2 steps. A white solid, mp 179–181°C. 1H-NMR (CDCl₃) δ: 2.33 (3H, s), 2.69–2.79 (2H, m), 2.80–2.98 (4H, m), 3.58–3.67 (1H, m), 5.05–5.15 (2H, s), 5.54 (2H, s), 5.73 (2H, s), 6.10–6.15 (1H, m), 6.29–6.34 (1H, m), 6.34–6.44 (1H, m), 6.45–6.54 (1H, m), 6.58 (1H, d, J = 3.4Hz), 6.85 (1H, d, J = 14.8Hz), 6.90 (1H, s), 7.45–7.47 (1H, m), 7.51 (1H, d, J = 14.9Hz), 7.74 (1H, s), 11.70–11.85 (1H, br). IR (ATR) cm⁻¹: 1766, 1635. MS m/z: 513 [M − H]⁻.
and brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give 29 (7.30 g, quant.) as a solid. ¹H-NMR (CDCl₃) : δ 2.94–3.05 (2H, m), 3.39 (2H, s), 3.46 (4H, s), 3.83 (3H, s), 3.85–3.96 (2H, m), 4.78 (0.7H, s), 4.83 (1.3H, s), 6.89 (0.3H, s), 6.91 (0.7H, s), 7.81 (0.7H, s), 7.83 (0.3H, s).

Methyl 7-Dimethylcarbamoysulfanyl-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-6-carboxylate (22) Compound 29 (7.64 g, 19.6 mmol) was dissolved in n-tetradecane (80 mL) and the mixture was stirred at 250°C under a N₂ atmosphere for 15 h. After cooling to room temperature, the mixture was purified by silica gel column chromatography to give 22 (1.63 g, 22% yield) as an oil. ¹H-NMR (CDCl₃) : δ 2.92–3.20 (8H, m), 3.81–3.94 (5H, m), 4.77 (0.7H, s), 4.81 (1.3H, s), 7.38 (0.3H, s), 7.40 (0.7H, s), 7.72 (0.7H, s), 7.74 (0.3H, s).

Methyl tert-Butyoxycarbonyl-7-dimethylcarbamoysulfanyl-1,2,3,4-tetrahydroisoquinoline-6-carboxylate (30) A 0.50 M aqueous K₂CO₃ solution (30 mL, 15 mmol) was added to a solution of compound 22 (1.53 g, 3.92 mmol) in MeOH (15 mL) and the mixture was stirred at room temperature for 1 h. Boc₂O (1.71 g, 7.84 mmol) was added to the reaction mixture and stirred at 50°C for 18 h. After the addition of water, the mixture was extracted twice with AcOEt. The organic layer was washed with brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give 30 (1.22 g, 79% yield for 2 steps) as a solid. ¹H-NMR (CDCl₃) : δ 1.48 (9H, s), 2.71–2.91 (2H, m), 2.95–3.20 (6H, m), 3.60–3.70 (2H, m), 3.86 (3H, s), 4.59 (2H, s), 7.34 (1H, s), 7.70 (1H, s).

Methyl tert-Butyoxycarbonyl-7-mercapto-1,2,3,4-tetrahydroisoquinoline-6-carboxylate (23) A 28% solution of NaOMe in MeOH (0.71 mL, 3.7 mmol) was added to a solution of compound 30 (1.22 g, 3.09 mmol) in MeOH (12 mL) at room temperature under a N₂ atmosphere, and the mixture was stirred at 60°C for 6 h. After the addition of a 10% aqueous citric acid solution, the mixture was extracted twice with AcOEt. The organic layer was washed with brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give 23 (999 mg, quant.) as an oil. ¹H-NMR (CDCl₃) : δ 1.49 (9H, s), 2.12–2.35 (2H, m), 3.55–3.68 (2H, m), 3.91 (3H, s), 4.52 (2H, s), 4.69 (1H, s), 7.04 (1H, s), 7.79 (1H, s).

