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Optimization of (2,3-Dihydro-1-benzofuran-3-yl)acetic Acids: Discovery of a Non-Free Fatty Acid-Like, Highly Bioavailable G Protein-Coupled Receptor 40/Free Fatty Acid Receptor 1 Agonist as a Glucose-Dependent Insulinotropic Agent

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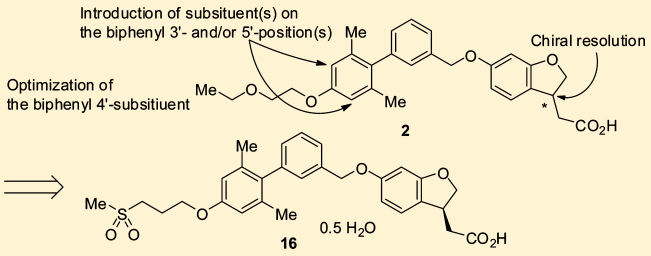
Supporting Information

ABSTRACT: G protein-coupled receptor 40 (GPR40)/free fatty acid receptor 1 (FFA1) is a free fatty acid (FFA) receptor that mediates FFA-amplified glucose-stimulated insulin secretion in pancreatic β-cells. We previously identified (2,3-dihydro-1-benzofuran-3-yl)acetic acid derivative **2** as a candidate, but it had relatively high lipophilicity. Adding a polar functional group on **2** yielded several compounds with lower lipophilicity and little effect on caspase-3/7 activity at 30 μM (a marker of toxicity in human HepG2 hepatocytes).



Three optimized compounds showed promising pharmacokinetic profiles with good in vivo effects. Of these, compound **16** had the lowest lipophilicity. Metabolic analysis of **16** showed a long-acting PK profile due to high resistance to β-oxidation. Oral administration of **16** significantly reduced plasma glucose excursion and increased insulin secretion during an OGTT in type 2 diabetic rats. Compound **16** (TAK-875) is being evaluated in human clinical trials for the treatment of type 2 diabetes.

INTRODUCTION

According to the International Diabetes Federation, approximately 285 million people worldwide suffer from diabetes as of 2010. Its incidence is increasing rapidly, and the number of patients will reach 438 million by the year 2030.[1] Type 2 diabetes accounts for almost 90% of total diabetes and is characterized by reduced insulin sensitivity combined with impaired insulin secretion. Persistent or uncontrolled hyperglycemia may cause a significantly increased risk of macrovascular and microvascular complications, including atherosclerosis, coronary heart disease, nephropathy, neuropathy, and retinopathy. Therefore, therapeutic control of glucose homeostasis is crucially important in the clinical management and treatment of diabetes. Drugs that enhance insulin secretion such as sulfonylureas and meglitinides are commonly used for the treatment of type 2 diabetes. However, these drugs enhance insulin secretion by directly closing KATP channel independent of blood glucose levels, leading to excess blood glucose-lowering, called hypoglycemia.[2] Furthermore, chronic exposure of them may induce β-cell apoptosis and reduction of the therapeutic effect, called secondary failure.[3,4] Hence, a novel drug that has a low risk of hypoglycemia and potent antidiabetic effects would be advantageous.

G protein-coupled receptor 40 (GPR40)/free fatty acid receptor 1 (FFA1) was identified as an orphan G protein-coupled receptor (GPCR),[5] and then deorphanized as a receptor for medium- to long-chain free fatty acids (FFAs).[6−8] Several reports have shown that GPR40/FFA1 is predominantly expressed in pancreatic β-cells, and the receptor mediates FFA-amplified insulin secretion.[6] The insulinotropic effects via GPR40/FFA1 are dependent on glucose concentration,[6,9,10] indicating that a selective agonist has a low risk of hypoglycemia and some supporting evidence has already been reported.[11,12] GPR40/FFA1 mainly couples with the $G_{\alpha q}$ protein, which activates phospholipase C, resulting in the production of inositol triphosphate and mobilization of intracellular Ca^{2+} from the endoplasmic reticulum.[9,10] The activation of GPR40/FFA1 also stimulates Ca^{2+} influx through voltage-gated Ca^{2+} channels,[13] and the resulting increase in

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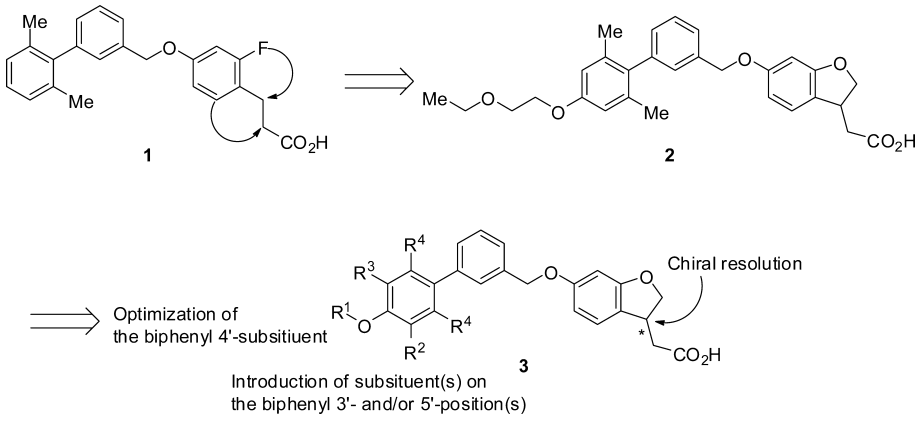
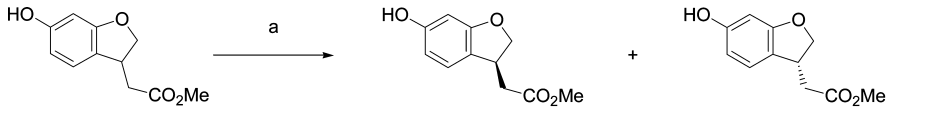


Figure 1. Design of (2,3-dihydro-1-benzofuran-3-yl)acetic acids.

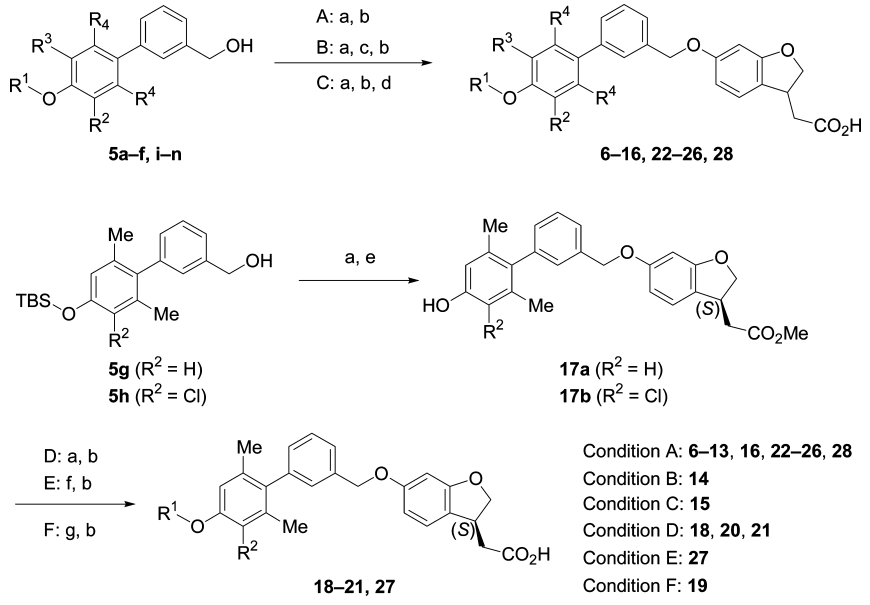
Scheme 1[a]



4a (racemate) **4b** (short retention time, *S*-isomer) **4c** (long retention time, *R*-isomer)

[a]Reagents and conditions: (a) CHIRALPAK AD HPLC separation, hexane/EtOH (88/12, v/v).

Scheme 2[a]



[a]Reagents and conditions: (a) **4a**–**c** (condition A) or R^1OH (condition D), ADDP, P(*n*-Bu)$_3$, toluene, rt, 60–95%; (b) 2 M NaOH aq, MeOH, THF, 50 °C, 32–94% except for **18**, **20**, **21**, 16–42% (from **17a** in 2 steps); **25**, 15% (from **43c** in 3 steps); **19** as a HCl-salt; (c) *m*-CPBA, AcOEt, 0 °C, 79%; (d) oxone, MeOH, H_2O, 0 °C to rt, 73%; (e) TBAF, THF, rt, 88–94%; (f) **30b**, K_3PO_4, DMF, 90 °C, 88%; (g) piperidine-4-ol, DEAD, PPh_3, toluene, rt, 63%.

intracellular Ca^{2+} concentrations enhances glucose-stimulated insulin secretion (GSIS).

A variety of synthetic GPR40/FFA1 agonists have been reported.[14] Importantly, some of them were designed based on FFAs as the endogenous ligands, so they inherit the feature of FFAs with the lipotoxic potential.[15,16] We postulated that reducing lipophilicity of compounds would be important to eliminate the undesirable off-target activities derived from highly lipophilic nature of FFAs.[17]

We independently identified several types of synthetic agonists and recently reported phenylpropanoic acid derivatives as represented by compound **1** with potent GPR40/FFA1 agonist activity and glucose-lowering effect during an oral glucose tolerance test (OGTT) in diabetic animal models.[18] However, this compound showed low sustainability in plasma due to its proposed vulnerability to β-oxidation at the propanoic acid moiety. Therefore, we intended to improve metabolic stability against β-oxidation by constructing a fused structure, leading to the identification of (2,3-dihydro-1-

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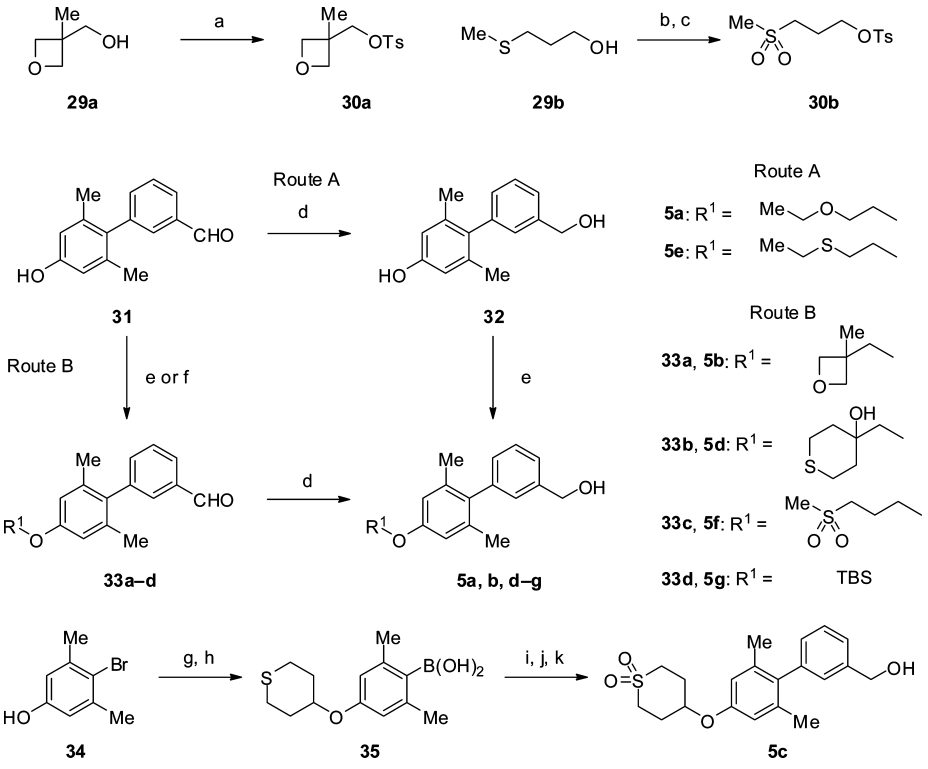
Scheme 3[a]



