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Phenylpropanoic acid derivatives bearing a benzothiazole ring as PPARδ-selective agonists

Hiroki Fujieda,[a] Shinya Usui,[a] Takayoshi Suzuki,[a] Hidehiko Nakagawa,[a] Michitaka Ogura,[b] Makoto Makishima[b] and Naoki Miyata[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467-8603, Japan*
[b]*Nihon University School of Medicine, 30-1 Oyaguchi-kamicho, Itabashi-ku, Tokyo 173-8610, Japan*

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Abstract—To find novel PPARδ-selective agonists, we designed and synthesized phenylpropanoic acid derivatives bearing 6-substituted benzothiazoles. Optimization of this series led to the identification of a potent and selective PPARδ agonist **17**. Molecular modeling suggested that compound **17** occupies the Y-shaped pocket of PPARδ appropriately.

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily and the PPAR subfamily consists of three members, PPARα, PPARγ, and PPARδ.[1] Many studies on PPARα and PPARγ have been performed and their roles are well established.[2,3] Further, these efforts led to the discovery of hypolipidemic agents[4] and insulin sensitizers.[5,6] Meanwhile, the role of PPARδ is just beginning to emerge. Several studies have suggested that PPARδ plays an important role in regulating lipid metabolism and energy homeostasis in muscle and adipose tissues[7–12] and the activation of PPARδ increases HDL levels, attenuates weight gain, and improves insulin sensitivity.[7,10] Thus, PPARδ-selective agonists are of interest not only as tools for elucidating the more intricate biological functions of PPARδ but also as candidate drugs for metabolic syndrome.

We previously reported compound **1** as a potent PPARγ ligand[13] and compound **2** as a potent PPARα ligand[14,15] (Fig. 1). In the course of our SAR studies on phenylpropanoic acid derivatives, we discovered that compound **4**, in which the pyridine ring of **1** is replaced by a benzothiazole ring, showed selective PPARδ activity as compared with the other aromatic compounds **1**, **3**, and **5**,[15] although the activity was not so strong (Fig. 2). Since PPARδ agonists having a benzothiazole ring have never been reported, we chose compound **4** as the lead compound for the exploration of novel PPARδ-selective agonists. We describe here the design, synthesis, and PPARδ selectivity of a series of phenylpropanoic acid derivatives bearing a 6-substituted benzothiazole ring.

The routes used for the synthesis of compounds **4**–**17** are illustrated in Schemes 1–5.

Preparation of compounds **3** and **4** is shown in Scheme 1. Ethyleneglycol **18** was allowed to react with *tert*-butyldimethylsilylchloride to give mono-alcohol **19**. Secondary amine **23**, the key intermediate for the preparation of **3** and **4**, was synthesized using a 2-nitrobenzenesulfonyl (nosyl) group[16,17]: *n*-Nonylamine **20** was treated with 2-nitrobenzenesulfonylchloride to afford *N*-nosyl nonylamine **21**. Mitsunobu reaction was applied to the conversion of **21** into *N*-alkyl compound **22**.[18] The nosyl group was removed by treating with benzenethiol in the presence of K_2CO_3 in anhydrous DMF to give a secondary amine **23**. Preparation of *N*-phenyloxazolyl compound **25a** and *N*-phenylthiazolyl compound **25b** was achieved by the method of Buchwald[19]: treatment of **23** with 2-chlorobenzoxazole or 2-chlorobenzothiazole **24**, $Pd_2(DBA)_3$, BINAP, and *tert*-BuONa in toluene. The TBS group of **25a** and **25b** was removed by treating with tetrabutylammonium fluoride (TBAF) in THF to give alcohols **26a** and **26b**,

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* Corresponding author. Tel.: +81 52 836 3407; fax: +81 52 836 3407; e-mail: miyata-n@phar.nagoya-cu.ac.jp

