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Design, synthesis, and evaluation of potent, structurally novel peroxisome proliferator-activated receptor (PPAR) δ-selective agonists

Jun-ichi Kasuga,[a] Izumi Nakagome,[b] Atsushi Aoyama,[a] Kumiko Sako,[a] Michiyasu Ishizawa,[c] Michitaka Ogura,[c] Makoto Makishima,[c] Shuichi Hirono,[b] Yuichi Hashimoto[a] and Hiroyuki Miyachi[a,*]

[a]*Institute of Molecular and Cellular Biosciences, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan*
[b]*School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan*
[c]*School of Medicine, Nihon University, 30-1 Oyaguchi-kamicho, Itabashi-ku, Tokyo 173-8610, Japan*

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Abstract—A series of 3-(4-alkoxyphenyl)propanoic acid derivatives was prepared as candidate peroxisome proliferator-activated receptor (PPAR) δ-selective agonists, based on our previously discovered potent human PPARα/δ dual agonist TIPP-401 as a lead compound. Structure–activity relationship studies clearly indicated the importance of the chain length of the alkoxy group at the 4-position, and the *n*-butoxy compound exhibited the most potent PPARδ transactivation activity and highest PPARδ selectivity. The (*S*)-enantiomer of a representative compound exhibited extremely potent PPARδ transactivation activity, comparable with or somewhat superior to that of the known PPARδ-selective agonist, GW-501516. The representative compound regulated the expression of genes involved in lipid and glucose homeostasis, and should be useful not only as a chemical tool to study PPARδ function, but also as a candidate drug for the treatment of metabolic syndrome.

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] and differences in genetic background, diet, physical activity, age, gender, nutrition, and so on, all affect its prevalence.[3] This 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, and obesity.[1,4] Based on these criteria, it is obvious that losing weight and restoration of serum glucose and serum lipid parameters to normal levels are of primary importance. From this point of view, the identification of molecular targets critically involved in the regulation of energy balance and glucose and lipid homeostasis is important for the creation of the new therapeutic agents for the treatment of metabolic syndrome. Nuclear receptors (NRs) are particularly attractive molecular targets to study, since they play a central role in maintaining energy balance and in cellular and whole-body glucose and lipid homeostasis. The NRs are activated by a wide variety of small physiological ligands, such as dietary unsaturated fatty acids, their metabolites, and various xenobiotics, thereby modulating the transcriptional networks of the target response genes. Among these receptors, special attention

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* Corresponding author. Tel.: + 81 03 5841 7848; fax: +81 03 5841 8495; e-mail: miyachi@iam.u-tokyo.ac.jp

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has been paid to the members of the peroxisome proliferator-activated receptor (PPAR) family (NR1Cs) for more than a decade.

PPARs are activated by endogenous saturated and unsaturated fatty acids and their metabolites, as well as synthetic ligands.[5] Three subtypes of PPARs have been identified to date, that is, PPARα, PPARγ, and PPARδ.[6] Each PPAR subtype is differentially expressed in a tissue-specific manner, and they have pivotal roles in lipid, lipoprotein, and glucose homeostasis.[7] PPARα is mostly expressed in the tissues involved in lipid oxidation, such as liver, kidney, skeletal, cardiac muscle, and adrenal glands.[8] PPARγ is expressed in adipose tissue, macrophages, and vascular smooth muscles.[9] In contrast, PPARδ is ubiquitously expressed, though it is mainly found in skeletal muscle and adipose tissues.[10] PPARs function after heterodimerization with another nuclear receptor partner, retinoid X receptor (RXR), and regulate gene expression by binding to a specific consensus DNA sequence, termed PPRE (peroxisome proliferator responsive element), located in the promoter region of target genes.[7]

Based on the findings that the glitazone-class antidiabetic agents and fibrate-class antidyslipidemic agents are ligands of PPARγ, and PPARα, respectively, much research interest has been focused on these two metabolic NR subtypes as therapeutic targets for the treatment of type II diabetes and dyslipidemia. In contrast, research interest in PPARδ has been limited, perhaps because of its ubiquitous distribution. However, the availability of PPARδ-knockout animals and selective ligands, especially GW-501516, developed by Glaxo-SmithKline, prompted us to examine the involvement of PPARδ in fatty acid metabolism, insulin resistance, reverse cholesterol transport, inflammation, and so on.[11] For example, ligand-mediated PPARδ activation significantly increased HDL-cholesterol levels, possibly in association with decreased lipoprotein lipase activity, in insulin-resistant middle-aged obese rhesus monkeys.[12] In a primate model of the metabolic syndrome, PPARδ activation lowered plasma insulin levels, without any adverse effect on glycemic control.[12] Similarly, in the case of ob/ob mice, PPARδ activation markedly improved glucose tolerance and insulin resistance,[12] although the underlying mechanism remains unclear.[13]

In addition to these animal-model studies, recent preliminary phase I/II clinical studies of GW-501516 demonstrated that a PPARδ agonist elevated HDL-cholesterol levels (phase I) and decreased triglyceride (phase I, phase I/II), total cholesterol (phase I/II), and apoB100 levels (phase I/II).[14] All these observations suggest that PPARδ may be an effective target for the treatment of metabolic syndrome.

Several PPARδ-selective agonists have been reported in the literature, though most are derivatives of GW-501516 and L-165041, that is, (2-methyl)phenoxyacetic acid derivatives.[15–20] We designed and synthesized a series of substituted phenylpropanoic acid derivatives as human PPARα/δ dual agonists, using KCL, a PPARα selective agonist with the 2-methoxybenzamide structural motif, as a lead.[21–25] As a part of our continuing research directed toward the structural development of characteristic subtype-selective PPAR agonists, we tried to construct structurally new PPAR/δ selective agonists.

Previously, we have designed and synthesized a potent human PPARα/δ dual agonist, TIPP401, based on KCL, a PPARα-selective agonist, as a lead compound (Fig. 1).[27–29] Two structural modifications of KCL, that is, a change of the linking group from $–CH_2NHCO–$ to $–CONHCH_2–$, and the introduction of a fluorine atom at the 2-position of the distal benzene ring, affording TIPP401, had increased the PPARδ transactivation

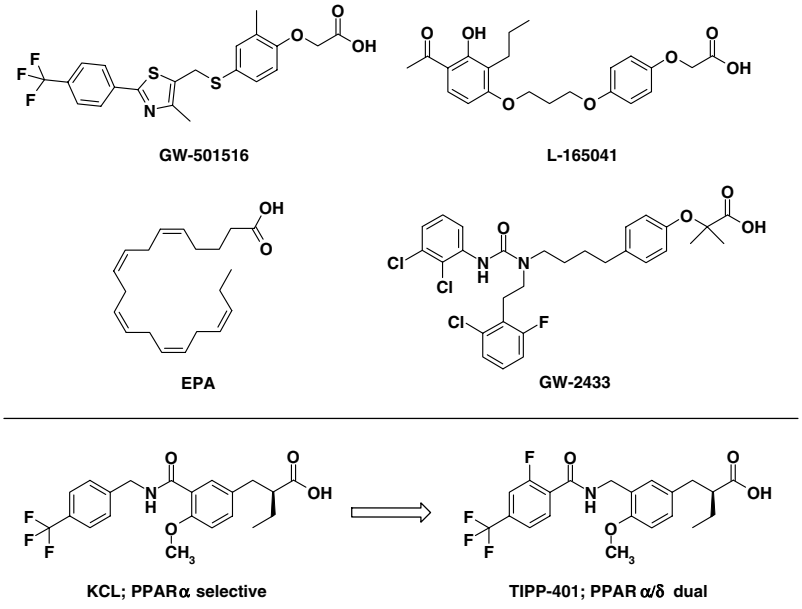


Figure 1. Structures of the representative ligands possessing PPARδ activity, GW-501516, L-165041, eicosapentaenoic acid (EPA), GW-2433, and our PPAR ligands KCL and TIPP-401.

activity with retention of the PPARα activity. We anticipated that if we could further increase the PPARδ activity of TIPP-401, while weakening its PPARα activity, we might obtain a structurally new type of PPARδ-selective agonist, distinct from the well-known phenoxyacetic acid derivative GW-501516.

In order to create PPARδ-selective agonists based on TIPP-401, we took into account the results of X-ray crystallographic analyses of PPARδ complexed with a natural unsaturated fatty acid, eicosapentaenoic acid (EPA), and the synthetic ligand GW-2433.[30] The PPAR ligand-binding pocket is reported to form a large Y-shaped cavity that extends from the C-terminal helix to the β-sheet lying between helices H3 and H6. EPA binds to the cavity in two distinct conformations, that is, 'tail-up' and 'tail-down' conformations. The carboxyl group and the first eight carbon units take almost the same configuration in both conformations. However, the distal hydrophobic tail part of the tail-up conformer of EPA was bent upwards into the upper cavity of the Y-shaped pocket, while in the tail-down conformer, the hydrophobic tail part was bent downwards into the bottom cavity of the Y-shaped pocket. In the case of the phenoxyacetic acid derivative GW-2433, which exhibited moderate PPARα/δ dual activity, there are two hydrophobic tail parts, and the two substituents project into the two cavities occupied by the hydrophobic tail part of EPA in the two different binding conformers. Contrary to the case of PPARδ, none of the PPARα and PPARγ agonists whose binding modes have been solved by X-ray crystallography takes the tail-up conformation, although the reason for this is not yet known. However, we speculated that the amino acid(s) forming the entrance to the upper cavity might be bulkier in PPARα and PPARγ than in PPARδ. We previously suggested that our PPARα-selective agonist KCL might take a tail-down conformation, based on molecular modeling studies of the KCL–PPARα complex and the results of site-directed mutagenesis studies of PPARα (Fig. 2).[31,32] TIPP-401 was also considered to dock into the downward cavity of PPARα, because Ile272, which is located on the lower half of helix 3, is critical for potent PPARα transactivation by TIPP-401.

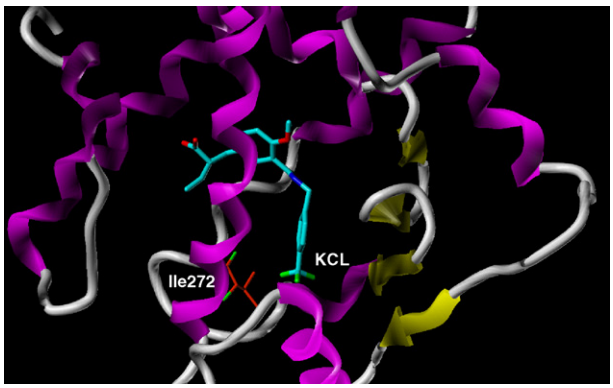


Figure 2. Molecular modeling of KCL docked to human PPARα ligand-binding domain.

