Regular Article

(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

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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 (PPAR γ) 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 (EC_{50} = 13 nM, maximal response 30%) and very weak protein tyrosine phosphatase 1B (PTP1B) inhibition (IC_{50} = 1100 nM), indicating a selective PPAR γ partial agonist. A computational docking calculation revealed that 20g bound to PPAR γ in a similar manner to that of known partial agonists. In male and female KK-A γ 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 PPAR γ partial agonists and 20g exerted anti-diabetic effects *via* the partial activation of PPAR γ .

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

Introduction

Peroxisome proliferator-activated receptor γ (PPAR γ) is a ligand-dependent transcription factor belonging to the nuclear receptor family. PPAR γ 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)} PPAR γ 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 (TNF α), an insulin resistance-evoking adipokine.²⁾ PPAR γ 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, congestive heart failure, and bone fracture.^{8–11)} Thus, extensive efforts have been made to develop safer PPAR γ agonists, and various PPAR α/γ dual agonists and PPAR pan-agonists have been reported. However, their development has mostly been suspended due to the potential risks of cardiovascular events, carcinogenicity, liver injury, and/or renal dysfunction.^{12–14)} The overactivation of PPAR with PPAR α/γ dual agonists and PPAR pan-agonists may lead to carcinogenesis and adverse effects in the liver, heart, and kidneys.^{15–19)}

PPAR γ partial agonists, such as INT-131 (Fig. 2), have been investigated as PPAR γ modulators and examined clinically.^{20–22)} A PPAR γ partial agonist is defined to partially activate PPAR γ and antagonize the activation of PPAR γ by a full agonist, such as farglitazar (Fig. 1). In the concept of PPAR γ

modulators, the partial activation of PPAR γ fully induces insulin sensitization without adverse effects. Although many structurally different PPAR γ partial agonists showed higher efficacy with lower toxicity in experimental diabetic animals, none have been successfully developed to date.^{10,20–23)} 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 PPAR γ partial agonists have different structures from those of known full agonists^{20–28)} (Fig. 2). We previously reported various types of PPAR γ agonists using the same scaffold, 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid: a selective PPAR γ full agonist, PPAR γ full agonist with protein tyrosine phosphatase 1B (PTP1B) inhibition, PPAR α/γ dual agonist with PTP1B inhibition, and PPAR γ partial agonist with PTP1B inhibition.^{25,26,29–31)} 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 PPAR γ activation on insulin sensitization.^{32–34)} One of the 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acids with PPAR α/γ agonist and PTP1B inhibitory activities has been reported to exhibit effective anti-diabetic activities with high safety,³⁵⁾ and this may be due to its weak PPAR γ activation and PTP1B inhibition. However, it was not a partial PPAR γ agonist because it did not antagonize a PPAR γ 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%).³¹⁾ As described above, our previous studies dem-

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Fig. 1. Chemical Structures of PPAR γ Full Agonists Fig. 2. Chemical Structures of PPAR γ Partial Agonists

onstrated that 3-carboxyl 1,2,3,4-tetrahydroisoquinoline derivatives exerted PPAR γ full agonist activity or PPAR γ 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 PPAR γ 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 amidated 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-ethylcarbodiimide 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-ylpropenonyl)tetrahydroisoquinoline derivatives (**20a–g**) is outlined in Chart 2.

The formulation of the starting material **11**³⁶⁾ 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 **16**³⁷⁾ was reduced by zinc and methylated by reductive alkylation to afford **17b**. The conversion of **17a**, **17b**, **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 **18c**), which was treated with *n*-Bu₃SnN₃ to give tetrazole **18g**. Compound **18c** was treated with hydroxylamine hydrochloride, which was cyclized to give the 4*H*-[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 by aqueous LiOH to afford **20a**. Compound **20a** was isolated as a *t*-butylamine salt, and **20b** and **20e** were isolated as hydrochloride salts.

The synthesis of (*E*)-2-[3-(2-furyl)acryloyl]-7-[2-(cyclopent-3-enyl)-5-methyloxazol-4-ylmethylsulfanyl]-6-(1*H*-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

Reagents and conditions: (i) SOCl_2 ; (ii) methyl 2-amino-3-oxobutanoate hydrochloride, Et_3N , CH_2Cl_2 ; (iii) I_2 , PPh_3 , Et_3N , CH_2Cl_2 ; (iv) LiAlH_4 , THF; (v) SOCl_2 ; (vi) K_2CO_3 , DMF; (vii) HCl in *i*-PrOH, HCO_2H ; (viii) (*E*)-3-(2-furyl)acrylic acid, EDC·HCl, CH_2Cl_2 ; (ix) LiOH aq., MeOH, THF; (x) *t*-BuNH $_2$, AcOEt.

Chart 1. Synthesis of 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**)

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 $\text{HCl}/\text{HCO}_2\text{H}$ 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.^{25,26,29–31} Among them, a series of 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid with a 7-bulky substituent exhibited PPAR γ partial agonist activity (EC_{50} = 48–193 nM); however, their maximal activation levels were relatively high (50–90%),³¹ which were 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.³¹ To find a PPAR γ partial agonist with low maximal activation and high water solubility, novel 3-unsubstituted 7-[2-(cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-2-[3-(2-furyl)acryloyl]-1,2,3,4-tetrahydroisoquinoline derivatives with an acidic, basic or neutral substituent at the 6-position were synthesized and evaluated for PPAR γ partial agonist activity. The PPAR γ agonist activities of synthesized compounds were assessed as transactivation activity in COS-1 cells containing the full-length human PPAR γ 1 plasmid co-transfected with the human retinoid X receptor α (RXR α) plasmid and reporter plasmid pGL3-PPREx4-tk-luc. Activation responses were expressed as relative values to the maximal activation by farglitazar, a PPAR γ full agonist. Antagonist activity against farglitazar (10^{-7}M) was also assessed. The effects of the compounds on PTP1B activities were also examined using a human PTP1B enzyme because reported 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid derivatives exhibit PTP1B inhibitory activities to some extent. Plasma concentrations after the oral administration of a selected compound at 10 mg/kg were evaluated and anti-diabetic effects were investigated in KK-A y mice, a type

2 diabetic animal. All animal experiments in the present study were conducted according to the guidelines for animal experiments of our institute and the guidelines for animal experimentation approved by the Japanese Association of Laboratory Animal Science.

The PPAR γ agonist and antagonist activities and PTP1B inhibitory activity of the compounds synthesized were shown in Table 1. The 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acid derivative (**10**) was a PPAR γ full agonist (EC_{50} = 528 nM, maximal activation 98.7%). Compound **20a** with 6-carboxyl exhibited PPAR γ partial agonist activity (EC_{50} = 179 nM, maximal activation 28.5%), indicating that 6-carboxyl is more suitable than 3-carboxyl for PPAR γ 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 γ 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 γ protein *via* ionic and/or hydrophobic interactions. Thioether derivative **26** showed lower activity for PPAR γ 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 γ partial agonist activities, suggesting selective PPAR γ agonists.