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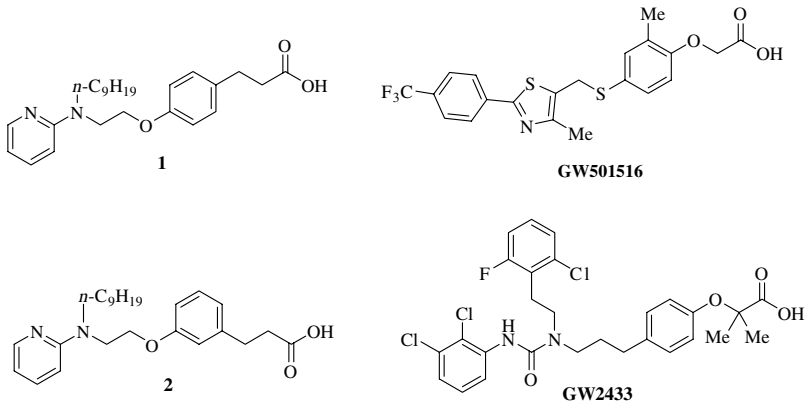


Figure 1. Structures of compounds **1** and **2**, GW501516, and GW2433.

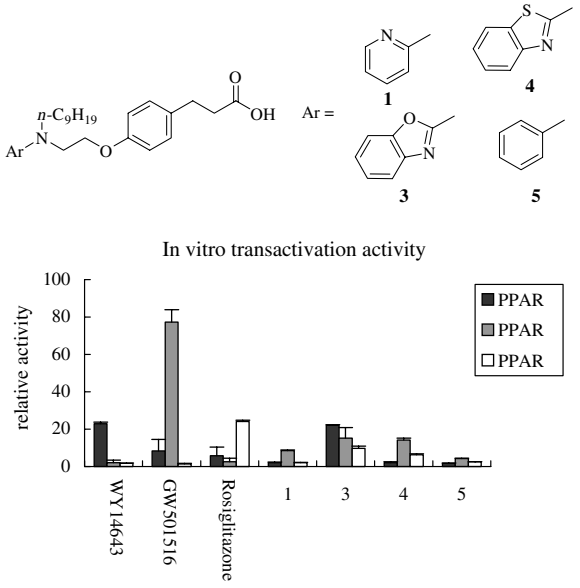
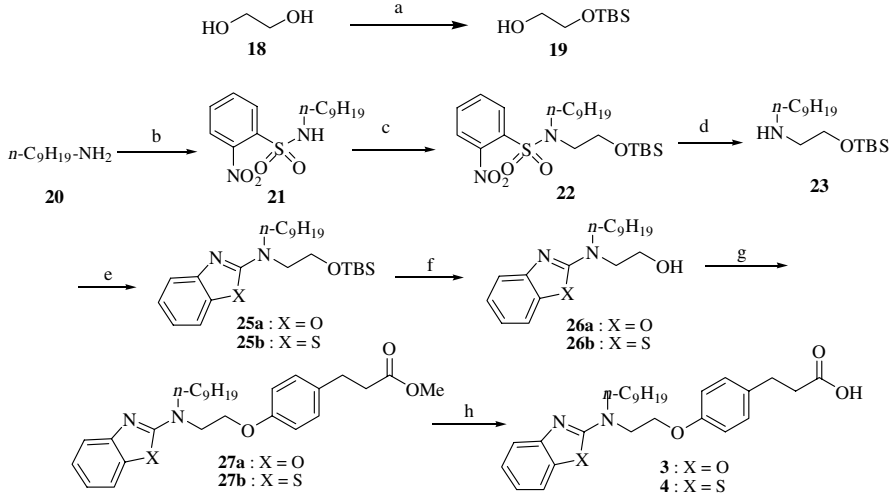


Figure 2. In vitro functional PPAR transactivation activity of compounds **1** and **3**–**5**. WY14643 (PPARα agonist) and GW501516 (PPARδ agonist), Rosiglitazone (PPARγ agonist) were used as reference compounds. GW501516 was used at 1 μM and others at 10 μM.