Based on these insights, our working hypothesis was that, if we could connect one more sterically bulky hydrophobic side chain to the backbone of TIPP-401, directed toward the upper cavity of PPARδ, it should have the effect of strengthening the PPARδ activity, while weakening the PPARα activity.

Based on our previously reported binding model of KCL,[32] a methoxy group at the 4-position was expected to be directed toward the upper cavity, so we prepared various 3-(4-alkoxyphenyl)propanoic acids (Table 1).

2. Chemistry

The synthetic routes to the present series of compounds are outlined in Schemes 1 and 2. Compounds (**5a**–**e**, **5g**–**i**) were prepared from 4-alkoxybenzaldehydes (**1a**–**f**) in six steps. 4-Alkoxybenzaldehydes were treated with triethyl 2-phosphonobutyrate in the presence of *t*-BuOK as a base, followed by hydrogenolysis, to afford ethyl phenylpropionate derivatives (**2a**–**f**). In the case of the preparation of **2f**, rebenzylation of phenolic hydroxyl group was needed. The formylation of **2a**–**f** to afford **3a**–**f**, which was amidealkylated with 4-(trifluoromethyl)benzamide derivatives. Subsequent alkaline hydrolysis afforded the desired products **5a**–**e**, **5g**–**i**. The 4-benzyloxy derivative (**5f**) was prepared by hydrogenolysis of **4f**, rebenzylation with benzyl bromide, and then alkaline hydrolysis.

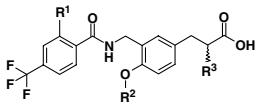
Compounds (**10a**–**g**), with various alkyl substituents at the α-position of the carboxylic acid, were prepared from 4-alkoxybenzaldehydes (**1a** and **1d**) in five steps, using a similar reaction sequence to that employed for the synthesis of compounds **5a**–**e**, **5g**–**i**.

An optically active phenylpropanoic acid derivative, (*S*)-**5i**, was prepared, based on Evans's asymmetric alkylation method as the key step, as depicted in Scheme 2. 5-Formylsalicylic acid (**11**) was benzylesterified (**12**) and *n*-butoxylated to give compound **13**. The formyl group of **13** was reduced with $NaBH_4$ (**14**) and bromination of the hydroxyl group afforded the bromomethyl derivative (**15**). (*R*)-*N*-Butyryl-4-benzyloxazolidinone was treated with **15** under Evans's asymmetric alkylation protocol,[26] followed by hydrogenolysis to afford the key synthetic intermediate **17**. This was reduced with BH_3–THF (**18**), and then oxidation with activated MnO_2 afforded the formyl derivative **19**. This was amidealkylated with 2-fluoro-4-(trifluoromethyl)benzamide, followed by the removal of the chiral auxiliary to afford the desired (*S*)-configuration product, (*S*)-**5i** (Scheme 2). The antipodal (*R*) enantiomer was prepared via similar procedures, using (*S*)-*N*-butyryl-4-benzyloxazolidinone as the reagent.

3. Results and discussion

As regards PPARα, introduction of a short-chain alkoxy group was found to be preferable for the

Table 1. PPARs transactivation activity of the present series of compounds



Compound	R^1	R^2	R^3	EC_{50} (nM)		
				PPARα	PPARδ	PPARγ
5a	H	Me	Et	18 ± 8	170 ± 40	2600 ± 400
5b	H	Et	Et	12 ± 3	15 ± 4	1800 ± 900
5c	H	*n*-Pr	Et	77 ± 11	6.8 ± 0.6	1300 ± 100
5d	H	*n*-Bu	Et	520 ± 120	4.8 ± 0.6	1300 ± 100
5e	H	*n*-Hexyl	Et	1200 ± 300	45 ± 5	2300 ± 200
5f	H	Bn	Et	>10,000	110 ± 20	6200 ± 1500
5g	F	Me	Et	8.2 ± 1.4	21 ± 1	2200 ± 500
5h	F	*n*-Pr	Et	41 ± 8	1.7 ± 0.1	650 ± 10
5i	F	*n*-Bu	Et	280 ± 40	1.9 ± 0.2	1400 ± 200
10a	H	Me	H	210 ± 40	3000 ± 300	>10,000
10b	H	Me	Me	19 ± 3	210 ± 30	>10,000
10c	H	Me	*n*-Pr	70 ± 21	120 ± 10	3600 ± 600
l0d	H	Me	*n*-Bu	230 ± 30	700 ± 150	2400 ± 100
l0e	F	Me	*n*-Pr	29 ± 5	40 ± 6	1500 ± 100
l0f	F	Me	*n*-Bu	140 ± 20	220 ± 20	1000
l0g	F	*n*-Bu	*n*-Pr	820 ± 60	5.1 ± 0.5	1000 ± 170
GW-501516				1000 ± 460	1.8 ± 0.3	8600 ± 1200

transactivation activity, that is, the ethoxy (**5b**) and methoxy (**5a**) derivatives exhibited the most potent activity. In contrast, a longer alkoxy group was preferable in the case of PPARδ transactivation activity, and the *n*-butoxy (**5d**) and *n*-propoxy (**5c**) derivatives were the most potent. These results are consistent with the idea that the shape and the environment of the hydrophobic cavity hosting the alkoxy group at the 4-position differ somewhat among these PPAR subtypes. These compounds were basically weak agonists for the PPARγ subtype, with each compound exhibiting an EC_{50} value of micromolar order or less.

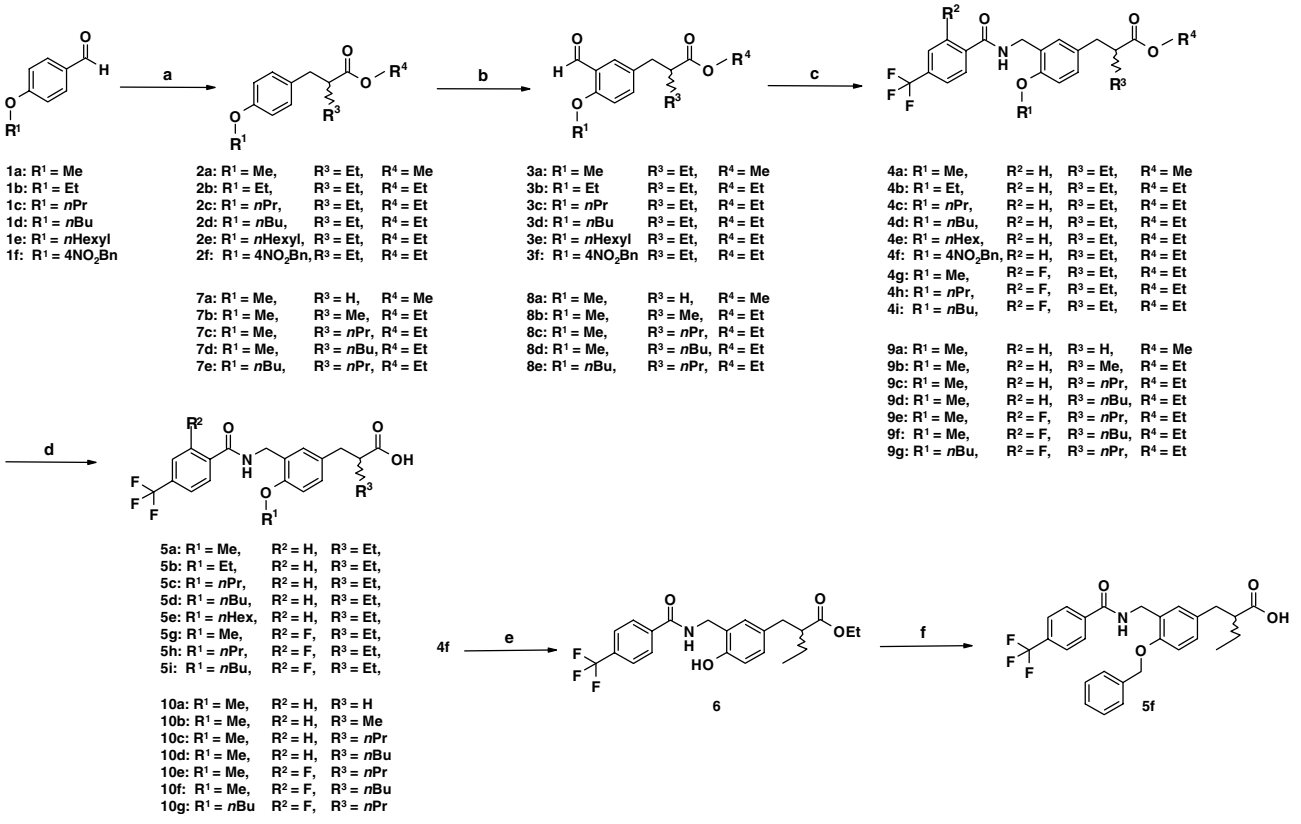
As the introduction of a fluorine atom on the distal benzene ring, especially at the 2-position, was reported to enhance the PPAR transactivation activity,[27,29] we prepared compounds **5g–i**. As expected, **5h** and **5i** exhibited enhanced PPARα and PPARδ transactivation activities as compared with the non-fluorinated compounds **5c** and **5d**. The PPARδ transactivation activity of **5i** is comparable with that of GW-501516 in our assay system, and the selectivity for PPARδ over both PPARα and PPARγ is more than 100-fold.

As already mentioned, the substituent at the α-position of the carboxyl group is also important for the potency in case of PPARα,[22,25] and therefore we investigated the effect of substitution at this position in the present series of compounds (Table 1). As regards PPARα, introduction of an ethyl group (**5a**) or a methyl group (**10b**) was favorable for the transactivation activity, and further elongation of the substituent decreased the activity. Similarly, an ethyl group (**5a**) or a *n*-propyl group (**10c**) was favorable for PPARδ transactivation activity, and further elongation of the substituent decreased the activity. These results may mean that the shape and the environment of the cavity hosting the alkyl group located at the α-position of the carboxyl group are similar in PPARα and PPARδ (Table 2).

