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Improvement of water-solubility of biarylcarboxylic acid peroxisome proliferator-activated receptor (PPAR) δ-selective partial agonists by disruption of molecular planarity/symmetry

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ABSTRACT

To elucidate the molecular basis of peroxisome proliferator-activated receptor (PPAR) δ partial agonism, X-ray crystal structures of complexes of the PPARδ ligand-binding site with partial agonists are required. Unfortunately, reported PPARδ partial agonists, biphenylcarboxylic acids **1** and **2**, possess insufficient aqueous solubility to allow such crystals to be obtained. To improve the aqueous solubility of **1** and **2**, substituents were introduced at the 2-position of the biaryl moiety, focusing on disruption of molecular planarity and symmetry. All 2-substituted biphenyl analogs examined showed more potent PPARδ agonistic activity with greater aqueous solubility than **1** or **2**. Among these biphenyls, **25** showed potent and selective PPARδ partial agonistic activity (EC_{50}: 5.7 nM), with adequate solubility in phosphate buffer (0.022 mg/mL). The 2-substituted pyridyl analog **27** showed weaker PPARδ partial agonistic activity (EC_{50}: 76 nM) with excellent solubility in phosphate buffer (2.7 mg/mL; at least 2700 times more soluble than **2**). Our results indicate that two strategies to improve aqueous solubility, that is, introduction of substituent(s) to modify the dihedral angle and to disrupt molecular symmetry, may be generally applicable to bicyclic molecules. Combination of these approaches with the traditional approach of reducing the molecular hydrophobicity may be particularly effective.

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily.[1] In the presence of a ligand, PPARs heterodimerize with retinoid X receptor (RXR), and the heterodimers modulate transcription of target genes by binding to PPAR response elements in the promoter region of target genes. Three different PPAR genes (α, β/δ referred to as δ, and γ) have been identified so far. Each PPAR isotype displays a distinct pattern of tissue distribution and a distinct pharmacological profile, suggesting that each has unique functions in different cell types.[2]

PPARα is highly expressed in tissues with high fatty acid oxidation and, upon stimulation, increases high-density lipoprotein cholesterol synthesis, promotes reverse cholesterol transport, induces cellular uptake of fatty acids, and reduces triglycerides.[3,4] The fibrate class of hypolipidemic drugs are synthetic PPARα ligands.[5,6] PPARγ plays a critical role in the differentiation of preadipocytes to adipocytes, promotes lipid storage, and enhances glucose disposal into peripheral tissues.[7,8] A group of clinically used insulin sensitizers called the thiazolidinediones are agonists for PPARγ.[9,10] PPARγ agonists enhance insulin sensitivity in target tissues and lower glucose and fatty acid levels in type 2 diabetic patients. However, despite their proven benefits, clinical application of these drugs has been plagued by certain adverse effects, such as weight gain, increased rate of bone fractures, fluid accumulation, and pulmonary edema, leading to increased frequency of congestive heart failure.[11–13] Therefore, partial agonists have been developed with the aim of retaining the beneficial effects while diminishing the adverse effects of full agonists. Metaglidasen, a PPARγ partial agonist, is the most advanced insulin sensitizer, and is currently in phase III clinical trials. The results of the phase II clinical trials showed that this compound, which is a prodrug ester that is rapidly and completely hydrolyzed in vivo to the circulating free acid form, significantly improved metabolic parameters without the side effects of fluid retention/edema or weight gain.[12] It has been proposed that the molecular basis of PPARγ partial agonism is stabilization of helix 3 in PPARγ, based on X-ray

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crystallographic and docking studies.[14,15] Furthermore, it has been reported that the reason for the differences in gene expression profiles induced by various PPARγ partial agonists is differences in conformational change of the receptor associated with selective cofactor recruitment.[16] In contrast, there was initially limited interest in PPARδ, probably due to its ubiquitous distribution, or the lack of selective agonists. However, the availability of PPARδ-knockout animals and selective agonists, especially GW501516 (Fig. 1) has revealed that PPARδ is involved in fatty acid metabolism, insulin resistance, reverse cholesterol transport and other biological pathways.[17–19] Recently, a PPARδ partial agonist showed full efficacy on free fatty acid oxidation in vitro and corrected the plasma lipid parameters and insulin sensitivity in vivo.[20]

So far, we have discovered several PPARδ-selective agonists, including TIPP-204.[21] We have also elucidated the X-ray crystal structure of the complex of TIPP-204 and human PPARδ ligand-binding domain.[22] Based on the X-ray crystal structure, biphenylcarboxylic acids **1** and **2** were designed and synthesized as PPARδ-selective partial agonists/antagonists (Fig. 1).[23] Compound **1** shows antagonistic activity with EC_{50} of 170 nM and 8% maximum efficacy, whereas **2** shows partial agonistic activity with EC_{50} of 29 nM and 48% maximum efficacy. The molecular mechanism of PPARδ partial agonism remains unclear. We thought that it might be clarified by comparing the X-ray crystal structures of PPARδ-partial agonist complexes with those with PPARδ-full agonist and PPARδ-antagonist complexes. Unfortunately, the reported PPARδ partial agonists, biphenylcarboxylic acids **1** and **2**, possess insufficient aqueous solubility to allow the preparation of suitable crystals. Herein, we describe the design and synthesis of novel PPARδ partial agonists with sufficient aqueous solubility for this purpose. To improve the aqueous solubility, we focused on the dihedral angle of the biaryl moiety and introduced substituent(s) to disrupt the molecular planarity and symmetry.

2. Results and discussion

2.1. Molecular design

In general, the aqueous solubility of drugs depends on their hydrophobicity (Log *P*).[24,25] Thus, the strategy of introducing hydrophilic group(s) into the molecule is widely used for increasing aqueous solubility. Therefore, as the first approach for increasing the aqueous solubility of **1** and **2**, we tried (i) introduction of an oxygen atom into the *n*-butyl group, and (ii) replacement of the phenyl ring with hetero rings. On the other hand, we also focused on the relationship between solubility and molecular planarity and/or symmetry. We hypothesized that introduction of substituent(s) into bicyclic structures with the aim of modifying the dihedral angle would result in disruption of the planarity and symmetry, leading to decreased crystal packing energy and lower melting point, and so in turn increasing the solubility. Following this hypothesis, we have previously increased the aqueous solubility of integrin antagonists[26–28] and aryl hydrocarbon receptor agonists.[29] We anticipated that introduction of methyl group(s) or fluorine atom(s) at the 2-position of the biaryl moiety of **1** and **2** might be effective, and we considered that this approach might be useful if the traditional methodology of introducing hydrophilic substituents into the PPARδ agonists resulted in a decrease of their activity. We also planned to analyze the relationship between aqueous solubility and molecular symmetry, focusing on the position of the carboxyl group.

2.2. Chemistry

Compounds **12–14**, in which an oxygen atom is introduced into the *n*-butyl group of **1**, were synthesized as shown in Scheme 1. Introduction of two methyl groups into 4′-hydroxybiphenyl-4-carboxylic acid (**3**) gave compound **4**. Compound **4** was formylated, followed by reductive amide alkylation to afford **6**. Simultaneous deprotection of the carboxyl and the hydroxyl group of **6** followed by selective benzylation of the carboxyl group[30] afforded **8**. Introduction of hydrophilic side chains into **8** gave **9–11**. Compounds **9–11** were deprotected to afford **12–14**.

Compounds in which the biphenyl moiety was modified were synthesized as outlined in Scheme 2. Briefly, Suzuki coupling reaction of bromide **15**[23] with appropriate boronic acids or pinacol borate afforded **1**, **2, 16**, and **17**. Compound **16** was hydrolyzed to afford **19**, while **17** was oxidized to afford **23**. As an alternative route, pinacol borate **18** was prepared from **15**. Then, borate **18** was coupled with the appropriate aryl bromides by means of Suzuki coupling reaction to afford **20–22** and **24–27**.

