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SAR-oriented discovery of peroxisome proliferator-activated receptor pan agonist with a 4-adamantylphenyl group as a hydrophobic tail

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Abstract—3-(4-Alkoxyphenyl)propanoic acid derivatives were prepared as candidate peroxisome proliferator-activated receptor (PPAR) α/δ/γ pan agonists, based on our previous SAR studies directed toward the development of subtype-selective PPAR agonists. Those studies indicated that the steric bulkiness of substituents introduced at the distal benzene ring had an important influence on PPAR activity. The finding that a 4-adamantyl derivative exhibited not only PPARα/δ activity but also significant PPARγ activity prompted us to search for structurally novel phenylpropanoic acid derivatives with more potent adipocyte differentiation activity than the well-known PPARγ agonist, rosiglitazone, as well as well-balanced PPARα and PPARδ agonistic activities. A representative phenylpropanoic acid derivative (**12**) bearing a 4-adamantylphenyl substituent proved to be a well-balanced PPAR-pan agonist with activities to regulate the expression of genes involved in lipid and glucose homeostasis, and should be useful as a candidate drug for the treatment of altered PPAR function.

The peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors belonging to the nuclear receptor superfamily; they are activated by endogenous fatty acids and their metabolites, and by synthetic ligands.[1] Three subtypes have been isolated to date: PPARα, PPARδ, and PPARγ. These subtypes share a high level of structural homology, but each has distinct physiological functions and shows a unique tissue distribution pattern. PPARα is expressed mostly in tissues involved in lipid oxidation, such as liver, kidney, skeletal and cardiac muscle, and adrenal glands. PPARγ is expressed in adipose tissue, macrophages, and vascular smooth muscles. In contrast to the specific distributions of PPARα and PPARγ, PPARδ is ubiquitously expressed.[2]

Upon ligand binding, PPARs heterodimerize with another nuclear receptor partner, retinoid X receptor (RXR), and the heterodimers regulate gene(s) expression by binding to specific consensus DNA sequences, termed peroxisome proliferator responsive element (PPRE). These elements are a direct repeat of the hexameric AGGTCA recognition motif, separated by one nucleotide (DR1), present in the promoter region of the target genes.[3]

Each of the PPAR subtypes plays a pivotal role in lipid, lipoprotein, and glucose homeostasis. PPARα regulates genes involved in fatty acid uptake, β-oxidation, and ω-oxidation. It down-regulates apolipoprotein C-III, and it also regulates genes involved in reverse cholesterol transport, such as apolipoprotein A-I and apolipoprotein A-II.[4] PPARγ is reported to be a master regulator of adipocyte differentiation, but more recent molecular-biological studies have indicated that its activation is also linked to the expression of many genes that affect energy metabolism, such as the TNF-α, leptin, and

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adiponectin genes.[5] PPARδ is the least well-defined subtype among the PPARs, but recent biological studies have disclosed that its activation significantly increases HDL cholesterol levels, and it influences glycemic control in a primate model of metabolic syndrome.[6–8] Recent studies also suggest that the overexpression of PPARδ in adipose tissue protects against diet-induced obesity in mice, and treatment with a PPARδ-selective agonist reduces weight gain without affecting food intake in fat-fed mice.

Considering the above-mentioned beneficial pharmacological effects of the PPAR subtypes, the concept of simultaneously activating all of the PPAR subtypes with a single compound, for example, a PPAR-pan agonist, is extremely attractive, especially for the treatment of metabolic syndrome, which consists of an accumulation of metabolic and cardiovascular risk factors that predispose to heart attack, stroke, heart failure, sudden cardiac death, and certain cancers. Simultaneous activation of all the PPARs might also reduce the occurence of adverse side effects, such as weight gain, fluid accumulation, and pulmonary and macular edemas (leading to increased frequency of congestive heart failure), which are often associated with PPARγ agonists, such as rosiglitazone and pioglitazone. Recently, some PPAR-pan agonists, including bezafibrate, a well-known fibrate class antilipidemic agent, have been disclosed in the literature (Fig. 1),[9–13] and among them sodelglitazar (**2**) is under phase II clinical study.[10]

Here, we present our structural development studies aimed at the discovery of novel PPAR-pan agonists based on the α-substituted phenylpropanoic acid structure as a versatile template for the creation of PPAR agonists.