[a]Reagents and conditions: (a) *p*-TsCl, pyridine, 0 °C, 70%; (b) *p*-TsCl, *N*,*N*,*N*′,*N*′-tetramethyl-1,6-hexanediamine, Et_3N, toluene, 0 °C, 94%; (c) oxone, MeOH, H_2O, 0 °C to rt, 96%; (d) $NaBH_4$, MeOH, THF, 0 °C to rt, 92–97%; (e) R^1Cl (for **5a** and **5e**), R^1OTs (for **5b** and **5f**), or 1-oxa-6-thiaspiro[2.5]octane (for **5d**), K_2CO_3, (KI), DMF, 70–100 °C, 47–98%; (f) TBSCl, imidazole, DMF, rt, 77%; (g) tetrahydro-4*H*-thiopyran-4-ol, DEAD, PPh_3, THF, rt, 86%; (h) (i) 1.6 M *n*-BuLi in hexanes, THF, −78 °C, (ii) $B(i\text{-}PrO)_3$, −78 °C to rt, (iii) 2 M HCl aq, rt, 71% (from **34** in 2 steps); (i) methyl 3-bromobenzoate, $Pd(PPh_3)_4$, 2 M Cs_2CO_3 aq, DME, reflux, 86%; (j) *m*-CPBA, AcOEt, 0 °C, 85%; (k) $LiAlH_4$, THF, 10 °C to rt, 93%.

benzofuran-3-yl)acetic acid derivative **2** with potent agonist activity for human GPR40/FFA1 and superior pharmacokinetic (PK) profile in rats.[19] Furthermore, **2** exhibited in vivo efficacy at a dose of 10 mg/kg in rats despite the inferior potency against rat GPR40/FFA1. On the other hand, the lipophilicity of **2** was still high (see Log *D* value: 3.83),[17] so we decided to explore the introduction of a polar substituent with the goal of reducing lipophilicity and assessed their toxic profiles in human hepatocyte HepG2 cells in which FFAs show their toxicity.[20] From our previous work on other chemical series, it was revealed that incorporation of the various functionalities into the 4′-position of the biphenyl ring was tolerated.[21] With these observations in mind, we devised the following strategies to identify new chemical entities with potent and durable glucose-lowering effect and favorable safety profiles suitable for clinical development (Figure 1). First, in an attempt to decrease the lipophilicity, we conducted an extensive evaluation of the 4′-substituent on the biphenyl group. Second, to determine the importance of the stereochemistry, we separated the enantiomers. Finally, we expected to improve in vitro activities and PK profiles by appending substituents on the biphenyl core. Herein, we describe the identification and development of (2,3-dihydro-1-benzofuran-3-yl)acetic acid derivatives leading to the discovery of TAK-875, the hemihydrate of [(3*S*)-6-({2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}-methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic acid (**16**), as a promising anti-diabetic drug candidate.[22]

Chemistry. The racemic dihydrobenzofuran intermediate **4a** was synthesized by the reported method in our previous publications.[19,22] Preparative chiral HPLC separation of **4a** using a CHIRALPAK AD column afforded enantiomers **4b** and **4c** (Scheme 1). The absolute stereochemistry was determined by X-ray crystallography of eutomer **16** derived from **4b** as described hereinafter.

The target GPR40/FFA1 agonists were prepared as outlined in Scheme 2 (for R groups: see Tables 1–3). Phenols **4a–c** were condensed with biphenylylmethanols **5a–f** and **5i–n** (vide infra) by Mitsunobu reaction or with the corresponding mesylates by alkylation, followed by ester hydrolysis of the coupling products to afford the carboxylic acids **6–16**, **22–26**, and **28**, including oxidation of sulfide to sulfone for **14** and **15**. Conversion of 4′-alkoxy substituents on the biphenyl scaffold was also performed in an alternative method. Alkylation of the key intermediates **17a** and **17b**, derived from **5g** and **5h**, with alcohols or tosylates followed by hydrolysis of the ester, furnished the desired compounds **18–21** and **27**.

Biphenylylmethanols **5a–g** were synthesized as depicted in Scheme 3. Tosylate **30a** was prepared by a conventional method. Meanwhile, tosylation of (3-methylthio)-1-propanol (**29b**) was performed by Tanabe's procedure[23] with a catalytic amount of diamine to avoid the self-cyclization reaction, followed by oxidation to give the tosylate **30b**. Intermediate **31**[19] was converted to alcohols **5a**, **5b**, and **5d–g** in two different approaches. Reduction of **31** with $NaBH_4$ to alcohol **32**, followed by alkylation, afforded **5a** and **5e** (Route A).

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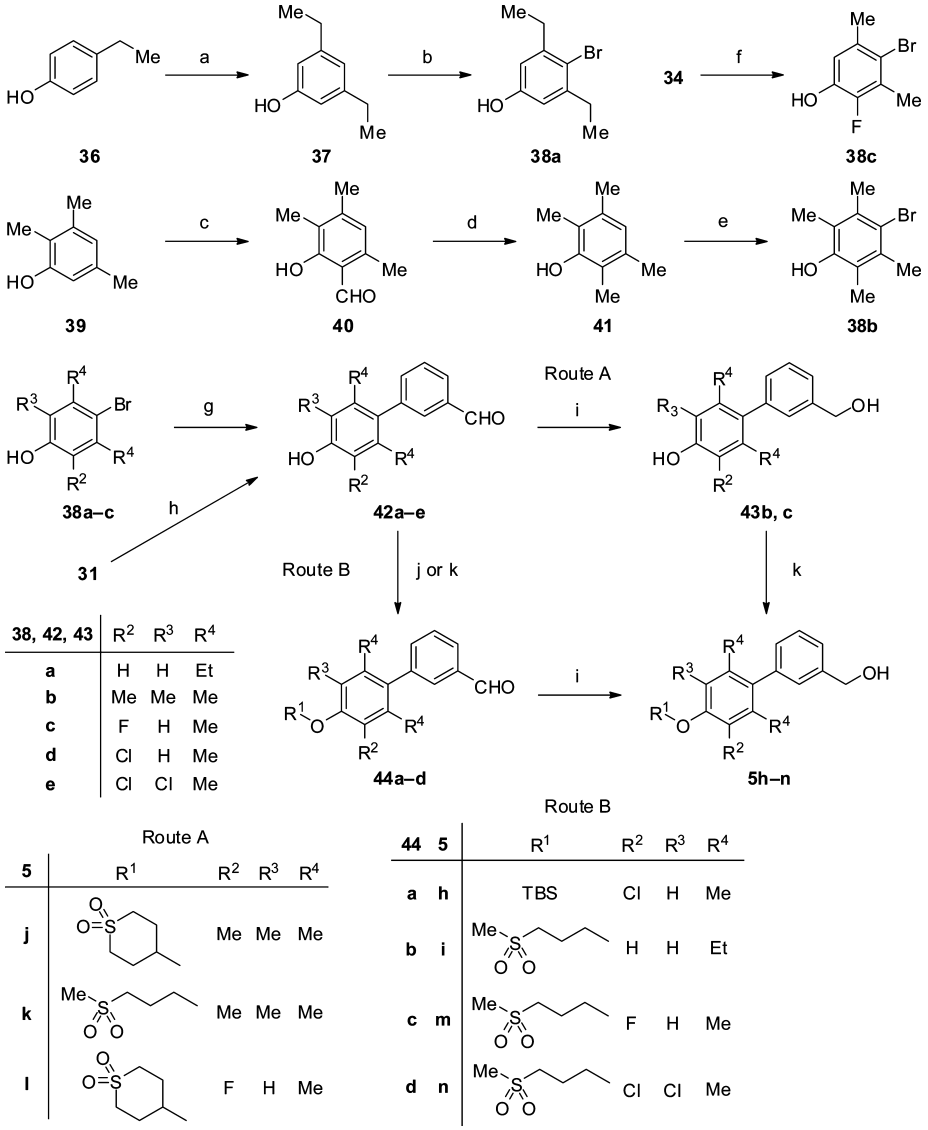
Scheme 4[a]



[a]Reagents and conditions: (a) $AlCl_3$, 115 °C, 78%; (b) *n*-Bu_4NBr_3, MeOH, rt, 72%; (c) dichloromethyl methyl ether, $TiCl_4$, CH_2Cl_2, 0 °C, 40%; (d) H_2, Pd/C, MeOH, toluene, rt, 97%; (e) Br_2, AcOH, rt, 83%; (f) 1-fluoropyridinium triflate, 1,2-dichloroethane, reflux, 36%; (g) 3-formylphenylboronic acid, $PdCl_2(dppf)\cdot CH_2Cl_2$, K_3PO_4, THF, 80 °C, 49–79%; (h) NCS, DMF, rt, 60–65%; (i) $NaBH_4$, MeOH, THF, 0 °C, 65–98%; (j) TBSCl, imidazole, DMF, rt, 88%; (k) R^1OTs, K_2CO_3, (KI), DMF, 90–95 °C, 53–95%.

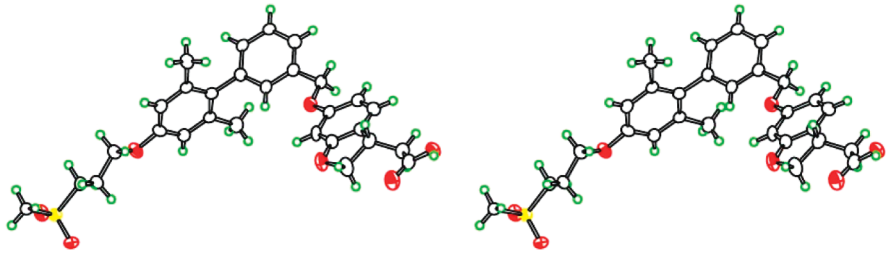


Figure 2. Stereoscopic molecular view of compound **16**.

Alternatively, **31** was alkylated with **30a**, 1-oxa-6-thiaspiro[2.5]-octane, or **30b**, or silylated with *tert*-butyldimethylchlorosilane, followed by reduction to provide alcohols **5b**, **5d**, **5f**, and **5g**. Synthesis of alcohol **5c** with 1,1-dioxidotetrahydrothiopyranyl group was accomplished using another way. Phenol **34** was alkylated under Mitsunobu condition to give tetrahydrothiopyranyl ether, which was converted to boronic acid **35**. Suzuki cross-coupling of **35** with methyl 3-bromobenzoate, followed by oxidation of the thioether, gave sulfone, which was treated with lithium aluminum hydride to give the desired alcohol **5c**.

The 3′- and/or 5′-substituted biphenylylmethanols **5h–n** were prepared following the routes illustrated in Scheme 4.

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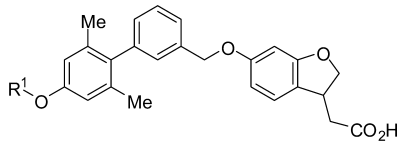
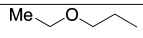
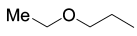
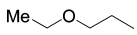
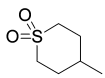
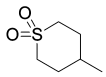
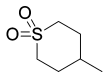
Table 1. In Vitro Activities of (2,3-Dihydro-1-benzofuran-3-yl)acetic Acids



compd	R^1	stereo	FLIPR human EC_{50} (μM)[a]	binding human K_i (μM)[b]	binding rat K_i (μM)[b]	Caspase-3/7[c] % at 30 μM	Log$D$[d] at pH7.4
2		*rac*	0.030 (0.019−0.047)	0.032	0.30	3.2	3.83
6		*S*	0.016 (0.012−0.023)	0.023	0.24	1.5	3.86
7		*R*	0.29 (0.19−0.44)	0.28	7.4	10.5	3.86
8		*rac*	0.024 (0.016−0.035)	0.021	0.083	23.6	3.86
9		*S*	0.018 (0.013−0.024)	0.025	0.11	21.1	3.88
10		*R*	0.27 (0.18−0.42)	0.27	2.2	20.9	3.88
11		*rac*	0.039 (0.024−0.065)	0.083	0.21	0.4	2.77
12		*S*	0.022 (0.016−0.030)	0.036	0.17	−2.5	2.75
13		*R*	0.29 (0.20−0.43)	0.30	0.51	1.3	2.84

[a]All values are average of $n = 3$ in the presence of 0.1% BSA. EC_{50} values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad). Efficacies of compounds at 10 μM were 103−113% of γ-linolenic acid at 10 μM. [b]All values are average of $n = 2$ in the presence of 0.2% BSA. [c]Percent of activation was compared to maximal activity of staurosporine as a reference compound. [d]The Log D values were determined at pH 7.4 according to the reported method.[28]

According to the procedure of Baddeley,[24] 4-ethylphenol (**36**) was converted to 3,5-diethylphenol (**37**), which underwent selective bromination at the *para*-position with *n*-Bu_4NBr_3 to yield **38a**. Formylation of 2,3,5-trimethylphenol (**39**) with $TiCl_4$ and dichloromethyl methyl ether[25] afforded a mixture of desired *o*-formylated product **40** and *p*-formylated product as a regioisomer (ca. 2:1). Subsequent catalytic hydrogenation proceeded smoothly to give phenol **41**, which was then treated with bromine to afford bromide **38b**. Monofluorination of **34** with 1-fluoropyridinium triflate gave **38c** in moderate yield. The obtained bromophenols **38a−c** were subjected to Suzuki coupling condition to afford biphenyls **42a−c**. Mono- and dichlorinated biphenyls **42d** and **42e** were synthesized by treatment of **31** with NCS. Biphenyls **42a−e** were converted to the corresponding alcohols **5h−n** by the same reaction sequence as described in Scheme 3.