To gain structural insights into the mechanisms underlying partial agonism by our compounds, we performed computa-

Chart 2. Synthesis of 6-Substituted-7-(2-cyclopentenyl-5-methyloxazol-4-ylmethoxy)-2-(3-furan-2-yl-propenonyl)tetrahydroisoquinoline Derivatives

PPAR γ full agonists, such as thiazolidinediones (TDZ), are known to interact with Helix 12 in the activation function 2 (AF-2) pocket of PPAR γ 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 ethylpyridyl groups formed hydrophobic interactions with Cys285

Reagents and conditions: (i) MeI, K_2CO_3 , DMF; (ii) H_2 , Pd-C, MeOH; (iii) *N,N*-dimethylthiocarbamoyl chloride, Et_3N , DMAP, 1,4-dioxane; (iv) *n*-tetradecane; (v) K_2CO_3 aq., MeOH then Boc_2O ; (vi) MeONa in MeOH, MeOH; (vii) **6**, Cs_2CO_3 , CH_3CN ; (viii) LiOH aq., MeOH, THF; (ix) $ClCO_2i-Bu$, *N*-methylmorpholine, NH_3 aq., THF; (x) $POCl_3$, Et_3N , CH_2Cl_2 ; (xi) *n*-Bu $_3$ Sn $_3$, toluene; (xii) HCl in *i*-PrOH, HCO_2H ; (xiii) (*E*)-3-(2-furyl)acrylic acid, $(COCl)_2$, DMF, Et_3N , CH_2Cl_2 ; (xiv) LiOH aq., MeOH, THF.

Chart 3. Synthesis of (*E*)-2-[3-(2-Furyl)acryloyl]-7-[2-(cyclopent-3-enyl)-5-methyloxazol-4-ylmethylsulfanyl]-6-(1*H*-tetrazol-5-yl)-1,2,3,4-tetrahydroisoquinoline (**26**)

(Helix3), Leu330 (Helix 4/5), Ile281 (Helix3), and Ile341 (β -strand 3). These interactions were considered to stabilize Helix 12 into an active conformation.

Among previously reported 2,7-substituted 1,2,3,4-tetrahydroisoquinoline 3-carboxylic acids, compound **1** exhibited PPAR γ full agonist activity (EC_{50} = 22 nM, maximal activation 104%) and weak PPAR α agonist activity (EC_{50} = 142 nM),²⁵ and compound **2** exhibited PPAR γ full agonist activity (EC_{50} = 70 nM, maximal activation 102%)²⁶; 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 around Tyr473 (Helix 12) and His449 (Helix 10/11) (Fig. 3c). Oxazole interacted with Ser289 (Helix 3) in compounds **1** and **2**, and the carbonyl oxygen of the 2-position acyl group in compound **1** interacted with Ser342 (β -strand 3) *via* hydrogen bonds, whereas the furan ring in compound **2** formed aromatic and/or hydrophobic interactions along Helix 3 and β -strand 3. In compound **10** (EC_{50} = 528 nM, maximal activation 98.7%), the carbonyl oxygen of the 2-position acyl group formed hydrogen bonds with Ser342 (β -strand 3) and the 7-position substituent interacted around Tyr473 (Helix 12) and His449 (Helix 10/11), whereas the linker length at the 7-position of compound **10** (methoxy) was shorter than those of compounds **1** and **2** (ethoxy) (Fig. 3d). Therefore, the interaction may be weaker than compounds **1** and **2**, resulting in weak agonist activity.

On the other hand, unlike full agonists, partial agonists are

known to preferentially stabilize other regions of the ligand-binding domain, particularly the β -sheet, and partially recruit a coactivator, resulting in PPAR γ partial agonism.⁴⁰ 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 carbonyl 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 PPAR γ 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 (EC_{50} = 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 PPAR γ protein.

Among the derivatives, compound **20g** with the lowest lipophilicity ($c\log D_{7.4}$ = 0.95) showed the most potent PPAR γ par-

Table 1. Chemical Structures, Molecular Weights, $\text{clog} D_{7.4}$, and PTP1B Inhibitory and PPAR γ Agonist Activities of 6-Substituted-7-(2-cyclopentenyl-5-methyloxazol-4-ylmethoxy)-2-(3-furan-2-yl-propenonyl)tetrahydroisoquinoline Derivatives

Compound	R ¹	R ²	X	Mol.Wt. ^{a)}	clog D _{7,4}	PPAR γ		PPAR γ		PTP1B
						(agonist) ^{b)}		(antagonist) ^{b)}		
						EC ₅₀	Max	IC ₅₀	Max	
						(nM)	(%)	(nM)	(%)	(nM) ^{c)}
10^{d)}	H		O	474.51	1.01	528	98.7	-	-	N. D. ^{g)}
20a^{d)}		H	O	474.51	1.01	179	28.5	594	63.4	8,200
20b^{e)}		H	O	473.56	3.94	-	(21.4) ^{d)}	253	84.8	>10,000
20c		H	O	455.51	3.52	-	-	-	-	>10,000
20d		H	O	473.52	2.82	-	(26.9) ^{d)}	690	90.4	>10,000
20e^{e)}		H	O	496.56	3.50	-	(26.0) ^{d)}	-	-	>10,000
20f		H	O	514.53	2.06	44	27.6	419	76.7	3,500
20g		H	O	498.53	0.95	13	30.0	512	76.1	1,100
26		H	S	514.60	1.39	584	25.9	51	52.5	N. D. ^{g)}

a) Molecular weight as the free form. b) $n = 2$. c) IC₅₀ value calculated from another test ($n = 3$). d) *t*-Butylamine salt. e) HCl salt. f) The activation level at 1000 nM. The maximal activation level was not observed due to cytotoxicity at higher concentrations and the EC₅₀ value was not obtained. g) Not determined.

tial agonist properties in the transactivation assay. **20g** showed no PPAR α or PPAR δ agonist activity (EC₅₀ > 10000 nM). The plasma C_{max} of **20g** at 10 mg/kg after oral administration reached $7.0 \pm 2.1 \mu\text{g/mL}$ in Sprague-Dawley (SD) rats and $19.8 \pm 0.6 \mu\text{g/mL}$ in KK-A^y 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-(2-furyl)acryloyl]-1,2,3,4-tetrahydroisoquinoline derivatives substituted with an acidic group at the 6-position are selective PPAR γ 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-enecarbonyl)amino]-3-oxobutyrates (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

Fig. 3. Docking Poses of Full Agonists to PPAR γ

(a) Crystal structure of pioglitazone (PDB code 5Y2O). (b–d) Docking poses of compounds **1** (b), **2** (c), and **10** (d). Compounds and PPAR γ residues described in the manuscript are shown as sticks and labeled. Hydrogen bonds and an aromatic interaction are depicted as cyan and green dotted lines, respectively. Secondary structures (Helix (H) and β -strand (S)) of PPAR γ were predicted and depicted by Maestro (Schrödinger Suite 2018-1) and H3 is partially hidden for visibility (corresponding to residues 286–288).

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. ¹H-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. ¹H-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.

