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Design, synthesis, and evaluation of a novel series of α-substituted phenylpropanoic acid derivatives as human peroxisome proliferator-activated receptor (PPAR) α/δ dual agonists for the treatment of metabolic syndrome

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Abstract—A series of α-alkyl-substituted phenylpropanoic acids was prepared as dual agonists of peroxisome proliferator-activated receptors alpha and delta (PPARα/δ). Structure–activity relationship studies indicated that the shape of the linking group and the shape of the substituent at the distal benzene ring play key roles in determining the potency and the selectivity of PPAR subtype transactivation. Structure–activity relationships among the amide series (**10**) and the reversed amide series (**13**) are similar, but not identical, especially in the case of the compounds bearing a bulky hydrophobic substituent at the distal benzene ring, indicating that the hydrophobic tail part of the molecules in these two series binds at somewhat different positions in the large binding pocket of PPAR. α-Alkyl-substituted phenylpropanoic acids of (*S*)-configuration were identified as potent human PPARα/δ dual agonists. Representative compounds exhibited marked nuclear receptor selectivity for PPARα and PPARδ. Subtype-selective PPAR activation was also examined by analysis of the *m*RNA expression of PPAR-regulated genes.

1. Introduction

The increasing incidences of obesity, type 2 diabetes, and dyslipidemia, and their consequences in terms of cardiovascular morbidity and mortality, represent a considerable public health problem.[1] The metabolic syndrome is composed of an accumulation of metabolic and cardiovascular risk factors that predispose to heart attack, stroke, heart failure, and sudden cardiac death.[2] Differences in genetic background, diet, physical activity, age, gender, and nutrition all affect the prevalence of the metabolic syndrome.[3] Metabolic syndrome is an extremely important diagnostic indication for the identification of high-risk patients for multiple risk factor modification to prevent or delay adverse cardiovascular events. Affected individuals have visceral obesity, impaired glucose tolerance, elevated blood pressure, elevated triglycerides, and low HDL-cholesterol.

According to the current unifying definition, key elements of the metabolic syndrome include insulin resistance, abnormal glucose metabolism, hypertension, atherogenic dyslipidemia (low HDL cholesterol or high triglycerides), and obesity. For clinical diagnosis of the metabolic syndrome, there are currently two similar sets of diagnostic criteria. The World Health Organization definition requires at least one of the three major features, i.e., type 2 diabetes, impaired glucose tolerance, and insulin resistance, plus at least two of the minor features, which include hypertension, obesity, hypertriglyceridemia, and microalbuminuria.[1] The National Cholesterol Education Program Adult Treatment Panel III Guidelines require three of five clinical criteria, including abdominal adiposity, hypertriglyceridemia, low HDL, hypertension, and fasting hyperglycemia, to make a diagnosis.[4] From both sets of criteria, it is clear that losing weight and restoration of serum glucose and

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lipid parameters to normal levels are of primary importance for treatment.

Therefore, identification of the molecular targets of the transducers critically involved in the control of glucose and lipid homeostasis is crucial for developing new therapeutic agents for the treatment of metabolic syndrome. Metabolic nuclear receptors (NR), are particularly attractive target molecules, since they have been found to play a central role in maintaining cellular and whole-body glucose and lipid homeostasis. These NRs are activated by a wide variety of physiological ligands, including dietary fatty acids, cholesterol metabolites, and xenobiotic compounds, thereby modulating the transcriptional networks of the target response genes. Among these receptors, special attention has been paid for more than a decade to the members of the peroxisome proliferator-activated receptor (PPAR) family.

PPARs are members of the nuclear receptor superfamily, and are activated by endogenous saturated and unsaturated fatty acids and their metabolites, as well as synthetic ligands.[5] PPARs are heterogeneous, and three subtypes have been identified to date: PPARα [officially NR1C1], PPARδ/β [NR1C2], and PPARγ [NR1C3]. Each PPAR subtype appears to be differentially expressed in a tissue-specific manner, and to play a pivotal role in lipid, lipoprotein, and glucose homeostasis.[6] PPARα is mostly expressed in the tissues involved in lipid oxidation, such as liver, kidney, skeletal, cardiac muscle, and adrenal glands. PPARγ is expressed in adipose tissue, macrophages, and vascular smooth muscles. PPARδ is mainly expressed in skeletal muscle and adipose tissues, but is ubiquitously expressed in other tissues in extremely small amounts. PPARs heterodimerize with another nuclear receptor partner, retinoid X receptor (RXR), and the heterodimers regulate gene expression by binding to a specific consensus DNA sequence, termed PPRE (peroxisome proliferator responsive element), which is a direct repeat of the hexameric AGGTCA recognition motif separated by single nucleotide (DR1), present in the promoter region of target genes.[7]

PPARγ was first identified as a master regulator of adipocyte differentiation, but more recent molecular-biological studies have indicated that PPARγ activation is also linked to the expression of many important genes that affect energy metabolism, such as the TNF-α, leptin, and adiponectin genes.[8]

PPARα regulates the expression of genes encoding for proteins involved in lipid and lipoprotein homeostasis.[9] For example, it regulates genes involved in fatty acid uptake (such as fatty acid binding protein, FABP), β-oxidation (acyl-CoA oxidase), and ω-oxidation (cytochrome P450). It down-regulates apolipoprotein C-III, a protein that inhibits triglyceride hydrolysis by lipoprotein lipase, and it also regulates genes involved in reverse cholesterol transport, such as apolipoprotein A-I and apolipoprotein A-II.[9]

Based on the findings that antidiabetic thiazolidine-2, 4-diones (glitazones) and antidyslipidemic fibrates are ligands of PPARγ and PPARα, respectively, much research has been focused on these metabolic nuclear receptor subtypes as therapeutic targets for the treatment of metabolic syndrome. In contrast, although PPARδ was discovered more than 15 years ago, research interest in it has been limited. However, after 2001, the availability of PPARδ-knockout animals and the selective ligands, such as GW-501516 (**3**), prompted us to examine the role of PPARδ in fatty acid metabolism.[10,11]

Studies using GW-501516 (**3**) as a chemical tool point to key roles for PPARδ in lipid metabolism, insulin resistance, and foam cell and macrophage activation in atherosclerosis. GW501516 (**3**) treatment significantly increased HDL cholesterol levels, possibly in association with decreased lipoprotein lipase activity, in insulin-resistant middle-aged obese rhesus monkeys.[12] The results showed that GW501516 (**3**) causes a dramatic dose-dependent increase in serum HDL cholesterol and a reduction in LDL cholesterol and triglycerides. In a primate model of the metabolic syndrome, GW501516 (**3**) dose-dependently lowered plasma insulin levels, without any adverse effect on glycemic control.[12] Similarly, in ob/ob mice, GW501516 (**3**) markedly improved glucose tolerance and insulin resistance,[12] although the underlying mechanism remains unclear.[13]

In vitro studies suggested that PPARδ activation by GW501516 (**3**) in cultured macrophages results in increased expression of the reverse cholesterol transporter ABC A1 and enhances the efflux of cholesterol.[12] All these observations suggest that PPARδ may also be an effective target for the treatment of metabolic syndrome.

Considering the above-mentioned findings, the metabolic function(s) of PPARδ seem to be mainly targeted to adipose tissue and smooth muscle, via fatty acid oxidation and energy uncoupling. If this is so, a compound that can effectively activate both PPARα and PPARδ might have additive and/or synergistic positive effect(s) in the treatment of metabolic syndrome, modulating both hepatic fatty acid oxidation through PPARα, and fatty acid oxidation and energy uncoupling in muscle and adipose tissue through PPARδ. There are a few examples of PPARα/δ dual agonists in the literature, including compounds **5**,[14] **6**,[15] and **7**[16] (Fig. 1), but their activities are not so high and the structural variety is poor. Therefore, there is considerable interest in creating novel PPARα/δ dual agonists from both basic scientific and clinical points of view.