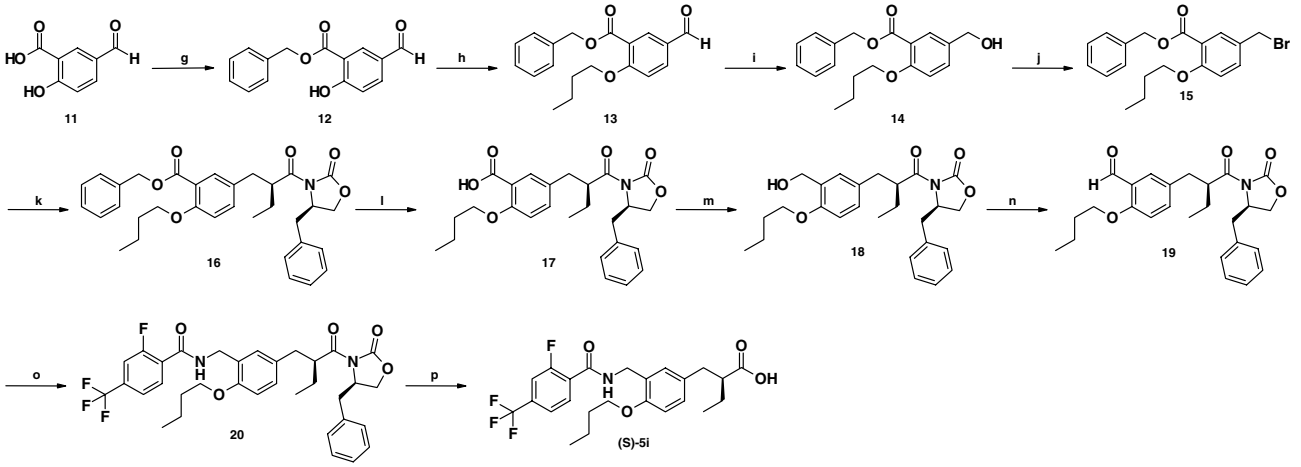
Considering the results obtained above, we then prepared the optically active derivatives (***S***)-**5i** and (***R***)-**5i**. Clear enantio-dependency of the transactivation activity toward the PPAR subtypes was found, and (***S***)-**5i**, which has *S* configuration, exhibited more potent transactivation activity than the antipodal *R* isomer, (***R***)-**5i**. Therefore, we concluded that the activity resides primarily in the (*S*)-enantiomer, but the enantio-selectivity is less apparent than in the case of the PPARα/δ dual agonist, TIPP-401.[29] (*S*)-**5i** exhibited extremely potent PPARδ transactivation activity, comparable with or somewhat superior to that of the known PPARδ-selective agonist GW-501516, and its PPAR subtype selectivity was also high (Fig. 3).

In order to investigate the nuclear receptor selectivity (cross-reactivity) of (*S*)-**5i**, we determined the transactivation activity of (*S*)-**5i** on representative nuclear receptors (PPARs, VDR, FXR, LXRα, RARα, and RXRα). As can be seen from Figure 4, (*S*)-**5i** seems to be specific for PPARδ (and to a lesser extent to PPARα) because it did not significantly activate VDR, PPARγ, LXRα, RARα or RXRα at concentrations up to 300 nM (more than 300-fold higher concentration as compared to that of EC_{50} of (*S*)-**5i**) 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 of (*S*)-**5i** to PPARδ.

Moreover, to assess the ability of (*S*)-**5i** to activate genes which have a peroxisome proliferator-responsive



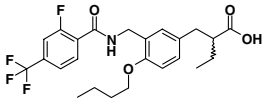
Scheme 1. Reagents and conditions: (a) (i) $(EtO)_2POCH(R^3)CO_2Et$, *t*-BuOK, THF, rt; (ii) H_2, 10% Pd–C, AcOEt, rt; (b) $TiCl_4$, $MeOCHCl_2$, CH_2Cl_2, −30 °C; (c) benzamide derivative, $(Et)_3SiH$, TFA, toluene, reflux; (d) aq LiOH, EtOH, rt; (e) H_2, 10% Pd–C, AcOEt, rt; (f) (i) BnBr, K_2CO_3, DMF, rt; (ii) same as in (d).



Scheme 2. Reagents and conditions: (g) BnBr, $KHCO_3$, DMF, rt; (h) *n*-BuI, K_2CO_3, DMF, rt; (i) $NaBH_4$, EtOH, rt; (j) PBr_3, ether, 0 °C; (k) LiHMDS, (*S*)-3-*n*-butyryl-4-benzyloxazolidine-2-one, THF, −30 to 0 °C; (l) H_2, 10% Pd–C, AcOEt, rt; (m) BH_3–THF, THF, 0 °C, rt; (n) MnO_2, CH_2Cl_2, rt; (o) 2-fluoro-4-trifluoromethylbenzamide, $(Et)_3SiH$, TFA, toluene, reflux; (p) $LiOH{\cdot}H_2O$, 30% H_2O_2, THF–H_2O.

element (PPRE) in the promoter region at the in vivo level, we examined changes in the PPAR-mediated expression of representative genes in mice. We chose peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) and uncoupling protein 3 (UCP3) as representative PPAR target genes, since the human genes were reported to possess PPRE in the promoter region. PGC-1α is a potent transcriptional coactivator for nuclear receptors.[33,34] PGC-1α, which is expressed in skeletal muscle, is able to induce mitochondrial myogenesis and to induce preferential synthesis of slow fibers.[35] UCP3 belongs to a family of mitochondrial uncoupling proteins which uncouple oxidative phosphorylation, thereby increasing thermogenesis and decreasing the formation of ATP.[36] Recent evidence also points to a role of UCP3 in the export of ionized fatty acids and lipid peroxides from the mitochondria.[37]

Table 2. Comparison of the enantio-selectivity of **5i** for transactivation of PPARs



Compound	EC_{50} (nM)		
	PPARα	PPARδ	PPARγ
(*S*)-**5i**	250 ± 40	0.91 ± 0.2	1100 ± 160
(*R*)-**5i**	620 ± 60	8.3 ± 1.5	7000 ± 1900
GW-501516	1000 ± 460	1.8 ± 0.3	8600 ± 1200

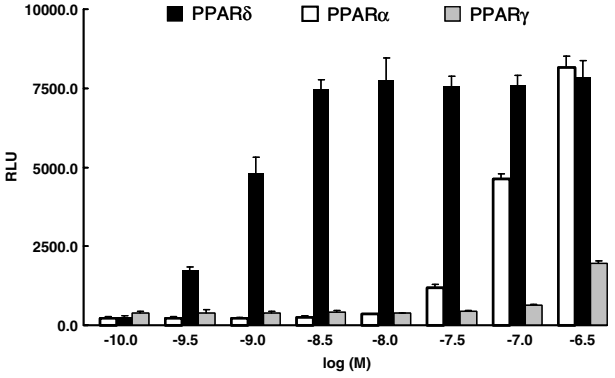


Figure 3. Dose-dependency of (*S*)-**5i** for transactivation of PPARs.

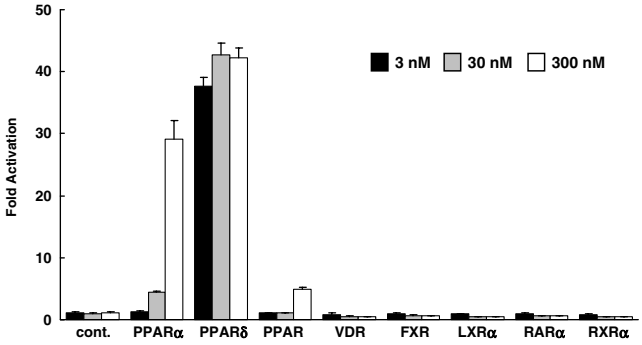


Figure 4. Nuclear receptor selectivity (cross-reactivity) of (*S*)-**5i**.

We first investigated the effects of bezafibrate (Beza; 30 mg, sc), which has PPARδ transactivation activity. As indicated in Figure 5, 30 mg Beza augmented expression of the PGC-1α and UCP3 genes by 2.5- and 10-fold, respectively. Treatment with 3 mg (*S*)-**5i**, about one-tenth of the amount of Beza, also augmented expression of the PGC-1α and UCP3 genes by 2.5- and 15-fold, respectively. These results indicate that the representative compound (*S*)-**5i** is an effective PPARδ-selective agonist at the in vivo level.

We have successfully obtained a potent and selective, structurally novel PPARδ agonist, (*S*)-**5i**. In order to investigate the structure–activity relationship, and the reason for the PPARδ selectivity, we analyzed the three-dimensional structure–activity relationship by means of the comparative molecular field analysis (CoMFA)[38,39] method, and the molecular modeling

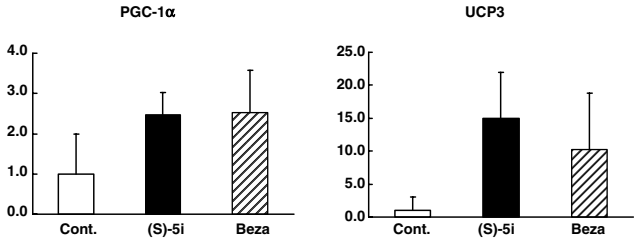


Figure 5. Assay of gene induction by (*S*)-**5i**. Tissues were harvested from C57BL/6 mice dosed for 3 days with vehicle, (*S*)-**5i** (3 mg/kg), or bezafibrate (30 mg/kg) by sc. The expression of PGC-1α and UCP3 genes was analyzed by real-time PCR analysis. Induction (fold) is reported relative to the vehicle control.

study (Figs. 6 and 7) (details will be reported elsewhere). Comparison of the CoMFA counterplots with the crystal structure of the PPARδ ligand-binding domain provided information about how the structural changes of the agonists affect their activities. As can be seen in Figure 6, hydrogen bonding interaction was observed between carbonyl oxygen of the reversed-amide type linker and threonine 288 (T288) of PPARδ (Fig. 6, left panel), while such a hydrogen-bonding interaction was not found between carbonyl oxygen of the amide-type linker and T288 (Fig. 6, right panel). This might be one of the reasons why the change of the linker from amide type to reversed-amide type enhanced the PPARδ transactivation activity by 10-fold.

In this CoMFA model (Fig. 7), the introduction of a sterically bulky group near the methoxy group at the 4-position in the present series favors the activity, and this was deduced to be related to the presence of the upper pocket in the Y-shaped ligand-binding domain of PPARδ. We speculate that the side chain *n*-butoxy group of (*S*)-**5i** fits into the upper pocket formed by the hydrophobic amino acids L330, K367, and F368. Further molecular modeling and X-ray crystallographic studies are on-going.

In summary, we have developed a series of potent human PPARδ-selective agonists, which possess potent PPARδ transactivation activity with high selectivity over the other PPAR subtypes. In vivo pharmacological evaluation of a representative compound is planned.

4. Experimental

4.1. General

Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-GX500 (500 MHz) spectrometer. Chemical shifts are expressed in parts per million relative to tetramethylsilane. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer.