Determination of the absolute stereochemistry in compound **16** was performed by X-ray diffraction analysis.[26] As shown in Figure 2, the absolute configuration of **16** was determined to be (*S*). Because **16** was derived from the key intermediate **4b**, the stereochemistry of **4b** was assigned as (*S*) and the stereochemistry of enantiomer **4c** assigned as (*R*) as described in Scheme 1.

RESULTS AND DISCUSSION

Agonist activities of the prepared compounds were measured by monitoring Ca^{2+} influx using the fluorometric imaging plate reader (FLIPR) system in the presence of 0.1% bovine serum albumin (BSA), and binding affinities for human and rat receptors were measured using the cell membranes in the presence of 0.2% BSA.[22] Compounds were evaluated for their apoptotic potential by measuring caspase-3/7 activity, which is a marker of apoptosis.[27] We utilized HepG2 cells in which FFAs, the endogenous ligands of GPR40/FFA1, have been reported to enhance the enzymatic activity.[20] The Log D values were measured at pH 7.4 with relative retention time over standard compounds of HPLC analysis.[28]

First, we briefly verified the tolerability of the substituent at the 4′-position of the biphenyl scaffold by use of other two functional groups (Table 1). (3-Methyloxetan-3-yl)methoxy derivative **8**, the cyclized analogue of 2-ethoxyethoxy derivative **2**, exhibited potent agonist activity; moreover, more bulky and polar (1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy derivative **11** still retained agonist activity and human/rat binding affinities. These results imply that the binding pocket encompassing the tail substituent can tolerate a wide range of groups including polar functionality. In parallel, we focused on the effect of stereochemistry at the 3-position of the dihydrobenzofuran ring. GPCRs are cell surface proteins known to dynamically change their conformations by ligand activation. As for GPR40/FFA1, a variety of long-chain fatty acids have been reported as natural ligands, suggesting that the ligand recognition site of this protein is not strict for binding to the lipophilic alkyl chain part. However, the binding pocket for

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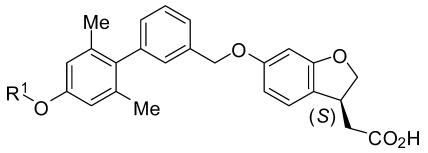
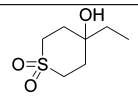
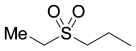
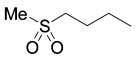
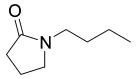
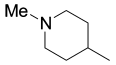
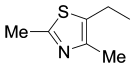
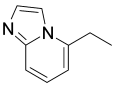
Table 2. In Vitro Activities of (2,3-Dihydro-1-benzofuran-3-yl)acetic Acids



compd	R^1	FLIPR human EC_{50} (μM)[a]	binding human K_i (μM)[b]	binding rat K_i (μM)[b]	Caspase-3/7[d] % at 30 μM	Log$D$[e] at pH7.4
14		0.013 (0.010–0.017)	0.024	0.17	–1.8	2.43
15		0.014 (0.011–0.019)	0.037	0.17	–1.3	2.73
16		0.016 (0.012–0.021)[c]	0.038	0.14	–2.1[c]	2.58[c]
18		0.017 (0.013–0.023)	0.031	0.17	3.9	3.22
19[f]		0.019 (0.014–0.025)	0.0088	0.066	–1.3	3.10
20		0.018 (0.013–0.025)	0.012	0.36	23.1	4.27
21		0.017 (0.013–0.022)	0.015	0.19	17.7	3.80

[a]All values are average of $n = 3$ in the presence of 0.1% BSA. EC_{50} values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad). Efficacies of compounds at 10 μM were 107–113% of γ-linolenic acid at 10 μM. [b]All values are average of $n = 2$ or 3 in the presence of 0.2% BSA. [c]The activity was measured with anhydrous **16**. [d]Percent of activation was compared to maximal activity of staurosporine as a reference compound. [e]The Log D values were determined at pH 7.4 according to the reported method.[28] [f]The activities were measured as hydrochloride.

the carboxylic acid moiety of the ligand is presumably restricted because this part is essential for signal transduction. Actually, previous SAR studies revealed that the electrostatic interaction of the carboxylic group and π–π interaction of the olefin group or the phenyl group are important for extension of GPR40/FFA1 activity.[6,18] Accordingly, we speculated that the absolute configuration of the chiral center neighboring the carboxylic acid in dihydrobenzofuran derivatives may be critical for both binding and signaling. It turned out that the (*S*)-enantiomer **6** showed more potent agonist activity to the human GPR40/FFA1 than the corresponding (*R*)-enantiomer **7**. Similarly, **6** had higher binding affinities against human and rat GPR40/FFA1 than **7**. Preference for (*S*)-stereochemistry over (*R*) was also observed in other 4′-alkoxybiphenyl series (**9** vs **10** and **12** vs **13**). These results indicate that the direction of the acetic acid moiety attached to the dihydrobenzofuran ring makes a significant impact on GPR40/FFA1 activity, namely, the relative position between the aromatic ring of the dihydrobenzofuran and the carboxylic acid moiety would affect the interaction with the GPR40/FFA1 receptor as expected. Having identified the preferable stereochemistry on the (2,3-dihydro-1-benzofuran-3-yl)acetic acid moiety, we employed the (*S*)-isomer for subsequent investigations.

Regarding caspase-3/7 activity, (1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy derivatives (**11**–**13**) with low Log D value (ca. 2.8) tend to be lower compared to the ether series (**2** and **7**–**10**). Among these compounds, **12** was selected for further evaluation.

As we expected, some polar functionalities such as ether or sulfone were tolerated at the 4′-position of the terminal biphenyl ring on the (2,3-dihydro-1-benzofuran-3-yl)acetic acid series. We then pursued replacement of the substituent at this position with the goal of optimizing lipophilicity of compounds (Table 2). Cyclic or linear sulfone analogues **14**–**16** with low lipophilicity (see Log D values: 2.43–2.73) exhibited potent agonist activity and binding affinities. Lactam analogue **18** was also tolerated in terms of agonist activity and binding affinities. Replacement of 1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl group on **12** with 1-methylpiperidin-4-yl group (**19**) increased binding affinities in human and rats. This effect might be derived from the enhanced interaction between the positive charge at the protonated piperidine ring and a polar functionality such as Ser8 (TM1).[22] Heteroaryl analogues such as thiazole **20** and imidazopyridine **21** showed potent agonist activity and affinities. However, heteroaryl analogues **20** and **21** showed caspase-3/7 activities, probably owing to their high lipophilicities (see Log D values: 4.27 and 3.80, respectively).

Various polar substituents such as sulfone, amide, amine, and heteroaromatics at the 4′-position of the biphenyl ring were tolerated for agonist activity and binding affinities regardless of their polarities, suggesting that human and rat GPR40/FFA1 receptors would have a large cavity in the ligand binding pocket located around the 4′-position of the biphenyl moiety. Thus, this position was thought to be suitable for modulating absorption, distribution, metabolism, excretion, and toxicology (ADME-Tox) properties. As can be seen, analogues **14**–**16**, **18**,

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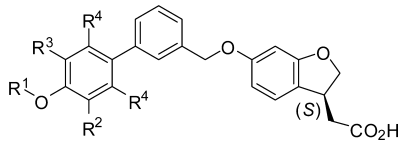
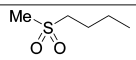
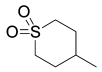
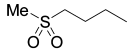
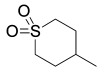
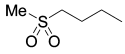
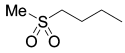
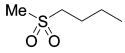
Table 3. In Vitro Activities of (2,3-Dihydro-1-benzofuran-3-yl)acetic Acids



comp	R^1	R^2	R^3	R^4	FLIPR human EC_{50} (μM)[a]	binding human K_i (μM)[b]	binding rat K_i (μM)[b]	Caspase-3/7[c] % at 30 μM	Log$D$[d] at pH7.4
22		H	H	Et	0.017 (0.013–0.023)	0.011	0.056	12.4	3.17
23		Me	Me	Me	0.029 (0.019–0.044)	0.040	0.40	12.3	3.35
24		Me	Me	Me	0.018 (0.012–0.027)	0.033	0.12	–1.1	3.12
25		F	H	Me	0.019 (0.014–0.026)	0.031	0.27	7.9	2.95
26		F	H	Me	0.016 (0.012–0.020)	0.018	0.20	–1.1	2.68
27		Cl	H	Me	0.016 (0.013–0.020)	0.019	0.11	8.3	3.07
28		Cl	Cl	Me	0.017 (0.013–0.022)	0.012	0.059	16.1	3.53

[a]All values are average of $n = 3$ in the presence of 0.1% BSA. EC_{50} values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad). Efficacies of compounds at 10 μM were 104–114% of γ-linolenic acid at 10 μM. [b]All values are average of $n = 2$ in the presence of 0.2% BSA. [c]Percent of activation was compared to maximal activity of staurosporine as a reference compound. [d]The Log D values were determined at pH 7.4 according to the reported method.[28]

and **19** with polar groups have good potency and minimum caspase activity, so they were selected for further investigation.

Last, we turned our attention to further increasing potency and modulating ADME-Tox profiles through introduction of hydrophobic substituent(s) such as a methyl group or a halogeno group on the biphenyl ring (Table 3) because most of the ligand binding pocket was thought to consist of hydrophobic amino acids.[22] Introduction of one or more small hydrophobic substituents (**22**–**28**) resulted in almost the same agonist activities and binding affinities or slight improvement in both. In this series, 2′,6′-diethyl analogue **22** exhibited marginally increased affinities in human and rat binding assays compared to the parent compound **16**.

In terms of caspase-3/7 activity, 1,1-dioxidotetrahydrothiopyran derivatives as with 2′,3′,5′,6′-tetramethyl analogue **23** and monofluoro analogue **25** tend to have a weak potential of activating caspase-3/7. Among 3-(methylsulfonyl)propoxy derivatives, 2′,3′,5′,6′-tetramethyl analogue **24** and monofluoro analogue **26** did not show any activities, whereas introduction of chloro group(s) (**27** and **28**) increased caspase-3/7 activity as the lipophilicity is increased (Log D value: 3.07 and 3.53, respectively). Thus, all compounds possessed comparable GPR40/FFA1 activity, but some of them slightly induced caspase-3/7 activities with increasing lipophilicity. The threshold of Log D value for apoptosis induction would be estimated as 2.9–3.2 according to these results. Consequently, we selected two compounds (**24** and **26**) in Table 3 for rat PK study.