2.3. Biological activity and structure–activity relationships

To investigate the cell-level PPARδ-agonistic activity of the biaryl series, we utilized a PPARδ-responsive reporter gene assay with CMX-GAL4N-hPPARδ LBD as the recombinant receptor gene, TK-MH100x4-LUC as the reporter gene, and the CMX β-galactosidase gene for normalization, as previously reported.[31] After incubation, cells were assayed for luciferase reporter gene and β-galactosidase activities. None of the compounds evaluated in our experiments reduced β-galactosidase activity in the concentration range investigated. Percent efficacy of PPARδ partial agonists is estimated at the maximal stimulatory response in relation to the maximal activity of GW501516. Similar PPARα and PPARγ receptor agonist assay systems were also utilized, with fenofibric acid as the positive control for PPARα and ciglitazone for PPARγ.

Initially, designed analogs possessing hydrophilic side chains were evaluated (Table 1). Introduction of an oxygen atom into the *n*-butyl group (**12–14**) abrogated the PPARδ partial agonistic activity. This result indicated that introduction of hydrophilic substituents into the *n*-butyl group blocks the PPARδ–drug interaction.

Next, the effect of replacement of the phenyl group in the biphenyl moiety with hetero rings was investigated (Table 2). 2-Pyridyl derivative **22** and 2-furanyl derivative **23** lacked PPARδ affinity. 3-Pyridyl derivative **21** showed about two times weaker PPARδ agonistic activity than **1**, and its efficacy was similar to that of **1**. The other 3-pyridyl derivative **26** showed about eight times

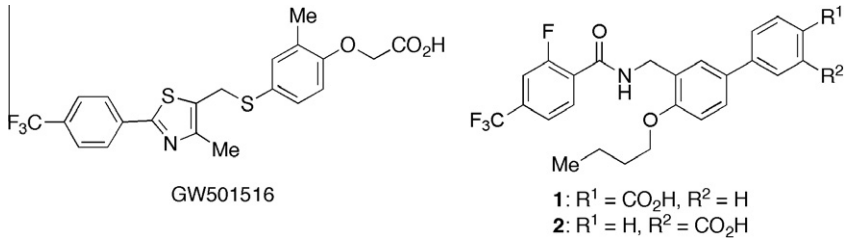
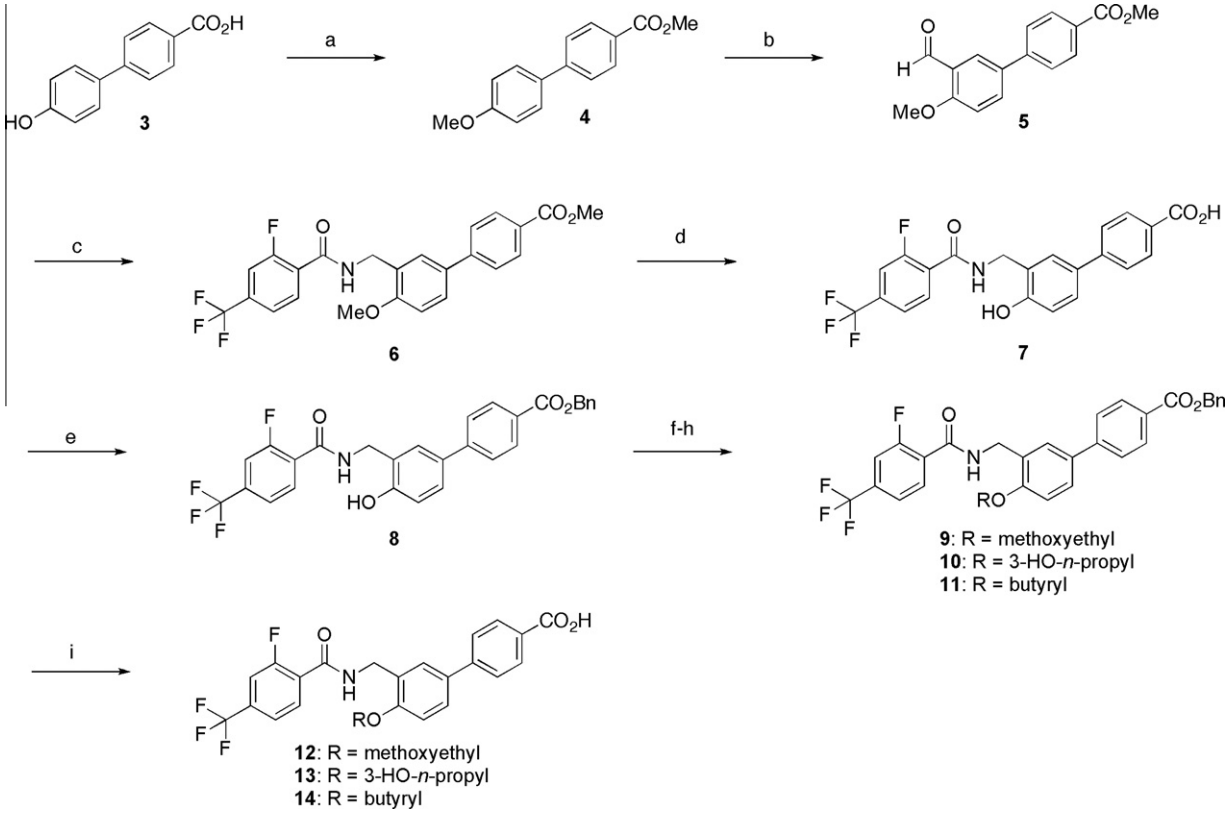


Figure 1. Chemical structures of PPARδ ligands.



Scheme 1. Reagents and conditions: (a) MeI, K_2CO_3, DMF, rt, 98%; (b) $TiCl_4$, $CHCl_2OCH_3$, DCM, −78 °C to rt, 86%; (c) 2-F-4-CF_3-benzamide, Et_3SiH, TFA, toluene, reflux, 73%; (d) BBr_3, DCM, −78 °C to rt, 82%; (e) BnBr, CsF–Celite, CH_3CN, reflux, 74%, (f) $CH_3OCH_2CH_2Cl$, K_2CO_3, DMF, 80 °C, 91% (for **9**); (g) $HOCH_2CH_2CH_2Br$, K_2CO_3, DMF, 100 °C, 63% (for **10**); (h) butyryl chloride, Et_3N, DCM, 0 °C, 99% (for **11**); (i) 10% Pd/C, H_2, AcOEt, rt, 82–90%.

weaker PPARδ agonistic activity than **2**. Overall, these approaches to decrease the hydrophobicity of the molecules led to decreased PPARδ partial agonistic activity.

Next, we examined an alternative approach to increase aqueous solubility, that is, disruption of molecular planarity and/or symmetry (Table 3). Introduction of a methyl group (**19**) or fluorine atom (**20**) at the 2-position of **1** led to improved PPARδ partial agonistic activity with similar efficacy. In particular, methyl analog **19** showed 15 times stronger PPARδ partial agonistic activity than **1**.

The effect of substitution with a *meta*-carboxyl group (**2**, **24–27**) was similar to that in the case of a *para*-carboxyl group (**1**, **19**, and **20**). Methyl analog **24** and difluoro analog **25** showed eighteen and five times stronger activity than **2**, respectively. These results indicate that hydrophobic biaryl structures are preferable for PPARδ partial agonistic activity as compared with hydrophilic biaryl structures.

Pyridyl analog **26** showed increased aqueous solubility (vide infra), although the PPARδ agonistic activity was weak. We hypothesized that introduction of methyl group(s) into the biaryl moiety in the pyridyl analog **26** would improve both the activity and aqueous solubility. As expected, dimethylpyridyl analog **27** showed three times stronger activity than **26**, and comparable activity to **2**.