4.1.1. Ethyl 2-[1-(4-methoxyphenyl)methyl]butyrate (2a). Potassium *t*-butoxide (5.6 g, 49.9 mmol) was suspended

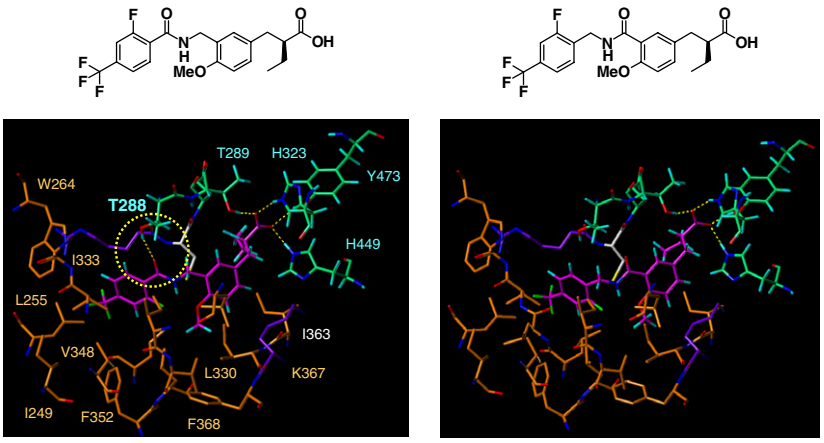


Figure 6. Predicted mode of binding of the amide derivative and the reversed-amide derivative to PPARδ. The steric map is shown in green and yellow, and the electrostatic map in blue and red. Orange, hydrophobic amino acids; green, hydrophilic amino acids; magenta, amide derivative and reversed-amide derivative. Hydrogen bonds are shown as yellow dotted lines.

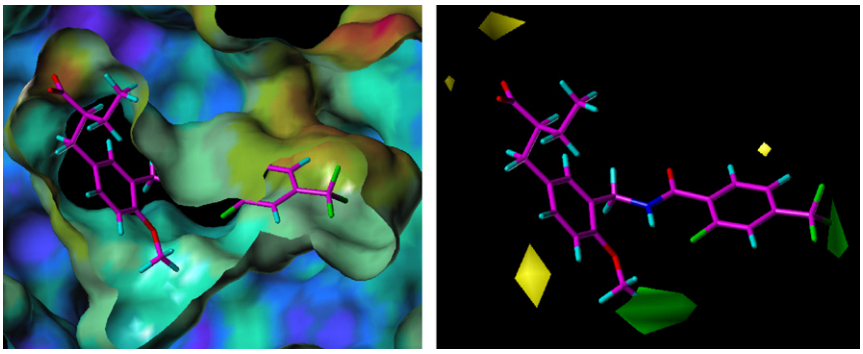


Figure 7. Comparison of the CoMFA counterplot of the steric field based on TIPP-401 (right) and the superimposition of TIPP-401 on the ligand-binding domain of PPARδ (left). The CoMFA steric counter map is shown in green and yellow. Green, areas in which bulky atomic groups are sterically favorable for the activity; yellow, areas in which bulky atomic groups are unfavorable for the activity.

in 15 mL of dehydrated tetrahydrofuran under argon and cooled with ice. Triethyl 2-phosphonobutyrate (12.0 mL, 50.4 mmol) dissolved in 15 mL of dehydrated tetrahydrofuran was added dropwise. When the addition was completed, the mixture was stirred for 1 h, then 4-methoxybenzaldehyde (5.45 g, 40.0 mmol) was added dropwise. The mixture was stirred for 6 h at room temperature. The reaction mixture was concentrated and mixed with ice-water. The whole was extracted with ethyl acetate, washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluant; *n*-hexane/ethyl acetate = 3:1, v/v) to afford 9.31 g (99%) of the title compound as colorless crystals (mixture of the geometric isomers). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.36 (d, J = 8.5 Hz, 2H), 6.92 (d, J = 8.5 Hz, 2H), 4.26 (q, J = 7.3 Hz, 2H), 3.83 (s, 3H), 2.57 (q, J = 7.3 Hz, 2H), 1.34 (t, J = 7.3 Hz, 3H), 1.18 (t, J = 7.3 Hz, 3H); MS (FAB) 235 $(M+H)^+$.

This compound (9.31 g, 39.7 mmol) was hydrogenated with 10% Pd–C (1.00 g) in 100 mL of ethanol affording 9.38 g (y; quant.) of the title compound. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.07 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 4.07 (m, 2H), 3.78 (s, 3H), 2.86 (dd, J = 13.7, 8.5 Hz, 1H), 2.69 (dd, J = 13.7, 6.4 Hz, 1H), 2.53 (m, 1H), 1.63 (m, 1H), 1.55 (m, 1H), 1.16 (t, J = 7.3 Hz, 3H), 0.91 (t, J = 7.3 Hz, 3H); MS (FAB) 237 $(M+H)^+$.

4.1.2. Ethyl 2-[1-(4-ethoxyphenyl)methyl]butyrate (2b). This compound was prepared from 4-ethoxybenzaldehyde and triethyl 2-phosphonobutyrate by means of a procedure similar to that used for **2a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06 (d, J = 8.8 Hz, 2H), 6.79 (d, J = 8.8 Hz, 2H), 4.09–4.04 (m, 2H), 4.00 (q, J = 7.1 Hz, 2H), 2.86 (dd, J = 13.9, 8.6 Hz, 1H), 2.68 (dd, J = 13.9, 6.8 Hz, 1H), 2.55–2.49 (m, 1H), 1.66–1.52 (m, 2H), 1.39 (t, J = 7.1 Hz, 3H), 1.16 (t, J = 7.3 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H); MS (FAB) 250 (M^+).

4.1.3. Ethyl 2-[1-(4-*n*-propoxyphenyl)methyl]butyrate (2c). This compound was prepared from 4-*n*-propoxybenzaldehyde and triethyl 2-phosphonobutyrate by means of a procedure similar to that used for **2a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 4.07 (m, 2H), 3.88 (t, J = 6.8 Hz, 2H), 2.86 (dd, J = 13.7, 8.5 Hz, 1H), 2.68

(dd, J = 13.7, 6.8 Hz, 1H), 2.52 (m, 1H), 1.79 (m, 2H), 1.63 (m, 1H), 1.55 (m, 1H), 1.17 (t, J = 7.3 Hz, 3H), 1.02 (t, J = 7.7 Hz, 3H), 0.91 (t, J = 7.3 Hz, 3H); MS (FAB) 264 (M^+).

4.1.4. Ethyl 2-[1-(4-*n*-butoxyphenyl)methyl]butyrate (2d). This compound was prepared from 4-*n*-butoxybenzaldehyde and triethyl 2-phosphonobutyrate by means of a procedure similar to that used for **2a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (d, J = 8.5 Hz, 2H), 6.79 (d, J = 8.5 Hz, 2H), 4.07 (m, 2H), 3.93 (t, J = 6.4 Hz, 2H), 2.76 (m, 2H), 2.52 (m, 1H), 1.75 (m, 2H), 1.62 (m, 2H), 1.48 (m, 2H), 1.16 (t, J = 7.3 Hz, 3H), 0.97 (t, J = 7.3 Hz, 3H), 0.91 (t, J = 7.3 Hz, 3H); MS (FAB) 278 (M^+).

4.1.5. Ethyl 2-[1-(4-*n*-hexyloxyphenyl)methyl]butyrate (2e). This compound was prepared from 4-*n*-hexyloxybenzaldehyde and triethyl 2-phosphonobutyrate by means of a procedure similar to that used for **2a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06 (d, J = 8.5 Hz, 2H), 6.79 (d, J = 8.5 Hz, 2H), 4.07 (m, 2H), 3.92 (t, J = 6.8 Hz, 2H), 2.86 (dd, J = 13.9, 8.5 Hz, 1H), 2.67 (dd, J = 13.9, 6.4 Hz, 1H), 2.52 (m, 1H), 1.76 (m, 2H), 1.63 (m, 1H), 1.55 (m, 1H), 1.45 (m, 2H), 1.33 (m, 4H), 1.17 (t, J = 7.3 Hz, 3H), 0.91 (m, 6H); MS (FAB) 306 (M^+).

4.1.6. Ethyl 2-{1-[4-(4-nitrophenyl)methoxyphenyl]methyl} butyrate (2f). This compound was prepared from 4-benzyloxybenzaldehyde and triethyl 2-phosphonobutyrate by means of a procedure similar to that used for **2a**, and subsequent 4-nitrobenzylation. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, J = 8.6 Hz, 2H), 7.59 (d, J = 8.6 Hz, 2H), 7.09 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.6 Hz, 2H), 5.14 (s, 2H), 4.04–4.09 (m, 2H), 3.92 (t, J = 6.8 Hz, 2H), 2.86 (dd, J = 13.9, 8.5 Hz, 1H), 2.67 (dd, J = 13.9, 6.4 Hz, 1H), 2.87 (dd, J = 14,1, 9.0 Hz, 1H), 2.70 (dd, J = 14.1, 6.8 Hz, 1H), 2.50–2.56 (m, 1H), 1.51–1.67 (m, 2H), 1.16 (t, J = 7.3 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H); MS (FAB) 357 (M^+).

4.1.7. Ethyl 2-[1-(3-formyl-4-methoxyphenyl)methyl]-butyrate (3a). To a solution of **2a** (9.38 g, 39.7 mmol) and 500 mL of anhydrous dichloromethane was added titanium tetrachloride (30.0 mL), followed by dichloromethylmethylether (12.0 mL) at −20 °C, under argon atmosphere. The mixture was stirred overnight at room temperature. The mixture was poured into 30 mL of 1.5 mol/L HCl and separated the dichloromethane layer. The organic layer was washed with satd $NaHCO_3$ solution and brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluant; *n*-hexane/ethyl acetate = 4:1, v/v) to afford 10.5 g (y; quant.) of the title compound as a pale brown oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.43 (s, 1H), 7.62 (d, J = 2.6 Hz, 1H), 7.35 (dd, J = 8.5, 2.6 Hz, 1H), 6.90 (d, J = 8.5 Hz, 1H), 4.06 (m, 2H), 3.90 (s, 3H), 2.88 (dd, J = 13.7, 9.0 Hz, 1H), 2.72 (dd, J = 13.7, 6.4 Hz, 1H), 2.54 (m, 1H), 1.63 (m, 1H), 1.55 (m, 1H), 1.16 (t, J = 7.3 Hz, 3H), 0.91 (t, J = 7.3 Hz, 3H); MS (FAB) 265 (M+H)$^+$.