Potent compounds without caspase-3/7 activity were further evaluated for oral PK profiles through cassette dosing experiments in fasted rats. With in vivo evaluation of drug efficacy in mind, plasma concentrations at 1 and 4 h (C_{1h} and C_{4h}) after oral administration are shown in Table 4 in addition to the PK parameters (C_{max}, T_{max}, AUC_{0-8h}). The 1,1-dioxidotetrahydrothiopyran derivative **12** presented a highly desirable PK profile, namely, rapid absorption (T_{max} = 0.7 h), high maximum concentration (C_{max} = 2667.7 ng/mL), and good plasma duration (C_{4h} = 1621.0 ng/mL), leading to a high plasma exposure (AUC_{0-8h} = 13007.6 ng·h/mL). The more polar analogue **14** with tertiary alcohol functionality showed lower C_{max} and AUC_{0-8h} compared to **12**. One of the reasons for this decrease was probably due to its decline in membrane permeability (data not shown). The ring-opened analogue **15**, with the flexible side chain, exhibited a good PK profile, but it showed somewhat lower plasma concentration relative to **12**. In

Table 4. Pharmacokinetic Profiles for (2,3-Dihydro-1-benzofuran-3-yl)acetic Acids[a]

compd	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-8h} (ng·h/mL)	C_{1h} (ng/mL)	C_{4h} (ng/mL)
12	2667.7	0.7	13007.6	2614.2	1621.0
14	1082.4	1.0	5210.4	1082.4	638.8
15	1941.3	1.17	9621.7	1737.7	1240.0
16	1883.5	2.00	11840.4	1855.6	1601.7
18	626.4	1.83	3474.9	566.8	477.3
19	743.6	1.58	4372.3	578.0	574.5
24	1275.2	0.67	3963.9	1152.2	340.5
26	2033.3	0.50	8036.1	1541.1	966.6

[a]Rat cassette dosing at 1 mg/kg, po (fasted). Test compounds were suspended in 0.5% methylcellulose aqueous solution. All values are averages of three rats.

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the study on the analogous compound, we have confirmed that the 2-(ethylsulfonyl)ethoxy group was degraded by β-elimination under basic conditions. It remains possible that similar decomposition could occur on **15** in vivo. In the meantime, the 3-(methylsulfonyl)propoxy analogue **16**, whose sulfonyl group was shifted to terminal side for one carbon, eliminates the possible concern for β-elimination and possesses an excellent PK profile as expected. Although the AUC_{0-8h} of lactam **18** and cyclic amine **19** were lower compared to the sulfone derivatives, their C_{1h} and C_{4h} were found to be at the same level near C_{max}, indicating favorable duration in plasma. Compared to the corresponding unsubstituted analogue, 2′,3′,5′,6′-tetramethyl derivative **24** exhibited lowered AUC_{0-8h}, likely due to relatively high clearance with increased lipophilicity. The 3′-Fluoro analogue **26** showed good absorption (high C_{max} and rapid T_{max}) but relatively low AUC_{0-8h} and C_{4h}, indicating that its clearance from plasma might be faster than that of the parent compound **16**.

As a whole, sulfonyl derivatives possessed favorable PK profiles, and introduction of lipophilic substituents at the 3′- and/or 5′-position on the biphenyl scaffold tended to decrease the plasma concentration. On the basis of the above results, compounds (**12**, **14**−**16**, **19**, and **26**) possessing favorable PK profile, notably long duration in plasma, were selected for in vivo pharmacological study.

In vivo efficacies of the selected compounds were evaluated by OGTT in female Wistar fatty rats, where compounds were orally administered 1 h or 4 h prior to glucose challenge (these tests were referred to 1H−OGTT or 4H−OGTT, respectively). The 1H−OGTT assessed intrinsic efficacy of compounds at 1 mg/kg dose, while the 4H−OGTT assessed their durability at 3 mg/kg dose. Among the tested compounds, **12**, **16**, and **26**, with especially desirable PK profiles, produced significantly improved glucose intolerance in both 1H−OGTT and 4H−OGTT (Table 5). Tertiary alcohol derivative **14** with relatively lower plasma concentration showed efficacy only in the 4H−OGTT and did not exhibit significant activity in the 1H−OGTT. Although ring-opened analogue **15** significantly reduced plasma glucose excursions in the 4H−OGTT, the potencies of these compounds were relatively lower compared to that of the parent compound **12**. The reason for insufficient efficacy of 1-methylpiperidine derivative **19** would be its relatively lower plasma concentration.

Table 5. Effects of Selected Compounds during an OGTT in Female Wistar Fatty Rats[a]

	ED (mg/kg)[b]	
compd	1H−OGTT	4H−OGTT
12	1	3
14	NE[c]	3
15	NT[d]	3
16	1	3
19	NT[d]	NE[c]
26	1	3

[a]Effects of compounds during an OGTT in female Wistar fatty rats (n = 6). See Experimental Section for details. [b]Effective dose (ED) was determined by statistical significance on AUC of plasma glucose. [c]NE: not effective. [d]NT: not tested.

Despite their lower affinities for the rat receptor as previously mentioned, a series of dihydrobenzofuran derivatives exhibited rapid and long-lasting in vivo efficacy by virtue of their prominent PK profiles. Among three compounds (**12**, **16**, and **26**) with pronounced in vivo efficacies, **16** possessed the lowest log D value (2.58). On the basis of the above information (i.e., in vitro and in vivo potencies, ADME-Tox profiles, and PK parameters), including drug-likeness (Log D), **16** was selected as a candidate for further investigation.

As reported in the previous communication,[22] **16** showed excellent PK profiles in rats and dogs. In an attempt to rationalize its PK profile, **16** was further characterized by performing additional PK studies using [^{14}C]-labeled **16** in rats and dogs. The observed metabolic pathways of **16** are summarized in Figure 3. The major component in plasma was unchanged **16** in rats and dogs, and a small amount of biphenylylcarboxylic acid **45** was detected in rats. In addition, taurine conjugate **46** and acyl glucuronide **47** were detected in rat bile, and these three metabolites (**45**−**47**) were also detected in feces of both species. Thus, the β-oxidation metabolite of **16** was not observed in rats and dogs. These results demonstrate that our design to introduce a fused structure into the phenylpropanoic acid indeed decreased β-oxidation, leading to an improved PK profile. The favorable metabolic process of **16** would contribute to a desirable PK profile suitable for once-daily dosing in humans.[29,30] The more detailed metabolic analysis of **16** will be presented elsewhere.

To assess the pharmacological effects of **16** in detail, we performed an OGTT in male Goto-Kakizaki (GK) rats, which are a spontaneous type 2 diabetic model with impaired insulin secretion in response to glucose. While insulin secretion after glucose challenge rapidly occurred in Wistar Kyoto rats as healthy control, the early phase insulin secretion was impaired in GK rats (Figure 4C) and the impaired insulin secretion was reflected in the increased glucose excursion (Figure 4A). Oral administration of **16** (1−10 mg/kg) 1 h prior to oral glucose challenge dose-dependently augmented insulin secretion (Figure 4C) and suppressed plasma glucose excursion (Figure 4A) in GK rats. The areas under the curves of plasma glucose ($AUC_{0-120min}$) (Figure 4B) and plasma insulin ($AUC_{0-120min}$) (Figure 4D) showed that the minimum effective dose for glucose-lowering and for increasing insulin secretion were 3 and 10 mg/kg, respectively. Moreover, the **16**-treated group had already augmented insulin secretion at glucose charge (0 min) as the GK rats have high level of fasting plasma glucose. We have already reported that **16**, even at an oral dose of 30 mg/kg, had no impact on fasting plasma glucose levels in Sprague−Dawley (SD) rats with normal glucose homeostasis and did not significantly promote insulin secretion.[12] Our present and previous results indicate that the insulinotropic action of **16** is strictly dependent on blood glucose levels. From the unique features of **16**, we believe that the compound may pose a low risk of hypoglycemia, while showing potent glucose-lowering effects in diabetic pathology caused by the dysfunction of pancreatic β-cells.

CONCLUSION

We have made considerable efforts to identify novel GPR40/FFA1 agonists with reduced undesirable hydrophobic and toxic profiles of FFAs as endogenous ligands while exhibiting potent GPR40/FFA1 agonist activity. Optimization of (2,3-dihydro-1-benzofuran-3-yl)acetic acid lead compound **2** culminated in the discovery of **16**. Introduction of various polar substituents at the 4′-position of the biphenyl ring resulted in: (1) maintained GPR40/FFA1 agonist activity, (2) reduced toxic profile (as measured by caspase-3/7 activity) accompanied by decreased

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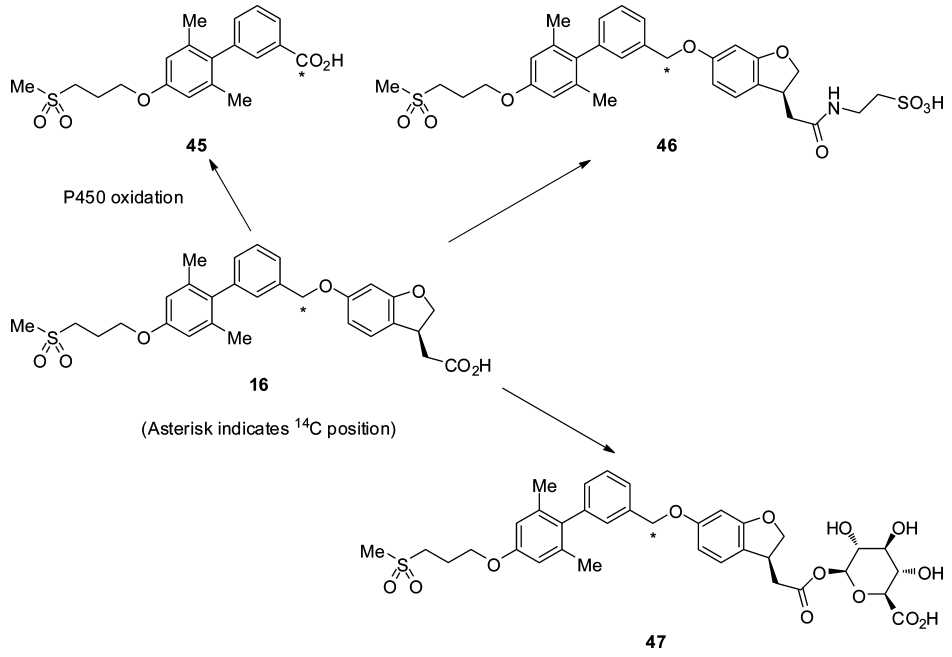

Figure 3. Presumed metabolic pathways of **16**.

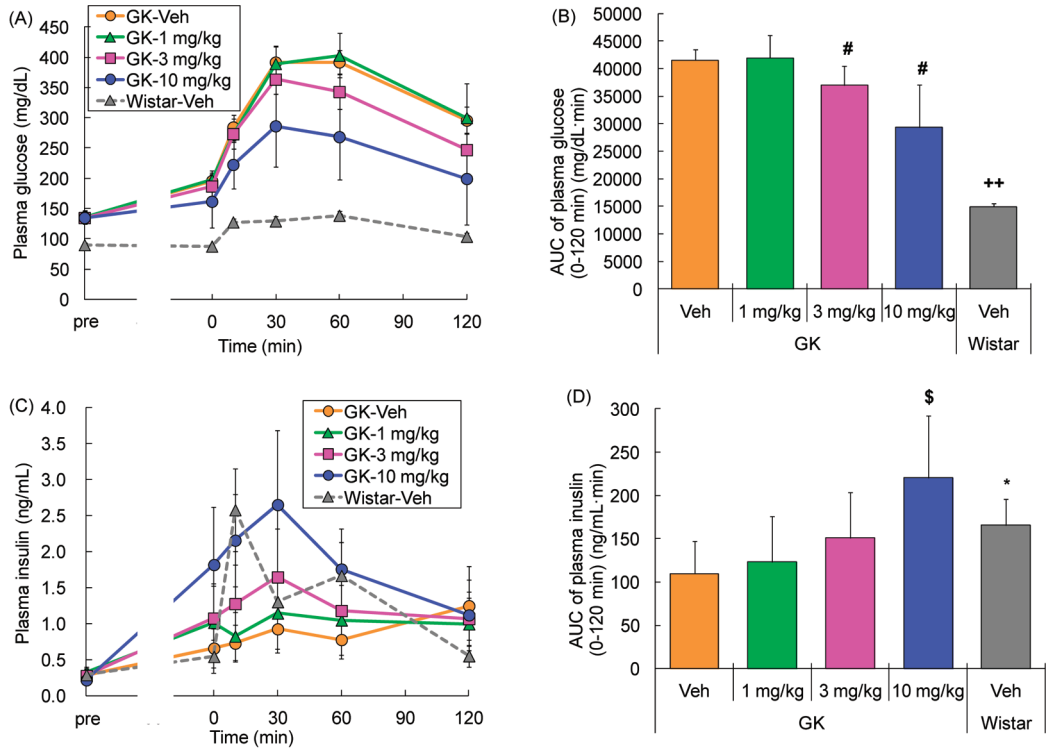

Figure 4. Effects of **16** during an OGTT in male GK rats. (A) and (C) show time-dependent changes of plasma glucose (PG) and plasma insulin after oral administration of **16**, followed by 1 g/kg oral glucose challenge, respectively. Data in (B) and (D) represent $AUC_{0-120min}$ of PG levels and $AUC_{0-120min}$ of plasma insulin levels shown in (A) and (C), respectively. Values are mean ± SD (n = 6). # $P \leq 0.025$ compared to vehicle-treated GK rats by one-tailed Shirley–Williams test. $ $P \leq 0.025$ compared to vehicle-treated GK rats by one-tailed Williams' test. ++ $P \leq 0.01$ compared to vehicle-treated GK rats by Aspin–Welch test. * $P \leq 0.05$ compared to vehicle-treated GK rats by Student's t test.