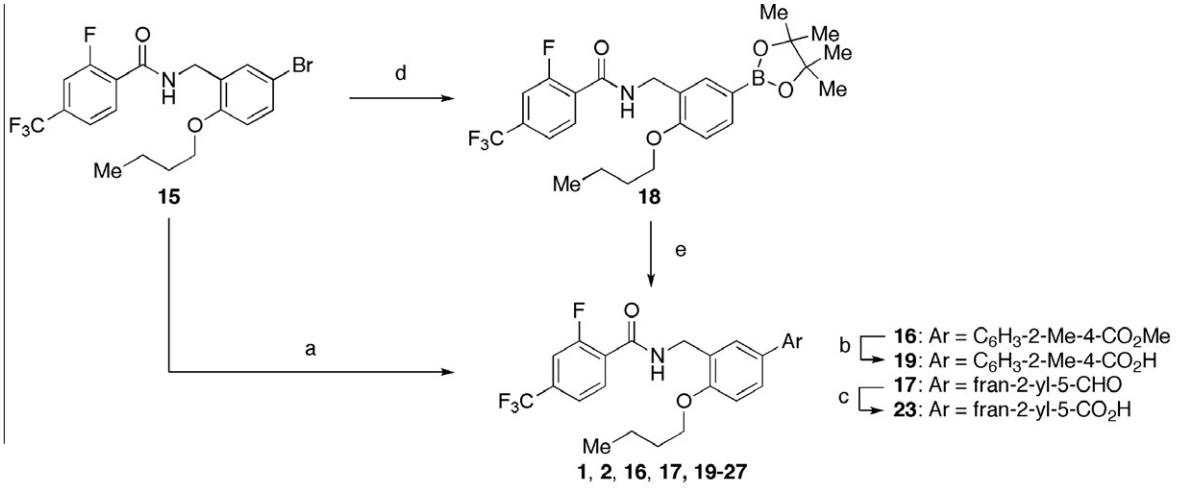
Interestingly, the PPAR activity profile was highly dependent on the position of the carboxyl group. The *meta*-carboxylic acid analogs (**2**, **24–27**) exhibited an efficacy of 40–68% compared to the full agonist GW501516. The *para*-carboxylic acid analogs (**1**, **19**, and **20**) exhibited very weak efficacy of around 10%.

Finally, all compounds in Table 3 showed improved selectivity for PPARδ over PPARα and PPARγ. *para*-Carboxylic acids **1**, **19**, and **20** proved to be PPARδ modulators with complete selectivity at the tested concentrations. The *meta*-carboxylic acids **2** and **24–27** showed agonistic activity with sufficiently high selectivity for PPARδ over PPARα and PPARγ (at least 55 times), although some of these compounds possess weak PPARα full agonistic activity. All these results (Table 3) indicate that introduction of substituents into the biaryl moiety enhanced PPARδ agonistic activity with broadly similar levels of maximum efficacy and selectivity.

2.4. Physico-chemical properties

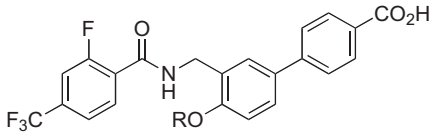
Thermodynamic aqueous solubility (solubility of a compound as a saturated solution in equilibrium) of potent partial agonists **1**, **2**, **19**, **20**, and **24–27** was evaluated according to Avdeef and Testa.[32] The aqueous solubility of **1** and **2** in 1:15 M phosphate buffer (pH 7.4) was quite low (<0.001 mg/mL). So, a mixture of an equal volume of 1:15 M phosphate buffer (pH 7.4) and EtOH was also used as an aqueous medium for the evaluation of thermodynamic solubility.

All compounds shown in Table 4 had higher solubility than the parent compounds. In the *para*-carboxyl series **1**, **19**, and **20**, introduction of a methyl group (**19**) or fluorine atom (**20**) into the biphenyl moiety resulted in better solubility than **1**, as expected. The fluoro analog **20** was nine times more soluble (3.22 mg/mL) than **1** in aqueous EtOH. Although compounds **19** and **20** were more soluble in aqueous EtOH than **1**, they were still essentially insoluble in phosphate buffer. In the case of the *meta*-carboxyl series **2** and **24–27**, introduction of a methyl group (**24**) or two fluorine atoms (**25**) resulted in seven times greater solubility than **2** in aqueous EtOH. Furthermore, **25** showed moderate solubility in phosphate buffer (0.0217 mg/mL) for the first time among these PPAR ligands. Pyridyl analog **26** showed better solubility in both 50% EtOH and phosphate buffer than the parent compound **2**. Dimethylpyridyl analog **27** showed two times better solubility than **26** in aqueous EtOH. It is noteworthy that **27** also has greatly improved solubility in phosphate buffer (2.70 mg/mL), that is, it



Scheme 2. Reagents and conditions: (a) $ArB(OH)_2$ or aryl boronic acid pinacol ester, $(PPh_3)_2PdCl_2$, Na_2CO_3, DME, EtOH, H_2O, 60 °C (for **1**, **2**, **16**, **17**); (b) 6 M HCl, AcOH, 100 °C, 96%; (c) 2-methyl-2-butene, $NaClO_2$, NaH_2PO_4, *t*-BuOH, H_2O, 80 °C, 84%; (d) bis(pinacolate)diborane, $(PPh_3)_2PdCl_2$, AcOK, 1,4-dioxane, 100 °C, 87%; (e) ArBr, $(PPh_3)_2PdCl_2$, Na_2CO_3, DME, EtOH, H_2O, 60 °C (for **20**–**22**, **24**–**27**).

Table 1
PPARδ transactivation activities of biphenyl compounds modified at the butoxy side chain



	R	PPARδ	
		EC_{50}[a] (nM)	% Efficacy
1	*n*-Bu	170	8
12	$CH_3O(CH_2)_2$	N.A.[b]	—
13	$HO(CH_2)_3$	N.A.[b]	—
14	$CH_3(CH_2)_2CO$	N.A.[b]	—

[a] EC_{50} values and % efficacy are given relative to the positive control, GW501516, for PPARδ.
[b] N.A. means not active at 1 μM as an agonist.

is at least 2700 and 350 times more soluble than **2** and **26**, respectively.

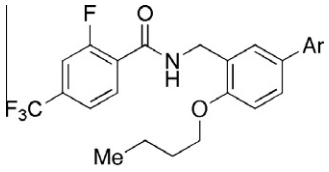
Among the synthesized compounds, **19** and **20** were more potent and more soluble PPARδ-selective partial agonists/antagonists than **1**. In the *meta*-carboxyl series, **24** and **25** were more potent PPARδ-selective partial agonists than **2**, with improved solubility. Two PPAR partial agonists, **25** and **27**, showed excellent overall profiles and appeared to be suitable for preparing crystals for the X-ray crystallographic study. Difluoro analog **25** was five times more potent and eight times more soluble than **2** in aqueous EtOH, and was soluble in phosphate buffer (0.0217 mg/mL). Dimethyl pyridyl analog **27** had comparable activity to **2** and was highly soluble in phosphate buffer (2.70 mg/mL).

To confirm the mechanism of the improved solubility of the biaryl analogs, physico-chemical parameters, that is, melting point, calculated dihedral angle, *C* Log *P* and retention time on reversed-phase HPLC, were evaluated (Table 4). Dihedral angles in optimized structures of simplified models **28** (Fig. 2) were obtained by means of density functional theory (DFT) calculations (B3LYP/6-31G*).[33]

Concerning the compounds with methyl group(s) introduced at the 2-position of the biaryl moiety, all compounds (**19**, **24**, and **27**) possess increased hydrophobicity, larger dihedral angle and lower melting point than those of the parent compounds **1**, **2**, and **26**, respectively. The most soluble analog **27** possesses the lowest melting point and the largest dihedral angle in this series. The only one exception was the smaller *C* Log *P* of **24** compared with **2**, although the other hydrophobicity parameter, retention time, was larger than that of **2**. These results suggested that introduction of methyl group(s) into biaryl molecules results in disruption of the planarity, increasing the dihedral angle and leading in turn to decreased crystal packing energy and lower melting point, with a consequent increase of solubility. Our strategy to improve solubility by focusing on dihedral angle, as presented in this paper, is quite distinct from the general/classical strategy based on decreasing the hydrophobicity of the molecule.