Fig. 4. Docking Poses of Partial Agonists to PPAR γ

(a) Crystal structure of amorfrutin 2 (PDB code 4A4V). (b–e) Docking poses of compounds **20a** (b), **20f** (d), and **20g** (e), and the superposition of those of **10** (orange) and **20a** (magenta) (c). Compounds and PPAR γ residues described in the manuscript are shown as sticks and labeled. Hydrogen bonds and a salt bridge are depicted as cyan and orange dotted lines, respectively. Secondary structures (Helix (H) and β -strand (S)) of PPAR γ were predicted and depicted by Maestro (Schrödinger Suite 2018-1) and H3 is partially hidden for visibility (corresponding to residues 286 and 287).

Table 2. Effects of **20g** Administered Orally for 1 Week to KK-A^y Mice

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

Mean $\pm$ 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 Na_2SO_4 , and then evaporated under reduced pressure.

The residue was dissolved in SOCl_2 (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. $^1\text{H-NMR}$ (CDCl_3) 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-Butoxycarbonyl-7-(2-cyclopent-3-enyl-5-methyloxazol-4-ylmethoxy)-3,4-dihydro-1H-isoquinoline-3-carboxylate (8) K_2CO_3 (1.35 g, 9.75 mmol) was added to a solution of **7**²⁹ (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 Na_2SO_4 . The solvent was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give **8** (1.38 g, 91% yield) as an oil. $^1\text{H-NMR}$ (CDCl_3) δ : 1.45 (4.5H, 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-enyl-5-methyloxazol-4-ylmethoxy)-2-(3-furan-2-ylacryloyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (9) A total of 9.1 M HCl in *i*-PrOH (0.34 mL, 3.1 mmol) was added to a solution of **8** (490 mg, 1.05 mmol) in HCO_2H (1.5 mL) under ice-cooling, and the mixture was stirred at room temperature for 30 min. The reaction mixture was neutralized with saturated aqueous NaHCO_3 solution and extracted with CHCl_3 . The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure.

The obtained residue was dissolved in CH_2Cl_2 (3.0 mL), (*E*)-3-(2-furyl)acrylic acid (160 mg, 1.16 mmol) and EDC·HCl (262 mg, 1.37 mmol) were added, and the mixture was stirred at room temperature for 2 h. After the addition of a 10% aqueous citric acid solution, the reaction mixture was extracted with CHCl_3 and washed with saturated aqueous NaHCO_3 solution and brine and then dried over Na_2SO_4 . The solvent was evaporated under reduced pressure, and the obtained residue was purified by column chromatography to give **9** (450 mg, 88% yield, 2 steps) as an oil. $^1\text{H-NMR}$ (CDCl_3) δ : 2.32 (3H, s), 2.69–2.86 (4H, m), 3.05–3.37 (2H, m), 3.55–3.64 (4H, m), 4.55–4.64 (0.3H, m), 4.78–5.03 (3.7H, m), 5.04–5.08 (0.3H, m), 5.57–5.64 (0.7H, m), 5.74 (2H, s), 6.44–6.52 (1H, s), 6.56–6.63 (1H, m), 6.68 (0.3H, d, $J=15.1$ Hz), 6.77–6.87 (2H, m), 6.91 (0.7H, d, $J=15.1$ Hz), 7.04–7.12 (1H, m), 7.43–7.57 (2H, m).

7-(2-Cyclopent-3-enyl-5-methyloxazol-4-ylmethoxy)-2-(3-furan-2-ylacryloyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid *t*-Butylamine Salt (10) A 1.0 M aqueous LiOH solution (2.8 mL, 2.8 mmol) was added to a solution of **9** (450 mg, 0.921 mmol) in MeOH (8.3 mL) and THF (2.8 mL), and the mixture was stirred at room temperature for 40 min. The reaction mixture was washed with Et_2O , and the aqueous layer was acidified with a 1.0 M aqueous HCl solution, which was extracted with AcOEt. The organic layer was washed with saturated brine and dried over Na_2SO_4 . The solvent was evaporated under reduced pressure to give carboxylic acid as a foam (420 mg). *tert*-Butylamine (49 μM , 0.46 mmol) 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 *t*-BuOMe, the precipitate was filtered to give **10** (178 mg, 71% yield) as a solid, mp 111–114°C. $^1\text{H-NMR}$ (dimethyl sulfoxide (DMSO)- d_6) 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). IR (ATR) cm^{-1} : 1646, 1604. MS m/z : 473 $[\text{M} - \text{H}]^-$, 475 $[\text{M} + \text{H}]^+$.

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 **11**³⁶ (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 NaHCO_3 , and extracted with AcOEt. The organic layer was washed with saturated brine, dried over Na_2SO_4 , and then evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give **12** (74.0 g, 65% yield) as a solid. $^1\text{H-NMR}$ (CDCl_3) 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), 10.82–10.86 (0.45H, m), 11.93–11.98 (0.55H, m).

7-Benzyloxy-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 K_2CO_3 (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 Na_2SO_4 . 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).

NaClO_2 (124 g, 1.37 mol) was added to a solution of NaH_2PO_4 (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 *t*-BuOH–THF (2:1) (1800 mL) and 2-methyl-2-butane (275 mL, 2.59 mol) at the same temperature, and was then 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. $^1\text{H-NMR}$ (CDCl_3) 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-Benzyloxy-2-tert-butoxycarbonyl-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. Boc_2O (69.2 g, 0.317 mol) 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 citric acid solution, water, and saturated brine, and then dried over Na_2SO_4 . 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 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-Benzoyloxy-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 (2 L) under ice-cooling, which was stirred at the same temperature for 20 min. A 28% aqueous NH₃ solution (97 mL, 1.4 mol) was added dropwise to the obtained mixture, and the mixture was stirred further at the same temperature for 2 h. AcOEt was added to the reaction mixture, which was washed with water and saturated brine, and then dried over Na₂SO₄. 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-Benzoyloxy-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 **14** (3.20 g, 8.35 mmol) in THF (25 mL) under ice-cooling, and the mixture was stirred at room temperature for 2 h. The reaction mixture was added to a 1.0 M aqueous NaOH solution (30 mL, 30 mmol), which was stirred at room temperature for 1 h. CHCl₃ was added to the mixture, separated into two layers, and the organic layer was washed with water and dried over Na₂SO₄. The solvent was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give **15h** (3.20 g, quant.) as an oil. ¹H-NMR (CDCl₃) δ: 1.49 (9H, s), 2.25 (1H, t, *J* = 6.6 Hz), 2.71–2.80 (2H, m), 3.57–3.69 (2H, m), 4.53 (2H, s), 4.69 (2H, d, *J* = 6.6 Hz), 6.68 (1H, s), 7.07 (1H, s), 7.30–7.44 (5H, m).

Methyl 2-tert-Butoxycarbonyl-7-hydroxy-1,2,3,4-tetrahydroisoquinolin-6-carboxylate (17a) MeI (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 16 h. AcOEt was added to the reaction mixture, washed with water and saturated brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure to give **14a** as a colorless oil (crude).