Previously, we designed and synthesized a series of substituted phenylpropanoic acid derivatives as human PPARα-selective agonists, using KRP-297,[17] a PPARγ/α dual agonist with the 2-methoxybenzamide structural motif, as a lead compound. We found that KCL (**2**), an α-ethylphenylpropanoic acid derivative, exhibited potent, human PPARα-selective agonistic activity.[18–21] As a part of our continuing research directed toward the structural development of subtype-selective PPAR agonists, we tried to construct structurally new

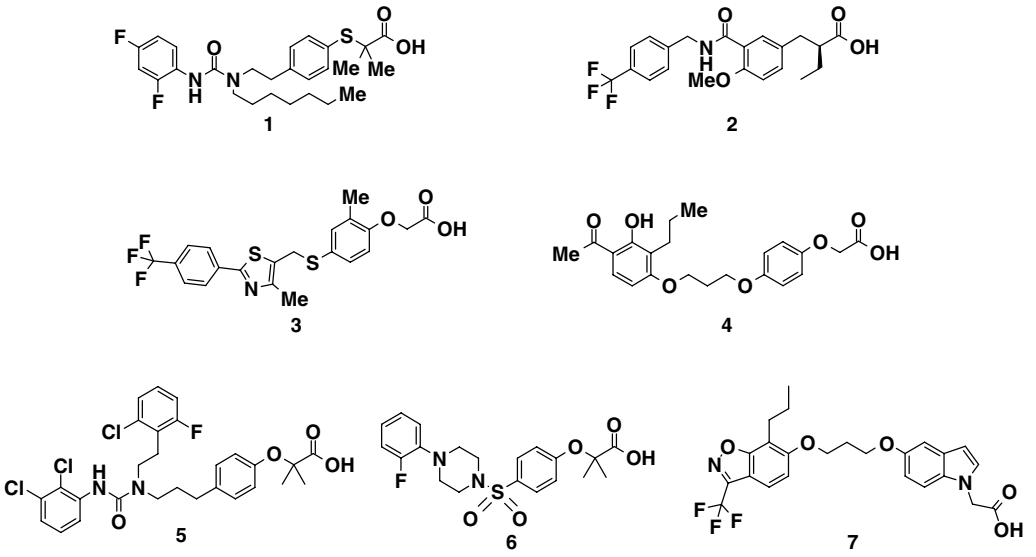


Figure 1. Structures of representative PPARα (**1** (GW-9645), **2** (KCL)), PPARδ (**3** (GW-501516), **4**), and dual PPARα/δ (**5** (GW-2433), **6, 7**) agonists.

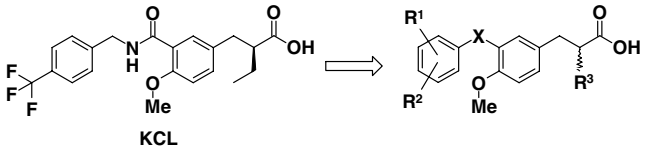


Figure 2. Structural development of KCL.

PPARα/δ dual agonists, using KCL with the 2-methoxybenzamide structure as a lead compound (Fig. 2). It was anticipated that small manipulations of the structure of KCL would open up characteristic new PPAR subtype selectivities.

2. Chemistry

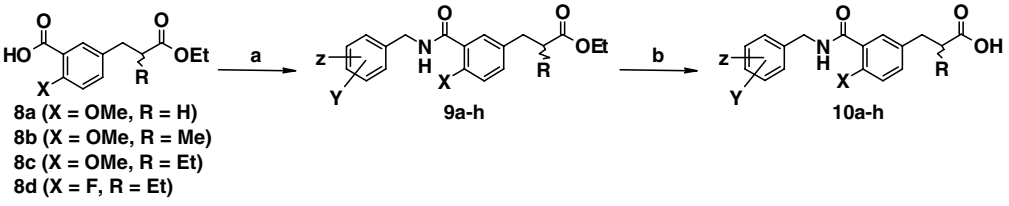
The synthetic routes to the present series of α-substituted phenylpropanoic acids are outlined in Schemes 1 and 2.

Compounds (**10a–h**) were prepared from substituted benzoic acid derivatives (**8a–d**) in 2 steps. Compounds **8a–d**[21] were condensed with substituted benzylamine, followed by alkaline hydrolysis to afford the desired products (Scheme 1).[22] The reversed amide derivatives (**13b–h**, **13l–m**) and the sulfonamide derivative **13a** were prepared from substituted benzylamine derivatives (**11a–d**) by means of procedures similar to those used for the preparation of compounds **10** (Scheme 2).

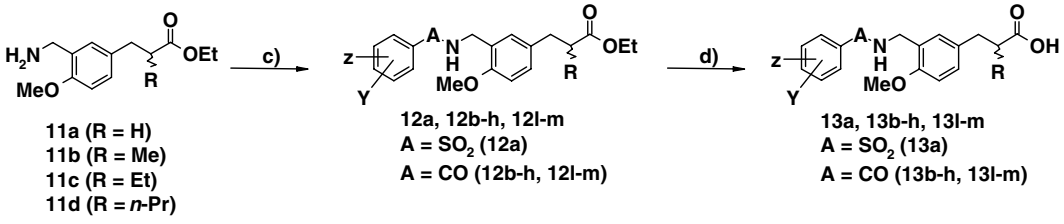
Optically active α-ethyl-substituted phenylpropanoic acid derivatives with the reversed amide linkage (**13i–k**, **13n**) were prepared as per a previously described method.[23]

3. Results and discussion

The transactivation activity of the present series of compounds towards each of the PPARs is summarized

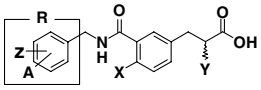


Scheme 1. Synthesis of **10a–h**. Reagents: (a) substituted benzylamine, $ClCO_2Et$, TEA, CH_2Cl_2; (b) aq-NaOH, EtOH.



Scheme 2. Synthesis of **13a–h**, **13l–m**. Reagents: (c) Substituted benzoyl chloride (or substituted benzenesulfonyl chloride), TEA, CH_2Cl_2; (d) aq-NaOH, EtOH.

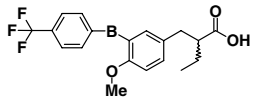
Table 1. In vitro functional PPAR transactivation activity of substituted phenylpropanoic acids



Compound	X	Y	Z	A	Stereo	EC_{50}[a] (nM)		
						PPARα	PPARδ	PPARγ
2	MeO	Et	H	4-CF_3	Rac	70	700	3000
10a	F	Et	H	4-CF_3	Rac	ia[b]	ia[b]	ia[b]
10b	MeO	Et	2-F	4-CF_3	Rac	73	730	870
10c	MeO	Et	3-F	4-CF_3	Rac	32	590	1700
10d	MeO	H	3-F	4-CF_3	Rac	500	ia[b]	ia[b]
10e	MeO	Me	3-F	4-CF_3	Rac	19	1900	8200
Compound	X	Y		R	Stereo	PPARα	PPARδ	PPARγ
10f	MeO	Et		4-phenoxybenzyl	Rac	15	1200	2000
10g	MeO	Et		fluoren-2-yl-methyl	Rac	500	3000	7000
10h	MeO	Et		pyrene-1-yl-methyl	Rac	ia[b]	ia[b]	ia[b]

[a] Compounds were screened for agonist activity on PPAR-GAL4 chimeric receptors in transiently transfected HEK-293 cells as described. EC_{50} value is the molar concentration of the test compound that affords 50% of the maximal reporter activity.
[b] "ia" means inactive (no apparent activity) at the concentration of 10 μM.