When **27** was compared with **2**, two kinds of modifications (replacement of the phenyl group with pyridine ring and introduc-

Table 2
PPARs transactivation activities of heterocyclic compounds

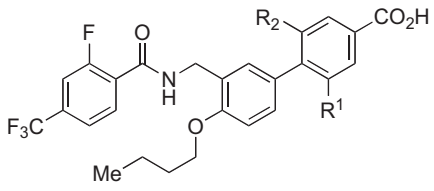


1, **2**, **21-23**, **26**

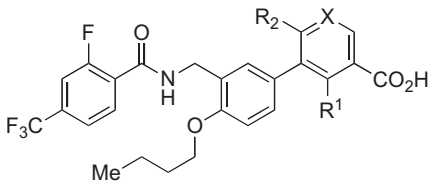
	Ar	PPARδ		PPARα		PPARγ	
		EC_{50}[a] (nM)	% Efficacy	EC_{50}[a] (nM)	% Efficacy	EC_{50}[a] (nM)	% Efficacy
1	4-CO_2H–C_6H_4	170	8	N.A.[b]	—	N.A.[b]	—
21	6-CO_2H-pyridin-3-yl	400	9	N.A.[b]	—	N.A.[b]	—
22	5-CO_2H-pyridin-2-yl	N.A.[b]	—	N.A.[b]	—	N.A.[b]	—
23	5-CO_2H-furan-2-yl	N.A.[b]	—	N.A.[b]	—	N.A.[b]	—
2	3-CO_2H–C_6H_4	29	48	790	100	N.A.[b]	—
26	5-CO_2H-pyridin-3-yl	220	62	4000	—	N.A.[b]	—

[a] EC_{50} values and % efficacy are given relative to the positive controls, GW501516 for PPARδ, fenofibric acid for PPARα, and ciglitazone for PPARγ.
[b] N.A. means not active at 1 μM as an agonist.

Table 3
PPAR transactivation activities of compounds possessing substituents at the 2-position



1, **19**, **20**



2, **24-27**

	R^1	R^2	X	PPARδ		PPARα		PPARγ	
				EC_{50}[a] (nM)	% Efficacy	EC_{50}[a] (nM)	% Efficacy	EC_{50}[a] (nM)	% Efficacy
1	H	H	—	170	8	N.A.[b]	—	N.A.[b]	—
19	Me	H	—	11	9	N.A.[b]	—	N.A.[b]	—
20	F	H	—	53	11	N.A.[b]	—	N.A.[b]	—
2	H	H	CH	29	48	790	100	N.A.[b]	—
24	Me	H	CH	1.6	40	130	100	N.A.[b]	—
25	F	F	CH	5.7	68	N.A.[b]	—	N.A.[b]	—
26	H	H	N	220	62	4000	—	N.A.[b]	—
27	Me	Me	N	76	55	4200	100	N.A.[b]	—

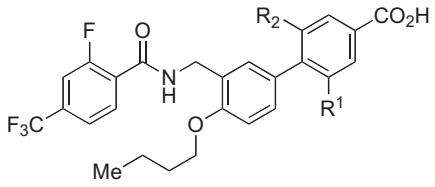
[a] EC_{50} values and % efficacy are given relative to the positive controls, GW501516 for PPARδ, fenofibric acid for PPARα, and ciglitazone for PPARγ.
[b] N.A. means not active at 1 μM as an agonist.

tion of methyl groups) led more than 2700 times higher solubility in phosphate buffer. We consider that this improvement of solubility can be ascribed to two factors, that is, decrease of hydrophobicity by introduction of the pyridine ring (**2** vs **26**) and disruption of molecular planarity by introduction of the methyl groups (**26** vs **27**). Thus, these results indicated that a combination of strategies for improving aqueous solubility is an effective approach.

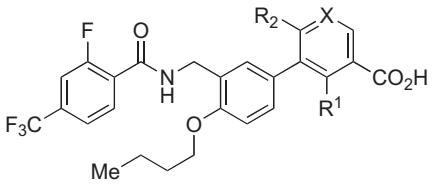
In the case of compounds containing fluorine atom(s), the reason for the improvement of aqueous solubility is not entirely clear. Compound **20** possessed a smaller calculated dihedral angle, lower melting point and higher hydrophobicity than **1**. A possible explanation of the small dihedral angle would be interaction between the fluorine lone pair and hydrogen at the 2′-position. Lack of molecular symmetry of **20** might lead to a lower melting point and greater solubility, or the changes of electron density arising from the introduction of fluorine might have resulted in increased solubility. Compound **25** possessed the same melting point as **2**, but had a larger calculated dihedral angle and higher hydrophobicity. We have reported that fluorination of β-naphthoflavone leads to improved solubility, a relatively small calculated dihedral angle and relatively high hydrophobicity.[29] Taken together, these results indicate that introduction of fluorine atom(s) might improve aqueous solubility not only by disrupting molecular planarity, but also via other mechanism(s). A recent report noted that a larger difference between the highest occupied and lowest unoccupied molecular orbitals (HOMO and LUMO, respectively), or larger molecular polarizability, is associated with lower solubility.[34] Among the compounds reported here, however, the aqueous solubility was not related to the difference between calculated HOMO and LUMO, or calculated dipole moment (data not shown).

On the other hand, we have previously reported examples where disruption of symmetry led to increased aqueous solubility.[27,28] Thus, we analyzed the relationship between aqueous solubility and molecular symmetry, focusing on the position of the carboxyl group. When the position of the carboxyl group was changed from *para* to *meta*, the solubility increased (four times: **1** vs **2**, and 10 times: **19** vs **24**). The melting points of *meta*-carboxylic acids **2** and **24** were lower than those of the corresponding *para*-derivatives **1** and **19**, respectively. These results suggest that disruption of symmetry results in decreased crystal packing energy

Table 4
Physico-chemical data of PPARδ modulators



1, **19**, **20**



2, **24-27**

	R^1	R^2	X	Solubility (mg/mL)		Melting point (°C)	Calculated dihedral angle[b] (°)	*C* Log *P*[c]	HPLC retention time[d] (min)
				50% EtOH[a]	Phosphate buffer (pH7.4)				
1	H	H	—	0.375	<0.001	259–262	43.5	7.05	7.98
19	Me	H	—	0.985	<0.001	241–243	52.5	7.25	9.46
20	F	H	—	3.22	<0.001	221–223	36.1	7.29	8.72
2	H	H	CH	1.35	<0.001	177–178	36.9	7.05	7.79
24	Me	H	CH	9.95	<0.001	146–149	57.5	6.95	8.65
25	F	F	CH	10.4	0.0217	177	46.2	7.10	7.42
26	H	H	N	9.03	0.00762	152	37.4	6.02	3.61
27	Me	Me	N	17.7	2.70	104–106	78.1	6.12	4.36

[a] Solubility in a mixture of equal volumes of EtOH and 1:15 M phosphate buffer (pH 7.4).
[b] Calculated dihedral angles of simplified models **28** (Fig. 2) were estimated by using GAUSSIAN 03.
[c] *C* Log *P* values were estimated with ChemDraw Ultra version 10.0.
[d] Waters μBondapak reversed-phase column (3.9 mm × 150 mm).

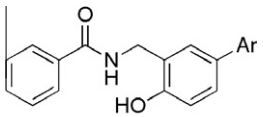


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Figure 2. Simplified models of PPARδ modulators for calculation of dihedral angle.

and lower melting point, leading to increased solubility. Determination of the crystal structure of these molecules will be needed to take this discussion further.

3. Conclusion

To elucidate the molecular basis of PPARδ partial agonism, PPARδ partial agonists possessing sufficient aqueous solubility to permit the preparation of crystals suitable for X-ray structure analysis are required. Focusing on disruption of molecular planarity and symmetry of PPARδ partial agonists **1** and **2**, substituents were introduced at the 2-position of the biaryl moiety. Structure–activity relationship study revealed that (1) a hydrophobic biaryl moiety enhances PPARδ agonistic activity, (2) substituents at the 2-position of the biaryl moiety improve aqueous solubility, (3) PPARδ efficacy was highly dependent on the position of the carboxyl group, and (4) the substituted PPARδ modulators possess high selectivity over other PPARs. As a result of structure development, we obtained PPARδ-selective partial agonists **25** and **27** which are sufficiently soluble in phosphate buffer. We are currently investigating the X-ray crystal structure of the complexes of these PPARδ selective partial agonists with the PPARδ ligand-binding domain. In addition, more potent PPARδ selective partial agonists/antagonists **19** and **20** with improved solubility were obtained.