We have been engaged in structural development studies of NR ligands (agonists and antagonists), based on our working hypothesis concerning the nature of the NR ligand superfamily,[14] and we have already successfully designed and synthesized a series of substituted phenylpropanoic acid human PPARα-selective agonists,[15] a PPARα/δ dual agonist,[16] and PPARδ-selective agonists.[17] Based on the SAR results obtained, our substituted-α-phenylpropanoic acid scaffold shows affinity preferentially for PPARα and PPARδ. PPAR agonists can be structurally divided into three parts, for example, (1) an acidic head part, (2) a linker part, and (3) a hydrophobic tail part, and our SAR studies clearly indicated that chemical modification of the acidic head part and the linker part of phenylpropanoic acid derivatives greatly influences the activity toward both PPARα and PPARδ, but has little effect on the activity toward PPARγ. Therefore, we turned our attention to the SAR of the hydrophobic tail part. Based on limited data, it appeared that the PPAR transactivation activity was influenced by the structural bulkiness of the substituent at the 4-position of the distal benzene ring. Therefore, we anticipated that the introduction of a bulkier substituent than a cyclohexyl group would enhance the activity toward PPARs, including PPARγ. We therefore designed and synthesized compounds bearing an adamantyl group for further study.

The synthetic routes were as follows (Chart 1). 4-Adamantyltoluene (**6**) was oxidized with O_2 in the presence of a catalytic amount of both $Co(OAc)_2$ and $Mn(OAc)_2$[18] to afford 4-adamantylbenzoic acid (**7**). Compound (**7**) was treated with thionyl chloride, followed by aqueous ammonia, to afford 4-adamantylbenzamide (**8**). This compound was amidealkylated with substituted benzaldehyde in the presence of triethylsilane and trifluoroacetic acid, followed by alkaline hydrolysis to afford the racemic target compounds (**10f**, **10h**–**10l**). Other racemic compounds were prepared by similar procedures to those used for compound (**10f**). Optically active derivatives (**12**, **13**) were prepared by the amidealkylation of compound (**8**) with an optically active benzaldehyde derivative, followed by LiOOH hydrolysis of the chiral oxazolidinone.

The PPAR transactivation activities of these compounds are summarized in Table 1. As regards PPARα and PPARδ, introduction of a bulky substituent at the 4-position of the hydrophobic tail part of the benzene ring

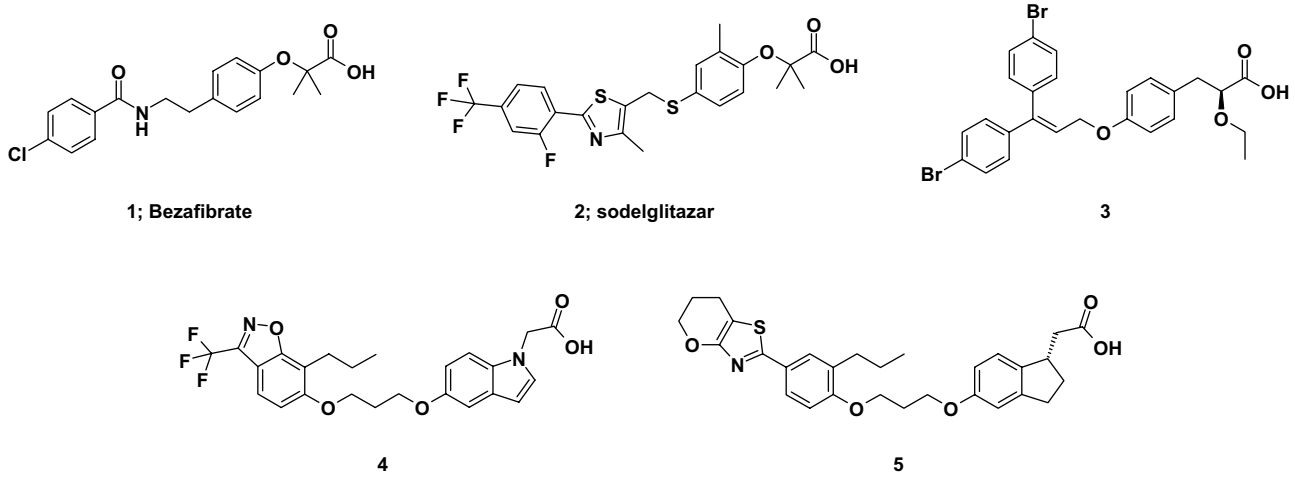


Figure 1. Chemical structures of representative PPAR-pan agonists.