A solution of crude **14a** in MeOH (50 mL) was hydrogenated at 0.4 MPa in the presence of 10% Pd–C (100 mg) at room temperature for 86 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give **17a** (730 mg, 91% yield for 2 steps) as a white solid. ¹H-NMR (CDCl₃) δ: 1.49 (9H, s), 2.72–2.82 (2H, m), 3.57–3.67 (2H, m), 3.94 (3H, s), 4.55 (2H, s), 6.73 (1H, s), 7.60 (1H, s), 10.50–10.55 (1H, br).

tert-Butyl 6-Carbamoyl-7-hydroxy-1,2,3,4-tetrahydroisoquinolin-2-carboxylate (17d) A solution of **15d** (28.7 g, 75.0 mmol) in MeOH (560 mL) was hydrogenated at 0.3 MPa in the presence of 10% Pd–C (2.9 g) at room temperature for 75 min. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give **17d** (8.8 g, 40% yield) as an oil. NMR (CDCl₃) δ: 1.49 (9H, s), 2.76 (2H, t, *J* = 5.8 Hz), 3.63 (2H, t, *J* = 5.8 Hz), 4.54 (2H, s), 5.56–6.22 (1H, br), 6.74 (1H, s), 7.13 (1H, s), 11.83–11.99 (1H,

br).

tert-Butyl 7-Hydroxy-6-hydroxymethyl-1,2,3,4-tetrahydroisoquinolin-2-carboxylate (17h) Compound **17h** 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**³⁷⁾ (0.88 g, 3.0 mmol) in acetic acid (5 mL), and the mixture was stirred at same temperature for 0.5 h. 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. ¹H-NMR (CDCl₃) δ: 1.48 (9H, s), 2.61–2.73 (2H, m), 3.52–3.65 (2H, m), 4.41 (2H, s), 6.52 (1H, s), 6.57 (1H, s).

tert-Butyl 6-Dimethylamino-7-hydroxy-1,2,3,4-tetrahydroisoquinolin-2-carboxylate (17b) A total of 37% formalin (8.49 mL, 113 mmol) and NaBH(OAc)₃ (2.87 g, 13.6 mmol) were added to a solution of **27** (597 mg, 2.26 mmol) in THF (12 mL), and the mixture was 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. ¹H-NMR (CDCl₃) δ: 1.48 (9H, s), 2.63 (6H, s), 2.69–2.77 (2H, m), 3.54–3.69 (2H, m), 4.48 (2H, s), 6.67 (1H, s), 6.90 (1H, s).

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).

tert-Butyl 7-[2-(Cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-6-dimethylamino-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (18b) Compound **18b** was synthesized from **17b** and **6** according to the procedure for the synthesis of **8**. Yield 35%. A brown oil. ¹H-NMR (CDCl₃) δ: 1.48 (9H, s), 2.30 (3H, s), 2.68–2.84 (12H, m), 3.50–3.70 (3H, m), 4.48 (2H, s), 4.90 (2H, s), 5.72 (2H, s), 6.64 (1H, s), 6.73 (1H, s).

tert-Butyl 6-Cyano-7-[2-(cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (18c) Et₃N (8.27 mL, 59.3 mmol) and POCl₃ (3.32 mL, 35.6 mmol) were added to a solution of **18d** (4.60 g, 11.9 mmol) in CH₂Cl₂ (90 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into water, neutralized with K₂CO₃, and separated into two layers. The organic layer was dried over Na₂SO₄, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give **18c** (3.16 g, 72% yield) as a solid. ¹H-NMR (CDCl₃) δ: 1.49 (9H, s), 2.36 (3H, s), 2.67–2.86 (6H,

m), 3.51–3.67 (3H, m), 4.57 (2H, s), 5.00 (2H, s), 5.74 (2H, s), 6.91 (1H, s), 7.32 (1H, s).

tert-Butyl 6-Carbamoyl-7-[2-cyclopent-3-enyl-5-methyloxazol-4-ylmethoxy]-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (18d) Compound **18d** was synthesized from **17d** and **6** according to the procedure for the synthesis of **8**. Yield 64%. A white solid. $^1\text{H-NMR}$ (CDCl_3) δ : 1.50 (9H, s), 2.32 (3H, s), 2.67–2.86 (6H, m), 3.52–3.68 (3H, m), 4.59 (2H, s), 4.97 (2H, s), 5.58–5.67 (1H, br), 5.74 (2H, s), 6.81 (1H, s), 7.93–8.02 (2H, m).

tert-Butyl 7-[2-(Cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-6-hydroxymethyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (18h) Compound **18h** was synthesized from **17h** and **6** according to the procedure for the synthesis of **8**. Yield 91%. A white solid. $^1\text{H-NMR}$ (CDCl_3) 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-methyloxazol-4-ylmethoxy)-6-formyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (28) DMSO (0.93 mL, 13 mmol), *i*-Pr₂NEt (2.2 mL, 13 mmol), and SO₃·Py (1.25 g, 7.83 mmol) were added to a solution of **18h** (1.15 g, 2.61 mmol) in CH₂Cl₂ (20 mL), which was then stirred at room temperature for 30 min. After the addition of a 1.0 M aqueous HCl solution, the mixture was extracted with CHCl₃. The organic layer was dried over Na₂SO₄ and evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give **28** (1.10 g, 96% yield) as a solid. $^1\text{H-NMR}$ (CDCl_3) δ : 1.50 (9H, s), 2.32 (3H, s), 2.69–2.86 (6H, m), 3.53–3.68 (3H, m), 4.59 (2H, s), 4.97 (2H, s), 5.74 (2H, s), 6.87 (1H, s), 7.60 (1H, s), 10.40 (1H, s).

tert-Butyl 7-[2-(Cyclopent-3-enyl)-5-methyloxazol-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 NH₃ solution (0.15 mL, 2.3 mmol) were added to a solution of **28** (200 mg, 0.465 mmol) in MeOH (1 mL), which was then stirred at room temperature for 14 h. After the addition of saturated aqueous NaHCO₃ 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. $^1\text{H-NMR}$ (CDCl_3) 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-methyloxazol-4-ylmethoxy]-6-(5-oxo-[1,2,4]oxadiazolidin-3-yl)-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (18f) Et₃N (1.19 mL, 8.54 mmol) was added to a suspension of NH₂OH·HCl (594 mg, 8.54 mmol) in DMSO (1 mL) and stirred at room temperature for 10 min. The precipitate was removed by filtration and washed with THF. After the removal of THF under a vacuum, the obtained residue was added to **18c** and stirred at 85°C for 15 h. After the addition of water, the mixture was extracted twice with AcOEt. The organic layer was washed with water and brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure.

Pyridine (0.13 mL, 1.6 mmol) and isobutyl chloroformate (0.18 mL, 1.4 mmol) were added to a solution of the obtained

residue in DMF (3.0 mL) under ice-cooling and the mixture was stirred at the same temperature for 1 h. After the addition of water, the mixture was extracted twice with AcOEt. The organic layer was washed with water and brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure.