The reasons why introduction of substituents to the 2-position of the biaryl moiety led to improve aqueous solubility were discussed. Analogs in which a methyl group(s) was introduced at the 2-position of the biaryl moiety showed increased hydrophobicity, a larger dihedral angle and a lower melting point than those of the parent compounds. These results suggested that these substituents disrupt the molecular planarity, increasing the dihedral angle and leading in turn to decreased crystal packing energy and lower melting point, thereby increasing the solubility. We suggest that the strategy to improve aqueous solubility by introduction of substituent(s) that modify the dihedral angle of bicyclic molecules could have general applicability. We also suggest that the use of a combination of strategies for improving aqueous solubility, that is, both reduction of hydrophobicity and disruption of molecular planarity, is effective. We also found examples in which disruption of molecular symmetry led to increased aqueous solubility. Further studies on the mechanisms involved are needed.

4. Experimental

4.1. General methods

^{1}H NMR spectra were recorded on a JEOL JNMGX500 (500 MHz) spectrometer. Chemical shifts are expressed in parts per million relative to tetramethylsilane. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer. Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on Merck 60 F254 pre-coated silica gel plates. Flash chromatography was performed on a column of Kanto Silica Gel N 60. HPLC analyses were performed on an analytical column (Waters μBondapak reversed-phase column (C18, 125 Å, 10 μm, 3.9 mm × 150 mm) eluted with a mobile phase consisting of 0.1% TFA in 55% CH_3CN at a flow rate of 1.0 mL/min, with UV monitoring at 262–295 nm, at 40 °C.

4.2. Chemistry

4.2.1. Methyl 4′-methoxybiphenyl-4-carboxylate (4)

A solution of 4′-hydroxy-4-biphenylcarboxylic acid (**3**, 1.02 g, 4.76 mmol) in DMF (30 mL) was treated with MeI (3.0 g) followed by K_2CO_3 (2.0 g) at rt, and the mixture was stirred at rt for 16 h. The reaction was quenched with aqueous NH_4Cl, and the whole was extracted with DCM. The organic layer was dried over $MgSO_4$

and concentrated in vacuo, and the residue was purified by silica gel chromatography (hexane/EtOAc = 10:1) to give **4** (1.13 g, 4.66 mmol, 98%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H), 7.57 (d, J = 8.5 Hz, 2H), 7.00 (d, J = 8.5 Hz, 2H), 3.93 (s, 3H), 3.86 (s, 3H). MS (FAB) 242 $(M)^+$.

4.2.2. Methyl 3′-formyl-4′-methoxybiphenyl-4-carboxylate (5)

A solution of **4** (1.13 g, 4.66 mmol) in DCM (100 mL) was cooled to −78 °C, then treated with $TiCl_4$ (3.0 mL) followed by $CHCl_2OCH_3$ (1.5 mL), and the mixture was stirred for 30 min at that temperature, warmed to rt, and stirred for 6 h. The reaction was quenched with 2 M aqueous HCl, and the whole was extracted with DCM and AcOEt. The organic layer was dried over $MgSO_4$ and concentrated in vacuo, and the residue was purified by silica gel chromatography (hexane/EtOAc = 10:1 to 3:1) to give **5** (1.08 g, 4.00 mmol, 86%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.53 (s, 1H), 8.12 (d, J = 2.4 Hz, 1H), 8.10 (d, J = 8.2 Hz, 2H), 7.84 (dd, J = 8.8, 2.4 Hz, 1H), 7.65 (d, J = 8.2 Hz, 2H), 7.11 (d, J = 8.8 Hz, 1H), 4.00 (s, 3H), 3.94 (s, 3H). MS (FAB) 271 $(M+H)^+$.

4.2.3. Methyl 3′-{[2-fluoro-4-(trifluoromethyl)phenylamido] methyl}-4′-methoxybiphenyl-4-carboxylate (6)

A solution of **5** (1.08 g, 4.00 mmol) and 2-fluoro-4-trifluoromethylbenzamide (1.66 g, 8.01 mmol) in toluene (30 mL) was treated with TFA (0.5 mL) followed by Et_3SiH (3.0 mL) and the mixture was refluxed for 24 h, then concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc = 5:1 to 3:1) to give **6** (1.35 g, 2.93 mmol, 73%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (t, J = 7.9 Hz, 1H), 8.07 (d, J = 7.9 Hz, 2H), 7.64 (d, J = 1.8 Hz, 1H), 7.62 (d, J = 7.9 Hz, 2H), 7.57 (dd, J = 8.5, 1.8 Hz, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.42 (m, 1H), 7.39 (d, J = 11.6 Hz, 1H), 7.00 (d, J = 8.5 Hz, 1H), 4.74 (d, J = 5.5 Hz, 2H), 3.96 (s, 3H), 3.93 (s, 3H). MS (FAB) 461 $(M)^+$.

4.2.4. 3′-{[2-Fluoro-4-(trifluoromethyl)phenylamido]methyl}-4′-hydroxybiphenyl-4-carboxylic acid (7)

A solution of **6** (1.15 g, 2.49 mmol) in DCM (80 mL) was cooled to −78 °C, then treated with BBr_3 (10 mmol, 1.0 M solution in DCM) at the same temperature, and the mixture was warmed to rt, and stirred for 3 h. The reaction was quenched with brine, and the whole was extracted with DCM and AcOEt. The organic layer was dried over $MgSO_4$ and concentrated in vacuo, and the residue was purified by silica gel chromatography (hexane/EtOAc = 2:1 to 0:1) to give **7** (880 mg, 2.03 mmol, 82%) as a white solid.

^{1}H NMR (500 MHz, DMSO) δ 9.96 (s, 1H), 8.99 (t, J = 5.5 Hz, 1H), 7.96 (d, J = 8.5 Hz, 2H), 7.84–7.81 (m, 2H), 7.68–7.66 (m, 3H), 7.59 (d, J = 2.4 Hz, 1H), 7.50 (dd, J = 8.5, 2.4 Hz, 1H), 6.94 (d, J = 8.5 Hz, 1H), 4.49 (d, J = 5.5 Hz, 2H). MS (FAB) 434 $(M+H)^+$.

4.2.5. Benzyl 3′-{[2-fluoro-4-(trifluoromethyl)phenylamido] methyl}-4′-hydroxybiphenyl-4-carboxylate (8)

A solution of **7** (396 mg, 0.914 mmol) in CH_3CN (80 mL) was treated with CsF–Celite (0.36 g) followed by benzyl bromide (0.66 g, 3.9 mmol) and the mixture was refluxed for 5 h, then concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc = 5:1 to 2:1) to give **8** (353 mg, 0.674 mmol, 74%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.29 (t, J = 8.2 Hz, 1H), 8.11 (d, J = 8.2 Hz, 2H), 7.64 (m, 1H), 7.60 (d, J = 8.2 Hz, 2H), 7.56 (d, J = 8.2 Hz, 1H), 7.52 (dd, J = 8.5, 2.4 Hz, 1H), 7.47–7.34 (m, 7H), 7.06 (d, J = 8.5 Hz, 1H), 5.38 (s, 2H), 4.67 (d, J = 6.1 Hz, 2H). MS (FAB) 524 $(M+H)^+$.

4.2.6. Benzyl 3′-{[2-fluoro-4-(trifluoromethyl)phenylamido] methyl}-4′-(2-methoxyethoxy)biphenyl-4-carboxylate (9)

A solution of **8** (30.2 mg, 57.7 μmol) and $CH_3OCH_2CH_2Cl$ (20 mg, 0.21 mmol) in DMF (1 mL) was treated with K_2CO_3 (10 mg) and the mixture was stirred at 80 °C for 2 h. The reaction was quenched with brine, and the whole was extracted with DCM. The organic layer was dried over $MgSO_4$ and concentrated in vacuo, and the residue was purified by silica gel chromatography (hexane/EtOAc = 5:1 to 2:1) to give **9** (30.5 mg, 52.4 μmol, 91%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (t, J = 7.9 Hz, 1H), 8.11 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 2.4 Hz, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.55–7.34 (m, 9H), 6.99 (d, J = 8.5 Hz, 1H), 5.38 (s, 2H), 4.75 (d, J = 4.9 Hz, 2H), 4.24 (t, J = 4.7 Hz, 2H), 3.79 (t, J = 4.7 Hz, 2H), 3.35 (s, 3H). MS (FAB) 582 $(M+H)^+$.