Methyl tert-Butyoxycarbonyl-7-[2-(cyclopent-3-yl)-3-(2-furyl) acrylic acid (23) 290 mg, 1.13 mmol) in HCO₂H (2 mL), and the mixture was stirred at 40°C for 2 h. The reaction mixture was purified by silica gel column chromatography to give 23 (1.26 g, 88% yield) as a white solid. ¹H-NMR (CDCl₃) : δ 1.49 (9H, s), 2.17 (3H, s), 2.56–2.79 (4H, m), 2.79–2.87 (2H, m), 3.46–3.58 (1H, m), 3.59–3.69 (2H, m), 3.96 (2H, s), 4.55 (2H, s), 5.70 (2H, s), 7.20 (1H, s), 7.72 (1H, s).

tert-Butyl 6-Carbamoyl-7-[2-(cyclopent-3-yl)-5-methyl-oxazol-4-ylmethylsulfanyl]-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (24) Compound 24 was synthesized from compound 23 according to the procedure for the synthesis of 15d. Yield 84%. A white solid. ¹H-NMR (CDCl₃) : δ 1.49 (9H, s), 1.91 (3H, s), 2.62–2.80 (4H, m), 2.80–2.87 (2H, m), 3.43–3.55 (1H, m), 3.59–3.68 (2H, m), 3.88 (2H, s), 4.51 (2H, s), 5.71 (2H, s), 7.20 (1H, s), 7.65 (1H, s).

tert-Butyl 7-[2-(cyclopent-3-yl)-5-methyl-oxazol-4-ylmethylsulfanyl]-6-(1H-tetrazol-5-yl)-3,4-dihydro-1Hisoquinoline-2-carboxylate (33) Compound 33 was synthesized from compound 24 according to the procedure for the synthesis of 18c. Yield quant. A white solid. ¹H-NMR (CDCl₃) : δ 1.49 (9H, s), 2.12 (3H, s), 2.62–2.78 (4H, m), 2.79–2.87 (2H, m), 3.46–3.58 (1H, m), 3.59–3.68 (2H, m), 3.98 (2H, s), 4.54 (2H, s), 5.72 (2H, s), 7.30 (1H, s), 7.39 (1H, s).

tert-Butyl 7-[2-(cyclopent-3-yl)-5-methyl-oxazol-4-ylmethylsulfanyl]-6-(1H-tetrazol-5-yl)-3,4-dihydro-1H-isquinoline-2-carboxylate (34) Compound 34 was synthesized from compound 33 according to the procedure for the synthesis of 18g. Yield 75%. A white solid. ¹H-NMR (CDCl₃) : δ 1.50 (9H, s), 2.14 (3H, s), 2.65–2.75 (2H, m), 2.89 (2H, t, J = 5.6 Hz), 3.54–3.64 (1H, m), 3.66 (2H, t, J = 5.6 Hz), 3.96 (2H, s), 4.61 (2H, s), 5.71 (2H, s), 7.49 (1H, s), 7.91 (1H, s).

[(E)-3-(2-Furlylacryloyl)-7-[2-(cyclopent-3-yl)-5-methyl-oxazol-4-ylmethylsulfanyl]-6-(1H-tetrazol-5-yl)-1,2,3,4-tetrahydroisoquinoline (26) A total of 8.6 M HCl in i-PrOH (0.39 mL, 3.4 mmol) was added to a solution of 34 (558 mg, 1.13 mmol) in HCO₂H (2 mL), and the mixture was stirred at room temperature for 15 min. After the addition of Et₃O, the precipitate formed was collected by filtration to give a crude intermediate.