4.1.8. Ethyl 2-[1-(4-ethoxy-3-formylphenyl)methyl]butyrate (3b). This compound was prepared from **2b** by means of a procedure similar to that used for **3a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.5 (s, 1H), 7.62 (d, J = 2.6 Hz, 1H), 7.33 (dd, J = 8.6 Hz, 2.6 Hz, 1H), 6.88 (d, J = 8.6 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 4.09–4.03 (m, 2H), 2.88 (dd, J = 14.1, 8.6 Hz, 1H), 2.72 (dd, J = 14.1, 6.4 Hz, 1H), 2.57–2.51 (m, 1H), 1.67–1.51 (m, 2H), 1.46 (t, J = 7.1 Hz, 3H), 1.17 (t, J = 7.3 Hz, 3H), 0.92 (t, J = 7.7 Hz, 3H); MS (FAB) 279 (M+H)$^+$.

4.1.9. Ethyl 2-[1-(3-formyl-4-*n*-propoxyphenyl)methyl]-butyrate (3c). This compound was prepared from **2c** by means of a procedure similar to that used for **3a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.49 (s, 1H), 7.63 (d, J = 2.3 Hz, 1H), 7.33 (dd, J = 8.5, 2.3 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 4.06 (m, 2H), 4.01 (t, J = 6.4 Hz, 2H), 2.88 (dd, J = 13.9, 8.5 Hz, 1H), 2.71 (dd, J = 13.9, 6.4 Hz, 1H), 2.54 (m, 1H), 1.86 (m, 2H), 1.63 (m, 1H), 1.54 (m, 1H), 1.17 (t, J = 7.3 Hz, 3H), 1.06 (t, J = 7.3 Hz, 3H), 0.91 (t, J = 7.3 Hz, 3H); MS (FAB) 293 (M+H)$^+$.

4.1.10. Ethyl 2-[1-(4-*n*-butoxy-3-formylphenyl)methyl]-butyrate (3d). This compound was prepared from **2d** by means of a procedure similar to that used for **2a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.5 (s, 1H), 7.62 (d, J = 2.6 Hz, 1H), 7.33 (dd, J = 8.6, 2.6 Hz, 1H), 6.88 (d, J = 8.6 Hz, 1H), 4.06 (m, 4H), 2.79 (m, 2H), 2.54 (m, 1H), 1.82 (m, 2H), 1.64 (m, 2H), 1.52 (m, 2H), 1.17 (t, J = 7.3 Hz, 3H), 0.99 (t, J = 7.3 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H); MS (FAB) 307 (M+H)$^+$.

4.1.11. Ethyl 2-[1-(3-formyl-4-*n*-hexyloxyphenyl) methyl]-butyrate (3e). This compound was prepared from **2e** by means of a procedure similar to that used for **3a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.48 (s, 1H), 7.62 (d, J = 2.1 Hz, 1H), 7.33 (dd, J = 8.5, 2.1 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 4.01–4.03 (m, 4H), 2.88 (dd, J = 13.9, 8.5 Hz, 1H), 2.71 (dd, J = 13.9, 6.4 Hz, 1H), 2.54 (m, 1H), 1.82 (m, 2H), 1.64 (m, 1H), 1.55 (m, 1H), 1.47 (m, 2H), 1.34 (m, 4H), 1.17 (t, J = 7.3 Hz, 3H), 0.93–0.89 (m, 6H); MS (FAB) 355 (M+H)$^+$.

4.1.12. Ethyl 2-{1-[3-formyl-4-(4-nitrophenyl)methoxyphenyl]methyl}butyrate (3f). This compound was prepared from **2f** by means of a procedure similar to that used for **3a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.5 (s, 1H), 8.24 (d, J = 9.0 Hz, 2H), 7.65 (d, J = 2.1 Hz, 1H), 7.60 (d, J = 9.0 Hz, 2H), 7.33 (dd, J = 8.6, 2.6 Hz, 1H), 6.88 (d, J = 8.6 Hz, 1H), 5.25 (s, 1H), 4.01–4.10 (m, 2H), 2.88 (dd, J = 14,1, 9.0 Hz, 1H), 2.72 (dd, J = 14,1, 6.0 Hz, 1H), 2.50–2.56 (m, 1H), 1.50–1.66 (m, 2H), 1.14 (t, J = 7.3 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H); MS (FAB) 357 (M^+).

4.1.13. 2-{4-Methoxy-3-[(4-trifluoromethylbenzoylamino)-methyl]phenylmethyl}butyric acid (5a). A mixture of **3a** (750 mg, 2.83 mmol), 4-trifluoromethylbenzamide (1.75 g, 8.48 mmol), triethylsilane (1.36 mL, 8.48 mmol), trifluoroacetic acid (0.65 mL, 8.48 mmol), and 50 mL of dehydrated toluene was refluxed for 24 h. The mixture was evaporated, and the residue was purified by silica

gel column chromatography (eluant; *n*-hexane/ethyl acetate = 2:1, v/v) to obtain 900 mg (73%) of **4a** as a colorless oil. A mixture of **4a** (900 mg, 2.06 mmol), 15 mL of ethanol, and 15 mL of a 1 mol/L aqueous solution of lithium hydroxide was stirred overnight at 50 °C, then concentrated under reduced pressure. The residue was suspended in water and acidified with dil HCl. The precipitate formed was collected by filtration, dried, and recrystallized from *n*-hexane–ethyl acetate to afford 620 mg (74%) of the title compound as colorless prisms. 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.

4.1.14. 2-{4-Ethoxy-3-[(4-trifluoromethylbenzoylamino)-methyl]phenylmethyl}butyric acid (5b). This compound was prepared from **3b** by means of a procedure similar to that used for **5a**. Mp 94–95 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, J = 8.1 Hz, 2H), 7.66 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 2.1, 1H), 7.08 (dd, J = 8.3, 2.1 Hz, 1H), 6.84 (s, 1H), 6.79 (d, J = 8.3 Hz, 1H), 4.61 (d, J = 6.0 Hz, 2H), 4.07 (q, J = 6.8 Hz, 2H), 2.89 (dd, J = 14.1, 2.6 Hz, 1H), 2.71 (dd, J = 14.1, 6.4 Hz, 1H), 2.59–2.53 (m, 1H), 1.69–1.55 (m, 2H), 1.43 (d, J = 6.8 Hz, 3H), 0.95 (d, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{22}H_{25}F_3NO_4$ 424.1736; found: 424.1736 $(M+H)^+$.

4.1.15. 2-{4-*n*-Propoxy-3-[(4-trifluoromethylbenzoylamino)methyl]phenylmethyl}butyric acid (5c). This compound was prepared from **3c** by means of a procedure similar to that used for **5a**. Mp 124 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, J = 8.1 Hz, 2H), 7.66 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 1.9 Hz, 1H), 7.07 (dd, J = 8.5, 1.9 Hz, 1H), 6.79 (m, 2H), 4.61 (d, J = 5.6 Hz, 2H), 3.96 (t, J = 6.4 Hz, 2H), 2.88 (dd, J = 13.7, 8.5 Hz, 1H), 2.71 (dd, J = 13.7, 6.4 Hz, 1H), 2.56 (m, 1H), 1.82 (m, 2H), 1.66 (m, 1H), 1.58 (m, 1H), 1.04 (t, J = 7.3 Hz, 3H), 0.95 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{23}H_{27}F_3NO_4$ 438.1892; found: 438.1903 $(M+H)^+$.

4.1.16. 2-{4-*n*-Butoxy-3-[(4-trifluoromethylbenzoylamino)methyl]phenylmethyl}butyric acid (5d). This compound was prepared from **3d** by means of a procedure similar to that used for **5a**. Mp 91–94 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, J = 8.4 Hz, 2H), 7.66 (d, J = 8.4, 2H), 7.14 (m, 1H), 7.07 (m, 1H), 6.83 (m, 1H), 6.79 (d, J = 8.1 Hz, 1H), 4.60 (d, J = 5.6 Hz, 2H), 3.99 (t, J = 6.4 Hz, 2H), 2.88–2.70 (m, 2H), 2.55 (m, 1H), 1.78 (m, 2H), 1.64–1.57 (m, 2H), 1.48 (m, 2H), 0.95 (m, 6H); HRMS (FAB) calcd for $C_{24}H_{29}F_3NO_4$ 452.2049; found: 452.2046 $(M+H)^+$.

4.1.17. 2-{4-*n*-Hexyloxy-3-[(4-trifluoromethylbenzoylamino)methyl]phenylmethyl}butyric acid (5e). This compound was prepared from **3e** by means of a procedure similar to that used for **5a**. Mp 92–94 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, J = 8.3 Hz, 2H), 7.66 (d, J = 8.3 Hz, 2H), 7.15 (d, J = 2.1 Hz, 1H), 7.08 (dd, J = 8.5, 2.1 Hz, 1H), 6.79 (m, 2H), 4.60 (d, J = 5.6 Hz, 2H), 3.99 (t, J = 6.4 Hz, 2H), 2.88 (dd, J = 13.7, 8.5 Hz, 1H), 2.71 (dd, J = 13.7, 6.4 Hz, 1H), 2.56 (m, 1H), 1.79 (m, 2H), 1.65 (m, 1H), 1.59 (m, 1H), 1.43 (m, 2H), 1.29 (m, 4H), 0.95 (t, J = 7.3 Hz, 3H), 0.86 (t, J = 6.8 Hz, 3H). HRMS (FAB) calcd for $C_{26}H_{33}F_3NO_4$ 480.2362; found: 480.2378 $(M+H)^+$.

4.1.18. 2-{3-[(2-Fluoro-4-trifluoromethylbenzoylamino)-methyl]-4-methoxyphenylmethyl}butyric acid (5g). This compound was prepared from **3a** by means of a procedure similar to that used for **5a**. 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. Calcd for $C_{21}H_{21}F_4NO_4$, C 59.02, H 4.95, N 3.28. Found: C, 59.05; H, 5.21; N, 3.14.

4.1.19. 2-{3-[(2-Fluoro-4-trifluoromethylbenzoylamino)-methyl]-4-*n*-propoxyphenylmethyl}butyric acid (5h). This compound was prepared from **3c** by means of a procedure similar to that used for **5a**. Mp 124–125 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (t, J = 8.1 Hz, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.39 (m, 2H), 7.16 (d, J = 1.9 Hz, 1H), 7.07 (dd, J = 8.5, 1.9 Hz, 1H), 6.79 (d, J = 8.5 Hz, 1H), 4.64 (d, J = 5.6 Hz, 2H), 3.97 (t, J = 6.4 Hz, 2H), 2.89 (dd, J = 13.9, 8.1 Hz, 1H), 2.71 (dd, J = 13.9, 6.4 Hz, 1H), 2.57 (m, 1H), 1.85 (m, 2H), 1.65 (m, 1H), 1.59 (m, 1H), 1.06 (t, J = 7.7 Hz, 3H), 0.95 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{23}H_{26}F_4NO_4$ 456.1798; found: 456.1792 $(M+H)^+$.