The residue was dissolved in xylene (5.0 mL) and the mixture was refluxed for 3 h. After cooling to room temperature, the mixture was purified by silica gel column chromatography to give **18f** (470 mg, 67% yield for 3 steps) as an oil. $^1\text{H-NMR}$ (CDCl_3) δ : 1.50 (9H, s), 2.33 (3H, s), 2.68–2.96 (6H, m), 3.58–3.79 (3H, m), 4.60 (2H, s), 5.01 (2H, s), 5.74 (2H, s), 6.83 (1H, s), 7.72 (1H, s), 11.65–11.83 (1H, br).

tert-Butyl 7-[2-Cyclopent-3-enyl-5-methyloxazol-4-ylmethoxy]-6-(1H-tetrazol-5-yl)-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (18g) *n*-Bu₃SnN₃ (3.25 mL, 11.9 mmol) was added to a solution of **18c** (1.15 g, 3.11 mmol) in toluene (12 mL), and the mixture was refluxed for 18 h. After cooling to room temperature, the reaction mixture was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give **18g** (1.15 g, 90% yield) as a solid. $^1\text{H-NMR}$ (CDCl_3) δ : 1.51 (9H, s), 2.36 (3H, s), 2.80–2.98 (6H, m), 3.62–3.75 (3H, m), 4.63 (2H, s), 5.08 (2H, s), 5.81 (2H, s), 6.89 (1H, s), 8.19 (1H, s).

Methyl (E)-7-(2-Cyclopent-3-enyl-5-methyloxazol-4-ylmethoxy)-2-(3-furan-2-yl-acryloyl)-1,2,3,4-tetrahydroisoquinoline-6-carboxylate (19a) Compound **19a** was synthesized from **18a** according to the procedure for the synthesis of **9**. Yield 85%, 2 steps. A yellow oil. $^1\text{H-NMR}$ (CDCl_3) δ : 2.34 (3H, s), 2.69–2.95 (6H, m), 3.45–3.55 (1H, m), 3.81–3.94 (5H, 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), 6.87 (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-methyloxazol-4-ylmethoxy)-2-(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 AcOEt. The organic layer was washed with saturated brine, and then dried over Na₂SO₄. The solvent was evaporated under reduced pressure. *tert*-Butylamine (90 μM , 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. $^1\text{H-NMR}$ (CDCl_3) δ : 1.24 (9H, 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, br), 4.89 (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 $[\text{M}-\text{H}]^-$.

(E)-7-[2-(Cyclopent-3-enyl)-5-methyloxazol-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 Na_2SO_4 . The solvent was evaporated under reduced pressure.

The obtained residue was dissolved in CH_2Cl_2 (5.0 mL) to which was added (*E*)-3-(2-furyl)acrylic acid (120 mg, 0.869 mmol) and EDC·HCl (210 mg, 1.10 mmol), and the mixture was stirred at room temperature for 2 h. After the addition of a 10% aqueous citric acid solution, the reaction mixture was extracted with CH_2Cl_2 , washed with saturated aqueous NaHCO_3 solution and brine, and then dried over Na_2SO_4 . The solvent was evaporated under reduced pressure, and the obtained residue was purified by column chromatography. A total of 7.3 M HCl in *i*-PrOH (0.13 mL, 0.95 mmol) and *i*-Pr₂O (2.0 mL) was added to the obtained residue in AcOEt (2.0 mL), and the precipitate was then filtered to give **20b** (281 mg, 55% yield for 3 steps) as a pale yellow solid, mp 91–94°C. ¹H-NMR (CDCl_3) δ : 2.42 (3H, s), 2.70–3.00 (6H, m), 3.23 (6H, s), 3.60–3.77 (1H, m), 3.80–3.96 (2H, m), 4.83 (2H, s), 5.05 (2H, s), 5.74 (2H, s), 6.47 (1H, dd, J = 3.4, 1.9 Hz), 6.58 (1H, d, J = 3.4 Hz), 6.84 (1H, d, J = 15.1 Hz), 7.01 (1H, s), 7.47 (1H, d, J = 1.9 Hz), 7.49 (1H, d, J = 15.1 Hz), 7.91 (1H, s). IR (ATR) cm^{-1} : 1644. MS m/z : 472 [$\text{M} - \text{H}$][−], 474 [$\text{M} + \text{H}$]⁺.

(*E*)-6-Cyano-7-[2-(cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-2-[3-(2-furyl)acryloyl]-1,2,3,4-tetrahydroisoquinoline (20c) A total of 8.6 M HCl in *i*-PrOH (0.24 mL, 2.1 mmol) was added to a solution of **18c** (300 mg, 0.689 mmol) in HCO_2H (1 mL), and the mixture was then stirred at room temperature for 15 min. The reaction mixture was basified with saturated aqueous NaHCO_3 solution, which was extracted with AcOEt. The organic layer was washed with water and saturated brine, and then dried over Na_2SO_4 . The solvent was evaporated under reduced pressure.

(COCl_2)₂ (0.089 mL, 1.0 mmol) and 1 drop of DMF were added to a solution of (*E*)-3-(2-furyl)acrylic acid (140 mg, 1.04 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred at room temperature for 0.5 h. The obtained mixture and Et₃N (0.48 mL, 3.5 mmol) were added to a solution of the crude intermediate in CH_2Cl_2 (5 mL), which was stirred at the same temperature for 2 h. The reaction mixture was washed with water, saturated aqueous NaHCO_3 solution, and saturated brine, and then dried over Na_2SO_4 . The obtained residue was added to *i*-Pr₂O (2.0 mL), and the precipitate was then filtered to give **20b** (244 mg, 78% yield for 2 steps) as a white solid, mp 159–161°C. ¹H-NMR (CDCl_3) δ : 2.36 (3H, s), 2.67–2.94 (6H, m), 3.50–3.63 (1H, m), 3.80–3.98 (2H, m), 4.84 (2H, s), 5.01 (2H, s), 5.73 (2H, s), 6.44–6.51 (1H, m), 6.57 (1H, d, J = 3.2 Hz), 6.83 (1H, d, J = 15.1 Hz), 6.99 (1H, s), 7.34 (1H, s), 7.43–7.47 (1H, m), 7.50 (1H, d, J = 15.1 Hz). IR (ATR) cm^{-1} : 2217, 1600. MS m/z : 456 [$\text{M} + \text{H}$]⁺.

(*E*)-6-Carbamoyl-7-[2-(cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-2-[3-(2-furyl)acryloyl]-1,2,3,4-tetrahydroisoquinoline (20d) Compound **20d** was synthesized from **18d** according to the procedure for the synthesis of **20c**. Yield 36% for 2 steps. A white solid. mp 172–174°C. ¹H-NMR (CDCl_3) δ : 2.32 (3H, s), 2.67–2.86 (4H, m), 2.86–3.00 (2H, m), 3.50–3.63 (1H, m), 3.80–3.97 (2H, m), 4.85 (2H, s), 4.98 (2H, s), 5.65 (1H, s), 5.74 (2H, s), 6.44–6.51 (1H, m), 6.56 (1H, d, J = 3.2 Hz), 6.86 (1H, d, J = 15.1 Hz), 6.88 (1H, s), 7.44–7.47 (1H, m), 7.50 (1H, d, J = 15.1 Hz), 7.90–8.00 (1H, br), 8.00 (1H, s). IR (ATR) cm^{-1} : 1602, 1421. MS m/z : 474 [$\text{M} - \text{H}$][−].