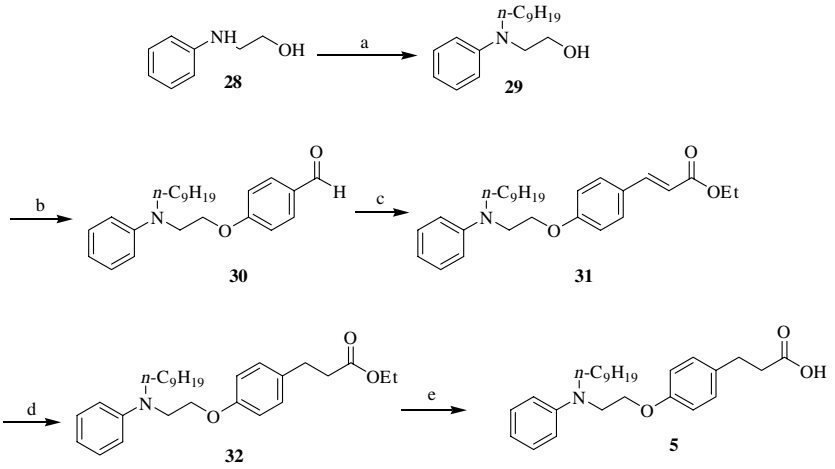
which were converted into ethers **27a** and **27b** by Mitsunobu reaction. Treatment of **27a** and **27b** with aqueous NaOH gave the desired carboxylic acids **3** and **4**.

The preparation of compound **5** is outlined in Scheme 2. *N*-phenylaminoethanol **28** was allowed to react with 1-iodononane to give *N*-alkyl compound **29**. Nucleophilic aromatic substitution by treatment of **29** with 4-fluorobenzaldehyde in the presence of sodium hydride gave ether **30**. Conversion of aldehyde **30** into **31** was achieved by Horner–Wadsworth–Emmons reaction.[20] The double bond of **31** was hydrogenated and subsequent hydrolysis gave carboxylic acid **5**.

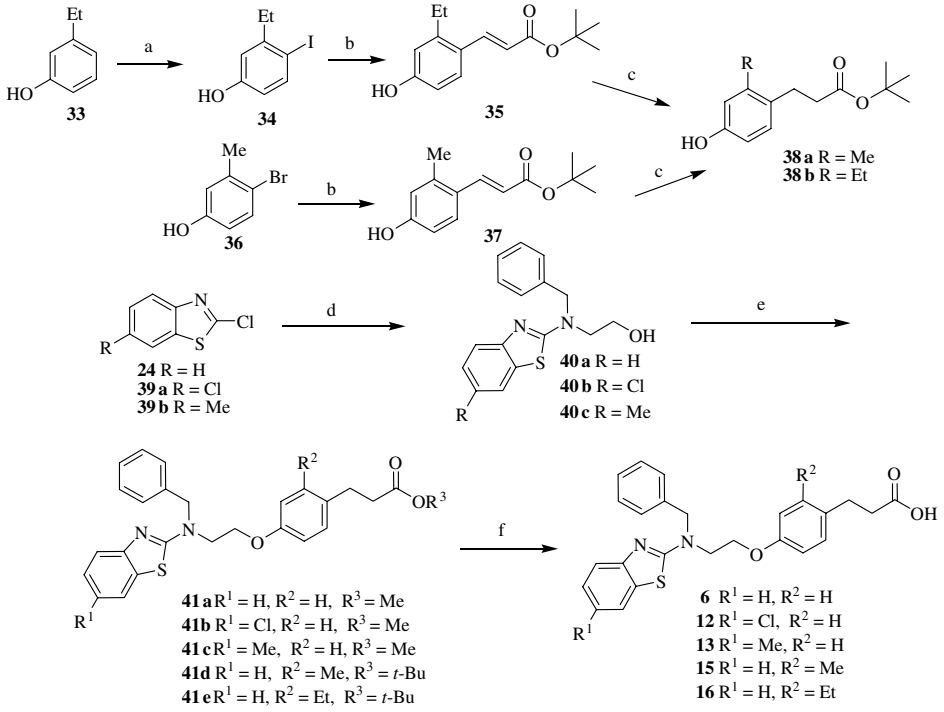
Preparation of compounds **6**, **12**, **13**, **15**, and **16** is shown in Scheme 3. 4-Bromo-3-ethylphenol **33** was allowed to react with KI, KIO_3, and HCl to give 4-iodo-3-ethylphenol **34**.[21] The Heck reaction was applied to the conversion of **34** into **35**, and **36** into **37**.[22] Compounds **24**, **39a**, and **39b** were allowed to react with *N*-benzylaminoethanol to give tertiary amines **40a**–**c**. The conversion of **40a**–**c** into **41a**–**e** was achieved by Mitsunobu reaction, and subsequent hydrolysis or treatment with TFA afforded carboxylic acids **6**, **12**, **13**, **15**, and **16**.