Table 2. In vitro functional PPAR transactivation activity of substituted phenylpropanoic acids



Compound	B	EC_{50}[a] (nM)		
		PPARα	PPARδ	PPARγ
2	CH_2NHCO	70	700	3000
13a	SO_2NHCH_2	ia[b]	ia[b]	ia[b]
13b	$CONHCH_2$	19	200	2600

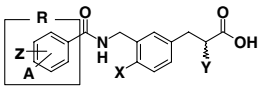
[a] See footnote (a) in Table 1.
[b] See footnote (b) in Table 1.

in Tables 1–3, together with the results for the lead compound, racemic KCL. First, we investigated the effect of the introduction of a fluorine atom in racemic KCL, because we expected that a fluorinated compound would bind more tightly to PPAR by effectively excluding the water molecule located in the hydrophobic binding pocket of PPAR. Compound **10a**, which has a fluorine atom on the central benzene ring, instead of a methoxyl group, however, showed considerably decreased PPARs transactivation activity as compared with racemic KCL; it did not exhibit apparent PPAR transactivation activity at the concentration of 10 μM. This result might indicate that the methoxyl group located at the *ortho* position of the amide carbonyl group is involved in a hydrogen-bonding interaction that restricts the conformation of the central benzene part of the molecule. As for substituents at the hydrophobic tail part, introduction of fluorine apparently did not affect PPAR transactivation activity or selectivity. Compounds **10b** and **10c**, which have a fluorine atom at the 2 or 3 position of the distal benzene ring, respectively, exhibited PPARα and PPARδ transactivation activities that were comparable with those of the non-fluorinated compound **10a** (although **10b** shows somewhat increased activity toward PPARγ, its activity is still low). Interestingly, some steric features of the hydrophobic tail are critically important for PPARα activity and the selectivity in the present series. The 4-phenoxybenzyl derivative (**10f**) exhibited potent PPARα transactivation activity and about 100-fold PPARα selectivity (vs. PPARδ and PPARγ), while the conformationally restricted fluoren-2-ylmethyl derivative (**10g**) and bulky pyren-1-ylmethyl derivative (**10h**) exhibited only weak activity or did not exhibit PPARα transactivation activity at the concentration of 10 μM.

As already mentioned, the substituent at the α-position of the carboxyl group is important for the potency for PPARα,[21] i.e., **10d**, which does not have an α-substituent, exhibited only weak PPARα transactivation activity (though it showed more than 100-fold PPARα selectivity), while introduction of a methyl group (**10e)** increased the PPARα activity by more than 10-fold, though the PPARδ and PPARγ transactivation activities are still weak, so that this compound retains PPARα selectivity. As a whole, the amide-type derivatives (**10**) tended to exhibit a PPARα-selective transactivation profile, and we did not find PPARα/δ dual agonists in this series of compounds.

Previously, we found that the 3-atom unit linker –CH_2–NH–CO– imparted potent PPARα transactivation activity, but shortening or lengthening of the linking group considerably decreased the activity.[23] We also noted that a flexible linker, such as –CH_2–CH_2–CH_2– or –CH_2–CH_2–O–, decreased PPARα transactivation activity, but caused PPARδ transactivation activity to appear. Therefore, we focused our attention on a hybrid type linker, i.e., –CO–NH–CH_2– (**13b**), and found that this linker increased both PPARα and PPARδ transactivation activity to some extent, as compared with the amide type (–CH_2–NH–CO–) linker (**2**). We speculated that

Table 3. In vitro functional PPAR transactivation activity of substituted phenylpropanoic acids



Compound	X	Y	Z	A	Stereo	EC_{50}[a] (nM)		
						PPARα	PPARδ	PPARγ
13b	MeO	Et	H	4-CF_3	rac	19	200	2600
13c	MeO	Et	2-F	4-CF_3	rac	10	24	2200
13d	MeO	Et	3-F	4-CF_3	rac	11	51	6000
13e	MeO	Et	3-CF_3	4-F	rac	250	2000	ia[(b)]
13f	MeO	H	H	4-CF_3	rac	200	ia[b]	ia[b]
13g	MeO	Me	H	4-CF_3	rac	19	210	ia[b]
13h	MeO	*n*-Pr	H	4-CF_3	rac	63	120	3600
13i	MeO	Et	2-F	4-CF_3	S	10	12	1900
13j	MeO	Et	3-F	4-CF_3	*S*	12	23	4900
13k	MeO	Et	3-F	4-CF_3	*R*	150	840	ia[b]
Compound	X	Y	R		Stereo	PPARα	PPARδ	PPARγ
13l	MeO	Et	4-phenoxyphenyl		rac	8.5	120	800
13m	MeO	Et	pyrene-1-yl		rac	24	66	ia[b]
13n	MeO	Et	pyrene-1-yl		*S*	10	40	ia[b]

[a] See footnote (a) in Table 1.
[b] See footnote (b) in Table 1.

the appearance of a conformationally flexible group and/or atom, such as $–CH_2–$ or –O–, adjacent to the central benzene ring might favor both PPARα and PPARδ activity, while introduction of a more conformationally restricted group (–CO–) next to the central benzene ring might be unfavorable for PPARδ activity. These results might reflect differences in the shape and environment of the hydrophobic cavity hosting the distal benzene ring between PPARα and PPARδ.

We then prepared the sulfonamidemethyl linker derivative **13a**, but this compound did not exhibit apparent PPAR transactivation activity. The hydrophilic sulfonamide moiety might interact unfavorably with the hydrophobic pocket of each PPAR ligand binding domain. Based on the above results, we further prepared the 'reversed amide' series of compounds (**13c–n**). As can be seen in Table 3, some noteworthy results were obtained. In contrast to the amide series, the introduction of fluorine at the 2 or 3 position of the distal benzene ring increased the PPARδ transactivation activity. The position of the distal benzene substituents is crucial, since compound **13e**, which has a fluorine atom at the 4 position and a trifluoromethyl group at the 3 position, showed considerably decreased PPAR transactivation activity. This is consistent with the previously obtained structure–activity relationship result that steric bulkiness at the 4-position is an important factor for potent PPARα transactivation activity.[21]

Considering the results obtained above, we then prepared optically active derivatives, **13i**, **13j**, and **13k**. As can be seen from Table 3, a clear enantio-dependency of the transactivation activity toward the PPARα and PPARδ isoforms was found. Compound **13j**, which has (*S*) configuration, exhibited potent transactivation activity on both PPARα and PPARδ, while the antipodal (*R*) isomer **13k** exhibited far less potency. Therefore, we concluded that both PPARα and PPARδ transactivation activities reside exclusively in the (*S*)-enantiomer, and both **13i** and **13j** show dual-agonist activity toward PPARα and PPARδ.

Interestingly, steric tolerance of the hydrophobic tail part is somewhat different between the amide series of compounds and the reversed amide series of compounds. In the case of R = 4-phenoxybenzyl (**10f**, **13l**), **10f** and **13l** exhibited almost equipotent PPARα transactivation activity. As for PPARδ, **13l** still exhibited a submicromolar order EC_{50}, while the activity of **10f** was about 10 times less potent than that of **13l**. As a result, the PPARδ/α selectivity ratio of **10f** is superior to that of **13l** (80-fold selective vs. 10-fold selective). The difference in the sterical factor is extremely evident in the case of R = pyren-1-yl group (**10h**, **13m–13n**). Although **10h** did not exhibit apparent PPARs transactivation activity

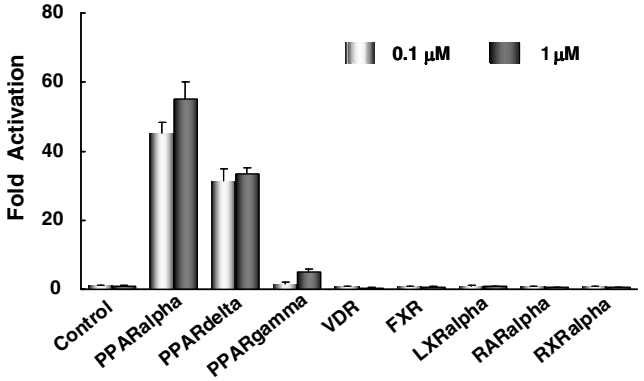


Figure 3. Cross-nuclear receptor reactivity of 0.1 μM, and 1 μM **13i**.