4.2.7. Benzyl 3′-{[2-fluoro-4-(trifluoromethyl)phenylamido] methyl}-4′-(3-hydroxypropoxy)biphenyl-4-carboxylate (10)

A solution of **8** (58.1 mg, 111 μmol) and 3-bromo-1-propanol (28 mg, 0.20 mmol) in DMF (3 mL) was treated with K_2CO_3 (26 mg) and the mixture was stirred at 100 °C for 2 h. The reaction was quenched with aqueous NH_4Cl, and the whole was extracted with DCM and AcOEt. The organic layer was dried over $MgSO_4$ and concentrated in vacuo, and the residue was purified by silica gel chromatography (hexane/EtOAc = 1:1) to give **10** (40.6 mg, 69.8 μmol, 63%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (t, J = 7.9 Hz, 1H), 8.11 (d, J = 8.5 Hz, 2H), 7.62–7.33 (m, 12H), 7.01 (d, J = 8.5 Hz, 1H), 5.38 (s, 2H), 4.74 (d, J = 4.9 Hz, 2H), 4.25 (t, J = 6.1 Hz, 2H), 3.91 (t, J = 6.1 Hz, 2H), 2.13 (m, 2H). MS (FAB) 582 $(M+H)^+$.

4.2.8. Benzyl 4′-(butanoyloxy)-3′-{[2-fluoro-4-(trifluoromethyl) phenylamido]methyl}biphenyl-4-carboxylate (11)

A solution of **8** (44.3 mg, 84.6 μmol) in DCM (5 mL) was cooled to 0 °C, then treated with butyryl chloride (24 mg) followed by Et_3N (0.1 mL), and the mixture was stirred at rt for 1 h. The reaction was quenched with aqueous $NaHCO_3$, and the whole was extracted with DCM. The organic layer was dried over $MgSO_4$ and concentrated in vacuo, and the residue was purified by silica gel chromatography (hexane/EtOAc = 5:1) to give **11** (49.6 mg, 83.6 μmol, 99%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (t, J = 7.7 Hz, 1H), 8.13 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 2.2 Hz, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.58 (dd, J = 8.4, 2.2 Hz, 1H), 7.53 (d, J = 7.7 Hz, 1H), 7.47–7.34 (m, 6H), 7.19 (d, J = 8.4 Hz, 1H), 7.06 (m, 1H), 5.38 (s, 2H), 4.68 (d, J = 4.9 Hz, 2H), 2.63 (t, J = 7.3 Hz, 2H), 1.80 (m, 2H), 1.05 (t, J = 7.3 Hz, 3H). MS (FAB) 594 $(M+H)^+$.

4.2.9. 3′-{[2-Fluoro-4-(trifluoromethyl)phenylamido]methyl}-4′-(2-methoxyethoxy)biphenyl-4-carboxylic acid (12)

A solution of **9** (30.5 mg, 52.4 μmol) and 10% Pd-C (20 mg) in AcOEt (5 mL) under an H_2 atmosphere was stirred for 1 h, then filtered through Celite. The filtrate was concentrated in vacuo to give **12** (21.0 mg, 42.7 μmol, 82%) as a white solid.

^{1}H NMR (500 MHz, DMSO) δ 2.92 (s, 1H), 8.94 (t, J = 5.5 Hz, 1H), 7.99 (d, J = 7.9 Hz, 2H), 7.86–7.81 (m, 2H), 7.73–7.64 (m, 5H), 7.14 (d, J = 8.5 Hz, 1H), 4.53 (d, J = 5.5 Hz, 2H), 4.21 (t, J = 4.0 Hz, 2H), 3.72 (t, J = 4.0 Hz, 2H), 3.31 (s, 3H). MS (FAB) 492 $(M+H)^+$.

4.2.10. 3′-{[2-Fluoro-4-(trifluoromethyl)phenylamido]methyl}-4′-(3-hydroxypropoxy)biphenyl-4-carboxylic acid (13)

A solution of **10** (40.6 mg, 69.8 μmol) and 10% Pd–C (10 mg) in AcOEt (10 mL) under H_2 atmosphere was stirred for 1 h. The reaction mixture was filtered through Celite, the filtrate was concentrated in vacuo, and the residue was purified by silica gel chromatography (hexane/EtOAc = 1:1 to 0:1) to give **13** (29.4 mg, 59.8 μmol, 86%) as a white solid.

^{1}H NMR (500 MHz, DMSO) δ 2.92 (s, 1H), 8.98 (t, J = 5.7 Hz, 1H), 7.98 (d, J = 8.5 Hz, 2H), 7.85–7.80 (m, 2H), 7.71 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 9.2 Hz, 1H), 7.65–7.63 (m, 2H), 7.13 (d, J = 9.2 Hz, 1H), 4.57 (t, J = 5.2 Hz, 1H), 4.52 (d, J = 5.7 Hz, 2H), 4.14 (t, J = 6.1 Hz, 2H), 3.61 (m, 2H), 1.91 (m, 2H).

4.2.11. 4′-(Butanoyloxy)-3′-{[2-fluoro-4-(trifluoromethyl)phenylamido]methyl}biphenyl-4-carboxylic acid (14)

Compound **14** (37.9 mg, 75.3 μmol, 90%) was synthesized as a white solid from **11** (49.6 mg, 83.6 μmol) following the general procedure for **12**.

^{1}H NMR (500 MHz, DMSO) δ 13.01 (s, 1H), 9.06 (t, J = 5.7 Hz, 1H), 8.03 (d, J = 8.5 Hz, 2H), 7.84 (d, J = 9.2 Hz, 1H), 7.79–7.67 (m, 6H), 7.26 (d, J = 7.9 Hz, 1H), 4.47 (d, J = 5.7 Hz, 2H), 2.65 (t, J = 7.3 Hz, 2H), 1.68 (m, 2H), 0.98 (t, J = 7.3 Hz, 3H).

4.2.12. 4′-Butoxy-3′-{[2-fluoro-4-(trifluoromethyl)phenylamido]methyl}biphenyl-4-carboxylic acid (1)

To a solution of **15** (60 mg, 0.13 mmol) and 4-carboxyphenylboronic acid (50 mg, 0.30 mmol) in 6 mL of DME, 4 mL of ethanol and 2 M aq Na_2CO_3 were added $(Ph_3P)_2PdCl_2$ (10 mg, 0.014 mmol). The reaction mixture was stirred at 50 °C for 5 h. The reaction was quenched by the addition of aq NH_4Cl, and the whole was extracted with AcOEt. The organic layer was dried over $MgSO_4$ and concentrated, and the residue was purified by silica gel chromatography (hexane/ethyl acetate = 3:1) to give **1** (30 mg, 0.061 mmol, 47%) as a white solid.

Mp 259–262 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.96 (t, J = 5.7 Hz, 1H), 7.98 (d, J = 8.5 Hz, 2H), 7.83 (m, 2H), 7.71 (d, J = 8.5 Hz, 2H), 7.69–7.62 (m, 3H), 7.12 (d, J = 9.2 Hz, 1H), 4.52 (d, J = 5.7 Hz, 2H), 4.08 (t, J = 6.1 Hz, 2H), 1.75 (m, 2H), 1.49 (m, 2H), 0.95 (t, J = 7.6 Hz, 3H). MS (FAB) 490 $(M+H)^+$. UV monitoring of HPLC at 295 nm.

4.2.13. 4′-Butoxy-3′-{[2-fluoro-4-(trifluoromethyl)phenylamido]methyl}biphenyl-3-carboxylic acid (2)

Compound **2** (29 mg, 59 μmol, 67%) was synthesized as a white solid from **15** and 3-carboxyphenyl boronic acid following the general procedure for **1**.

Mp 177–178 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.28 (t, J = 1.8 Hz, 1H), 8.26 (t, J = 7.9 Hz, 1H), 8.03 (d, J = 7.9 Hz, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.63 (d, J = 1.8 Hz, 1H), 7.56–7.50 (m, 3H), 7.42 (m, 1H), 7.40 (d, J = 11.6 Hz, 1H), 6.99 (d, J = 7.9 Hz, 1H), 4.76 (d, J = 5.5 Hz, 2H), 4.11 (t, J = 6.7 Hz, 2H), 1.87 (m, 2H), 1.55 (m, 2H), 1.01 (t, J = 7.6 Hz, 3H). MS (FAB) 490 $(M+H)^+$. UV monitoring of HPLC at 270 nm.