Scheme 1. Reagents and conditions: (a) *tert*-butyldimethylsilyl chloride, Et_3N, CH_2Cl_2, DMAP, rt, 75%; (b) 2-nitrobenzenesulfonyl chloride, K_2CO_3, CH_2Cl_2, rt, 93%; (c) **19**, DEAD, PPh_3, anhydrous THF, 0 °C to rt; (d) benzenethiol, K_2CO_3, anhydrous DMF, rt, 83% (2 steps); (e) 2-chlorobenzoxazole or 2-chlorobenzothiazole (**24**), $Pd_2(DBA)_3$, rac-BINAP, *tert*-BuONa, anhydrous toluene, 105 °C; (f) TBAF, THF, rt, 62–67% (2 steps); (g) methyl 3-(4-hydroxyphenyl)propionate, DEAD, PPh_3, anhydrous THF, 0 °C to rt, 70–72%; (h) 2 N aq NaOH, MeOH, THF, rt, 99–100%.



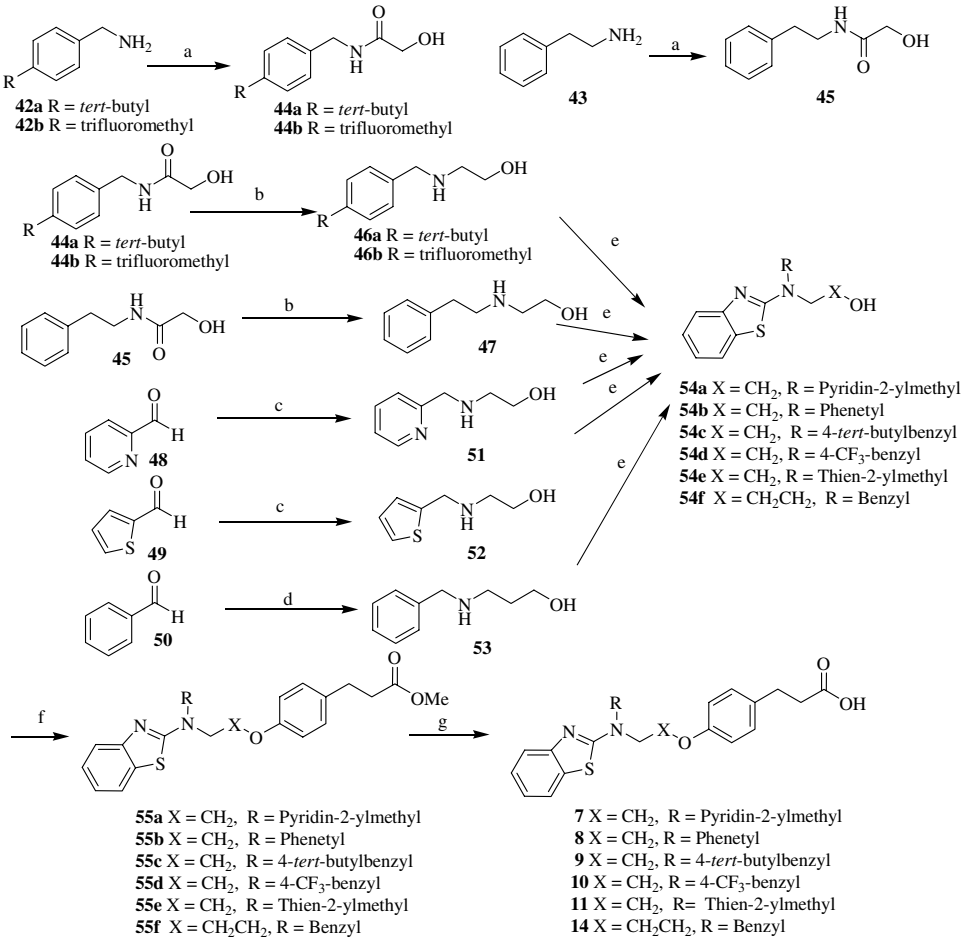
Scheme 2. Reagents and conditions: (a) 1-iodononane, 1,4-dioxane, 100 °C, 81%; (b) i—NaH, anhydrous DMF, 0 °C to 50 °C, ii—4-fluorobenzaldehyde, anhydrous DMF, rt, 26%; (c) $(EtO)_2P(O)CH_2CO_2Et$, NaH, anhydrous THF, 0 °C to rt, 42%; (d) H_2, Pd/C, MeOH, rt, 79%; (e) 2 N aq NaOH, EtOH–THF, rt, 97%.