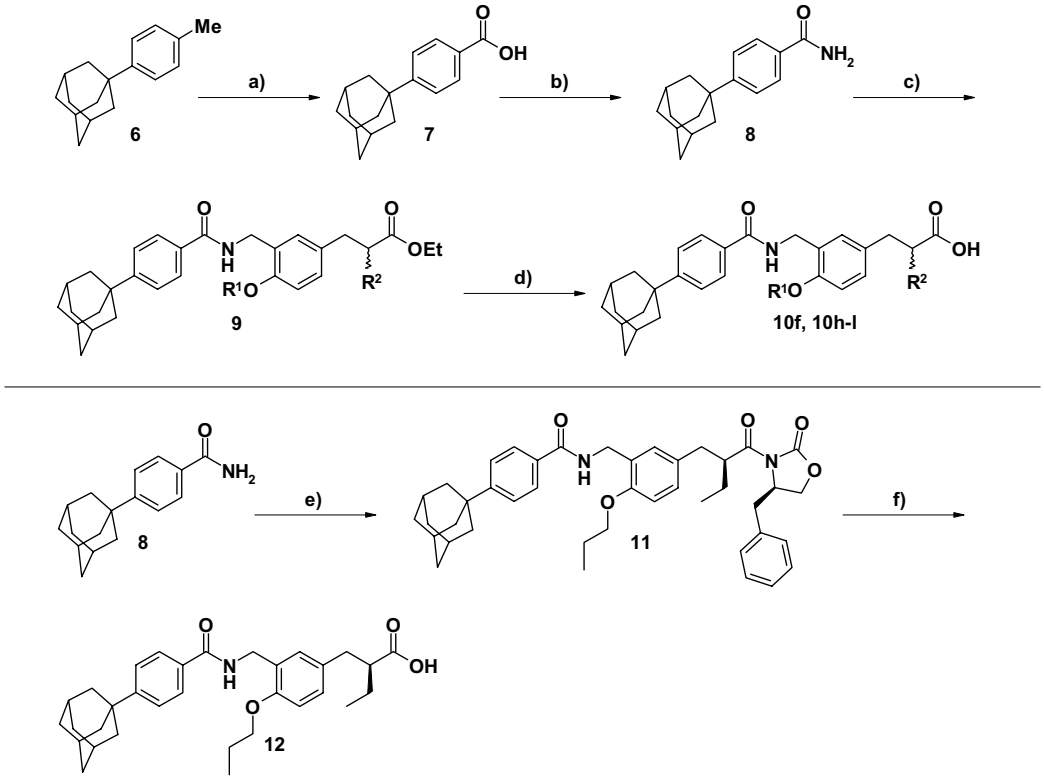


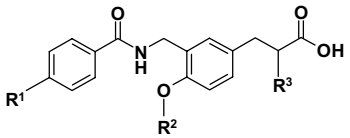
Chart 1. Synthetic route to present series of phenylpropanoic acid derivatives. Reagents and conditions: (a) $Co(OAc)_2$, $Mn(OAc)_2$, NaBr, O_2, AcOH, 1,4-dioxane, 90 °C, overnight, 94%; (b) 1—$SOCl_2$, reflux, 4 h, 2—aqNH_3, acetone, rt, overnight, quant (two steps); (c) ethyl 2-alkyl-3(4-alkoxy-3-formyl)phenylpropanoate, triethylsilane, tifluoroacetic acid, toluene, reflux, 24 h, 70%; (d) LiOHaq, EtOH, 50 °C, 6 h, 85%; (e) 5-[2-((*S*)-(*R*)-4-benzyl-2-oxooxazolidine-3-carbonyl)butyl]-2-propoxybenzaldehyde, triethylsilane, trifluoroacetic acid, toluene, reflux, 24 h, 70%; (f) LiOH H_2O, 30% H_2O_2, THF:H_2O = 4:1 (V/V), 0 °C, 85%.