(*E*)-7-[2-(Cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-2-[3-(2-furyl)acryloyl]-6-(1*H*-imidazol-2-yl)-

1,2,3,4-tetrahydroisoquinoline Hydrochloride (20e) Compound **20e** was synthesized from **18e** according to the procedure for the synthesis of **20b**. Yield 33% for 3 steps. A white solid, mp not determined. ¹H-NMR (CDCl_3) δ : 2.30 (3H, s), 2.53–2.63 (2H, m), 2.68–2.78 (2H, m), 2.78–2.95 (2H, m), 3.49–3.60 (1H, m), 3.78–3.92 (2H, m), 4.77–4.96 (2H, m), 5.21 (2H, s), 5.72 (2H, s), 6.60–6.65 (1H, m), 6.89 (1H, d, J = 3.0 Hz), 7.01 (1H, d, J = 15.1 Hz), 7.39 (1H, d, J = 15.1 Hz), 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 [$\text{M} - \text{H}$][−], 497 [$\text{M} + \text{H}$]⁺.

(*E*)-7-[2-(Cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-2-[3-(2-furyl)acryloyl]-6-(5-oxo-[1,2,4]oxadiazolidin-3-yl)-1,2,3,4-tetrahydroisoquinoline (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 (CDCl_3) δ : 2.33 (3H, s), 2.68–2.78 (2H, m), 2.82–2.98 (4H, m), 3.68–3.76 (1H, m), 3.84–3.95 (2H, m), 4.87 (2H, s), 5.02 (2H, s), 5.74 (2H, s), 6.45–6.49 (1H, m), 6.58 (1H, d, J = 3.4 Hz), 6.85 (1H, d, J = 14.9 Hz), 6.90 (1H, s), 7.45–7.47 (1H, m), 7.51 (1H, d, J = 14.9 Hz), 7.74 (1H, s), 11.70–11.85 (1H, br). IR (ATR) cm^{-1} : 1766, 1635. MS m/z : 513 [$\text{M} - \text{H}$][−].

(*E*)-7-[2-(Cyclopent-3-enyl)-5-methyloxazol-4-ylmethoxy]-2-[3-(2-furyl)acryloyl]-6-(1*H*-tetrazol-5-yl)-1,2,3,4-tetrahydroisoquinoline (20g) Compound **20g** was synthesized from **18g** according to the procedure for the synthesis of **9**. Yield 59% for 2 steps. A white solid, mp 178–180°C. ¹H-NMR (CDCl_3) δ : 2.37 (3H, s), 2.85–3.06 (6H, m), 3.70 (1H, quintet, J = 8.0 Hz), 3.84–4.03 (2H, m), 4.90 (2H, m), 5.10 (2H, s), 5.81 (2H, s), 6.44–6.52 (1H, m), 6.58 (1H, d, J = 3.2 Hz), 6.88 (1H, d, J = 14.9 Hz), 6.95 (1H, s), 7.47 (1H, s), 7.51 (1H, d, J = 14.9 Hz), 8.21 (1H, s). IR (ATR) cm^{-1} : 1651, 1608. MS m/z : 497 [$\text{M} - \text{H}$][−], 499 [$\text{M} + \text{H}$]⁺. Anal. Calcd for $\text{C}_{27}\text{H}_{31}\text{N}_5\text{O}_3$: C, 65.06; H, 5.26; N, 16.86. Found: C, 65.19; H, 5.31; N, 16.71.

Methyl 7-Hydroxy-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-6-carboxylate (21) MeI (1.99 mL, 32.0 mmol) and K_2CO_3 (5.90 g, 42.7 mmol) were added to a solution of **13** (8.07 g, 21.3 mmol) in DMF (80 mL) under ice-cooling, which was then stirred at room temperature for 3 h. The reaction mixture was added to water and extracted twice with AcOEt. The organic layer was washed with water and brine, and then dried over Na_2SO_4 . The solvent was evaporated under reduced pressure.

A solution of the obtained residue in MeOH (217 mL) was hydrogenated at 0.3 MPa in the presence of 10% Pd-C (2.17 g) at room temperature for 1 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give **21** (5.67 g, 88% yield for 2 steps) as a solid. NMR (CDCl_3) δ : 1.49 (9H, s), 2.76 (2H, t, J = 5.8 Hz), 3.63 (2H, t, J = 5.8 Hz), 4.54 (2H, s), 5.56–6.22 (1H, br), 6.74 (1H, s), 7.13 (1H, s), 11.83–11.99 (1H, br).

Methyl 7-Dimethylthiocarbamoyloxy-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-6-carboxylate (29) Et₃N (5.33 mL, 37.4 mmol), DMAP (0.46 g, 3.7 mmol), and *N,N*-dimethylthiocarbamoyl chloride (4.62 g, 37.4 mmol) were added to a solution of compound **21** (5.67 g, 18.7 mmol) in 1,4-dioxane (60 mL) and the mixture was stirred at 80°C for 15 h. After the addition of water, the mixture was extracted twice with AcOEt. The organic layer was washed with water

and brine, and then dried over Na_2SO_4 . 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. $^1\text{H-NMR}$ (CDCl_3) 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-Dimethylcarbamoylsulfanyl-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 N_2 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. $^1\text{H-NMR}$ (CDCl_3) 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-Butoxycarbonyl-7-dimethylcarbamoylsulfanyl-1,2,3,4-tetrahydroisoquinoline-6-carboxylate (30) A 0.50 M aqueous K_2CO_3 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. Boc_2O (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 Na_2SO_4 . 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. $^1\text{H-NMR}$ (CDCl_3) δ : 1.48 (9H, s), 2.80–2.91 (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-Butoxycarbonyl-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 N_2 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 Na_2SO_4 . 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. $^1\text{H-NMR}$ (CDCl_3) δ : 1.48 (9H, s), 2.71–2.85 (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-Butoxycarbonyl-7-[(2-cyclopent-3-enyl)-5-methyloxazol-4-ylmethylsulfanyl]-1,2,3,4-tetrahydroisoquinoline-6-carboxylate (31) Cs_2CO_3 (1.83 g, 5.61 mmol) was added to a solution of **23** (1.21 g, 3.74 mmol) and **6** (890 mg, 4.49 mmol) in DMF (25 mL), 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 then dried over Na_2SO_4 . The solvent was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography to give **31** (1.48 g, 82% yield) as an oil. $^1\text{H-NMR}$ (CDCl_3) δ : 1.49 (9H, s), 2.23 (3H, s), 2.66–2.78 (4H, m), 2.78–2.88 (2H, m), 3.47–3.59 (1H, m), 3.60–3.68 (2H, m), 3.88 (3H, s), 3.96 (2H, s), 4.56 (2H, s), 5.72 (2H, s), 7.19 (1H, s), 7.71 (1H, s).