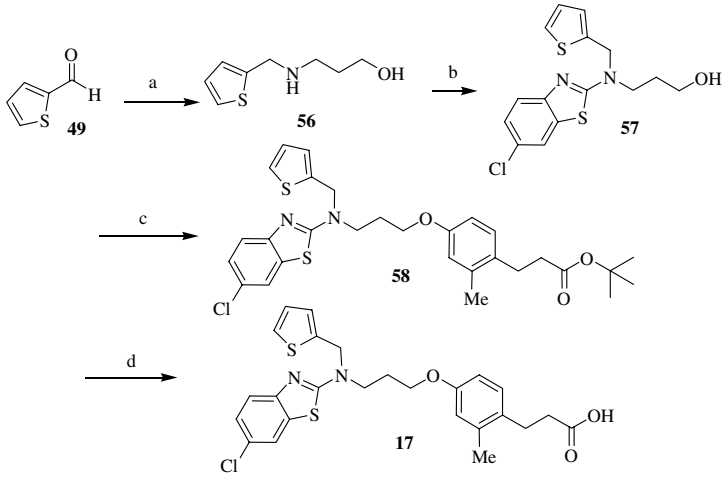
Scheme 3. Reagents and conditions: (a) KI, KIO_3, HCl, 60%; (b) *tert*-butylacrylate, $Pd(OAc)_2$, P(*o*-tol)$_3$, Et_3N, 110 °C, 58–83%; (c) H_2, Pd/C, MeOH, rt, 75–81%; (d) *N*-benzylaminoethanol, $Pd_2(DBA)_3$, rac-BINAP, *tert*-BuONa, anhydrous toluene, 80 °C or *N*-benzylaminoethanol, Et_3N, 100 °C, 44–79%; (e) methyl 3-(4-hydroxyphenyl)propionate or **38a** or **38b**, DEAD, PPh_3, anhydrous THF, 0 °C to rt, 24–77%; (f) 2 N aq NaOH, EtOH, THF, rt or TFA, CH_2Cl_2, rt, 22–81%.

Compounds **7**–**11** and **14** were prepared as shown in Scheme 4. Coupling between glycolic acid and amines **42a**, **42b**, and **43** afforded amides **44a**, **44b**, and **45**. Amides **44a**, **44b**, and **45** were reduced by $LiAlH_4$ to give secondary amines **46a**, **46b**, and **47**. Reductive aminoalkylation of 2-aminoethanol or 3-amino-1-propanol with **48**–**50** gave secondary amines **51**, **52**, and **53**. Amines **46a**, **46b**, **47**, and **51**–**53** were allowed to react with 2-chlorobenzothiazole **24** to give tertiary amines **54a**–**f**. Alcohols **54a**–**f** were converted into **7**–**11** and **14** in the same way as described for the synthesis of **3** and **4**.