was apparently effective in increasing the transactivation activity, especially for PPARα. The rank order of the activity increased in the order H (**10a**) < Me (**10b**) < *t*-Bu (**10c**) < Ph (**10d**) < cyclohexyl (**10e**). Compound **10e** exhibited nanomolar-order activity toward PPARα. The activity toward PPARγ also appeared to correlate with the sterical bulkiness of the 4-substituent on the benzene ring in the hydrophobic tail part. Although they were less active toward PPARα and PPARδ, **10d** and **10e** exhibited submicromolar PPARγ activity. Overall, compounds **10d** and **10e** exhibited potent PPARα-selective agonistic activity, combined with submicromolar PPARδ and PPARγ agonistic activity. Based on these SAR, we anticipated that the introduction of a bulkier substituent would further enhance the transactivation activity toward PPARs, including PPARγ. Therefore, we prepared the 4-adamantyl derivative **10f** and 4-(trimethyl)adamantyl derivative **10g**. As we expected, the PPARγ transactivation activity of **10f** was increased as compared with that of **10e**, while the activity toward PPARδ decreased to some extent, and that toward PPARα was greatly decreased. These results prompted us to speculate that the volume of the hydrophobic pocket hosting the 4-position of the distal benzene ring differs among the three PPAR subtypes, with PPARγ having the largest pocket, and PPARα the smallest. In order to enhance the PPARγ activity, we prepared the bulkier trimethyl adamantyl derivative **l0g**, but this showed decreased PPARγ activity. The PPARα and PPARδ activities of **10g** were also decreased as compared to those of **10f**. These results indicated that the pocket of PPARγ is not large enough to accommodate this substituent, and so an adamantyl group is preferable.

Compound **10f** proved to be PPARα/γ dual agonist with somewhat superior activity to the initial lead, KRP-297[19] (the EC_{50} values of the transactivation activity of KRP-297 for PPARα, PPARδ, and PPARγ were 300 nM, 10,000 nM, and 300 nM, respectively, in our assay system). According to our previous SAR data,[17] PPARδ activity correlated positively with the length of the alkoxyl group at the center benzene ring, and the length of the alkyl substituent at the α-position of the head part carboxylic acid moiety.

Therefore, we prepared further compounds, focusing on variation at these two positions, and we found that compound **10k** with an *n*-propoxy group at the center benzene ring exhibited improved PPARδ activity without apparent loss of PPARα and PPARγ activities as compared with those of **10f**. Compound **10k** was found to be a well-balanced PPAR-pan agonist (although compound **10j** exhibited more potent PPARγ activity than **10k**, we did not go forward with this compound, because the balance of PPAR-pan agonism was worse than that of **10k**).

Table 1. PPAR transactivation activity of phenylpropanoic acid derivatives



Compound	R^1	R^2	R^3	Stereo	EC_{50}[a] (nM)		
					PPARα	PPARδ	PPARγ
10a	H	Me	Et	rac	1700	8100	ia[b]
10b	Me	Me	Et	rac	100	3800	9400
10c	*t*-Bu	Me	Et	rac	37	720	2300
10d	Phenyl	Me	Et	rac	6	210	320
10e	Cyclohexyl	Me	Et	rac	4	300	220
10f	Adamantyl	Me	Et	rac	95	1100	120
10g	Trimethyladamantyl	Me	Et	rac	210	>10,000	340
10h	Adamantyl	Me	Me	rac	41	ia[b]	280
10i	Adamantyl	Me	*n*-Pr	rac	140	1400	120
10j	Adamantyl	Et	Et	rac	28	250	50
10k	Adamantyl	*n*-Pr	Et	rac	140	220	70
10l	Adamantyl	*n*-Hex	Et	rac	1400	810	620
12	Adamantyl	*n*-Pr	Et	*S*	61	120	43
13	Adamantyl	*n*-Pr	Et	*R*	500	870	83

[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.

Our SAR data[15–17] indicated that the (*S*)-conformer is more potent than the antipodal (*R*)-conformer in the case of our phenylpropanoic acid PPAR agonists. So, we prepared both enantiomers and reconfirmed that the (*S*)-conformer **12** is more potent than the (*R*)-conformer **13**. However, although the activity resided primarily in the (*S*)-enantiomer, the enantioselectivity was less marked than in the case of the PPARα/δ dual agonist, TIPP-401.

Finally, based on the accumulated SAR data, we prepared the human PPARα/δ/γ pan agonist **12**, which proved able to activate all three PPAR subtypes with EC_{50} values of about 100 nM or less in our assay system. The dose–response relationship of **12** is depicted in Figure 2.

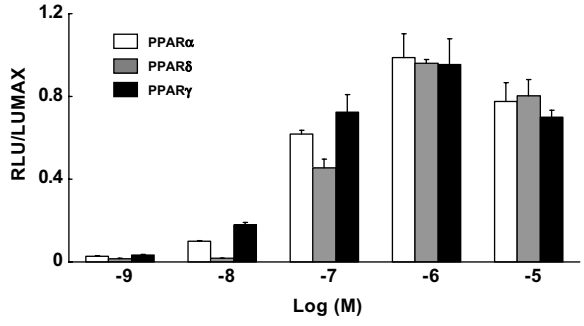


Figure 2. Dose–response relationship of **12** for PPAR transactivation. The vertical line represents the relative luciferase unit versus the maximal luciferase unit of each representative PPAR agonist. The horizontal axis represents the log scale concentration of compound **12**.