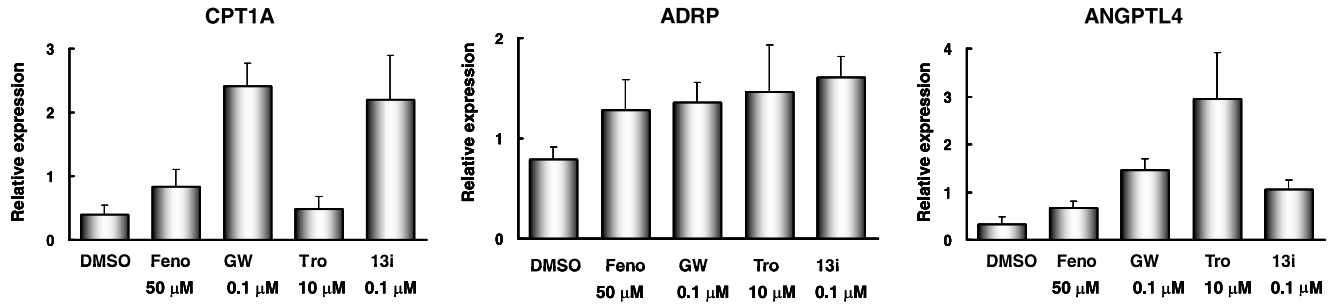


Figure 4. Regulation of PPAR targeted gene expression by various PPAR ligands.

at the concentration of 10 μM, **13m** still exhibited potent dual PPARα/δ transactivation activity, comparable with those of **13c** and **13d**. These differences might be a result of interaction of the distal hydrophobic tail part of **10h** and **13m** with different amino acids in the large hydrophobic ligand binding pocket, probably due to the difference in the conformational flexibility of these linker groups. In the case of the pyren-1-yl derivative, PPARα and PPARδ transactivation activities also reside in the (*S*)-enantiomer, **13n**.

In order to investigate the nuclear receptor selectivity (cross-reactivity) of the representative compound **13i**, we determined the transactivation activity of **13i** on representative nuclear receptors (PPARs, VDR, FXR, LXRα, RARα, and RXRα). As can be seen from Figure 3, **13i** is specific for PPARα and PPARδ because it did not significantly activate VDR, PPARγ, LXRα, RARα or RXRα at concentrations up to 1 μM under the experimental conditions used. These results indicate that, although the ligand binding domains of nuclear receptors are similar, there are distinct structural requirements for preferential binding to both PPARα and PPARδ.

To demonstrate the ability of compound **13i** to activate the genes which have peroxisome proliferator responsive element (PPRE) in the promoter region in the cell level, we examined changes in expression of representative PPAR-mediated genes in human hepatocellular carcinoma Huh-7. We chose carnitine palmitoyl acyl-CoA transferase 1A (CPT1A), angiopoietin-like protein 4 (ANGPTL4), and adipocyte differentiation-related protein (ADRP) as the representative PPAR target genes. For each gene of human were reported to possess PPRE in the promoter region.[24–26] CPT1A is the key enzyme in the carnitine-dependent transport across the mitochondrial inner membrane and its deficiency results in a decreased rate of fatty acid β-oxidation. ANGPTL4 is a member of the angiopoietin/angiopoietin-like gene family and encodes a glycosylated, secreted protein with a fibrinogen C-terminal domain. This gene is the target of peroxisome proliferation activators, such as fibrate class antihyperlipidemic agents. ADRP is associated with the globule surface membrane material. This protein is a major constituent of the globule surface. Increase in the level of ADRP *m*RNA is one of the earliest indications of adipocyte differentiation.

At first, we investigated the effects of the representative PPAR-subtype selective agonists, fenofibrate (Feno; a PPARα-selective agonist), GW-501516 (GW; a PPARδ-selective agonist), and troglitazone (Tro; a PPARγ-selective agonist), to transactivate these genes in Huh-7. As indicated in Figure 4, when Huh-7 cells were treated with 50 μM Feno, 0.1 μM GW, and 10 μM Tro (the indicated concentrations of each compound were enough to exhibit subtype-selective transactivation activity), these three genes *m*RNA expressions were augmented subtype selectively. These results indicated that each PPAR-subtype in Huh-7 is functionally active. ADRP gene expression was significantly augmented with these three PPAR agonists. CPT1A gene expression was greatly augmented by Feno and GW, indicating that expression of this gene is mediated through the PPARα and PPARδ pathways. ANGPTL4 gene expression was greatly augmented only by Tro, which indicates that expression of this gene is mediated through the PPARγ pathway. Treatment with 0.1 μM **13i** augmented CPT1A gene expression to an extent comparable with that obtained with 0.1 μM GW, and had little effect on expression of ANGPTL4 gene, probably due to the weak activity of **13i** toward PPARγ.

These results indicate that the representive compound **13i** is an effective PPARα/δ dual agonist at the cellular level.

In summary, we have developed a series of potent human PPARα/δ dual agonists, which possess potent PPARα/δ transactivation activity with high selectivity over PPARγ. In vivo pharmacological evaluation of representative compounds is awaited.

4. Experimental

4.1. General

Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and were within plus or minus 0.3% of the theoretical values. NMR spectra were recorded on a JEOL JNM-GX500 (500 MHz) spectrometer. Chemical shifts are expressed in ppm relative to tet-

ramethylsilane. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer.

4.1.1. 2-Ethyl-3-[4-methoxy-3-[*N*-[[2-fluoro-4-(trifluoromethyl)phenyl]methyl]carbamoyl]phenyl] propanoic acid (10b). Ethyl 3-(3-carboxy-4-methoxyphenyl)-2-ethylpropionate (**8c**)[21] (400 mg, 1.43 mmol) was dissolved in 10 mL of dry dichloromethane, and the solution was cooled to −10 °C to −15 °C. Triethylamine (0.5 mL, 3.58 mmol) was added under stirring, followed by the addition of ethyl chlorocarbonate (185 mg, 1.71 mmol) dissolved in 5 mL of dry dichloromethane. The mixture was stirred for 10 min at −10 °C, then 2-fluoro-4-(trifluoromethyl)benzylamine (392 mg, 1.71 mmol) dissolved in 5 mL of dry dichloromethane was added dropwise. Stirring was continued for 30 min at −10 °C, then for 7 h at room temperature and finally the mixture was left to stand overnight. It was washed with aqueous citric acid, aqueous sodium hydrogen carbonate, and brine, then dried over anhydrous sodium sulfate and concentrated. The residue was purified by silica gel column chromatography (eluant *n*-hexane: AcOEt = 1:1 v/v) to afford 270 mg of ethyl 2-ethyl-3-[4-methoxy-3-[*N*-[[4-(trifluoromethyl)phenyl]methyl]carbamoyl]phenyl] propionate (**9b**) as a yellow oil.

A mixture of **9b** (270 mg), 15 mL ethanol, and 15 mL of a 1 mol/L aqueous solution of sodium hydroxide was stirred for 4 h at 50 °C, then concentrated under reduced pressure. The residue was dissolved in water and acidified with dil. HCl. The precipitate formed was collected by filtration, dried, and recrystallized from ethyl acetate to afford 165 mg (27%) of the title compound as colorless prisms: mp 102–103 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.96 (t, 3H, *J* = 7.3 Hz), 1.53–1.72 (m, 2H), 2.59–2.66 (m, 1H), 2.77 (dd, 1H, *J* = 13.7, 6.8 Hz), 2.96 (dd, 1H, *J* = 13.7, 8.3 Hz), 3.92 (s, 3H), 4.73 (d, 2 H, *J* = 5.9 Hz), 6.90 (d, 1H, *J* = 8.3 Hz), 7.29 (dd, 1H, *J* = 8.3, 2.4 Hz), 7.47 (d, 1H, *J* = 8.3 Hz), 7.59 (d, 2H, *J* = 7.8 Hz), 8.08 (d, 1H, *J* = 2.4 Hz), 8.32 (t, 1H, *J* = 5.9 Hz); HRMS (FAB^+) calcd for $C_{21}H_{22}F_4NO_4$ $(M+H)^+$; 428.1485. Found 428.1453. Anal. ($C_{21}H_{21}F_4NO_4$) C, H, N.