2-tert-Butoxycarbonyl-7-[(2-cyclopent-3-enyl)-5-methyl-

oxazol-4-ylmethylsulfanyl]-1,2,3,4-tetrahydroisoquinoline-6-carboxylic Acid (32) A 1.0 M aqueous LiOH solution (9.0 mL, 9.0 mmol) was added to a solution of **31** (1.48 g, 3.05 mmol) in MeOH (9.0 mL) and THF (27 mL), and the mixture was stirred at 40°C for 2 h. The reaction mixture was acidified with a 1.0 M aqueous HCl solution, which was extracted with AcOEt. The organic layer was washed with saturated brine, and dried over Na_2SO_4 . The solvent was evaporated under reduced pressure to give **32** (1.26 g, 88% yield) as a white solid. $^1\text{H-NMR}$ (CDCl_3) 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-enyl)-5-methyloxazol-4-ylmethylsulfanyl]-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (24) Compound **24** was synthesized from **32** according to the procedure for the synthesis of **15d**. Yield 84%. A white solid. $^1\text{H-NMR}$ (CDCl_3) 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 6-Cyano-7-[2-(cyclopent-3-enyl)-5-methyloxazol-4-ylmethylsulfanyl]-1,2,3,4-tetrahydroisoquinoline-2-carboxylate (33) Compound **33** was synthesized from **24** according to the procedure for the synthesis of **18c**. Yield quant. A white solid. $^1\text{H-NMR}$ (CDCl_3) 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-enyl-5-methyl-oxazol-4-ylmethylsulfanyl)-6-(1H-tetrazol-5-yl)-3,4-dihydro-1H-isoquinoline-2-carboxylate (34) Compound **34** was synthesized from **33** according to the procedure for the synthesis of **18g**. Yield 75%. A white solid. $^1\text{H-NMR}$ (CDCl_3) δ : 1.50 (9H, s), 2.14 (3H, s), 2.65–2.75 (2H, m), 2.77–2.87 (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).

2-[(E)-3-(2-Furyl)acryloyl]-7-[2-(cyclopent-3-enyl)-5-methyloxazol-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 HCO_2H (2 mL), and the mixture was stirred at room temperature for 15 min. After the addition of Et_2O , the precipitate formed was collected by filtration to give a crude intermediate.

$(\text{COCl})_2$ (0.145 mL, 1.7 mmol) and 1 drop of DMF were added to a solution of (E)-3-(2-furyl)acrylic acid (230 mg, 1.70 mmol) in CH_2Cl_2 (20 mL), and the mixture was stirred at room temperature for 0.5 h. The obtained mixture and Et_3N (0.79 mL, 5.7 mmol) were added to a solution of the crude intermediate in CH_2Cl_2 (5 mL), which was stirred at the same temperature for 3 h. The reaction mixture was washed with water, saturated aqueous NaHCO_3 solution, and saturated brine, and then dried over Na_2SO_4 . 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. $^1\text{H-NMR}$ (CDCl_3) 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), 7.93 (1H, s). IR (ATR) cm^{-1} : 1637, 1536. MS m/z : 513 $[\text{M}-\text{H}]^-$, 515 $[\text{M}+\text{H}]^+$.

PPAR γ , PPAR α , and PPAR δ Agonist Activities COS-1 cells (Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan) were cultured in Dulbecco's modified Eagle's medium (DMEM, Nissui Pharmaceutical, Co., Ltd., Tokyo, Japan) supplemented with 10% (v/v) fetal bovine serum (FBS, JRH Bioscience, Inc., Lenexa, KS), 0.3% NaHCO_3 , and 1% penicillin-streptomycin under 5% CO_2 at 37°C.

The full-length human PPAR γ 1 plasmid (Open Biosystems, Huntsville, U.S.A.), human PPAR α plasmid (GeneCopoeia Inc., Rockville, U.S.A.), or human PPAR δ plasmid (GeneCopoeia Inc.) were electroporated into COS-1 cells with the human RXR α plasmid (GeneCopoeia Inc.) and the reporter plasmid pGL3-PPREx4-tk-luc using Nucleofector II (AAD-1001S, Lonza Group Ltd., Basel, Switzerland). Cells were incubated for 24 h in the presence and absence of test compounds in DMEM containing 10% FBS under 5% CO_2 at 37°C. The medium was removed and luciferase activities were measured using a commercial kit (PicaGene LT7.5; TOYO B-Net Co., Ltd., Tokyo, Japan) and microplate luminescence reader (Dainippon Sumitomo Pharma Co., Ltd.). EC_{50} values were assessed from dose-response curves. The maximal activation level relative to the level maximally activated by farglitazar, a PPAR γ agonist (10^{-7}M), Wy-14643, a PPAR α agonist (10^{-5}M), and GW-501516, a PPAR δ agonist (10^{-7}M), were evaluated.

PTP1B Inhibitory Activity Inhibitory activities against recombinant human PTP1B (Enzo Life Sciences, Inc., Farmingdale, NY, U.S.A.) were measured using *p*-nitrophenyl phosphate (*p*NPP) as a substrate. Briefly, PTP1B inhibitory activities were assessed in the absence and presence of the test compounds in 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.2) containing the enzyme, 1 mM dithiothreitol (DTT), and 1 mM ethylenediaminetetraacetic acid (EDTA). The reaction was started by the addition of *p*NPP and stopped by the addition of 1 M NaOH after a 30-min incubation at 37°C. The absorbance of *p*-nitrophenol produced was measured at 405 nm.

Plasma Concentration after Oral Administration in Male SD Rats and KK-A y Mice Male SD rats (7 weeks old; Japan SLC, Inc., Hamamatsu, Japan) and male KK-A y mice (10 weeks old; Clea Japan, Inc., Tokyo, Japan) were used. The test compound at 10 mg/kg suspended in 0.5% methylcellulose solution was administered orally and then blood was taken from the external jugular vein after 0.5, 1, 3, 5, and 8 h in rats, and from the tail vein after 0.5, 1, 4, and 8 h in KK-A y mice. The plasma concentrations of the compounds were assessed using HPLC equipment consisting of a pump (PU-980; JASCO, Tokyo, Japan), UV detector (UV-970; JASCO), autoinjector (AS-950; JASCO), and STR-ODS-II column (5 μm , 4.6 $\times$ 150 mm; Shimadzu GLC Ltd., Kyoto, Japan).

Hypoglycemic and Hypotriglyceridemic Effects in Male and Female KK-A y Mice Male KK-A y mice (12 weeks old; Clea Japan, Inc.) and female KK-A y mice (19 weeks old; Clea Japan, Inc., Tokyo, Japan) were allocated into three groups: control group, 20g-treated group, and pioglitazone-treated group ($n = 7$). Mice were given normal powdered food or food mixed with the test compounds for 7 d. 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 $\leq 2.0\text{\AA}$, no mutated residue around the ligand-binding site and no distortion by crystal contacts^{41,42}) X-ray crystal structures of human PPAR γ 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 PPAR γ . 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 conformer 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 $\text{\AA}$ 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 ConfGen (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 ConfGen 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/ $\text{\AA}$ of convergence with a distance-dependent dielectric constant ($\epsilon = 4$).

Conflict of Interest The authors declare no conflicts of interest.