To characterize the PPARγ agonistic profile of **12**, we examined the ability of MCC-555, rosiglitazone (which are well-known PPARγ agonists), and compound **12** to stimulate adipocyte differentiation of murine preadipocyte 3T3-L1 cells (Fig. 3). PPARγ agonists promote the conversion of a variety of preadipocyte and stem cell lines into mature adipocytes.[20] All three compounds in the concentration range from 1 nM to 100 nM dose-dependently stimulated adipocyte differentiation and induced triglyceride accumulation, as indicated by Oil Red O staining and by measurements of triglyceride content (data not shown). The ability of **12** to differentiate preadipocytes is similar to that of rosiglitazone, and far more potent than that of MCC-555.

To further characterize **12** as a PPAR-pan agonist, we examined its activating effect on representative genes having a peroxisome proliferator responsive element (PPRE) in the promoter region at the cellular level in human hepatocellular carcinoma Huh-7 (Fig. 4). Carnitine palmitoyl acyl-CoA transferase 1A (CPT1A), HMG-CoA synthase 2 (HMGCS2), adipocyte differentiation-related protein (ADRP), and angiopoietin-like protein 4 (ANGPTL4) were selected, as the human genes were reported to possess PPRE in the promoter region.[21–23] CPT1A is the key enzyme in carnitine-dependent transport across the mitochondrial inner membrane and its deficiency results in a decreased rate of fatty acid β-oxidation. HMGCS2 is a potential regulatory enzyme in the pathway that converts acetyl-CoA to ketone bodies. This enzyme condenses acetyl-CoA with acetoacetyl-CoA to form HMG-CoA, which is the substrate for HMG-CoA reductase. Defects in HMGCS2 are the cause of HMG-CoA synthase deficiency, which leads to severe hypoketotic hypoglycemia,

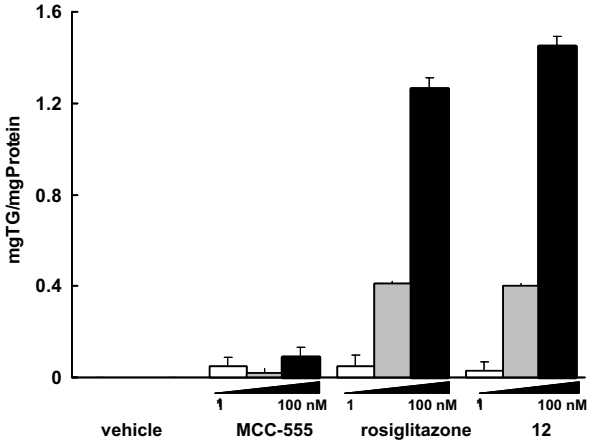


Figure 3. Induction of adipocyte differentiation by PPAR agonists: MCC-555, rosiglitazone, and compound **12**. MCC-555 and rosiglitazone are well-known PPARγ agonists. Estimation of triacylglycerol and protein content was performed as follows: 3T3-L1 cell monolayers were rinsed twice with phosphate-buffered saline (PBS-) and were dried briefly before extraction of lipids with isopropanol. Triacylglycerol was estimated by acetylacetone colorimetry as described by Fletcher.[24] The lipid cleared cell sheet was then solubilized with 1 mol/L NaOH and assayed for protein content according to the method of Lowry et al.[25] Each compound was added to medium from day 1 until day 10. ST 13 cells were harvested 10 days after cell seeding and evaluated for triacylglycerol and protein content. Values are means of triplicate wells.

mild hepatomegaly, or fatty liver. 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 the fibrate class of antihyperlipidemic agents. ADRP is associated with the globule surface membrane material, and is a major constituent of the globule surface; an increase of ADRP mRNA is one of the earliest indicators of adipocyte differentiation.