4.1.20. 2-{3-[4-*n*-Butoxy-(2-fluoro-4-trifluoromethylbenzoylamino)methyl]phenylmethyl}butyric acid (5i). This compound was prepared from **3d** by means of a procedure similar to that used for **5a**. Mp 109–110 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (t, J = 7.9 Hz, 1H), 7.51 (d, J = 7.9 Hz, 1H), 7.39 (m, 2H), 7.16 (d, J = 2.1 Hz, 1H), 7.07 (dd, J = 8.3, 2.1 Hz, 1H), 6.79 (d, J = 8.3 Hz, 1H), 4.63 (d, J = 6.0 Hz, 2H), 4.00 (t, J = 6.4 Hz, 2H), 2.89 (dd, J = 13.9, 8.5 Hz, 1H), 2.70 (dd, J = 13.9, 6.4 Hz, 1H), 2.56 (m, 1H), 1.81 (m, 2H), 1.69–1.48 (m, 4H), 0.99–0.94 (m, 6H). HRMS (FAB) calcd for $C_{24}H_{28}F_4NO_4$ 470.1954; found: 470.1940 $(M+H)^+$.

4.1.21. Ethyl 2-{4-hydroxy-3-[(4-trifluoromethylbenzoylamino)methyl}butyrate (6). This compound was prepared from **3f** by means of a procedure similar to that used for **5a**, and subsequent hydrogenolysis. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.97 (s, 1H), 7.87 (d, J = 8.5 Hz, 2H), 7.67 (d, J = 8.1 Hz, 2H), 7.01 (dd, J = 8.1, 2.1 Hz, 1H), 6.93 (d, J = 2.1 Hz, 1H), 6.85 (d, J = 8.5 Hz, 1H), 4.52 (d, J = 6.4 Hz, 1H), 4.00–4.06 (m, 2H), 2.80 (dd, J = 13.7, 8.5 Hz, 1H), 2.64 (dd, J = 13.7, 6.4 Hz, 1H), 2.49–2.55 (m, 1H), 1.53–1.66 (m, 2H), 1.12 (t, J = 6.4 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H); MS (FAB) 424 $(M+H)^+$.

4.1.22. Ethyl 2-{4-benzyloxy-3-[(4-trifluoromethylbenzoylamino)methyl]benzyl}butyrate. A mixture of **6** (400 mg, 0.944 mmol), anhydrous cesium carbonate (301 mg, 0.924 mmol), benzyl bromide (0.110 mL, 0.926 mmol), and 10 mL of dehydrated *N*,*N*-dimethylformamide was stirred for 16 h at room temperature. The reaction mixture was diluted with AcOEt, washed with 2 mol/L of HCl, satd $NaHCO_3$, water, brine, and evaporated. The residue was purified by silica gel column chromatography (eluant; *n*-hexane/ethyl acetate = 4:1–1:1, v/v) to obtain 298 mg (62%) of the title compound as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (d, J = 8.1 Hz, 2H), 7.59 (d, J = 8.1 Hz, 2H), 7.36–7.43 (m, 5H), 7.15 (d, J = 2.1 Hz, 1H), 7.08 (dd, J = 8.1, 2.1 Hz, 1H), 6.90 (d, J = 8.5 Hz, 1H), 6.79 (s, 1H), 5.09 (s, 1H), 4.65 (d, J = 5.5 Hz, 2H), 4.04–4.10 (m, 2H), 2.87 (dd, J = 12.1, 8.5 Hz, 1H), 2.69 (dd, J = 12.1, 6.4 Hz, 1H), 2.51–2.57 (m, 1H), 1.52–1.67 (m, 2H), 1.16 (t, J = 7.3 Hz, 3H), 0.91 (t, J = 7.3 Hz, 3H); MS (FAB) 514 $(M+H)^+$.

4.1.23. 2-{4-benzyloxy-3-[(4-trifluoromethylbenzoylamino)methyl]benzyl}butyric acid (5f). This compound was prepared from ethyl 2-{4-benzyloxy-3-[(4-trifluoromethylbenzoylamino)methyl]benzyl}butyrate by means of a procedure similar to that used for **5a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 8.1 Hz, 2H), 7.37–7.40 (m, 5H), 7.09 (d, J = 8.1 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 5.07 (s, 2H), 4.60 (d, J = 5.5 Hz, 2H), 2.88 (dd, J = 14.1, 9.0 Hz, 1H), 2.71 (dd, J = 14.1, 6.4 Hz, 1H), 2.51–2.57 (m, 1H), 1.54–1.69 (m, 2H), 0.94 (t, J = 7.7 Hz, 3H); MS (FAB) 486 $(M+H)^+$.

4.1.24. Ethyl 3-(4-methoxyphenyl)-2-methylpropionate (7b). This compound was prepared from 4-methoxybenzaldehyde and triethyl 2-phosphonopropionate by means of a procedure similar to that used for **2a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 (d, J = 8.5 Hz, 2H), 6.81 (d, J = 8.5 Hz, 2H), 4.08 (q, J = 7.3 Hz, 2H), 3.78 (s, 3H), 2.95 (dd, J = 13.3, 6.4 Hz, 1H), 2.69–2.59 (m, 2H), 1.19 (t, J = 7.3 Hz, 3H), 1.13 (t, J = 6.8 Hz, 3H); MS (FAB) 222 (M^+).

4.1.25. Ethyl 2-[(4-methoxyphenyl)methyl]pentanoate (7c). This compound was prepared from 4-methoxybenzaldehyde and triethyl 2-phosphonopentanoate by means of a procedure similar to that used for **2a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06 (d, J = 8.5 Hz, 2H), 6.81 (d, J = 8.5 Hz, 2H), 3.78 (s, 3H), 3.59 (s, 3H), 2.86 (dd, J = 13.7, 8.1 Hz, 1H), 2.68 (dd, J = 13.7, 6.4 Hz, 1H), 2.62 (m, 1H), 1.60 (m, 1H), 1.46 (m, 1H), 1.30 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H); MS (FAB) 236 (M^+).

4.1.26. Ethyl 2-[(4-methoxyphenyl)methyl]hexanoate (7d). This compound was prepared from 4-methoxybenzaldehyde and triethyl 2-phosphonohexanoate by means of a procedure similar to that used for **2a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06 (d, J = 8.5 Hz, 2H), 6.81 (d, J = 8.5 Hz, 2H), 3.78 (s, 3H), 3.60 (s, 3H), 2.86 (dd, J = 13.7, 8.5 Hz, 1H), 2.68 (dd, J = 13.7, 6.4 Hz, 1H), 2.60 (m, 1H), 1.62 (m, 1H), 1.49 (m, 1H), 1.27 (m, 4H), 0.87 (t, J = 6.4 Hz, 3H); MS (FAB) 250 $(M)^+$.

4.1.27. Ethyl 2-[(4-*n*-butoxyphenyl)methyl]pentanoate (7e). This compound was prepared from 4-*n*-butoxybenzaldehyde and triethyl 2-phosphonopentanoate by means of a procedure similar to that used for **2a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.04 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 3.93 (t, J = 6.4 Hz, 2H), 3.59 (s, 3H), 2.86 (dd, J = 13.7, 8.1 Hz, 1H), 2.67 (dd, J = 13.7, 6.8 Hz, 1H), 2.62 (m, 1H), 1.75 (m, 2H), 1.60 (m, 1H), 1.52–1.42 (m, 3H), 1.30 (m, 2H), 0.97 (t, J = 7.7 Hz, 3H), 0.88 (t, J = 7.3 Hz, 3H); MS (FAB) 278 $(M)^+$.

4.1.28. Ethyl (3-formyl-4-methoxyphenyl)propanoate (8a). This compound was prepared from **7a** by means of a procedure similar to that used for **3a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.4 (s, 1H), 7.63 (d, J = 2.6 Hz, 1H), 7.38 (dd, J = 8.6 Hz, 2.6 Hz, 1H), 6.90 (d, J = 8.6 Hz, 1H), 3.86 (s, 2H), 3.64 (s, 2H), 2.89 (t, J = 7.7 Hz, 2H), 2.59 (t, J = 7.7 Hz, 2H).

4.1.29. Ethyl (3-formyl-4-methoxyphenyl)-2-methylpropanoate (8b). This compound was prepared from **7b** by means of a procedure similar to that used for **3a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.42 (s, 1H), 7.61 (s, 1H), 7.35 (s, 1H), 6.89 (d, J = 8.5 Hz, 1H), 4.07 (m, 2H), 3.89 (s, 3H), 2.96 (m, 1H), 2.66 (m, 2H), 1.15 (m, 6H); MS (FAB) 251 $(M+H)^+$.

4.1.30. Ethyl [(3-formyl-4-methoxyphenyl)methyl]pentanoate (8c). This compound was prepared from **7c** by means of a procedure similar to that used for **3a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.44 (s, 1H), 7.62 (d, J = 2.1 Hz, 1H), 7.34 (dd, J = 8.5, 2.1 Hz, 1H), 6.90 (d, J = 8.5 Hz, 1H), 3.91 (s, 3H), 3.60 (s, 3H), 2.88 (dd, J = 13.7, 9.0 Hz, 1H), 2.72 (dd, J = 13.7, 6.4 Hz, 1H), 2.64 (m, 1H), 1.62 (m, 1H), 1.45 (m, 1H), 1.31 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H); MS (FAB) 265 $(M+H)^+$.

4.1.31. Ethyl [(3-formyl-4-methoxyphenyl)methyl]hexanoate (8d). This compound was prepared from **7d** by means of a procedure similar to that used for **3a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.44 (s, 1H), 7.62 (d, J = 2.3 Hz, 1H), 7.34 (dd, J = 8.3, 2.3 Hz, 1H), 6.90 (d, J = 8.3 Hz, 1H), 3.91 (s, 3H), 3.60 (s, 3H), 2.88 (dd, J = 13.7, 9.0 Hz, 1H), 2.72 (dd, J = 13.7, 6.0 Hz, 1H), 2.60 (m, 1H), 1.63 (m, 1H), 1.49 (m, 1H), 1.28 (m, 4H), 0.87 (t, J = 6.8 Hz, 3H); MS (FAB) 279 $(M+H)^+$.