4.1.2. 2-Ethyl-3-[4-fluoro-3-[*N*-[[4-(trifluoromethyl)phenyl]methyl]carbamoyl]phenyl]propanoic acid (10a). This compound was prepared from **8b**[21] and 4-(trifluoromethyl)benzylamine by means of a procedure similar to that used for **10b**. Mp 99–100 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.18 (s, 1H), 7.81 (d, 1H, *J* = 7.9 Hz), 7.72 (d, 1H, *J* = 5.5 Hz), 7.55 (d, 1H, *J* = 7.9 Hz), 7.41 (s, 1H), 7.26 (m, 5H), 6.46 (d, 1H, *J* = 5.5 Hz), 5.00 (m, 1H), 4.76 (s, 2H), 4.22 (s, 2H), 3.95 (m, 1H), 3.61 (m, 1H), 1.80 (m, 3H), 1.56 (m, 3H); FAB-MS *m*/*z* 398 $(M+H)^+$; Anal. ($C_{20}H_{19}F_4NO_4$) C, H, N.

4.1.3. 2-Ethyl-3-[4-methoxy-3-[*N*-[[3-fluoro-4-(trifluoromethyl)phenyl]methyl]carbamoyl]phenyl] propanoic acid (10c). This compound was prepared from **8c**[21] and 3-fluoro-4-(trifluoromethyl)benzylamine by means of a procedure similar to that used for **10b**. Mp 120–121 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.18 (s, 1H), 7.81 (d, 1H, *J* = 7.9 Hz), 7.72 (d, 1H, *J* = 5.5 Hz), 7.55 (d, 1H, *J* = 7.9 Hz), 7.41 (s, 1H), 7.26 (m, 5H), 6.46 (d, 1H, *J* = 5.5 Hz), 5.00 (m, 1H), 4.76 (s, 2H), 4.22 (s, 2H), 3.95 (m, 1H), 3.61 (m, 1H), 1.80 (m, 3H), 1.56 (m, 3H); HRMS (FAB^+) calcd for $C_{21}H_{22}F_4NO_4$ $(M+H)^+$; 470.1779. Found 470.1762. Anal. ($C_{21}H_{21}F_4NO_4$) C, H, N.

4.1.4. 3-[4-Methoxy-3-[*N*-[[4-(trifluoromethyl)phenyl]-methyl]carbamoyl]phenyl]propanoic acid (10d). This compound was prepared from **8a**[21] and 4-(trifluoromethyl)benzylamine by means of a procedure similar to that used for **10b**. Mp 141–143 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.18 (s, 1H), 7.81 (d, 1H, *J* = 7.9 Hz), 7.72 (d, 1H, *J* = 5.5 Hz), 7.55 (d, 1H, *J* = 7.9 Hz), 7.41 (s, 1H), 7.26 (m, 5H), 6.46 (d, 1H, *J* = 5.5 Hz), 5.00 (m, 1H), 4.76 (s, 2H), 4.22 (s, 2H), 3.95 (m, 1H), 3.61 (m, 1H), 1.80 (m, 3H), 1.56 (m, 3H); HRMS (FAB^+) calcd for $C_{19}H_{18}F_4NO_4$ $(M+H)^+$; 400.1169. Found 400.1172.

4.1.5. 3-[4-Methoxy-3-[*N*-[[4-(trifluoromethyl)phenyl]-methyl]carbamoyl]phenyl]-2-methylpropanoic acid (10e). This compound was prepared from **8b**[21] and 4-(trifluoromethyl)benzylamine by means of a procedure similar to that used for **10b**. Mp 125–127 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.04 (d, 1H, *J* = 2.6 Hz), 7.54 (t, 1H, *J* = 7.7 Hz), 7.29 (dd, 1H, *J* = 7.7, 2.6 Hz), 7.20 (d, 1H, *J* = 7.2 Hz), 7.17 (d, 1H, *J* = 7.2 Hz), 6.90 (d, 1H, *J* = 7.0 Hz), 4.69 (d, 2H, *J* = 6.0 Hz), 3.93 (s, 3H), 3.00–3.04 (m, 1H), 2.73–2.78 (m, 1H), 2.66–2.71 (m, 1H), 1.16 (d, 3H, *J* = 6.8 Hz); HRMS (FAB^+) calcd for $C_{20}H_{20}F_4NO_4$ $(M+H)^+$; 414.1339. Found 414.1328. Anal. ($C_{20}H_{20}F_4NO_4$) C, H, N.

4.1.6. 2-Ethyl-3-[4-methoxy-3-[*N*-[[4-(phenoxy)phenyl]-methyl]carbamoyl]phenyl] propanoic acid (10f). This compound was prepared from **8c**[21] and 4-(phenoxy)benzylamine by means of a procedure similar to that used for **10b**. Mp 123–124 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.18 (s, 1H), 7.81 (d, 1H, *J* = 7.9 Hz), 7.72 (d, 1H, *J* = 5.5 Hz), 7.55 (d, 1H, *J* = 7.9 Hz), 7.41 (s, 1H), 7.26 (m, 5H), 6.46 (d, 1H, *J* = 5.5 Hz), 5.00 (m, 1H), 4.76 (s, 2H), 4.22 (s, 2H), 3.95 (m, 1H), 3.61 (m, 1H), 1.80 (m, 3H), 1.56 (m, 3H); HRMS (FAB^+) calcd for $C_{26}H_{28}NO_5$ $(M+H)^+$; 434.1970. Found 434.1967. Anal. ($C_{26}H_{27}NO_5$) C, H, N.

4.1.7. 2-Ethyl-3-[4-methoxy-3-[*N*-[[fluoren-1-yl]methyl]-carbamoyl]phenyl] propanoic acid (10g). This compound was prepared from **8c**[21] and fluoren-1-yl-methylamine by means of a procedure similar to that used for **10b**. Mp 151–153 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.10 (s, 1H), 7.75 (m, 2H), 7.53 (m, 2H), 7.36 (m, 2H), 7.26 (m, 2H), 6.88 (d, *J* = 7.7 Hz, 1H), 4.74 (d, *J* = 5.6 Hz, 2H), 3.88 (s, 5H), 2.94 (m, 1H), 2.80 (m, 1H), 2.63 (m, 1H), 1.62 (m, 2H), 0.96 (t, *J* = 6.4 Hz, 3H); MS *m*/*z* 430 $(M+H)^+$; Anal. ($C_{27}H_{24}NO_4$) C, H, N.

4.1.8. 2-Ethyl-3-[4-methoxy-3-[*N*-[[pyren-1-yl]methyl]carbamoyl]phenyl] propanoic acid (10h). This compound was prepared from **8c**[21] and pyren-1-yl-methylamine by means of a procedure similar to that used for **10b**. Mp 132–134 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (d, 2H, J = 8.8 Hz), 8.30 (s, 1H), 8.13–8.19 (m, 5H), 7.99–8.05 (m, 3H), 7.23 (d, 1H, J = 8.1 Hz), 6.78 (d, 1H, J = 8.8 Hz), 5.36 (d, 2H, J = 5.1 Hz), 3.65 (s, 3H), 2.94–2.98 (m, 1H), 2.76—2.80 (m, 1H), 2.64–2.65 (m, 1H), 1.64–1.69 (m, 1H), 1.57–1.61 (m, 1H), 0.96 (t, 3H, J = 7.5 Hz); FAB-MS m/z 393 $(M+H)^+$; Anal. ($C_{30}H_{27}NO_4$) C, H, N.