Preparation of compound **17** is shown in Scheme 5. Reductive aminoalkylation of 3-amino-1-propanol with aldehyde **49** gave **56**. Amine **56** was converted to compound **17** by the same method described for the preparation of **15** and **16**.



Scheme 4. Reagents and conditions: (a) glycolic acid, EDCI, DMAP, THF, rt, 51–73%; (b) $LiAlH_4$, anhydrous THF, 0 °C to reflux; (c) 2-aminoethanol, anhydrous MeOH, 0 °C, $NaBH_4$; (d) 1-amino-3-propanol, anhydrous MeOH, 0 °C, $NaBH_4$; (e) 2-chlorobenzothiazole, Et_3N, 100 °C, 10–30% (2 steps); (f) methyl 3-(4-hydroxyphenyl)propionate, DEAD, PPh_3, anhydrous THF, 0 °C to rt, 36–96%; (g) 2 N aq NaOH, EtOH, THF, rt, 15–80%.

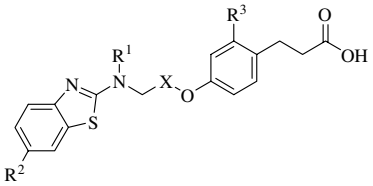


Scheme 5. Reagents and conditions: (a) 3-amino-1-propanol, MeOH, $NaBH_4$; (b) 2,6-dichlorobenzothiazole, Et_3N, 100 °C, 83% (2 steps); (c) **38a**, DEAD, PPh_3, anhydrous THF, 0 °C to rt, 64%; (d) TFA, CH_2Cl_2, rt, 73%.

Compounds **6**–**17** were tested in an in vitro transactivation assay against human PPAR subtypes and the results are listed in Table 1[23,24]. GW501516 (Fig. 1) was used as a reference compound.

In previous reports, the co-crystal structure of PPARδ with pan-agonist GW2433 (Fig. 1) has revealed that PPARδ has a unique Y-shaped pocket and GW2433 fills all three legs of the pocket.[25,26] The crystal structure also

Table 1. In vitro functional PPAR transactivation activity of compounds **4** and **6–17**



Compound	X	R^1	R^2	R^3	EC_{50}[a]		
					α (μM)	δ (μM)	γ (μM)
4	CH_2	*n*-C_9H_{19}	H	H	N.E.[b]	N.E.[b]	N.E.[b]
6	CH_2	Benzyl	H	H	N.E.[b]	2.81	N.E.[b]
7	CH_2	Pyridin-2-ylmethyl	H	H	N.E.[b]	N.E.[b]	N.E.[b]
8	CH_2	Phenethyl	H	H	N.E.[b]	N.E.[b]	N.E.[b]
9	CH_2	4-*tert*-Butylbenzyl	H	H	N.E.[b]	4.88	6.34
10	CH_2	4-CF_3-benzyl	H	H	4.46	0.94	3.69
11	CH_2	Thien-2-ylmethyl	H	H	N.E.[b]	1.74	N.E.[b]
12	CH_2	Benzyl	Cl	H	N.E.[b]	1.36	N.E.[b]
13	CH_2	Benzyl	Me	H	N.E.[b]	2.61	N.E.[b]
14	CH_2CH_2	Benzyl	H	H	N.E.[b]	2.55	N.E.[b]
15	CH_2	Benzyl	H	Me	N.E.[b]	1.17	N.E.[b]
16	CH_2	Benzyl	H	Et	N.E.[b]	2.97	N.E.[b]
17	CH_2CH_2	Thien-2-ylmethyl	Cl	Me	N.E.[b]	0.39	N.E.[b]
GW501516					N.E.[c]	0.085	N.E.[c]

[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 maximal reporter activity.
[b] N.E., did not have sufficient activity to determine EC_{50} values up to 20 μM.
[c] N.E., did not have sufficient activity to determine EC_{50} values up to 10 μM.