4.2.14. 4′-Methyl butoxy-3′-{[2-fluoro-4-(trifluoromethyl) phenylamido]methyl}-2-methylbiphenyl-4-carboxylate (16)

Compound **16** (32.1 mg, 0.0620 mmol, 60%) was synthesized as a white solid from **15** and methyl 3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate following the general procedure for **1**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (t, J = 7.6 Hz, 1H), 7.93 (s, 1H), 7.86 (dd, J = 7.9, 1.8 Hz, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.42 (m, 1H), 7.39 (d, J = 11.6 Hz, 1H), 7.31 (d, J = 2.2 Hz, 1H), 7.27 (m, 1H), 7.23 (dd, J = 8.3, 2.2 Hz, 1H), 6.95 (d, J = 8.3 Hz, 1H), 4.73 (d, J = 5.5 Hz, 2H), 4.10 (t, J = 6.7 Hz, 2H), 3.92 (s, 3H), 2.32 (s, 3H), 1.86 (m, 2H), 1.55 (m, 2H), 1.01 (t, J = 7.6 Hz, 3H). MS (FAB) 517 $(M)^+$.

4.2.15. *N*-[2-Butoxy-5-(5-formylfuran-2-yl)benzyl]-2-fluoro-4-(trifluoromethyl)benzamide (17)

Compound **17** (58.4 mg, 0.126 mmol, 93%) was synthesized as a yellow oil from **15** and 5-formyl-2-furanboronic acid following the general procedure for **1**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.60 (s, 1H), 8.25 (t, J = 7.3 Hz, 1H), 7.78–7.77 (m, 2H), 7.52 (d, J = 8.5 Hz, 1H), 7.40 (d, J = 11.0 Hz, 1H), 7.37 (m, 1H), 7.29 (d, J = 3.7 Hz, 1H), 6.95 (d, J = 9.2 Hz, 1H), 6.73 (d, J = 3.7 Hz, 1H), 4.71 (d, J = 5.5 Hz, 2H), 4.09 (t, J = 6.4 Hz, 2H), 1.85 (m, 2H), 1.53 (m, 2H), 1.00 (t, J = 7.3 Hz, 3H). MS (FAB) 464 $(M+H)^+$.

4.2.16. 4′-Butoxy-3′-{[2-fluoro-4-(trifluoromethyl)phenylamido]methyl}-2-methylbiphenyl-4-carboxylic acid (19)

A solution of intermediate **16** (32.1 mg, 62.0 μmol) in 6 M aqueous HCl (3 mL) and AcOH (8 mL) was stirred at 100 °C for 2 h. The reaction mixture was poured into water, and the precipitated solid was collected by filtration to give **19** (30.1 mg, 59.8 μmol, 96%) as a white solid.

Mp 241–243 °C. ^{1}H NMR (500 MHz, DMSO) δ 12.88 (s, 1H), 8.94 (t, J = 5.9 Hz, 1H), 7.84–7.77 (m, 4H), 7.66 (d, J = 7.9 Hz, 1H), 7.28–7.25 (m, 3H), 7.08 (m, 1H), 4.50 (d, J = 5.9 Hz, 2H), 4.07 (t, J = 6.1 Hz, 2H), 2.28 (s, 3H), 1.76 (m, 2H), 1.50 (m, 2H), 0.95 (t, J = 7.6 Hz, 3H). MS (FAB) 504 $(M+H)^+$. UV monitoring of HPLC at 280 nm.

4.2.17. 5-(4-Butoxy-3-{[2-fluoro-4-(trifluoromethyl)phenylamido]methyl}phenyl)furan-2-carboxylic acid (23)

A solution of **17** (58.4 mg, 0.126 mmol) and NaH_2PO_4 (18 mg, 0.15 mmol) in *t*-BuOH (6 mL) and H_2O (2 mL) was treated with 2-methyl-2-butene (1 mL) followed by sodium chlorite (45 mg, 0.50 mmol), and the mixture was stirred at 80 °C for 5 h. The reaction was quenched with aqueous HCl and the whole was extracted with DCM. The organic layer was dried over $MgSO_4$ and concentrated in vacuo, and the residue was purified by silica gel chromatography (hexane/AcOEt = 2:1 to 0:1) to give **23** (51.0 mg, 0.106 mmol, 84%) as a yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (t, J = 7.6 Hz, 1H), 7.77 (d, J = 2.4 Hz, 1H), 7.75 (dd, J = 8.5, 2.4 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.41–7.36 (m, 3H), 6.94 (d, J = 8.5 Hz, 1H), 6.67 (d, J = 3.7 Hz, 1H), 4.72 (d, J = 5.5 Hz, 2H), 4.09 (t, J = 6.7 Hz, 2H), 1.85 (m, 2H), 1.53 (m, 2H), 1.00 (t, J = 7.3 Hz, 3H). MS (FAB) 479 $(M)^+$.

4.2.18. *N*-[2-Butoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl]-2-fluoro-4-(trifluoromethyl)benzamide (18)

A mixture of **15** (145 mg, 0.323 mmol), bis(pinacolate)diboron (120 mg, 0.473 mmol), $(Ph_3P)_2PdCl_2$ (18 mg, 0.039 mmol), and KOAc (140 mg, 1.43 mmol) was dissolved in 1,4-dioxane (3 mL) and the whole was stirred at 100 °C for 3 h, then diluted with AcOEt and H_2O, and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and concentrated in vacuo, and the residue was purified by silica gel chromatography (hexane/EtOAc = 10:1) to give **18** (139 mg, 0.281 mmol, 87%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (t, J = 7.9 Hz, 1H), 7.78 (d, J = 1.4 Hz, 1H), 7.74 (dd, J = 8.0, 1.4 Hz, 1H), 7.51 (d, J = 8.5 Hz, 1H), 7.37 (d, J = 11.6 Hz, 1H), 7.31 (m, 1H), 6.89 (d, J = 8.0 Hz, 1H), 4.69 (d, J = 4.9 Hz, 2H), 4.06 (t, J = 6.7 Hz, 2H), 1.82 (m, 2H), 1.50 (m, 2H), 1.32 (s, 12H), 0.97 (t, J = 7.6 Hz, 3H). MS (FAB) 495 $(M)^+$.

4.2.19. 4′-Butoxy-2-fluoro-3′-{[2-fluoro-4-(trifluoromethyl) phenylamido]methyl}biphenyl-4-carboxylic acid (20)

A solution of **18** (30.8 mg, 62.2 μmol) and 4-bromo-3-fluorobenzoic acid (19.5 mg, 89.0 μmol) in DME (3 mL), ethanol (2 mL), and 2 M aqueous Na_2CO_3 (3 mL) was treated with $(Ph_3P)_2PdCl_2$ (10 mg, 0.014 mmol) and the mixture was stirred at 60 °C for 3 h, then diluted with AcOEt. The reaction was quenched with brine, and the whole was extracted with AcOEt. The organic layer was dried over $MgSO_4$ and concentrated in vacuo, and the residue was purified by silica gel chromatography (hexane/AcOEt = 3:1) to give **20** (22.4 mg, 44.1 μmol, 71%) as a white solid.

Mp 221–223 °C. ^{1}H NMR (500 MHz, DMSO) δ 13.25 (s, 1H), 8.96 (t, J = 5.7 Hz, 1H), 7.83–7.78 (m, 3H), 7.72 (dd, J = 11.3, 1.5 Hz, 1H), 7.68 (d, J = 7.9 Hz, 1H), 7.60 (t, J = 7.9 Hz, 1H), 7.52–7.49 (m, 2H), 7.14 (d, J = 9.2 Hz, 1H), 4.51 (d, J = 5.7 Hz, 2H), 4.09 (t, J = 6.1 Hz, 2H), 1.76 (m, 2H), 1.49 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H). MS (FAB) 508 $(M+H)^+$. UV monitoring of HPLC at 290 nm.