(COCl₂) (0.145 mL, 1.7 mmol) and 1 drop of DMF were added to a solution of (E)-3-(2-furylacrylic acid (230 mg, 1.7 mmol) in CH₂Cl₂ (20 mL), and the mixture was stirred at room temperature for 0.5 h. The obtained mixture and Et₃N (0.79 mL, 5.7 mmol) were added to a solution of the crude intermediate in CH₂Cl₂ (5 mL), which was stirred at the same temperature for 3 h. The reaction mixture was washed with water, saturated aqueous NaHCO₃ solution, and saturated brine, and then dried over Na₂SO₄. The residue obtained was purified by silica gel column chromatography to give 26 (290 mg, 50% yield for 2 steps) as a pale yellow solid, mp 158–161°C. ¹H-NMR (CDCl₃) : δ 2.15 (3H, s), 2.64–2.75 (2H, m), 2.77–2.90 (2H, m), 2.94–3.08 (2H, m), 3.47–3.60 (1H, m), 3.83–3.94 (5H, m), 3.97 (2H, s), 4.88 (2H, s), 5.71 (2H, s), 6.43–6.52 (1H, m), 6.58 (1H, d, J = 3.4 Hz), 6.85 (1H, d, J = 14.8 Hz), 7.46 (1H, s), 7.51 (1H, d, J = 14.8 Hz), 7.55 (1H, s).
agonist (10 μm) was administered orally and then blood was taken from the tail vein after 0.5, 1, 4, and 8 h in KK-Ay male and female KK-Ay mice. Blood samples were taken from the tail veins of non-fasted mice 24 h after the final administration. Plasma glucose and triglyceride levels in mice were assessed using commercial kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Computational Analyses High-quality (resolution ≤2.0 Å, no mutated residue around the ligand-binding site and no distortion by crystal contacts) X-ray crystal structures of human PPARγ complexed with non-covalent agonists were retrieved from the PDB. The structures obtained were also curated by the crystallization condition (pH 7.5 ± 1.0) for the following computational docking calculations under mild pH conditions. Sixteen complex structures were found (PDB codes 1ZGY, 2HWQ, 2YFE (chain A), 3B1M, 3ET3, 3LMP, 3V9T, 3V9V, 3VSO, 4A4V (chain A), 4A4W (chain A), 4F9M, 5GTN, 5HZC (chain A), 5Y2O and 5Y2T (chain A)). These structures were manually divided into two groups according to whether the ligand interacted with Helix 10/11 and/or Helix 12 in the AF-2 pocket of PPARγ. A structural similarity analysis was then performed for each group by calculating the atomic root-mean-square deviation (RMSD) of amino acid residues around the ligand-binding site using the conformation cluster script of Schrödinger Suite 2018-1 (Schrödinger, LLC, New York, U.S.A.). One representative structure was selected from each of the two groups, as the nearest one to the centroid, respectively. Protein structures were then preprocessed using Protein Preparation Wizard in Maestro (Schrödinger Suite 2018-1), which added hydrogen atoms, repaired side chains of incomplete residues, and optimized the hydrogen bonding network. The restrained minimization of protein structures were performed with an RMSD cut-off value of 0.3 Å using the OPLS3 force field. Receptor grids for computational ligand docking were then generated for both of the two protein structures using the grid generation feature of Glide (Schrödinger Suite 2018-1). LigPrep (Schrödinger Suite 2018-1) was used to define the possible 3D structures of compounds with various ionization states at pH 7.0 ± 2.0, tautomers, and ring conformations. Multiple conformations of compounds were then generated using ConGen (Schrödinger Suite 2018-1). All docking calculations were performed with Glide and the run in standard precision (SP) mode. The ligand van der Waals radius for non-polar atoms was scaled by factors of 0.8 and 1.0. The conformers generated by ConGen were docked into the receptor grids, and the top pose for each compound, assessed by the Glide docking score, was collected. Each docked structure was then energy-minimized using the OPLS3 force field and 0.05 kcal/mol Å of convergence with a distance-dependent dielectric constant (ε = 4).

Conflict of Interest The authors declare no conflicts of interest.

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