4.1.9. 2-Ethyl-3-[4-methoxy-3-[*N*-[4-(trifluoromethyl)benzenesulfonylamino]methyl]phenyl]propanoic acid (13a). Ethyl 3-(3-aminomethyl-4-methoxyphenyl)-2-ethylpropionate HCl (**11c**)[21] (212 mg, 0.70 mmol) was dissolved in 20 mL of dry dichloromethane and 1.2 equiv of triethylamine was added under stirring, followed by the addition of 4-(trifluoromethyl)benzenesulfonyl chloride (177 mg, 0.70 mmol) dissolved in 5 mL of dry dichloromethane. Stirring was continued overnight at room temperature. The mixture was washed with aqueous citric acid, aqueous sodium hydrogen carbonate, and brine, then dried over anhydrous sodium sulfate and concentrated. The residue was purified by silica gel column chromatography (eluant *n*-hexane: AcOEt = 1:1 v/v) to afford 297 mg of ethyl 2-ethyl-3-[4-methoxy-3-[*N*-[4-(trifluoromethyl)benzenesulfonylamino]methyl]phenyl]propanoic acid (**12a**) as an oil. A mixture of **12a** (295 mg), 15 mL ethanol and 15 mL of 1 mol/L aqueous solution of sodium hydroxide was stirred for 4 h at 50 °C, then concentrated under reduced pressure. The residue was dissolved in water, and acidified with dil. HCl. The precipitate that formed was collected by filtration, dried, and recrystallized from ethyl acetate to afford 235 mg (85%) of the title compound: mp 130–131 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.1 Hz, 2H), 6.97 (dd, J = 8.1, 2.1 Hz, 1H), 6.85 (d, J = 2.1 Hz, 1H), 6.54 (d, J = 8.1 Hz, 1H), 5.45 (br, 1H), 4.16 (d, J = 6.0 Hz, 2H), 3.65 (s, 3H), 2.79 (dd, J = 13.6, 8.5 Hz, 1H), 2.60 (dd, J = 13.7, 6.4 Hz, 1H), 2.50 (m, 1H), 1.62 (m, 1H), 1.56 (m, 1H), 0.95 (t, J = 7.2 Hz, 3H); HRMS (FAB^+) calcd for $C_{20}H_{22}F_4NO_5S$ $(M+H)^+$; 445.1171. Found 445.1171.

4.1.10. 2-Ethyl-3-[4-methoxy-3-[*N*-[4-(trifluoromethyl)-benzoylamino]methyl]phenyl]propanoic acid (13b). This compound was prepared from **11c**[21] and 4-trifluoromethylbenzoyl chloride by means of a procedure similar to that used for **13a**. Mp 133–135 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, 2H, J = 8.7 Hz), 7.24 (d, 2H, J = 8.1 Hz), 7.17 (d, 1H, J = 2.1 Hz), 7.10 (d, 1H, J = 8.1 Hz), 6.80 (d, 1H, J = 8.7 Hz), 6.69 (s, 1H), 4.58 (d, 2H, J = 6.0 Hz), 3.85 (s, 3H), 2.86–2.90 (m, 1H), 2.70–2.74 (m, 1H), 2.55–2.59 (m, 1H), 1.63–1.70 (m, 1H), 1.55–1.60 (m, 1H), 0.95 (t, 3H, J = 7.3 Hz); FAB MS m/z 423 $(M+H)^+$; Anal. Calcd For $C_{21}H_{22}F_3NO_4$4/$5H_2O$ C, 59.51; H, 5.57; N, 3.30. Found: C, 59.27; H, 5.18; N, 3.21.

4.1.11. 2-Ethyl-3-[4-methoxy-3-[*N*-[2-fluoro-4-(trifluoromethyl)benzoylamino]methyl]phenyl]propanoic acid (13c). This compound was prepared from **11c**[21] and 2-fluoro-4-trifluoromethylbenzoyl chloride by means of a procedure similar to that used for **13a**. Mp 108–110 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (t, 1H, J = 7.7 Hz), 7.4 (m, 3H), 7.17 (s, 1H), 7.10 (d, 1H, J = 8.1 Hz), 6.81 (d, 1H, J = 8.1 Hz), 4.63 (d, 2H, J = 6.0 Hz), 3.87 (s, 3H), 2.89 (dd, 1H, J = 14.1, 8.5 Hz), 2.73 (dd, 1H, J = 14.1, 8.5 Hz, 1H), 2.57 (m, 1H), 1.80 (br, 1H), 1.66 (m, 1H), 1.59 (m, 1H), 0.96 (t, 3H, J = 7.5 Hz); MS (FAB); 428$(M+H)^+$; Anal. ($C_{21}H_{21}F_4NO_4$) C, H, N.

4.1.12. 2-Ethyl-3-[4-methoxy-3-[*N*-[3-fluoro-4-(trifluoromethyl)benzoylamino]methyl]phenyl]propanoic acid (13d). This compound was prepared from **11c**[21] and 4-fluoro-3-trifluoromethylbenzoyl chloride by means of a procedure similar to that used for **13a**. Mp 120–121 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (m, 3H), 7.14 (s, 1H), 7.10 (dd, J = 8.5, 2.1 Hz, 1H), 6.81 (d, J = 8.1 Hz, 1H), 6.77 (br, 1H), 4.57 (d, J = 6.0 Hz, 2H), 3.85 (s, 3H), 2.88 (dd, J = 13.7, 8.5 Hz, 1H), 2.72 (dd, J = 13.7, 6.0 Hz, 1H), 2.56 (m, 1H), 1.66 (m, 1H), 1.58 (m, 1H), 0.96 (t, J = 7.3 Hz, 3H); MS (FAB); 428 $(M+H)^+$; Anal. ($C_{21}H_{21}F_4NO_4$) C, H, N.

4.1.13. 2-Ethyl-3-[4-methoxy-3-[*N*-[4-fluoro-3-(trifluoromethyl)benzoylamino]methyl]phenyl]propanoic acid (13e). This compound was prepared from **11c**[21] and 3-fluoro-4-trifluoromethylbenzoyl chloride by means of a procedure similar to that used for **13a**. Oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (m, 1H), 8.00 (m, 1H), 7.26 (t, J = 9.4 Hz, 1H), 7.18 (m, 1H), 7.14 (dd, J = 8.1, 6.0 Hz, 1H), 6.85 (m, 2H), 5.70 (br, 1H), 4.61 (d, J = 5.6 Hz, 2H), 3.89 (s, 3H), 2.92 (dd, J = 13.7, 8.5 Hz, 1H), 2.75 (dd, J = 13.7, 7.7 Hz, 1H), 2.59 (m, 1H), 1.68 (m, 1H), 1.61 (m, 1H), 0.99 (t, J = 7.7 Hz, 3H); HRMS; $(M+H)^+$; calcd for $C_{21}H_{22}F_4NO_4$, 428.1485. Found 428.1488.

4.1.14. 3-[4-Methoxy-3-[*N*-[[4-(trifluoromethyl)benzoylamino]methyl]phenyl]propanoic acid (13f). This compound was prepared from **11a**[21] and 4-(trifluoromethyl)benzoyl chloride by means of a procedure similar to that used for **13a**. Mp 166–167 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (d, 2H, J = 8.3 Hz), 7.68 (d, 2H, J = 8.3 Hz), 7.20 (d, 1H, J = 2.1 Hz), 7.14 (dd, 1H, J = 8.1, 2.1 Hz), 6.84 (d, 1H, J = 8.1 Hz), 6.72 (s, 1H), 4.62 (d, 2H, J = 6.0 Hz), 3.87 (s, 3H), 2.91 (t, 2H, J = 7.7 Hz), 2.65 (d, 2H, J = 7.7 Hz); HRMS; $(M+H)^+$; calcd for $C_{21}H_{22}F_4NO_4$, 382.1236. Found 382.1266.

4.1.15. 3-[4-Methoxy-3-[*N*-[[4-(trifluoromethyl)benzoylamino]methyl]phenyl]-2-methylpropanoic acid (13g). This compound was prepared from **11b**[21] and 4-(trifluoromethyl)benzoyl chloride by means of a procedure similar to that used for **13a**. Mp 129–130 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, 2H, J = 8.1 Hz), 7.67 (d, 2H, J = 8.1 Hz), 7.18 (d, 1H, J = 2.1 Hz), 7.10 (dd, 1H, J = 8.1, 2.1 Hz), 6.82 (d, 1H, J = 8.1 Hz), 6.74 (s, 1H), 4.61 (d, 2H, J = 5.5 Hz), 3.87 (s, 3H), 2.96 (dd, 1H, J = 13.7, 6.8 Hz), 2.76–2.64 (m, 2H), 1.18 (d, 3H,

J = 6.8 Hz); HRMS; $(M+H)^+$; calcd for $C_{20}H_{21}F_3NO_4$, 396.1422. Found 396.1423.