4.2.20. 5-(4-Butoxy-3-{[2-fluoro-4-(trifluoromethyl)phenylamido]methyl}phenyl)pyridine-2-carboxylic acid (21)

Compound **21** (10.9 mg, 0.0222 mmol, 28%) was synthesized as a white solid from **18** and 5-bromopicolinic acid following the general procedure for **20**.

^{1}H NMR (500 MHz, DMSO) δ 8.98–8.94 (m, 2H), 8.16 (dd, J = 7.9, 2.4 Hz, 1H), 8.09 (d, J = 7.9 Hz, 1H), 7.86–7.81 (m, 2H), 7.73–7.68 (m, 3H), 7.17 (d, J = 8.5 Hz, 1H), 4.53 (d, J = 6.1 Hz, 2H),4.10 (t, J = 6.4 Hz, 2H), 1.76 (m, 2H), 1.49 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H). MS (FAB) 491 $(M+H)^+$.

4.2.21. 6-(4-Butoxy-3-{[2-fluoro-4-(trifluoromethyl)phenylamido]methyl}phenyl)pyridine-3-carboxylic acid (22)

Compound **22** (26.2 mg, 0.0534 mmol, 69%) was synthesized as a white solid from **18** and 6-bromonicotinic acid following the general procedure for **20**.

^{1}H NMR (500 MHz, DMSO) δ 13.24 (s, 1H), 9.08 (d, J = 2.4 Hz, 1H), 8.97 (t, J = 5.5 Hz, 1H), 8.27 (dd, J = 8.5, 2.4 Hz, 1H), 8.12 (d, J = 2.4 Hz, 1H), 8.07 (dd, J = 8.5, 2.4 Hz, 1H), 7.98 (d, J = 8.5 Hz, 1H), 7.85–7.81 (m, 2H), 7.70 (d, J = 7.3 Hz, 1H), 7.14 (d, J = 8.5 Hz, 1H), 4.52 (d, J = 5.5 Hz, 2H), 4.10 (t, J = 6.1 Hz, 2H), 1.76 (m, 2H), 1.49 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H). MS (FAB) 491 $(M+H)^+$.

4.2.22. 4′-Butoxy-3′-{[2-fluoro-4-(trifluoromethyl)phenylamido]methyl}-2-methylbiphenyl-3-carboxylic acid (24)

Compound **24** (17.5 mg, 0.0348 mmol, 56%) was synthesized as a white solid from **18** and 3-bromo-2-methylbenzoic acid following the general procedure for **20**.

Mp 146–149 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (t, J = 7.9 Hz, 1H), 7.94 (dd, J = 7.9, 1.2 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.47–7.27 (m, 5H), 7.19 (dd, J = 8.5, 2.4 Hz, 1H), 6.95 (d, J = 8.5 Hz, 1H), 4.73 (d, J = 5.5 Hz, 2H), 4.10 (t, J = 6.4 Hz, 2H), 2.47 (s, 3H), 1.87 (m, 2H), 1.55 (m, 2H), 1.01 (t, J = 7.3 Hz, 3H). MS (FAB) 504 $(M+H)^+$. UV monitoring of HPLC at 263 nm.

4.2.23. 4′-Butoxy-2,6-difluoro-3′-{[2-fluoro-4-(trifluoromethyl)phenylamido]methyl}biphenyl-3-carboxylic acid (25)

Compound **25** (33.1 mg, 0.0630 mmol, 53%) was synthesized as a white solid from **18** and 3-bromo-2,4-difluorobenzoic acid[35] following the general procedure for **20**.

Mp 177 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (t, J = 7.9 Hz, 1H), 8.02–7.97 (m, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.46 (s, 1H), 7.44–7.37 (m, 3H), 7.04 (t, J = 8.5 Hz, 1H), 6.99 (d, J = 8.5 Hz, 1H), 4.74 (d, J = 5.5, Hz, 2H), 4.10 (t, J = 6.1 Hz, 2H), 1.89–1.83 (m, 2H), 1.54 (tq, J = 7.9, 7.3 Hz, 2H), 1.01 (t, J = 7.3 Hz, 3H). MS (FAB) 526 $(M+H)^+$. UV monitoring of HPLC at 262 nm.

4.2.24. 5-(4-Butoxy-3-{[2-fluoro-4-(trifluoromethyl)phenylamido]methyl}phenyl)pyridine-3-carboxylic acid (26)

Compound **26** (55 mg, 0.11 mmol, 54%) was synthesized as a white solid from **18** and 5-bromonicotinic acid following the general procedure for **20**.

Mp 152 °C. ^{1}H NMR (500 MHz, DMSO) δ 9.04 (d, J = 1.8 Hz, 1H), 9.00–8.95 (m, 2H), 8.41 (d, J = 2.1 Hz, 1H), 7.85–7.79 (m, 2H), 7.74–7.66 (m, 3H), 7.15 (d, J = 8.5 Hz, 1H), 4.54 (d, J = 5.5, Hz, 2H), 4.09 (t, J = 6.1 Hz, 2H), 1.79–1.73 (m, 2H), 1.49 (tq, J = 7.9, 7.3 Hz, 2H), 0.95 (t, J = 7.3 Hz, 3H). MS (FAB) 491 $(M+H)^+$. UV monitoring of HPLC at 275 nm.

4.2.25. 5-(4-Butoxy-3-{[2-fluoro-4-(trifluoromethyl)phenylamido]methyl}phenyl)-4,6-dimethylpyridine-3-carboxylic acid (27)

Compound **27** (78 mg, 0.15 mmol, 71%) was synthesized as a white solid from **18** and 5-bromo-4,6-dimethylpyridine-3-carboxylic acid[36] following the general procedure for **20**.

Mp 104–106 °C. ^{1}H NMR (500 MHz, DMSO) δ 8.89 (br s, 2H), 7.82–7.75 (m, 2H), 7.65 (d, J = 7.9 Hz, 1H), 7.16–7.08 (m, 3H), 4.51 (dd, J = 5.5, 2.4 Hz, 2H), 4.08 (t, J = 6.1 Hz, 2H), 2.29 (s, 6H), 1.80–1.74 (m, 2H), 1.51 (tq, J = 7.3, 7.3 Hz, 2H), 0.96 (t, J = 7.3 Hz, 3H). MS (FAB) 519 $(M+H)^+$. UV monitoring of HPLC at 262 nm.

4.3. Reporter gene assay

Transfections of CMX-GAL4N-hPPARα, CMX-GAL4N-hPPARγ, CMX-GAL4N-hPPARδ and CMX-β-GAL into HEK 293 cells were performed by the calcium phosphate coprecipitation method. GW501516 (100 nM) for PPARδ, fenofibric acid (100 nM) for PPARα, and ciglitazone (100 nM) for PPARγ (positive controls) and test compounds were added 6 h later. After overnight incubation, luciferin was added and luminescence was measured on an ARVOTM SX microplate reader. β-Galactosidase was added and the absorbance was measured on the microplate reader with emission detection at 405 nm.

4.4. Thermodynamic aqueous solubility

The thermodynamic solubility determination was based on the method of Avdeef and Testa.[32] Briefly, about 1 mg of compound was ground with an agate mortar and taken up in 1.0 mL of an equal volume of a mixture of 1:15 M phosphate buffer (pH 7.4) and EtOH, or 1:15 M phosphate buffer (pH 7.4). The suspension was shaken for 48 h at 25 °C. An aliquot was filtered through a Millipore DIMEX-13 (0.22 μm). The filtrate was diluted in DMF and injected into an HPLC with UV detection; peak areas were recorded at 262–295 nm. The concentration of the sample solution was calculated using a previously determined calibration curve, corrected for the dilution factor of the sample.

4.5. DFT Calculations

All calculations were performed at the DFT level, using the hybrid Becke3LYP (B3LYP) function as implemented in Gaussian 2003. The 6-31G* basis set was used for H, C, N, O and F atoms. Geometry optimization and vibrational analysis were performed at the same level. All stationary points were optimized without any symmetry assumptions and characterized by normal coordinate analysis at the same level of the theory (number of imaginary frequencies, Nimag, 0).

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