made it clear that the two legs of the Y-shaped pocket are formed by hydrophobic amino acid residues and are not so large when compared with the hydrophobic regions of PPARα and PPARγ where the nonyl groups of compound **1** or compound **2** are estimated to be located.[13,14] Based on these information, compounds **6–11** in which the nonyl group of **4** is replaced by smaller lipophilic groups were designed and synthesized. Since compounds **6–11** could possibly have Y-shaped conformation and lack a long alkylchain which is needed for affinity to PPARα or PPARγ,[13,14] they were expected to bind PPARδ selectively. As shown in Table 1, compound **6** (R^1 = Bn), compound **9** (R^1 = 4-*tert*-butylbenzyl), compound **10** (R^1 = 4-CF_3-benzyl), and compound **11** (R^1 = thien-2-ylmethyl) were found to be PPARδ agonists more potent than lead compound **4**,

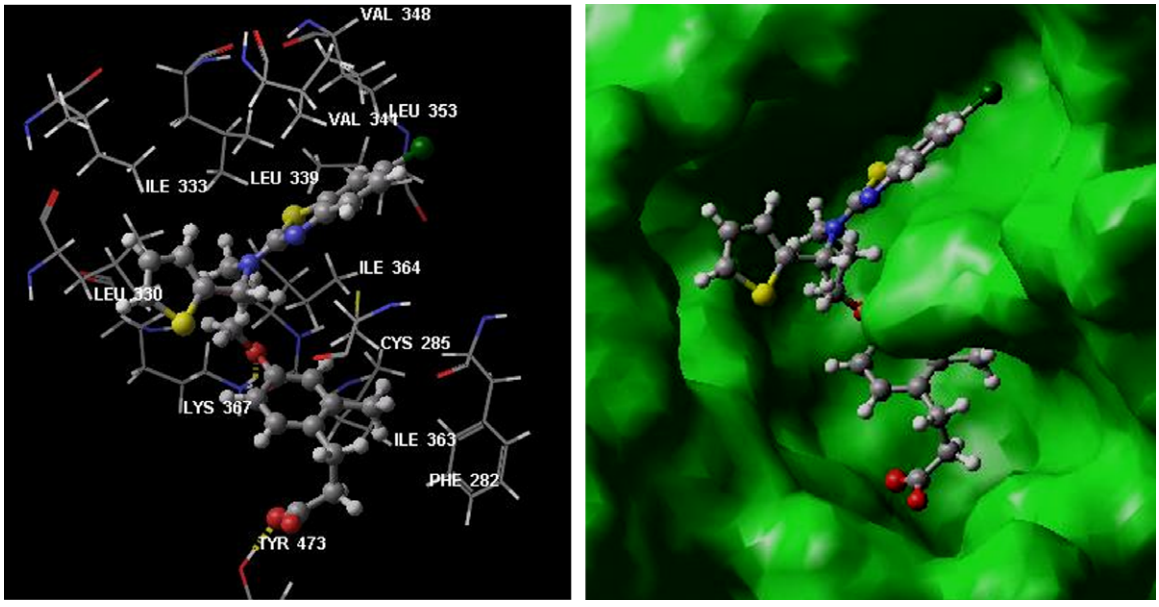


Figure 3. View of the conformation of **17** docked in PPARδ. Amino acid residues and hydrogen bonds are displayed as wires and dotted lines, respectively (left), and the surface of the PPARδ is displayed in the background (right).

and compounds **6** and **11** also showed selectivity towards PPARδ.

Having investigated the requirements for the R^1 group, we next turned our attention to the benzothiazole ring. The R^1 group was fixed as the benzyl group and the effect of a substituent at the 6-position of the benzothiazole ring (R^2 group) was examined. Among compounds **6**, **12**, and **13**, compound **12** (R^2 = Cl) showed transcriptional activity for PPARδ more potently than compound **6**, whereas methyl compound **13** displayed activity similar to **6**.