4.1.16. 3-[4-Methoxy-3-[*N*-[[4-(trifluoromethyl)benzoyl-amino]methyl]phenyl]-2-*n*-propylpropanoic acid (13h). This compound was prepared from **11d**[21] and 4-(trifluoromethyl)benzoyl chloride by means of a procedure similar to that used for **13a**. Mp 129—130 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, 2H, J = 8.3 Hz), 7.66 (d, 2H, J = 8.3 Hz), 7.16 (d, 1H, J = 2.1 Hz), 7.09 (dd, 1H, J = 8.5, 2.1 Hz), 6.81 (d, 1H, J = 8.5 Hz), 6.77 (s, 1H), 4.58 (d, 2H, J = 6.0 Hz), 3.85 (s, 3H), 2.87 (dd, 1H, J = 13.7, 9.0 Hz), 2.71 (dd, 1H, J = 13.7, 6.0 Hz), 2.63 (m, 2H), 1.63 (m, 1H), 1.48 (m, 1H), 1.36 (m, 2H), 0.90 (d, 3H, J = 7.3 Hz); HRMS; $(M+H)^+$; calcd for $C_{20}H_{21}F_3NO_4$, 424.1769. Found 424.1736.

4.1.17. (*S*)-2-Ethyl-3-[4-methoxy-3-[*N*-[3-fluoro-4-(trifluoro-methyl)benzoylamino]methyl]phenyl]-propanoic acid (13i). This compound was prepared according to the reported method.[23] Mp 120–121 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (m, 3H), 7.14 (s, 1H), 7.10 (dd, J = 8.5, 2.1 Hz, 1H), 6.81 (d, J = 8.1 Hz, 1H), 6.77 (br, 1H), 4.57 (d, J = 6.0 Hz, 2H), 3.85 (s, 3H), 2.88 (dd, J = 13.7, 8.5 Hz, 1H), 2.72 (dd, J = 13.7, 6.0 Hz, 1H), 2.56 (m, 1H), 1.66 (m, 1H), 1.58 (m, 1H), 0.96 (t, J = 7.3 Hz, 3H); HRMS; $(M+H)^+$; calcd for $C_{21}H_{21}F_4NO_4$, 428.1485. Found 428.1453. Anal. ($C_{21}H_{21}F_4NO_4$) C, H, N.

4.1.18. (*S*)-2-Ethyl-3-[4-methoxy-3-[*N*-[2-fluoro-4-(trifluoro-methyl)benzoylamino]methyl]phenyl]-propanoic acid (13j). This compound was prepared according to the reported method.[23] Mp 102–103 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (t, 1H, J = 7.7 Hz), 7.4 (m, 3H), 7.17 (s, 1H), 7.10 (d, 1H, J = 8.1 Hz), 6.81 (d, 1H, J = 8.1 Hz), 4.63 (d, 2H, J = 6.0 Hz), 3.87 (s, 3H), 2.89 (dd, 1H, J = 14.1, 8.5 Hz), 2.73 (dd, 1H, J = 14.1, 8.5 Hz, 1H), 2.57 (m, 1H), 1.80 (br, 1H), 1.66 (m, 1H), 1.59 (m, 1H), 0.96 (t, 3H, J = 7.5 Hz); HRMS; $(M+H)^+$; calcd for $C_{21}H_{21}F_4NO_4$, 428.1485. Found 428.1453. Anal. ($C_{21}H_{21}F_4NO_4$) C, H, N.

4.1.19. (*R*)-2-Ethyl-3-[4-methoxy-3-[*N*-[2-fluoro-4-(trifluoro-methyl)benzoylamino]methyl]phenyl]-propanoic acid (13k). This compound was prepared according to the reported method.[23] Mp 102–103 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (t, 1H, J = 7.7 Hz), 7.4 (m, 3H), 7.17 (s, 1H), 7.10 (d, 1H, J = 8.1 Hz), 6.81 (d, 1H, J = 8.1 Hz), 4.63 (d, 2H, J = 6.0 Hz), 3.87 (s, 3H), 2.89 (dd, 1H, J = 14.1, 8.5 Hz), 2.73 (dd, 1H, J = 14.1, 8.5 Hz, 1H), 2.57 (m, 1H), 1.80 (br, 1H), 1.66 (m, 1H), 1.59 (m, 1H), 0.96 (t, 3H, J = 7.5 Hz); HRMS; $(M+H)^+$; calcd for $C_{21}H_{21}F_4NO_4$, 428.1485. Found 428.1453. Anal. ($C_{21}H_{21}F_4NO_4$) C, H, N.

4.1.20. 2-Ethyl-3-[4-methoxy-3-[*N*-[[4-phenoxyphenyl]car-bonylaminomethyl]phenyl]propanoic acid (13l). This compound was prepared from **11c**[21] and 4-phenoxybenzoyl chloride by means of a procedure similar to that used for **13a**. Oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (d, 2H, J = 9.0 Hz,), 7.36 (t, 2H, J = 8.1 Hz), 7.16 (m, 2H), 7.07 (dd, 1H, J = 8.6, 2.1 Hz), 7.02 (d, 2H, J = 8.1 Hz), 6.97 (d, 2H, J = 8.5 Hz), 6.79 (d, 1H, J = 8.1 Hz), 6.66 (t, 1H, J = 4.6 Hz), 4.57 (d, 2H, J = 6.0 Hz), 3.84 (s, 3H), 3.50 (br, 1H), 2.87 (dd, 1H, J = 13.7, 8.6 Hz), 2.71 (dd, 1H, J = 13.7, 6.4 Hz), 2.55 (m, 1H), 1.65 (m, 1H), 1.58 (m, 1H), 0.95 (t, 3H, J = 7.3 Hz, 3H); MS (FAB); 434$(M+H)^+$; Anal. ($C_{26}H_{27}NO_5$) C, H, N.

4.1.21. 2-Ethyl-3-[4-methoxy-3-[*N*-[[pyren-1-yl]carbony-laminomethyl]phenyl]propanoic acid (13m). This compound was prepared from **11c**[21] and pyren-1-ylcarbonyl chloride by means of a procedure similar to that used for **13a** ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.08 (s, 1H), 9.04 (s, 1H), 8.50 (d, 1H, J = 9.4 Hz), 8.33–8.36 (m, 3H), 8.22–8.27 (m, 3H), 8.18 (d, 1H, J = 7.7 Hz), 8.12 (t, 1H, J = 7.7 Hz), 7.23 (s, 1H), 7.09 (d, 1H, J = 8.3 Hz), 6.94 (d, 1H, J = 8.3 Hz), 4.54 (d, 2H, J = 5.6 Hz), 3.84 (s, 3H), 2.80 (dd, 1H, J = 13.7, 7.3 Hz), 2.62 (dd, 1H, J = 13.7, 7.3 Hz), 2.44–2.49 (m, 1H), 1.45–1.55 (m, 2H), 0.85 (t, 3H, J = 7.3 Hz); HRMS; $(M+H)^+$; calcd for $C_{30}H_{28}NO_4$, 466.2018. Found 466.1984. Anal. ($C_{30}H_{27}NO_4$) C, H, N.

4.1.22. (*S*)-2-Ethyl-3-[4-methoxy-3-[*N*-[[pyren-1-yl]car-bonylaminomethyl]phenyl]propanoic acid (13n). This compound was prepared according to the reported method.[23] ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.46 (d, 1H, J = 9.4 Hz), 8.17 (m, 2H), 7.98 (m, 5H), 7.32 (s, 1H), 7.10 (d, 1H, J = 5.6 Hz), 6.80 (d, 1H, J = 5.6 Hz), 6.63 (m, 1H), 4.70 (m, 2H), 3.82 (s, 3H), 2.87 (m, 1H), 2.74 (m, 1H), 2.56 (m, 1H), 1.50 (m, 2H), 0.94 (t, 3H, J = 7.5 Hz); HRMS; $(M+H)^+$; calcd for $C_{30}H_{28}NO_4$, 466.2018. Found 466.2029. Anal. ($C_{30}H_{27}NO_4$) C, H, N.