Log D values, and (3) improved PK profile, in particular, the sulfonyl group is the best as for long-lasting plasma concentration. Metabolism studies of **16** showed that the compound was highly resistant to β-oxidation as we expected. Encouraged by the results of the safety studies in rats and dogs, **16** was selected as a candidate for human clinical trials.

EXPERIMENTAL SECTION

Chemistry. Reagents and solvents were obtained from commercial sources and used without further purification. Reaction progress was determined by thin layer chromatography (TLC) analysis on Merck Kieselgel 60 F254 plates or Fuji Silysia NH plates. Chromatographic purification was carried out on silica gel columns [(Merck Kieselgel 60, 70–230 mesh or 230–400 mesh Merck) or (Chromatorex NH-DM

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1020, 100−200 mesh)] or on Purif-Pack (SI: 60 μM or NH: 60 μM, Fuji Silysia Chemical, Ltd.). Melting points were determined on a BÜCHI B-545 melting point apparatus and were uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker Ultra Shield-300 (300 MHz) instruments. Chemical shifts are given in parts per million (ppm) with tetramethylsilane as an internal standard. Abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublets of doublet, br = broad. Coupling constants (J values) are given in hertz (Hz). Low-resolution mass spectra (MS) were determined on a Waters liquid chromatography−mass spectrometer system (MS), using a CAPCELL PAK UG-120 ODS (Shiseido Co., Ltd.) column (2.0 mm i.d. × 50 mm) with aqueous CH_3CN (10−95%) containing 0.05% trifluoroacetic acid (TFA) and an HP-1100 (Agilent Technologies) apparatus for monitoring at 220 nm. All MS experiments were performed using electrospray ionization (ESI) in positive ion mode. Analytical HPLC was performed on a Shimadzu LC-VP instrument, equipped with CAPCELL PAK C18 UG120 S-3 μm, 2.0 mm × 50 mm column with a 4 min linear gradient from 90/10 to 5/95 and subsequently with a 1.5 min isocratic elution 5/95 A/B, where A = H_2O−0.1% TFA, B = CH_3CN−0.1% TFA, at a flow rate of 0.5 mL/min, with UV detection at 220 nm, at column temperature of 25 °C. Elemental analyses were carried out by Takeda Analytical Laboratories Limited and were within 0.4% of the theoretical values unless otherwise noted. The purity of compounds was assessed by elemental analysis or analytical HPLC (>95%). Optical rotations were determined on a JASCO P-1030 polarimeter. Preparative purifications were performed using a Gilson pumping system in conjunction with a photodiode array detector (Hewlett-Packard 1100 series) and a Gilson 215 auto sampler. Separations were achieved using the following method, which utilized a YMC packed column (CombiPrep ODS-A, 5 μm, 20 mm i.d. × 50 mm) with a 1 min isocratic elution 10/90, a 3.7 linear gradient from 10/90 to 0/100, and then a 2.7 min isocratic elution 0/100 A/B at a flow rate of 25 mL/min.

Methyl [(3*S*)-6-Hydroxy-2,3-dihydro-1-benzofuran-3-yl]-acetate (4b) and Methyl [(3*R*)-6-Hydroxy-2,3-dihydro-1-benzofuran-3-yl]acetate (4c). Methyl (6-hydroxy-2,3-dihydro-1-benzofuran-3-yl)acetate (**4a**) was optically resolved using normal phase preparative HPLC [column, CHIRALPAK AD, 50 mm i.d. × 500 mmL; mobile phase, hexane/EtOH (88/12) (v/v) by isocratic elution; flow rate, 60 mL/min; detection, UV 220 nm; temperature, 30 °C]. Retention times of the two enantiomers were 16.3 and 18.7 min. The (*S*)-isomer **4b** (retention time 16.3 min) was thus obtained with a 99.7% ee [column, CHIRALPAK AD-H, 4.6 mm i.d. × 250 mmL; mobile phase, hexane/IPA (80/20) (v/v) by isocratic elution; flow rate, 0.5 mL/min; detection, UV 220 nm; temperature, 30 °C]. $[\alpha]_D$ +5.4° (*c* 0.92, $CHCl_3$). The (*R*)-isomer **4c** (retention time 18.7 min) was thus obtained with a 99.1% ee [column, CHIRALPAK AD-H, 4.6 mm i.d. × 250 mmL; mobile phase, hexane/IPA (80/20) (v/v) by isocratic elution; flow rate, 0.5 mL/min; detection, UV 220 nm; temperature: 30 °C]. $[\alpha]_D$ −6.4° (*c* 0.94, $CHCl_3$).

[(3*S*)-6-{[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]-methoxy}-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (6). Step 1: To a mixture of **4b** (0.250 g, 1.20 mmol), **5a** (0.360 g, 1.20 mmol), and P(*n*-Bu)$_3$ (0.388 g, 1.92 mmol) in toluene (20 mL) was added gradually ADDP (0.484 g, 1.92 mmol), and the mixture was stirred under nitrogen atmosphere at room temperature for 20 h. Hexane (10 mL) was added, and the insoluble material was removed by filtration. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (AcOEt/hexane = 5:95−40:60) to give methyl [(3*S*)-(6-{[4′-(2-ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]-methoxy}-2,3-dihydro-1-benzofuran-3-yl)acetate (0.456 g, 77%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.0 Hz, 3H), 1.98 (s, 6H), 2.50−2.61 (m, 1H), 2.70−2.79 (m, 1H), 3.62 (q, J = 7.0 Hz, 2H), 3.71 (s, 3H), 3.75−3.86 (m, 3H), 4.11−4.16 (m, 2H), 4.26 (dd, J = 9.1, 6.1 Hz, 1H), 4.75 (t, J = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44−6.51 (m, 2H), 6.69 (s, 2H), 7.01 (d, J = 8.1 Hz, 1H), 7.08 (dt, J = 7.1, 1.5 Hz, 1H), 7.16 (s, 1H), 7.34−7.45 (m, 2H). MS m/z 491 $(M + H)^+$. HPLC purity (220 nm) 100.0%. Step 2: To a solution of the obtained ester (0.451 g, 0.919 mmol) in MeOH (2 mL) and THF (4 mL) was added 2 M NaOH aqueous solution (0.750 mL, 1.50 mmol) at room temperature, and the mixture was stirred at 50 °C for 2 h. The mixture was diluted with water, acidified with 10% citric acid aqueous solution, and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70−80:20) to give an oil, which was triturated with hexane to give crystals. Recrystallization from hexane/AcOEt gave **6** (0.315 g, 72%) as colorless crystals; mp 58−59 °C. $[\alpha]_D$ +6.5° (*c* 0.30, CH_3CN). 99.7% ee [column, CHIRALPAK AD, 4.6 mm i.d. × 250 mmL; mobile phase, hexane/IPA/TFA (85:15:0.1) (v/v/v) by isocratic elution; flow rate, 0.5 mL/min; detection, UV 220 nm; temperature, room temperature]. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.0 Hz, 3H), 1.98 (s, 6H), 2.55−2.66 (m, 1H), 2.76−2.85 (m, 1H), 3.62 (q, J = 7.0 Hz, 2H), 3.75−3.86 (m, 3H), 4.14 (t, J = 5.0 Hz, 2H), 4.28 (dd, J = 9.1, 6.0 Hz, 1H), 4.75 (t, J = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44−6.52 (m, 2H), 6.68 (s, 2H), 7.02−7.10 (m, 2H), 7.16 (s, 1H), 7.34−7.45 (m, 2H). MS m/z 477 $(M + H)^+$. HPLC purity (220 nm) 100.0%. Anal. Calcd for $C_{29}H_{32}O_6$: C, 73.09; H, 6.77. Found: C, 73.02; H, 6.73.

[(3*S*)-6-({4′-[(4-Hydroxy-1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (14). The title compound was prepared from **4b** and **5d** by a method similar to that described for **6** except for step 2. Step 1: Methyl [(3*S*)-6-({4′-[(4-hydroxytetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}-methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate in 90% yield as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.77−1.89 (m, 2H), 1.99 (s, 6H), 2.06−2.15 (m, 2H), 2.18 (s, 1H), 2.42−2.60 (m, 3H), 2.70−2.79 (m, 1H), 3.04−3.17 (m, 2H), 3.72 (s, 3H), 3.74−3.86 (m, 3H), 4.26 (dd, J = 9.1, 6.0 Hz, 1H), 4.75 (t, J = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44−6.51 (m, 2H), 6.67 (s, 2H), 7.01 (d, J = 7.9 Hz, 1H), 7.04−7.09 (m, 1H), 7.15 (s, 1H), 7.35−7.45 (m, 2H). MS m/z 531 $(M - 18 + H)^+$. Step 2: To a solution of the obtained oil (0.586 g, 1.07 mmol) in AcOEt (5 mL) was added gradually *m*-CPBA (0.568 g, 2.14 mmol) at 0 °C, and the mixture was stirred at 0 °C for 2 h. The mixture was quenched with $Na_2S_2O_3$ aqueous solution and saturated $NaHCO_3$ aqueous solution and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt/hexane = 40:60−80:20) to give methyl [(3*S*)-6-({4′-[(4-hydroxy-1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)methoxy]-2′,6′-dimethylbiphenyl-3-yl}-methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.489 g, 79%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H), 2.16−2.33 (m, 4H), 2.46 (s, 1H), 2.50−2.61 (m, 1H), 2.69−2.80 (m, 1H), 2.90−3.01 (m, 2H), 3.43−3.57 (m, 2H), 3.72 (s, 3H), 3.74−3.86 (m, 1H), 3.88 (s, 2H), 4.26 (dd, J = 9.1, 6.1 Hz, 1H), 4.75 (t, J = 9.1 Hz, 1H), 5.06 (s, 2H), 6.43−6.51 (m, 2H), 6.67 (s, 2H), 6.99−7.10 (m, 2H), 7.15 (s, 1H), 7.35−7.47 (m, 2H). MS m/z 581 $(M + H)^+$. Step 3: **14** in 76% yield as colorless crystals (hexane/AcOEt); mp 198−201 °C. $[\alpha]_D$ +5.1° (*c* 0.30, CH_3CN). ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H), 2.17−2.33 (m, 4H), 2.56−2.67 (m, 1H), 2.76−2.85 (m, 1H), 2.90−3.01 (m, 2H), 3.43−3.56 (m, 2H), 3.75−3.86 (m, 1H), 3.88 (s, 2H), 4.29 (dd, J = 9.1, 6.0 Hz, 1H), 4.76 (t, J = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44−6.52 (m, 2H), 6.67 (s, 2H), 7.02−7.09 (m, 2H), 7.15 (s, 1H), 7.35−7.46 (m, 2H). MS m/z 567 $(M + H)^+$. HPLC purity (220 nm) 99.4%. Anal. Calcd for $C_{31}H_{34}O_8S$: C, 65.71; H, 6.05. Found: C, 65.69; H, 6.03.