We first investigated the effects of the PPAR-subtype-selective agonists GW-7647 (abbreviated as 7647; a PPARα-selective agonist), GW-501516 (abbreviated as 1516; a PPARδ-selective agonist), and troglitazone (abbreviated as Tro; a PPARγ-selective agonist). As indicated in Figure 4, when Huh-7 cells were treated with 1 μM 7647, 0.1 μM 1516, or 10 μM Tro (these concentrations were sufficient to induce subtype-selective transactivation activity), expression of the four *m*RNAs was augmented subtype-selectively, although the nature of the selectivity was not entirely clear: CPT1A affected mainly PPARα and to a lesser extent PPARδ, HMGCS2 affected mainly PPARγ and to a lesser extent PPARα and PPARδ, ADRP affected mainly PPARγ and to a lesser extent PPARα, and ANGPTL4 affected mainly PPARγ and PPARα, but also PPARδ. These results indicated that all three PPAR subtypes are functionally active in Huh-7, and are consistent with previous reports that these three PPAR subtypes show partially overlap-

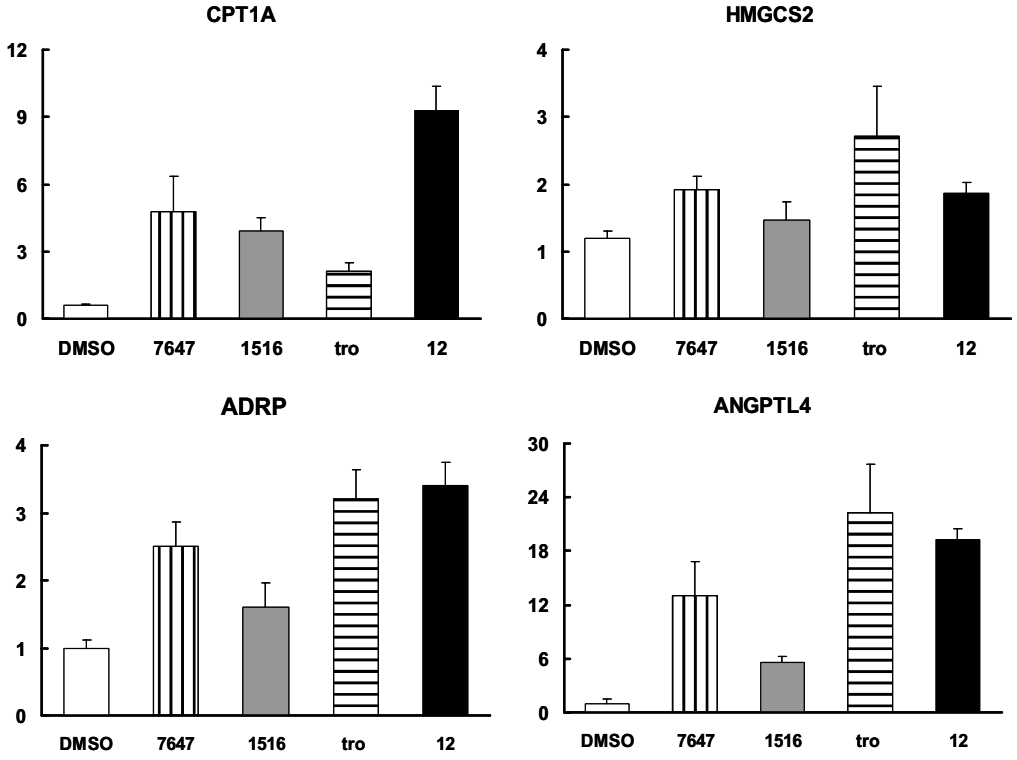


Figure 4. Induction of changes in the expression of selected genes (CPT1A, HMGCS2, ADRP, and ANGPTL4) in Huh-7 cells by several PPAR agonists. One micromolar GW-7647 (abbreviated as 7647; a PPARα-selective agonist), 0.1 μM GW-501516 (abbreviated as 1516; a PPARδ-selective agonist), and 10 μM troglitazone (abbreviated as Tro; a PPARγ-selective agonist) were used as positive control. These concentrations were sufficient to induce subtype-selective transactivation activity.

ping patterns of regulation of critical genes for lipid, lipoprotein, and glucose homeostasis.

Treatment with 1 μM **12**, a concentration sufficient to activate all three PPAR subtypes, augmented the expression of all four genes, to a similar extent to that obtained with the positive control agents. These results indicate that this representative compound (**12**) is an effective PPAR-pan agonist and can activate various kinds of PPAR-regulated genes at the cellular level.

In summary, we have developed potent human PPAR-pan agonists, which possess potent transactivation activity toward PPARs. We are planning an X-ray crystallographic analysis in combination with molecular modeling to understand the reason why an adamantyl group is favorable, as well as in vitro and in vivo pharmacological evaluations of a representative compound as a candidate anticancer and antimetabolic syndrome agent.

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