References

- 1) Bloomgarden Z. T., *Diabetes Care*, **28**, 488–493 (2005).
- 2) Lebovitz H. E., Banerji M. A., *Recent Prog. Horm. Res.*, **56**, 265–294 (2001).
- 3) Tack C. J. J., Smits P., *Neth. J. Med.*, **64**, 166–174 (2006).
- 4) Sohda T., Momose Y., Meguro K., Kawamatsu Y., Sugiyama Y., Ikeda H., *Arzneimittelforschung*, **40**, 37–42 (1990).
- 5) Sohda T., Kawamatsu Y., Fujita T., Meguro K., Ikeda H., *Yakugaku Zasshi*, **122**, 909–918 (2002).
- 6) Kummer M. P., Heneka M. T., *PPAR Res.*, **2008**, 403896 (2008).
- 7) Kallwitz E. R., McLachlan A., Cotler S. J., *World J. Gastroenterol.*, **14**, 22–28 (2008).
- 8) Lehrke M., Lazar M. A., *Cell*, **123**, 993–999 (2005).

- 9) Staels B., *Cell Metab.*, **2**, 77–78 (2005).
- 10) Krische D., *West. J. Med.*, **173**, 54–57 (2000).
- 11) Hussein Z., Wentworth J. M., Nankervis A., Proietto J., Colman P. G., *Med. J. Aust.*, **181**, 536–539 (2004).
- 12) Cho N., Momose Y., *Curr. Top. Med. Chem.*, **8**, 1483–1507 (2008).
- 13) Henke B. R., *J. Med. Chem.*, **47**, 4118–4127 (2004).
- 14) Nissen S. E., Wolski K., Topol E. J., *JAMA*, **294**, 2581–2586 (2005).
- 15) Fievet C., Fruchart J., Staels B., *Curr. Opin. Pharmacol.*, **6**, 606–614 (2006).
- 16) Lai D. Y., *J. Environ. Sci. Health C. Environ. Carcinog. Ecotoxicol. Rev.*, **22**, 37–55 (2004).
- 17) Cornwell P. D., De Souza A. T., Ulrich R. G., *Mutat. Res.*, **549**, 131–145 (2004).
- 18) Zungu M., Felix R., Essop M. F., *Mitochondrion*, **6**, 315–322 (2006).
- 19) Rubenstrunk A., Hanf R., Hum D. W., Fruchart J. C., Staels B., *Biochim. Biophys. Acta*, **1771**, 1065–1081 (2007).
- 20) Higgins L. S., Mantzoros C. S., *PPAR Res.*, **2008**, 936906 (2008).
- 21) Motani A., Wang Z., Weiszmann J., McGee L. R., Lee G., Liu Q., Staunton J., Fang Z., Fuentes H., Lindstrom M., Liu J., Biermann D. H., Jaen J., Walker N. P., Learned R. M., Chen J. L., Li Y., *J. Mol. Biol.*, **386**, 1301–1311 (2009).
- 22) Taygerly J. P., McGee L. R., Rubenstein S. M., *et al.*, *Bioorg. Med. Chem.*, **21**, 979–992 (2013).
- 23) Berger J. P., Petro A. E., Macnaul K. L., Kelly L. J., Zhang B. B., Richards K., Elbrecht A., Johnson B. A., Zhou G., Doebber T. W., Biswas C., Parikh M., Sharma N., Tanen M. R., Thompson G. M., Ventre J., Adams A. D., Mosley R., Surwit R. S., Moller D. E., *Mol. Endocrinol.*, **17**, 662–676 (2003).
- 24) Gampe R. T. Jr., Montana V. G., Lambert M. H., Miller A. B., Bledsoe R. K., Milburn M. V., Kliewer S. A., Willson T. M., Xu H. E., *Mol. Cell*, **5**, 545–555 (2000).
- 25) Otake K., Azukizawa S., Takahashi K., Fukui M., Shibabayashi M., Kamemoto H., Kasai M., Shirahase H., *Chem. Pharm. Bull.*, **59**, 876–879 (2011).
- 26) Otake K., Azukizawa S., Fukui M., Kunishiro K., Kamemoto H., Kanda M., Miike M., Kasai M., Shirahase H., *Bioorg. Med. Chem.*, **20**, 1060–1075 (2012).
- 27) de Groot J. C., Weidner C., Krausze J., Kawamoto K., Schroeder F. C., Sauer S., Büssow K., *J. Med. Chem.*, **56**, 1535–1543 (2013).
- 28) Ostberg T., Svensson S., Selén G., Uppenberg J., Thor M., Sundbom M., Sydow-Bäckman M., Gustavsson A. L., Jendeborg L., *J. Biol. Chem.*, **279**, 41124–41130 (2004).
- 29) Azukizawa S., Kasai M., Takahashi K., Miike T., Kunishiro K., Kanda M., Mukai C., Shirahase H., *Chem. Pharm. Bull.*, **56**, 335–345 (2008).
- 30) Otake K., Azukizawa S., Fukui M., Shibabayashi M., Kamemoto H., Miike T., Kunishiro K., Kasai M., Shirahase H., *Chem. Pharm. Bull.*, **59**, 1233–1242 (2011).
- 31) Otake K., Azukizawa S., Takeda S., Fukui M., Kawahara A., Kitao T., Shirahase H., *Chem. Pharm. Bull.*, **63**, 998–1014 (2015).
- 32) Thareja S., Aggarwal S., Bhardwaj T. R., Kumar M., *Med. Res. Rev.*, **32**, 459–517 (2012).
- 33) Ala P. J., Gonneville L., Hillman M., Becker-Pasha M., Yue E. W., Douty B., Wayland B., Polam P., Crawley M. L., McLaughlin E., Sparks R. B., Glass B., Takvorian A., Combs A. P., Burn T. C., Hollis G. F., Wynn R., *J. Biol. Chem.*, **281**, 38013–38021 (2006).
- 34) Wrobel J., Sredy J., Moxham C., Dietrich A., Li Z., Sawicki D. R., Seestaller L., Wu L., Katz A., Sullivan D., Tio C., Zhang Z. Y., *J. Med. Chem.*, **42**, 3199–3202 (1999).
- 35) Kubo M., Fukui M., Ito Y., Kitao T., Shirahase H., Hinoi E., Yoneda Y., *J. Pharmacol. Sci.*, **124**, 276–285 (2014).
- 36) Shandong Xuanzhu Pharma Co., Ltd., CN105524045 (2016).
- 37) Möcklinghoff S., van Otterlo W. A., Rose R., Fuchs S., Zimmermann T. J., Dominguez Seoane M., Waldmann H., Ottmann C., Brunsfeld L., *J. Med. Chem.*, **54**, 2005–2011 (2011).
- 38) Rose P. W., Prlić A., Altunkaya A., *et al.*, *Nucleic Acids Res.*, **45** (D1), D271–D281 (2017).
- 39) Lee M. A., Tan L., Yang H., Im Y. G., Im Y. J., *Sci. Rep.*, **7**, 16837 (2017).
- 40) Kroker A. J., Bruning J. B., *PPAR Res.*, **2015**, 816856 (2015).
- 41) Nolte R. T., Wisely G. B., Westin S., Cobb J. E., Lambert M. H., Kurokawa R., Rosenfeld M. G., Willson T. M., Glass C. K., Milburn M. V., *Nature* (London), **395**, 137–143 (1998).
- 42) dos Santos J. C., Bernardes A., Giampietro L., Ammazalorso A., De Filippis B., Amoroso R., Polikarpov I., *J. Struct. Biol.*, **191**, 332–340 (2015).