[(3*S*)-6-({4′-[2-(Ethylsulfonyl)ethoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (15). The title compound was prepared from **4b** and **5e** by a method similar to that described for **6** except for step 3. Step 1: Methyl [(3*S*)-6-({4′-[2-(ethylsulfanyl)ethoxy]-2′,6′-dimethylbiphenyl-3-yl}-methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate in 60% yield as a pale-yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.31 (t, J = 7.4 Hz, 3H), 1.99 (s, 6H), 2.50−2.79 (m, 4H), 2.92 (t, J = 7.0 Hz, 2H), 3.71 (s, 3H), 3.74−3.86 (m, 1H), 4.15 (t, J = 7.0 Hz, 2H), 4.26 (dd, J = 9.1, 6.1 Hz, 1H), 4.75 (t, J = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44−6.51 (m, 2H), 6.66 (s, 2H), 7.01 (d, J = 7.9 Hz, 1H), 7.05−7.10 (m, 1H), 7.16 (s, 1H), 7.34−7.45 (m, 2H). MS m/z 507 $(M + H)^+$. Step 2: [(3*S*)-6-({4′-[2-(Ethylsulfanyl)ethoxy]-2′,6′-dimethylbiphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic acid in 89% yield as a colorless oil. ^{1}H

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NMR ($CDCl_3$) δ 1.31 (t, J = 7.4 Hz, 3H), 1.99 (s, 6H), 2.56−2.71 (m, 3H), 2.76−2.86 (m, 1H), 2.92 (t, J = 7.0 Hz, 2H), 3.75−3.87 (m, 1H), 4.15 (t, J = 6.8 Hz, 2H), 4.28 (dd, J = 9.1, 6.1 Hz, 1H), 4.76 (t, J = 9.1 Hz, 1H), 5.05 (s, 2H), 6.44−6.52 (m, 2H), 6.66 (s, 2H), 7.02−7.10 (m, 2H), 7.16 (s, 1H), 7.34−7.45 (m, 2H). MS m/z 493 $(M + H)^+$. Step 3: To a solution of the obtained oil (0.304 g, 0.617 mmol) in MeOH (10 mL) was added dropwise a solution of oxone (0.569 g, 0.926 mmol) in water (5 mL) at 0 °C, and the mixture was stirred at 0 °C to room temperature for 12 h. MeOH was evaporated. The residue was diluted with water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by preparative HPLC to give crystals. Recrystallization from heptane/AcOEt gave **15** (0.237 g, 73%) as colorless crystals; mp 130−131 °C. $[\alpha]_D$ +6.6° (c 0.30, CH_3CN). ^1H NMR ($CDCl_3$) δ 1.47 (t, J = 7.5 Hz, 3H), 1.99 (s, 6H), 2.55−2.67 (m, 1H), 2.75−2.86 (m, 1H), 3.19 (q, J = 7.5 Hz, 2H), 3.42 (t, J = 5.4 Hz, 2H), 3.75−3.87 (m, 1H), 4.29 (dd, J = 9.1, 6.0 Hz, 1H), 4.44 (t, J = 9.1 Hz, 2H), 4.76 (t, J = 5.4 Hz, 1H), 5.06 (s, 2H), 6.44−6.52 (m, 2H), 6.64 (s, 2H), 7.02−7.09 (m, 2H), 7.15 (s, 1H), 7.35−7.46 (m, 2H). MS m/z 525 $(M + H)^+$. HPLC purity (220 nm) 99.8%. Anal. Calcd for $C_{29}H_{32}O_7S$: C, 66.39; H, 6.15. Found: C, 66.35; H, 6.15.

[(3*S*)-6-({2′,6′-Dimethyl-4′-[3-(methylsulfonyl)propoxy]-biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid Hemihydrate (16). Step 1: To a mixture of **4b** (0.208 g, 1.00 mmol), **5f** (0.348 g, 1.00 mmol), and $P(n\text{-}Bu)_3$ (0.324 g, 1.60 mmol) in toluene (15 mL) was added portionwise ADDP (0.404 g, 1.60 mmol), and the mixture was stirred under nitrogen atmosphere at room temperature for 1.5 h. Hexane (8 mL) was added, and the insoluble material was removed by filtration. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (AcOEt/hexane = 40:60−80:20) to give methyl [(3*S*)-6-({2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.442 g, 82%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.30−2.41 (m, 2H), 2.49−2.61 (m, 1H), 2.69−2.79 (m, 1H), 2.97 (s, 3H), 3.23−3.31 (m, 2H), 3.71 (s, 3H), 3.74−3.86 (m, 1H), 4.08−4.13 (m, 2H), 4.26 (dd, J = 9.1, 6.1 Hz, 1H), 4.75 (t, J = 9.1 Hz, 1H), 5.05 (s, 2H), 6.43−6.51 (m, 2H), 6.64 (s, 2H), 7.01 (d, J = 8.0 Hz, 1H), 7.07 (dt, J = 7.1, 1.6 Hz, 1H), 7.15 (s, 1H), 7.34−7.46 (m, 2H). MS m/z 539 $(M + H)^+$. Step 2: To a solution of the obtained oil (11.2 g, 20.8 mmol) in MeOH (40 mL) and THF (80 mL) was added 2 M NaOH aqueous solution (20.0 mL, 40.0 mmol), and the mixture was stirred at 50 °C for 2 h. The mixture was concentrated, diluted with water, acidified with 1 M hydrochloric acid aqueous solution, and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to give crystals, which were washed with heptane/AcOEt. Recrystallization from EtOH/H_2O gave **16** (9.31 g, 85%) as colorless crystals; mp 127−129 °C. $[\alpha]_D$ +5.3° (c 0.3085, CH_3CN). 99.6% ee [column, CHRALPAK AD-3 (NC002), 4.6 mm i.d. × 250 mmL; mobile phase, hexane/2-propanol/TFA = 500:500:1 (v/v/v) by isocratic elution; flow rate, 0.5 mL/min; detection, UV 220 nm; column temperature, 30 °C]. ^1H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.29−2.41 (m, 2H), 2.61 (dd, J = 16.9, 9.2 Hz, 1H), 2.81 (dd, J = 16.9, 5.5 Hz, 1H), 2.97 (s, 3H), 3.23−3.31 (m, 2H), 3.75−3.87 (m, 1H), 4.13 (t, J = 5.8 Hz, 2H), 4.28 (dd, J = 9.1, 6.0 Hz, 1H), 4.76 (t, J = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44−6.52 (m, 2H), 6.64 (s, 2H), 7.02−7.10 (m, 2H), 7.16 (s, 1H), 7.35−7.46 (m, 2H). MS m/z 525 $(M + H)^+$. HPLC purity (220 nm) 100.0%. Anal. Calcd for $C_{29}H_{32}O_7S{\cdot}0.5H_2O$: C, 65.27; H, 6.23. Found: C, 65.23; H, 6.15.

Methyl {(3*S*)-6-[(4′-Hydroxy-2′,6′-dimethylbiphenyl-3-yl)-methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetate (17a). Step 1: Methyl {(3*S*)-6-[(4′-{[*tert*-butyl(dimethyl)silyl]oxy}-2′,6′-dimethylbiphenyl-3-yl)methoxy]-2,3-dihydro-1-benzofuran-3-yl}acetate was prepared from **4b** and **5g** by a method similar to that described for **6**, step 1 in 85% yield as a colorless solid. ^1H NMR ($CDCl_3$) δ 0.23 (s, 6H), 1.00 (s, 9H), 1.95 (s, 6H), 2.55 (dd, J = 16.5, 9.3 Hz, 1H), 2.75 (dd, J = 16.5, 5.5 Hz, 1H), 3.71 (s, 3H), 3.74−3.88 (m, 1H), 4.26 (dd, J = 9.2, 6.0 Hz, 1H), 4.75 (t, J = 9.2 Hz, 1H), 5.05 (s, 2H), 6.44−6.51 (m, 2H), 6.57 (s, 2H), 7.01 (d, J = 7.9 Hz, 1H), 7.06−7.10 (m, 1H), 7.17 (s, 1H), 7.33−7.44 (m, 2H). MS m/z 533 $(M + H)^+$. Step 2: To a solution of the obtained solid (2.27 g, 4.26 mmol) in THF (25 mL) was added 1 M TBAF in THF (4.7 mL, 4.7 mmol) at room temperature, and the mixture was stirred under nitrogen atmosphere at room temperature for 1 h. The mixture was concentrated, and the residue was partitioned between water and AcOEt. The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80−60:40) to give **17a** (1.67 g, 94%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.97 (s, 6H), 2.55 (dd, J = 16.5, 9.8 Hz, 1H), 2.75 (dd, J = 16.5, 4.8 Hz, 1H), 3.72 (s, 3H), 3.74−3.86 (m, 1H), 4.26 (dd, J = 9.0, 6.2 Hz, 1H), 4.63 (s, 1H), 4.75 (t, J = 9.0 Hz, 1H), 5.05 (s, 2H), 6.43−6.50 (m, 2H), 6.59 (s, 2H), 7.01 (d, J = 8.1 Hz, 1H), 7.04−7.11 (m, 1H), 7.16 (s, 1H), 7.34−7.46 (m, 2H). MS m/z 419 $(M + H)^+$.

[(3*S*)-6-({2′,6′-Dimethyl-4′-[3-(2-oxopyrrolidin-1-yl)-propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (18). Step 1: To a mixture of **17a** (0.325 g, 0.777 mmol), 1-(3-hydroxypropyl)pyrrolidin-2-one (0.167 g, 1.16 mmol), and $P(n\text{-}Bu)_3$ (0.314 g, 1.55 mmol) in toluene (15 mL) was added ADDP (0.392 g, 1.55 mmol), and the mixture was stirred at room temperature for 15 h. Hexane (15 mL) was added, and the insoluble material was removed by filtration. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70−100:0) to afford methyl [(3*S*)-6-({2′,6′-dimethyl-4′-[3-(2-oxopyrrolidin-1-yl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.420 g, crude) as a colorless oil. The crude product was used for the next reaction without further purification. MS m/z 544 $(M + H)^+$. Step 2: Compound **18** was prepared from the obtained oil by a method similar to that described for **6**, step 2 in 42% yield (from **17a**) as colorless crystals (heptane/MeCN); mp 90−92 °C. $[\alpha]_D$ +5.2° (c 0.32, CH_3CN). ^1H NMR ($CDCl_3$) δ 1.94−1.99 (m, 6H), 1.99−2.10 (m, 4H), 2.41 (t, J = 8.1 Hz, 2H), 2.59 (dd, J = 16.5, 9.3 Hz, 1H), 2.78 (dd, J = 16.5, 5.4 Hz, 1H), 3.42−3.52 (m, 4H), 3.73−3.85 (m, 1H), 4.00 (t, J = 6.3 Hz, 2H), 4.28 (dd, J = 9.2, 5.8 Hz, 1H), 4.74 (t, J = 9.2 Hz, 1H), 5.06 (s, 2H), 6.43−6.50 (m, 2H), 6.64 (s, 2H), 7.02−7.09 (m, 2H), 7.13 (s, 1H), 7.33−7.44 (m, 2H). MS m/z 530 $(M + H)^+$. HPLC purity (220 nm) 99.7%. Anal. Calcd for $C_{32}H_{35}NO_6{\cdot}0.5H_2O$: C, 71.36; H, 6.74; N, 2.60. Found: C, 71.39; H, 6.60; N, 2.61.