4.1.32. Ethyl [(4-*n*-butoxy-3-formylphenyl)methyl]pentanoate (8e). This compound was prepared from **7e** by means of a procedure similar to that used for **3a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.47 (s, 1H), 7.61 (d, J = 2.3 Hz, 1H), 7.31 (dd, J = 8.5, 2.3 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 4.06 (t, J = 6.4 Hz, 2H), 3.60 (s, 3H), 2.88 (dd, J = 13.7, 8.5 Hz, 1H), 2.71 (dd, J = 13.7, 6.0 Hz, 1H), 2.64 (m, 1H), 1.82 (m, 2H), 1.62 (m, 1H), 1.52 (m, 2H), 1.45 (m, 1H), 1.31 (m, 2H), 0.99 (t, J = 7.7 Hz, 3H), 0.89 (t, J = 7.3 Hz, 3H); MS (FAB) 307 $(M+H)^+$.

4.1.33. 3-{4-Methoxy-3-[(4-trifluoromethylbenzoylamino)methyl]phenyl}propanoic acid (10a). This compound was prepared from **8a** by means of a procedure similar to that used for **5a**. Mp 166–167 °C. ^{1}H NMR

(500 MHz, $CDCl_3$) δ 7.86 (d, J = 8.3 Hz, 2H), 7.68 (d, J = 8.3 Hz, 2H), 7.20 (d, J = 2.1 Hz, 1H), 7.14 (dd, J = 8.1, 2.1 Hz, 1H), 6.84 (d, J = 8.1 Hz, 1H), 6.72 (s, 1H), 4.62 (d, J = 6.0 Hz, 2H), 3.87 (s, 3H), 2.91 (t, J = 7.7 Hz, 2H), 2.65 (d, J = 7.7 Hz, 2H); HRMS (FAB) calcd for $C_{19}H_{19}F_3NO_4$ 382.1266; found: 382.1236 $(M+H)^+$.

4.1.34. 3-{4-Methoxy-3-[(4-trifluoromethylbenzoylamino)-methyl]phenyl}-2-methylpropanoic acid (10b). This compound was prepared from **8b** by means of a procedure similar to that used for **5a**. Mp 129 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, J = 8.1 Hz, 2H), 7.67 (d, J = 8.1 Hz, 2H), 7.18 (d, J = 2.1 Hz, 1H), 7.10 (dd, J = 8.1, 2.1 Hz, 1H), 6.82 (d, J = 8.1 Hz, 1H), 6.74 (s, 1H), 4.61 (d, J = 5.5 Hz, 2H), 3.87 (s, 3H), 2.96 (dd, J = 13.7, 6.8 Hz, 1H), 2.76–2.64 (m, 2H), 1.18 (d, J = 6.8 Hz, 3H); MS (FAB) 396 $(M+H)^+$. HRMS (FAB) calcd for $C_{20}H_{20}F_3NO_4$ 396.1423 $(M+H)^+$; found: 396.1422.

4.1.35. 2-{4-Methoxy-3-[(4-trifluoromethylbenzoylamino)-methyl]phenylmethyl}pentanoic acid (10c). This compound was prepared from **8c** by means of a procedure similar to that used for **5a**. Mp 129–130 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, J = 8.3 Hz, 2H), 7.66 (d, J = 8.3 Hz, 2H), 7.16 (d, J = 2.1 Hz, 1H), 7.09 (dd, J = 8.5, 2.1 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 6.77 (s, 1H), 4.58 (d, J = 6.0 Hz, 2H), 3.85 (s, 3H), 2.87 (dd, J = 13.7, 9.0 Hz, 1H), 2.71 (dd, J = 13.7, 6.0 Hz, 1H), 2.63 (m, 2H), 1.63 (m, 1H), 1.48 (m, 1H), 1.36 (m, 2H), 0.90 (d, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{22}H_{25}F_3NO_4$ 424.1736; found: 424.1769 $(M+H)^+$.

4.1.36. 2-{4-Methoxy-3-[(4-trifluoromethylbenzoylamino)-methyl]phenylmethyl}hexanoic acid (10d). This compound was prepared from **8d** by means of a procedure similar to that used for **5a**. Mp 151 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, J = 8.1 Hz, 2H), 7.66 (d, J = 8.1 Hz, 2H), 7.16 (s, 1H), 7.09 (dd, J = 8.3, 2.1 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H), 6.76 (s, 1H), 4.59 (d, J = 6.0 Hz, 2H), 3.85 (s, 3H), 2.87 (dd, J = 13.9, 9.0 Hz, 1H), 2.72 (dd, J = 13.9, 5.6 Hz, 1H), 2.61 (m, 1H), 1.65 (m, 1H), 1.51 (m, 1H), 1.31 (m, 4H), 0.87 (t, J = 6.8 Hz, 3H). HRMS (FAB) calcd for $C_{23}H_{27}F_3NO_4$ 438.1892; found: 438.1909 $(M+H)^+$.

4.1.37. 2-{4-Methoxy-3-[(2-fluoro-4-trifluoromethylbenzoylamino)methyl]phenylmethyl}pentanoic acid (10e). This compound was prepared from **8c** by means of a procedure similar to that used for **5a**. Mp 95–97 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 8.21 (t, J = 8.1 Hz, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.42 (t, J = 5.8 Hz, 1H), 7.37 (d, J = 11.5 Hz, 1H), 7.15 (d, J = 2.1 Hz, 1H), 7.09 (dd, J = 8.1, 2.1 Hz, 1H), 6.80 (d, J = 8.1 Hz, 1H), 4.62 (d, J = 5.6 Hz, 2H), 3.86 (s, 3H), 2.89 (dd, J = 13.7, 8.1 Hz, 1H), 2.69 (dd, J = 13.7, 6.8 Hz, 1H), 2.63 (m, 1H), 1.61 (m, 1H), 1.47 (m, 1H), 1.41–1.30 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{22}H_{24}F_4NO_4$ 442.1641; found: 442.1643 $(M+H)^+$.

4.1.38. 2-{4-*n*-Butoxy-3-[(2-fluoro-4-trifluoromethylbenzoylamino)methyl]phenylmethyl}pentanoic acid (10f). This compound was prepared from **8d** by means of a procedure similar to that used for **5a**. Mp 135 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 8.22 (t, J = 8.1 Hz, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.39 (m, 2H), 7.15 (d, J = 2.1 Hz, 1H), 7.07 (dd, J = 8.3, 2.1 Hz, 1H), 6.79 (d, J = 8.3 Hz, 1H), 4.63 (d, J = 5.6 Hz, 2H), 4.00 (t, J = 6.4 Hz, 2H), 2.89 (dd, J = 13.7, 7.7 Hz, 1H), 2.68 (dd, J = 13.7, 6.4 Hz, 1H), 2.63 (m, 1H), 1.80 (m, 2H), 1.61 (m, 1H), 1.53–1.31 (m, 5H), 0.98 (t, J = 7.7 Hz, 3H), 0.89 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{23}H_{26}F_4NO_4$ 456.1798; found: 456.1779 $(M+H)^+$.

4.1.39. Benzyl 2-*n*-butoxy-5-formylbenzoate (13). A mixture of **11** (5.00 g, 30.0 mmol), benzyl bromide (3.56 mL, 30.0 mmol), potassium hydrogencarbonate (3.00 g, 30.0 mmol), and 40 mL of *N*,*N*-dimethylformamide was stirred for 24 h. The mixture was poured into ice-water and stirred for 2 h. The precipitate was redissolved in 100 mL of AcOEt, dried over anhyd $MgSO_4$, filtered, and concentrated to obtain **13**. A mixture of **12**, iodobutane (5.52 g, 30.0 mmol), potassium carbonate (5.53 g, 30.0 mmol), and 40 mL of *N*,*N*-dimethylformamide was stirred for 24 h. The reaction mixture was poured into water, and the whole was extracted with ethyl acetate, washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluant; *n*-hexane/ethyl acetate = 4:1, v/v) to afford 4.00 g (43%) of the title compound as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$) δ 9.87 (s, 1H), 8.30 (d, J = 2.6 Hz, 1H), 7.97 (dd, J = 8.6, 2.6 Hz, 1H), 7.43–7.44 (m, 2H), 7.32–7.38 (m, 3H), 7.05 (d, J = 8.6 Hz, 1H), 5.35 (s, 2H), 4.10 (t, J = 6.4 Hz, 2H), 1.74–1.80 (m, 2H), 1.42–1.46 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H).

4.1.40. Benzyl 5-bromomethyl-2-*n*-butoxybenzoate (15). To a solution of **13** (3.55 g, 11.4 mmol) and 70 mL of ethanol was added $NaBH_4$ (0.50 g, 13.3 mmol) portionwise at 0 °C, and stirred for 2 h at room temperature. The excess ethanol was evaporated, and the residue was poured into water and the whole was extracted with CH_2Cl_2. The extract was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluant; *n*-hexane/ethyl acetate = 4:1, v/v) to afford 3.26 g (91%) of the intermediate alcohol **14** as a colorless oil. A mixture of **14** (3.26 g, 10.4 mmol), phosphorus tribromide (0.50 mL, 5.32 mmol), and 70 mL of dehydrated ether was stirred for 1 h at 0 °C. The reaction mixture was poured into saturated ammonium chloride solution, and the whole was extracted with ether. The extract was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluant; *n*-hexane/ethyl acetate = 10:1, v/v) to afford 2.74 g (70%) of the title compound as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, J = 2.1 Hz, 1H), 7.48–7.32 (m, 6H), 6.92 (d, J = 9.0 Hz, 1H), 5.34 (s, 2H), 4.47 (s, 2H), 4.02 (t, J = 6.4 Hz, 2H), 1.75 (m, 2H), 1.44 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H); MS (FAB) 377, 379 $(M+H)^+$.

4.1.41. Benzyl 5-[(*S*)-2-((*R*)-4-benzyl-2-oxo-oxazolidine-3-carbonyl)butyl]2-*n*-butoxybenzoate (16). (*R*)-3-(1-Butyr-

yl)-4-benzyloxazolidin-2-one (1.30 g, 5.25 mmol) and 20 mL of dehydrated tetrahydrofuran were mixed under an argon atmosphere, and cooled to −60 °C. Under stirring, a 1 mol/L solution of sodium bis(trimethylsilyl)amide in dehydrated tetrahydrofuran (5.80 mL, 5.80 mmol) was added dropwise. After completion of the addition, the mixture was stirred for 1 h at −15 °C, recooled to −60 °C, and then a solution of **15** (1.81 g, 4.80 mmol) in dehydrated tetrahydrofuran (20 mL) was added dropwise. After completion of the addition, the mixture was further stirred for 1 h while gradually heated to room temperature. A saturated aqueous solution of ammonium chloride was added to the reaction mixture, and the whole was extracted with ethyl acetate. The extract was washed with water and brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by silica gel chromatography (eluant; *n*-hexane/ethyl acetate = 5:1, v/v) to afford 1.75 g (67%) of the desired compound as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (d, J = 2.1 Hz, 1H), 7.42–7.22 (m, 9H), 7.03 (d, J = 6.8 Hz, 2H), 6.87 (d, J = 8.5 Hz, 1H), 5.28 (s, 2H), 4.64 (m, 1H), 4.14–3.94 (m, 5H), 3.06 (dd, J = 13.2, 3.4 Hz, 1H), 3.01 (dd, J = 13.7, 8.1 Hz, 1H), 2.75 (dd, J = 13.7, 6.4 Hz, 1H), 2.44 (dd, J = 13.2, 9.8 Hz, 1H), 1.75 (m, 3H), 1.53 (m, 1H), 1.41 (m, 2H), 1.01–0.88 (m, 6H); MS (FAB) 544 $(M+H)^+$.