We also examined the effect of linker length. Compound **14**, where X = CH_2CH_2, modestly improved the PPARδ activity of compound **6**, where X = CH_2.

Since earlier studies revealed that the introduction of a methyl group at the *ortho* position of phenylpropanoic acid improved potency and selectivity toward PPARδ,[26,27] we looked at the effects of the R^3 group. The introduction of a methyl substituent at the *ortho* position of phenylpropanoic acid led to a 2.5-fold increase of PPARδ activity (**6** vs **15**). On the other hand, the introduction of ethyl substitution (compound **16**) was not effective.

Encouraged by these findings, we prepared compound **17** with the best combination of R^1–R^3 and X groups in this study. To our satisfaction, compound **17** showed the highest activity and selectivity for PPARδ in this series.[28]

Next, we studied the binding mode of compound **17**, the most active compound in this study, using Glide 3.5 and Macromodel 8.1 software.[29] As expected, inspection of the simulated PPARδ/**17** complex suggested that compound **17** had a Y-shaped conformation and filled the Y-shaped pocket of PPARδ appropriately (Fig. 3). Specifically, the 6-Cl-benzothiazole ring and the thiophene ring are estimated to occupy each of the two legs of the Y-shaped pocket which are formed by Val 341, Cys, 285, Val 348 and by Leu 330, Ile 333, Leu 339, respectively. In addition, it was shown that the Me group of **17** is located in the small hydrophobic pocket composed of Phe 282, Cys 285, and Ile 363. Interestingly, a hydrogen bond was observed between the oxygen atom of the ether linker and Lys 367. This hydrogen bond may be another important factor for PPARδ selectivity, because no such hydrogen bond has been observed between phenylpropanoic acid derivatives and PPARα or PPARγ.[13,14,30]

In summary, to explore novel PPARδ-selective agonists, we designed and prepared a series of phenylpropanoic acid derivatives. Compound **6** bearing a benzothiazole ring and a benzyl group showed PPARδ activity and selectivity. The introduction of a Cl group at the C-6 position of the benzothiazole ring and Me group at the *ortho* position of phenylpropanoic acid further improved PPARδ transcriptional activity. Compound **17**, which has the best R^1–R^3 and X groups, was found to be the most potent and selective PPARδ agonist in this series. Molecular modeling suggested that compound **17** fills the Y-shaped pocket of PPARδ appropriately. Currently, further detailed studies pertaining to compound **17** are under way.

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24. Human embryonic kidney (HEK) 293 cells were cultured in DMEM containing 5% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. Transfections of PPAR and reporter gene con-

structs were performed by calcium phosphate coprecipitation. Eight hours after transfection, ligands were added. Cells were harvested 12–16 h after treatment, and luciferase and β-galactosidase activities were assayed using a 1420 ARVO^{TM} MX multilabel counter (Perkin-Elmer, Boston, MA, U.S.A.). DNA cotransfection experiments included 58 ng of reporter plasmid, 12 ng of CMX-β-galactosidase, and 18 ng of each receptor expression plasmid per well in a 96-well plate. Luciferase data were normalized to an internal β-galactosidase control and reported values are means of triplicate assays.

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28. The relative efficacy of compound **17** was 86% of that of GW501516.
29. The X-ray structure of PPARδ complexed with GW2433 (PDB code 1GWX) was used as the target structure for docking. Protein preparation, receptor grid generation and ligand docking were performed using the software Glide 3.5. Compound **17** was docked into the ligand binding site of PPARδ. The extra precision mode of Glide was used to determine favorable binding poses, which allowed the ligand conformation to be flexibly explored while holding the protein as a rigid structure during docking. The predicted complex structure was then fully energy-minimized with both the protein and the ligand allowed to move using Macromodel 8.1 software. The conformation of **17** in the PPARδ ligand binding site was minimized by MM calculation based upon the OPLS-AA force field with each parameter set as follows; solvent: water, method: LBFGS, Max # Iterations: 10,000, Converge on: Gradient, Convergence Threshhold: 0.05.
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