[(3*S*)-6-({2′,6′-Dimethyl-4′-[(1-methylpiperidin-4-yl)oxy]-biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid Hydrochloride (19). Step 1: To a mixture of **17a** (0.350 g, 0.836 mmol), 1-methylpiperidin-4-ol (0.143 g, 1.25 mmol), and PPh_3 (0.351 g, 1.34 mmol) in toluene (15 mL) was added 40% DEAD in toluene (0.582 g, 1.34 mmol), and the mixture was stirred at room temperature for 15 h. Then, 1-methylpiperidin-4-ol (0.098 g, 0.851 mmol), PPh_3 (0.197 g, 1.05 mmol), and 40% DEAD in toluene (0.328 g, 0.750 mmol) were added to the mixture. After stirring at room temperature for 8 h, hexane (15 mL) was added to the mixture, and the insoluble material was removed by filtration. The filtrate was concentrated, and the residue was purified by basic silica gel column chromatography (AcOEt/hexane = 20:80−50:50) to afford methyl [(3*S*)-6-({2′,6′-dimethyl-4′-[(1-methylpiperidin-4-yl)oxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.270 g, 63%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.80−1.93 (m, 2H), 1.95−2.10 (m, 8H), 2.24−2.36 (m, 5H), 2.55 (dd, J = 16.5, 9.3 Hz, 1H), 2.66−2.80 (m, 3H), 3.71 (s, 3H), 3.74−3.87 (m, 1H), 4.21−4.39 (m, 2H), 4.75 (t, J = 9.0 Hz, 1H), 5.05 (s, 2H), 6.44−6.51 (m, 2H), 6.66 (s, 2H), 7.01 (d, J = 7.9 Hz, 1H), 7.06−7.11 (m, 1H), 7.17 (s, 1H), 7.33−7.45 (m, 2H). MS m/z 516 $(M + H)^+$. Step 2: To a stirred solution of the obtained oil (0.270 g, 0.52 mmol) in MeOH (4 mL) and THF (8 mL) was added 2 M NaOH aqueous solution (2.0 mL, 4.0 mmol). The mixture was stirred at room temperature for 15 h. Then the mixture was neutralized with saturated NH_4Cl aqueous solution. To the mixture was added sodium chloride, and the mixture was extracted with AcOEt/THF/CH_2Cl_2. The extract was dried over anhydrous $MgSO_4$ and concentrated. The resultant residue was treated with 4 M HCl in AcOEt (5 mL, 20 mmol). Then hexane (20 mL) was added to the mixture, and the resulting crystals were collected by filtration to afford **19** (91 mg, 32%) as colorless crystals; mp 165−167 °C. MS m/z

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502 (M + H)$^+$ as a free form. ^{1}H NMR (DMSO-d_6) δ 1.82−2.30 (m, 4H), 1.92 (s, 6H), 2.40−2.56 (m, 1H), 2.61−2.84 (m, 4H), 3.03−3.46 (m, 5H), 3.59−3.72 (m, 1H), 4.18 (dd, J = 9.1, 6.9 Hz, 1H), 4.67 (t, J = 9.1 Hz, 1H), 5.10 (s, 2H), 6.40−6.53 (m, 2H), 6.77 (s, 2H), 7.02−7.15 (m, 3H), 7.35−7.49 (m, 2H). HPLC purity (220 nm) 99.0%.

[(3*S*)-6-({3′-Chloro-2′,6′-dimethyl-4′-[3-(methylsulfonyl)-propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic Acid (27). Step 1: A mixture of **17b** (0.616 g, 1.36 mmol), **30b** (0.517 g, 1.77 mmol), and K_3PO_4 (0.433 g, 2.04 mmol) in DMF (2 mL) was stirred under nitrogen atmosphere at 90 °C for 2.5 h. The mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70−80:20) to give methyl [(3*S*)-6-({3′-chloro-2′,6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]-biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetate (0.681 g, 88%) as a colorless viscous oil. ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H), 2.05 (s, 3H), 2.35−2.46 (m, 2H), 2.55 (dd, J = 16.5, 9.1 Hz, 1H), 2.75 (dd, J = 16.5, 5.7 Hz, 1H), 2.99 (s, 3H), 3.31−3.41 (m, 2H), 3.71 (s, 3H), 3.75−3.86 (m, 1H), 4.20 (t, J = 5.9 Hz, 2H), 4.26 (dd, J = 9.2, 6.2 Hz, 1H), 4.75 (t, J = 9.2 Hz, 1H), 5.06 (s, 2H), 6.43−6.51 (m, 2H), 6.69 (s, 1H), 6.99−7.07 (m, 2H), 7.13 (s, 1H), 7.37−7.47 (m, 2H). MS m/z 573 (M + H)$^+$. Step 2: Compound **27** was prepared by a method similar to that described for **6**, step 2 in 63% yield as colorless crystals (Et_2O−AcOEt); mp 127−128 °C. $[\alpha]_D$ +5.6° (c 0.30, CH_3CN). ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H), 2.05 (s, 3H), 2.35−2.47 (m, 2H), 2.62 (dd, J = 16.8, 9.2 Hz, 1H), 2.81 (dd, J = 16.8, 5.5 Hz, 1H), 2.99 (s, 3H), 3.32−3.40 (m, 2H), 3.75−3.87 (m, 1H), 4.20 (t, J = 5.7 Hz, 2H), 4.29 (dd, J = 9.1, 6.0 Hz, 1H), 4.76 (t, J = 9.1 Hz, 1H), 5.06 (s, 2H), 6.44−6.52 (m, 2H), 6.69 (s, 1H), 7.02−7.08 (m, 2H), 7.13 (s, 1H), 7.37−7.47 (m, 2H). MS m/z 559 (M + H)$^+$. HPLC purity (220 nm) 99.63%. Anal. Calcd for $C_{29}H_{31}ClO_7S$: C, 62.30; H, 5.59. Found: C, 62.03; H, 5.58.

(3-Methyloxetan-3-yl)methyl 4-Methylbenzenesulfonate (30a). To a suspension of *p*-TsCl (14.3 g, 75.0 mmol) in pyridine (60 mL) was added slowly 3-methyl-3-oxetanemethanol (**29a**) (5.11 g, 50.0 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at 0 °C for 4 h. The mixture was added to ice water and stirred for 1 h. The precipitate was collected by filtration, washed with cold water, and dried to give **30a** (8.97 g, 70%) as colorless crystals; mp 60−61 °C. ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 3H), 2.47 (s, 3H), 4.11 (s, 2H), 4.32−4.39 (m, 4H), 7.37 (d, J = 8.4 Hz, 2H), 7.81 (d, J = 8.4 Hz, 2H). MS m/z 257 (M + H)$^+$. HPLC purity (220 nm) 98.6%. Anal. Calcd for $C_{12}H_{16}O_4S$: C, 56.23; H, 6.29. Found: C, 56.21; H, 6.22.

3-(Methylsulfonyl)propyl 4-Methylbenzenesulfonate (30b). Step 1: To a solution of 3-methylthio-1-propanol (**29b**) (5.30 g, 50.0 mmol), Et_3N (10.5 mL, 75.0 mmol), and *N*,*N*,*N*′,*N*′-tetramethyl-1,6-hexanediamine (0.861 g, 5.00 mmol) in toluene (50 mL) was added dropwise *p*-TsCl (14.3 g, 75.0 mmol) in toluene (50 mL) at 0 °C, and the mixture was stirred under nitrogen atmosphere at 0 °C for 3 h. The mixture was quenched with water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt/hexane = 10:90−40:60) to give 3-(methylthio)propyl 4-methylbenzenesulfonate (12.2 g, 94%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.87−1.98 (m, 2H), 2.04 (s, 3H), 2.45 (s, 3H), 2.51 (t, J = 7.1 Hz, 2H), 4.14 (t, J = 6.1 Hz, 2H), 7.35 (d, J = 8.2 Hz, 2H), 7.80 (d, J = 8.2 Hz, 2H). MS m/z 261 (M + H)$^+$. Step 2: To a solution of the obtained sulfonate (12.2 g, 46.9 mmol) in MeOH (250 mL) was added dropwise a solution of oxone (57.7 g, 93.8 mmol) in water (250 mL) at 0 °C, and the mixture was stirred at 0 °C to room temperature for 20 h. After evaporation of the solvent, the residue was diluted with water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The resulting crystals were washed with heptane/AcOEt to give **30b** (13.1 g, 96%) as colorless crystals; mp 88−89 °C. ^{1}H NMR ($CDCl_3$) δ 2.17−2.28 (m, 2H), 2.46 (s, 3H), 2.92 (s, 3H), 3.07−3.15 (m, 2H), 4.18 (t, J = 5.9 Hz, 2H), 7.37 (d, J = 8.3 Hz, 2H), 7.79 (d, J = 8.3 Hz, 2H). MS m/z 293 (M + H)$^+$. Anal. Calcd for $C_{11}H_{16}O_5S_2$: C, 45.19; H, 5.52. Found: C, 44.96; H, 5.53.

3′-(Hydroxymethyl)-2,6-dimethylbiphenyl-4-ol (32). To a solution of 4′-hydroxy-2′,6′-dimethylbiphenyl-3-carbaldehyde (**31**) (6.95 g, 30.7 mmol) in MeOH (30 mL) and THF (60 mL) was added gradually $NaBH_4$ (1.29 g, 30.7 mmol) at 0 °C, and the mixture was stirred under nitrogen atmosphere at 0 °C to room temperature for 20 h. The mixture was concentrated, quenched with water and 1 M HCl aqueous solution, and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated to give crystals. Recrystallization from heptane/AcOEt gave **32** (6.56 g, 93%) as colorless crystals; mp 175 °C. ^{1}H NMR ($CDCl_3$) δ 1.67 (t, J = 5.8 Hz, 1H), 1.98 (s, 6H), 4.65 (s, 1H), 4.74 (d, J = 5.8 Hz, 2H), 6.59 (s, 2H), 7.06 (dt, J = 7.3, 1.5 Hz, 1H), 7.12 (s, 1H), 7.33 (dt, J = 7.5, 1.5 Hz, 1H), 7.41 (t, J = 7.5 Hz, 1H). MS m/z 211 (M − 18 + H)$^+$. Anal. Calcd for $C_{15}H_{16}O_2$: C, 78.92; H, 7.06. Found: C, 78.76; H, 7.04.

[4′-(2-Ethoxyethoxy)-2′,6′-dimethylbiphenyl-3-yl]methanol (5a). A mixture of **32** (4.57 g, 20.0 mmol), 2-chloroethyl ethyl ether (3.29 mL, 30.0 mmol), K_2CO_3 (3.32 g, 24.0 mmol), and KI (0.332 g, 2.00 mmol) in DMF (30 mL) was stirred under nitrogen atmosphere at 80 °C for 70 h. The mixture was diluted with water and extracted with AcOEt. The organic layer was washed sequentially with 1 M NaOH aqueous solution and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by basic silica gel column chromatography (AcOEt/hexane = 20:80−60:40) and crystallized from heptane/AcOEt to give **5a** (4.43 g, 74%) as colorless crystals; mp 62−63 °C. ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.1 Hz, 3H), 1.66 (t, J = 5.9 Hz, 1H), 2.00 (s, 6H), 3.62 (q, J = 7.1 Hz, 2H), 3.80 (t, J = 5.1 Hz, 2H), 4.14 (t, J = 5.1 Hz, 2H), 4.73 (d, J = 5.9 Hz, 2H), 6.69 (s, 2H), 7.06 (d, J = 7.3 Hz, 1H), 7.12 (s, 1H), 7.33 (d, J = 7.3 Hz, 1H), 7.40 (t, J = 7.3 Hz, 1H). MS m/z 301 (M + H)$^+$. Anal. Calcd for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.75; H, 8.10.

4′-{*tert*-Butyl(dimethyl)silyl}oxy}-2′,6′-dimethylbiphenyl-3-carbaldehyde (33d). To a solution of **31** (9.0 g, 39.8 mmol) and imidazole (2.98 g, 43.8 mmol) in DMF (100 mL) was added TBSCl (6.6 g, 43.8 mmol) at room temperature, and the mixture was stirred at room temperature for 4 h. The mixture was diluted with AcOEt, washed sequentially with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt/hexane = 9:91−20:80) to give **33d** (10.5 g, 77%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.25 (s, 6H), 1.02 (s, 9H), 1.97 (s, 6H), 6.62 (s, 2H), 7.44 (dt, J = 7.5, 1.5 Hz, 1H), 7.59 (t, J = 7.5 Hz, 1H), 7.68 (t, J = 1.5 Hz, 1H), 7.86 (dt, J = 7.5, 1.5 Hz, 1H), 10.06 (s, 1H). MS m/z 341 (M + H)$^+$.