4.2. Cell culture and cotransfection assay

Human embryonic kidney HEK293 cells were cultured in DMEM containing 5% fetal bovine serum and antibiotic-antimycotic (Nacalai) at 37 °C in a humidified atmosphere of 5% CO_2 in air. Transfections were performed by calcium phosphate coprecipitation. Eight hours after transfection, ligands were added. Cells were harvested approximately 16–20 h after the treatment, and luciferase and ß-galactosidase activities were assayed using a luminometer and a microplate reader. DNA cotransfection experiments included 50 ng of reporter plasmid, 20 ng pCMX-ß-galactosidase, 15 ng each receptor expression plasmid, and pGEM carrier DNA to make a total of 150 ng of DNA per well in a 96-well plate. Luciferase data were normalized to an internal ß-galactosidase control and reported values are means of triplicate assays.

4.3. Quantitative real-time PCR study

Total RNA of human hepatocellular carcinoma Huh-7 cells was isolated using an RNA preparation kit (Isogen; Nippon Gene Corp.). Total RNA (6 μg) was treated with DNase I, then the DNase I was inactivated using DNase I inactivating reagent (Ambion; TURBO DNA-free™).

First-strand cDNA was synthesized from 4 μg of total RNA of each cell sample using the SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen) with oligo(dT) 12–18 primer.

The cDNAs were then used as templates for individual PCRs using specific primer sets, which were designed by the Primer3 program (Whitehead Institute). PCRs were carried out using a QuantiTect® SYBR Green PCR Kit (Qiagen). The quantitative PCR analysis was performed using the DNA Engine Opticon® System (Bio-Rad Laboratories). Amplification specificity was verified by visualizing PCR products on an ethidium bromide-stained 2% agarose gel. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalizing each expression data.

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References and notes

1. Alberti, K. G.; Zimmet, P. Z. *Diabet. Med.* **1998**, *15*, 539.
2. Ford, E. S.; Giles, W. H.; Mokdad, A. H. I. *Diabetes Care* **2004**, *27*, 2444.
3. Cameron, A. J.; Shaw, J. E.; Zimmet, P. Z. *Endocrinol. Metab. Clin. North Am.* **2004**, *33*, 351.
4. Kereiakes, D. J.; Willerson, J. T. *Circulation* **2003**, *108*, 1552.
5. Banner, C. D.; Gottlicher, M.; Widmark, E.; Sjovall, J.; Rafter, J. J.; Gustafsson, J. A. *J. Lipid Res.* **1993**, *34*, 1583.
6. Willson, T. M.; Brown, P. J.; Sternbach, D. D.; Henke, B. R. *J. Med. Chem.* **2000**, *43*, 527.
7. Keller, H.; Dreyer, C.; Medin, J.; Mahfoudi, A.; Ozato, K.; Wahli, W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2160.
8. Okuno, A.; Tamemoto, H.; Tobe, K.; Ueki, K.; Mori, Y.; Iwamoto, K.; Umesono, K.; Akanuma, Y.; Fujiwara, T.; Horikoshi, H.; Yazaki, Y.; Kadowaki, T. *J. Clin. Invest.* **1998**, *101*, 1354.
9. Staels, B.; Auwerx, J. *Curr. Pharmaceut. Des.* **1997**, *3*, 1.
10. Lim, H.; Gupta, R. A.; Ma, W. G.; Paria, B. C.; Moller, D. E.; Morrow, J. D.; DuBois, R. N.; Trzaskos, J. M.; Dey, S. K. *Genes Dev.* **1999**, *13*, 1561.
11. Sznaidman, M. L.; Haffner, C. D.; Maloney, P. R.; Fivush, A.; Chao, E.; Goreham, D.; Sierra, M. L.; LeGrumelec, C.; Xu, H. E.; Montana, V. G.; Lambert, M. H.; Willson, T. M.; Oliver, W. R., jr.; Sternbach, D. D. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1517.
12. Oliver, W. R., jr.; Shenk, J. L.; Snaith, M. R.; Russell, C. S.; Plunket, K. D.; Bodkin, N. L.; Lewis, M. C.; Winegar, D. A.; Sznaidman, M. L.; Lambert, M. H.; Xu, H. E.; Sternbach, D. D.; Kliewer, S. A.; Hansen, B. C.; Willson, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5306.
13. Tanaka, T.; Yamamoto, J.; Iwasaki, S.; Asaba, H.; Hamura, H.; Ikeda, Y.; Watanabe, M.; Magoori, K.; Ioka, R. X.; Tachibana, K.; Watanabe, Y.; Uchiyama, Y.; Sumi, K.; Iguchi, H.; Ito, S.; Doi, T.; Hamakubo, T.; Naito, M.; Auwerx, J.; Yanagisawa, M.; Kodama, T.; Sakai, J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 15924.
14. Brown, P. J.; Smith-Oliver, T. A.; Charifson, P. S.; Tomkinson, N. C.; Fivush, A. M.; Sternbach, D. D.; Wade, L. E.; Orband-Miller, L.; Parks, D. J.; Blanchard, S. G.; Kliewer, S. A.; Lehmann, J. M.; Willson, T. M. *Chem. Biol.* **1997**, *4*, 909.
15. Kalypsys Inc. WO2004092117-A1 (2004).
16. Mahindroo, N.; Huang, C. F.; Peng, Y. H.; Wang, C. C.; Liao, C. C.; Lien, T. W.; Chittimalla, S. K.; Huang, W. J.; Chai, C. H.; Prakash, E.; Chen, C. P.; Hsu, T. A.; Peng, C. H.; Lu, I. L.; Lee, L. H.; Chang, Y. W.; Chen, W. C.; Chou, Y. C.; Chen, C. T.; Goparaju, C. M.; Chen, Y. S.; Lan, S. J.; Yu, M. C.; Chen, X.; Chao, Y. S.; Wu, S. Y.; Hsieh, H. P. *J. Med. Chem* **2005**, *48*, 8194.
17. Nomura, M.; Kinoshita, S.; Satoh, H.; Maeda, T.; Murakami, K.; Tsunoda, M.; Miyachi, H.; Awano, K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 533.
18. Miyachi, H.; Nomura, M.; Tanase, T.; Takahashi, Y.; Ide, T.; Tsunoda, M.; Murakami, K.; Awano, K. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 77.
19. Miyachi, H.; Nomura, M.; Tanase, T.; Suzuki, M.; Murakami, K.; Awano, K. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 333.
20. Nomura, M.; Tanase, T.; Miyachi, H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2101.
21. Nomura, M.; Tanase, T.; Ide, T.; Tsunoda, M.; Suzuki, M.; Uchiki, H.; Murakami, K.; Miyachi, H. *J. Med. Chem.* **2003**, *46*, 3581.
22. Kasuga, J.; Makishima, M.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 554.
23. Kasuga, J.; Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 771.
24. Mascaro, C.; Acosta, E.; Ortiz, J. A.; Marrero, P. F.; Hegardt, F. G.; Haro, D. *J. Biol. Chem.* **1998**, *273*, 8560.
25. Mandard, S.; Zandbergen, F.; Tan, N. S.; Escher, P.; Patsouris, D.; Koenig, W.; Kleemann, R.; Bakker, A.; Veenman, F.; Wahli, W.; Müller, M.; Kersten, S. *J. Biol. Chem.* **2004**, *279*, 34411.
26. Tachibana, K.; Kobayashi, Y.; Tanaka, T.; Tagami, M.; Sugiyama, A.; Katayama, T.; Ueda, C.; Yamasaki, D.; Ishimoto, K.; Sumitomo, M.; Uchiyama, Y.; Kohro, T.; Sakai, J.; Hamakubo, T.; Kodama, T.; Doi, T. *Nucl. Recept.* **2005**, *3*, 3.