4.1.42. 5-[(*S*)-2-((*R*)-4-Benzyl-2-oxo-oxazolidine-3-carbonyl)butyl]2-*n*-butoxybenzaldehyde (19). Compound **16** (1.75 g, 3.22 mmol), 100 mg of 10% palladium on carbon, and 30 mL of ethyl acetate were mixed and catalytic hydrogenation was carried out at an initial hydrogen pressure of 98 kPa. After completion of the reaction, the catalyst was removed by filtration and the filtrate was washed with ethyl acetate. The reaction mixture and the washings were combined and concentrated. The residue was purified by silica gel chromatography (eluant; *n*-hexane/ethyl acetate = 3:1, v/v) to afford 1.37 g (94%) of the carboxylic acid derivative **17** as a colorless oil. To a solution of **17** (1.37 g, 3.02 mmol) and 25 mL of dehydrated tetrahydrofuran was added a 1 mol/L solution of borane–tetrahydrofuran complex (5.00 mL, 5.00 mmol) dropwise. After completion of the addition, the mixture was stirred overnight at room temperature. A saturated aqueous solution of ammonium chloride was added to the reaction mixture, and the whole was extracted with ethyl acetate. The extract was washed with water and brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by silica gel chromatography (eluant; *n*-hexane/ethyl acetate = 4:1, v/v) to afford 1.18 g (89%) of the hydroxymethyl derivative **18** as a colorless oil. A solution of **18** (1.18 g, 2.68 mmol), activated-MnO_2 (0.50 g, 5.75 mmol), and 30 mL of dehydrated dichloromethane was stirred overnight at room temperature. The catalyst was filtered, washed with dichloromethane, and concentrated. The residue was purified by silica gel chromatography (eluant; *n*-hexane/ethyl acetate = 4:1, v/v) to afford 855 g (73%) of the formyl derivative **20** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (d, J = 2.3 Hz, 1H), 7.52 (dd, J = 8.5, 2.3 Hz, 1H), 7.29–7.24 (m, 3H), 7.08 (d, J = 6.8 Hz, 2H), 6.97 (d, J = 8.5 Hz, 1H), 4.68 (m, 1H), 4.24–4.01 (m, 5H), 3.14 (dd, J = 13.2, 3.4 Hz, 1H), 3.08 (dd, J = 13.7, 7.7 Hz, 1H), 2.77 (dd, J = 13.7, 6.8 Hz, 1H), 2.58 (dd, J = 13.2, 9.8 Hz, 1H), 1.87 (m, 2H), 1.77 (m, 1H), 1.51 (m, 3H), 0.99 (t, J = 7.3 Hz, 3H), 0.93 (t, J = 7.3 Hz, 3H); MS (FAB) 454 $(M+H)^+$.

4.1.43. *N*-{5-[(*S*)-2-((*R*)-4-Benzyl-2-oxo-oxazolidine-3-carbonyl)butyl]-2-*n*-butoxybenzyl}-2-fluoro-4-trifluoromethylbenzamide (20). A mixture of **19** (393 mg, 0.898 mmol), 2-fluoro-4-trifluoromethylbenzamide (0.41 g, 1.98 mmol), triethylsilane (0.80 mL, 4.98 mmol), trifluoroacetic acid (0.24 mL, 3.12 mmol), and 15 mL of dehydrated toluene was stirred for 2 days at 80 °C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (eluant; *n*-hexane/ethyl acetate = 4:1, v/v) to obtain 376 mg (67%) of the title as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (t, J = 7.9 Hz, 1H), 7.45 (d, J = 7.9 Hz, 1H), 7.31–7.16 (m, 7H), 6.92 (m, 2H), 6.82 (d, J = 8.5 Hz, 1H), 4.69–4.57 (m, 3H), 4.12–3.97 (m, 5H), 2.99 (dd, J = 13.7, 8.5 Hz, 1H), 2.95 (dd, J = 13.5, 3.8 Hz, 1H), 2.75 (dd, J = 13.7, 6.4 Hz, 1H), 2.44 (dd, J = 13.5, 9.0 Hz, 1H), 1.77 (m, 3H), 1.57 (m, 1H), 1.49 (m, 2H), 0.98–0.92 (m, 6H); MS (FAB) 629 $(M+H)^+$.

4.1.44. (*S*)-2-{3-[4-*n*-Butoxy-(2-fluoro-4-trifluoromethylbenzoylamino)methyl]phenylmethyl}butyric acid ((*S*)-5i). Compound **20** (376 mg, 0.598 mol) was dissolved in 8 mL tetrahydrofuran and 2 mL of water under an argon atmosphere with ice-cooling. To this solution was added 30% aqueous hydrogen peroxide (2.4 mL, 23.7 mmol). Then 1.2 mL of a 1 mol/L solution of lithium hydroxide monohydrate was added, and the mixture was stirred further for 1 h under ice-cooling. 1.2 mL of a solution of sodium hydrogen sulfite was added dropwise to the mixture and the whole was stirred for 30 min. The reaction mixture was concentrated, poured into ice-water, acidified with dil HCl, and then extracted with methylene chloride. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was dissolved in ethyl acetate and *n*-hexane, and allowed to stand. The precipitated crystals were collected by filtration and dried. A second crop of crystals was obtained from the filtrate. The first and second crops were combined, washed with the mixed solvent of *n*-hexane and ethyl acetate (4:1–3:1, v/v), and dried to afford 155 mg (55%) of the title compound as a colorless crystalline powder. Mp 70–71 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (t, J = 8.1 Hz, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.40 (m, 2H), 7.16 (d, J = 2.1 Hz, 1H), 7.07 (dd, J = 8.3, 2.1 Hz, 1H), 6.79 (d, J = 8.3 Hz, 1H), 4.63 (d, J = 6.0 Hz, 2H), 4.00 (t, J = 6.8 Hz, 2H), 2.90 (dd, J = 13.9, 8.1 Hz, 1H), 2.69 (dd, J = 13.9, 6.4 Hz, 1H), 2.56 (m, 1H), 1.80 (m, 2H), 1.67–1.46 (m, 4H), 0.98 (t, J = 7.3 Hz, 3H), 0.95 (t, J = 7.3 Hz, 3H); MS (FAB) 470 $(M+H)^+$. $[\alpha]_D$ +20° (*c* 0.60, $CHCl_3$, 23 °C). Anal. Calcd for $C_{24}H_{27}F_4NO_4$ C, 61.40; H, 5.80; N, 2.98. Found: C, 61.12; H, 5.73; N, 2.98.

4.1.45. (*R*)-2-{3-[4-*n*-Butoxy-(2-fluoro-4-trifluoromethylbenzoylamino)methyl]phenylmethyl}butyric acid ((*R*)-5i). This compound was prepared from (*S*)-3-(1-Butyryl)-4-benzyloxazolidin-2-one by means of a procedure similar

to that used for (*S*)-**5j**. Mp 70–71 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65–7.57 (m, 3H), 7.14 (s, 1H), 7.10 (d, J = 8.3 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H), 6.77 (s, 1H), 4.57 (m, 2H), 3.85 (s, 3H), 2.88 (dd, J = 13.9, 8.5 Hz, 1H), 2.72 (dd, J = 13.9, 6.0 Hz, 1H), 2.56 (m, 1H), 1.67 (m, 1H), 1.58 (m, 1H), 0.96 (t, J = 7.6 Hz, 3H); MS (FAB) 470 $(M+H)^+$. $[\alpha]_D$ −25° (*c* 0.39, $CHCl_3$, 24 °C). Anal. Calcd for $C_{24}H_{27}F_4NO_4$ C, 61.40; H, 5.80; N, 2.98. Found: C, 61.04; H, 5.88; N, 2.85.

4.2. Cell culture and cotransfection assay

Human embryonic kidney HEK293 cells were cultured in DMEM containing 5% fetal bovine serum and antibiotic–antimycotic mixture (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 β-galactosidase activities were assayed using a luminometer and a microplate reader. DNA cotransfection experiments included 50 ng of reporter plasmid, 20 ng of pCMX-β-galactosidase, 15 ng of 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 β-galactosidase control and reported values are the means of triplicate assays.

4.3. Gene assay

C57BL/6 male mice (age 8–9 weeks, Charles River Laboratories) were subcutaneously injected once daily with 3 mg/kg (*S*)-**5i**, 30 mg/kg bezafibrate or the vehicle (PBS) for 3 days. On the 4th day, the mice were euthanized. Samples of liver were collected, rapidly frozen in dry ice, and stored at −80 °C. Total RNA was isolated from the tissues and real-time PCR was performed. For each sample, the quantity of the target gene was determined to obtain a normalized target value.

4.4. 3D-QSAR analysis

CoMFA fields were derived in a 3D cubic lattice with grid spacing of 2 Å and extending 4 Å beyond the aligned molecules in all directions to encompass the aligned molecules. CoMFA steric (Lennard–Jones 6–12 potential) field energies and CoMFA electrostatic (Coulombic potential) fields were calculated using a probe atom with the van der Waals properties of sp^3 carbon and a charge of +1.0. CoMFA electrostatic fields were calculated with a distance-dependent dielectric at each lattice point. The SYBYL energy cutoff of 30 kcal/mol was used. In CoMFA calculation, potential functions (a Lennard–Jones potential and a Coulombic potential) are very steep near the van der Waals surface, causing rapid change, so that the use of cut-off values is required.

The poses of ligands generated from the Glide program were used to carry out partial-least-squares (PLS) regression analyses. The CoMFA fields were used as independent variables and the logarithm of the reciprocal of EC_{50} was used as dependent variable in PLS regression analyses. The optimal number of components in the PLS model was determined using the cross-validation coefficient r^2 (called q^2) values obtained from the leave-one-out cross-validation technique. The PLS model with the highest q^2 values was then selected to derive 3D-QSAR models and the poses of ligands to the PPARδ LBD.

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