[2,6-Dimethyl-4-(tetrahydro-2*H*-thiopyran-4-yloxy)phenyl]-boronic Acid (35). Step 1: 4-(4-Bromo-3,5-dimethylphenoxy)-tetrahydro-2*H*-thiopyran was prepared from 3,5-dimethyl-4-bromophenol (**34**) and tetrahydro-4*H*-thiopyran-4-ol by a method similar to that described for **17**, step 1 in 86% yield as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.93−2.07 (m, 2H), 2.10−2.23 (m, 2H), 2.37 (s, 6H), 2.49−2.62 (m, 2H), 2.85−2.98 (m, 2H), 4.25−4.36 (m, 1H), 6.65 (s, 2H). Step 2: To a solution of the obtained solid (3.01 g, 10.0 mmol) in THF (50 mL) was added dropwise a solution of 1.6 M *n*-BuLi in hexane (6.57 mL, 10.5 mmol) under argon atmosphere at −78 °C. The mixture was stirred at −78 °C for 1.5 h, and then B(*i*-PrO)$_3$ (6.92 mL, 30.0 mmol) was added at the same temperature. The mixture was gradually warmed to room temperature and stirred for 16 h. After the mixture was cooled to 0 °C, 2 M HCl aqueous solution (50 mL) was added. The resulting mixture was stirred at 0 °C for 2.5 h. The phases were separated, and the aqueous phase was extracted with AcOEt (pH of the aqueous phase was adjusted to neutral with saturated $NaHCO_3$ aqueous solution). The combined organic phase was dried over anhydrous $MgSO_4$, and concentrated to give a white solid. The resulting solid was washed with cold hexane and dried to afford **35** (1.89 g, 71%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.90−2.06 (m, 2H), 2.09−2.23 (m, 2H), 2.35 (s, 6H), 2.48−2.62 (m, 2H), 2.83−2.98 (m, 2H), 4.28−4.40 (m, 1H), 6.51 (s, 2H), 6.59 (s, 2H). MS m/z not detected.

{4′-[(1,1-Dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-yl}methanol (5c). Step 1: A mixture of **35** (1.41 g, 5.30 mmol), methyl 3-bromobenzoate (1.14 g, 5.30 mmol), and

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$Pd(PPh_3)_4$ (0.306 g, 0.265 mmol) in 2 M Cs_2CO_3 (6.35 mL) and DME (20 mL) was stirred under argon atmosphere at 95 °C for 24 h. The mixture was diluted with water and extracted with AcOEt. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt/hexane = 0:100−25:75) to give crystals. Recrystallization from hexane−AcOEt gave methyl 2′,6′-dimethyl-4′-(tetrahydro-2*H*-thiopyran-4-yloxy)biphenyl-3-carboxylate (1.63 g, 86%) as colorless crystals; mp 69−71 °C. ^{1}H NMR ($CDCl_3$) δ 1.92−2.13 (m, 8H), 2.15−2.29 (m, 2H), 2.52−2.66 (m, 2H), 2.89−3.03 (m, 2H), 3.91 (s, 3H), 4.33−4.44 (m, 1H), 6.66 (s, 2H), 7.34 (dt, J = 7.8, 1.5 Hz, 1H), 7.49 (t, J = 7.8 Hz, 1H), 7.84 (t, J = 1.5 Hz, 1H), 8.01 (dt, J = 7.8, 1.5 Hz, 1H). MS m/z 379 $(M + Na)^+$. HPLC purity (220 nm) 99.7%. Anal. Calcd for $C_{21}H_{24}O_3S$: C, 70.75; H, 6.79. Found: C, 70.73; H, 6.80. Step 2: Methyl 4′-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)oxy]-2′,6′-dimethylbiphenyl-3-carboxylate was prepared from the obtained crystals by a method similar to that described for **14**, step 2 in 85% yield as colorless crystals; mp 180 °C. ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6H), 2.30−2.45 (m, 2H), 2.45−2.59 (m, 2H), 2.88−3.02 (m, 2H), 3.37−3.53 (m, 2H), 3.92 (s, 3H), 4.63−4.72 (m, 1H), 6.69 (s, 2H), 7.33 (dt, J = 7.6, 1.4 Hz, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.83 (t, J = 1.6 Hz, 1H), 8.02 (dt, J = 7.6, 1.4 Hz, 1H). MS m/z 389 $(M + H)^+$. HPLC purity (220 nm) 98.6%. Step 3: To a solution of the obtained crystals (0.128 g, 0.33 mmol) in THF (2 mL) was added gradually $LiAlH_4$ (80%, 15.7 mg, 0.33 mmol) at 0 °C. The mixture was stirred for 1.5 h at 0 °C, followed by gradually addition of $Na_2SO_4 \cdot 10H_2O$ at the same temperature. After stirring at room temperature for 1 h, the mixture was filtered through a pad of Celite. The filtrate was concentrated to afford **5c** (0.111 g, 93%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.76 (t, J = 5.6 Hz, 1H), 2.00 (s, 6H), 2.29−2.44 (m, 2H), 2.44−2.58 (m, 2H), 2.87−3.02 (m, 2H), 3.37−3.53 (m, 2H), 4.63−4.70 (m, 1H), 4.74 (d, J = 5.6 Hz, 2H), 6.68 (s, 2H), 7.05 (dt, J = 7.4, 1.5 Hz, 1H), 7.12 (s, 1H), 7.31−7.38 (m, 1H), 7.42 (t, J = 7.4 Hz, 1H). MS m/z 343 $(M - 18 + H)^+$. HPLC purity (220 nm) 97.1%.

3,5-Diethylphenol (37). A mixture of 4-ethylphenol (**36**) (25.7 g, 210 mmol) and $AlCl_3$ (62.5 g, 469 mmol) was stirred under nitrogen atmosphere at 115 °C for 4 h. The reaction mixture was cooled to 60 °C, poured onto crushed ice, and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt/hexane = 0:100−25:75) to give **37** (12.3 g, 78%) as a reddish-brown oil. ^{1}H NMR ($CDCl_3$) δ 1.21 (t, J = 7.7 Hz, 6H), 2.58 (q, J = 7.7 Hz, 4H), 4.66 (s, 1H), 6.49−6.52 (m, 2H), 6.60−6.63 (m, 1H). MS m/z 151 $(M + H)^+$.

4-Bromo-3,5-diethylphenol (38a). To a solution of **37** (3.00 g, 20.0 mmol) in MeOH (30 mL) was added n-Bu_4NBr_3 (9.64 g, 20.0 mmol) at room temperature, and the mixture was stirred for 1 h. After evaporation of the solvent, the residue was diluted with water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt/hexane = 0:100−25:75) to give **38a** (3.28 g, 72%). An analytical sample was obtained as colorless crystals (heptane). ^{1}H NMR ($CDCl_3$) δ 1.21 (t, J = 7.6 Hz, 6H), 2.73 (q, J = 7.6 Hz, 4H), 4.65 (s, 1H), 6.59 (s, 2H). MS m/z not detected. Anal. Calcd for $C_{10}H_{13}BrO$: C, 52.42; H, 5.72. Found: C, 52.22; H, 5.66.

2-Hydroxy-3,4,6-trimethylbenzaldehyde (40). To a solution of 2,3,5-trimethylphenol (**39**) (13.6 g, 100 mmol) in CH_2Cl_2 (20 mL) was added dropwise $TiCl_4$ (41.7 g, 220 mmol) under nitrogen atmosphere at 0 °C over 0.5 h, and the mixture was stirred at 0 °C for 1 h. To the mixture was added dropwise dichloromethyl methyl ether (11.5 g, 100 mmol), and the mixture was stirred at 0 °C for 6 h. The mixture was quenched with saturated NH_4Cl aqueous solution and extracted with CH_2Cl_2. The organic layer was washed sequentially with diluted HCl aqueous solution, $NaHCO_3$ aqueous solution and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt/hexane = 5:95−50:50) to give **40** (6.58 g, 40%) as pale-brown crystals. ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3H), 2.27 (s, 3H), 2.53 (s, 3H), 6.53 (s, 1H), 10.23 (s, 1H), 12.29 (s, 1H). MS m/z 165 $(M + H)^+$.

2,3,5,6-Tetramethylphenol (41). Compound **40** (6.58 g, 40.1 mmol) was hydrogenated on 10% Pd/C (1.0 g) in MeOH (120 mL) under hydrogen atmosphere (balloon pressure) at room temperature for 22 h. The catalyst was removed by filtration, and the filtrate was concentrated to give **41**(5.83 g, 97%). An analytical sample was obtained as colorless plates (MeOH). ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 6H), 2.22 (s, 6H), 4.59 (s, 1H), 6.60 (s, 1H). MS m/z 151 $(M + H)^+$. Anal. Calcd for $C_{10}H_{14}O$: C, 79.96; H, 9.39. Found: C, 80.02; H, 9.42.

4-Bromo-2,3,5,6-tetramethylphenol (38b). To a suspension of **41** (5.10 g, 34.0 mmol) in AcOH (90 mL) was added dropwise a solution of Br_2 (1.98 mL, 38.6 mmol) in AcOH (30 mL) at room temperature, and the mixture was stirred at room temperature for 5 h. The mixture was concentrated, and the residue was diluted with AcOEt, washed sequentially with $Na_2S_2O_3$ aqueous solution and brine, dried over anhydrous $MgSO_4$, and concentrated to give **38b** (6.48 g, 83%). An analytical sample was obtained as off-white crystals (petroleum ether). ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 6H), 2.40 (s, 6H), 4.59 (s, 1H). MS m/z not detected.

4-Bromo-2-fluoro-3,5-dimethylphenol (38c). A mixture of **34** (2.00 g, 9.95 mmol) and *N*-fluoropyridinium triflate (6.15 g, 24.9 mmol) in 1,2-dichloroethane (20 mL) was stirred at reflux for 7 h. The mixture was quenched with 1 M $Na_2S_2O_3$ aqueous solution and extracted with AcOEt. The extract was washed sequentially with water and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt/hexane = 0:100−30:70) to afford **38c** (0.790 g, 36%) as colorless crystals. ^{1}H NMR ($CDCl_3$) δ 2.29−2.36 (m, 6H), 5.04 (d, J = 4.0 Hz, 1H), 6.79 (d, J = 9.0 Hz, 1H). MS m/z not detected.

3′-Chloro-4′-hydroxy-2′,6′-dimethylbiphenyl-3-carbaldehyde (42d). To a solution of **31** (11.3 g, 50.0 mmol) in DMF (50 mL) was added gradually NCS (6.68 g, 50.0 mmol) at 0 °C, and the mixture was stirred at room temperature for 13 h. The mixture was heated to 50 °C, and stirred for 3 h. To the mixture was added NCS (1.34 g, 10.0 mmol), and the mixture was stirred at 50 °C for 3 h. To the mixture was added NCS (0.668 g, 5.00 mmol), and the resulting mixture was stirred at 50 °C for 1 h. The mixture was poured into water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (AcOEt/hexane = 5:95−40:60) to give crystals. Recrystallization from heptane/AcOEt gave **42d** (8.47 g, 65%) as colorless crystals; mp 85−86 °C. ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H), 2.05 (s, 3H), 5.61 (s, 1H), 6.84 (s, 1H), 7.36−7.42 (m, 1H), 7.57−7.66 (m, 2H), 7.85−7.91 (m, 1H), 10.06 (s, 1H). MS m/z 261 $(M + H)^+$. Anal. Calcd for $C_{15}H_{13}ClO_2$: C, 69.10; H, 5.03. Found: C, 69.16; H, 4.97.

3′,5′-Dichloro-4′-hydroxy-2′,6′-dimethylbiphenyl-3-carbaldehyde (42e). To a solution of **31** (11.3 g, 50.0 mmol) in DMF (50 mL) was added gradually NCS (13.4 g, 100 mmol) at 0 °C, and the mixture was stirred at room temperature for 14 h. The mixture was heated to 50 °C, and the mixture was stirred for 2 h. The mixture was poured into water and extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The resulting crystals were triturated with heptane/AcOEt to give **42e** (8.88 g, 60%) as colorless crystals; mp 116−117 °C. ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 6H), 6.00 (s, 1H), 7.35−7.40 (m, 1H), 7.60−7.66 (m, 2H), 7.88−7.94 (m, 1H), 10.06 (s, 1H). MS m/z 294 $(M + H)^+$. Anal. Calcd for $C_{15}H_{12}Cl_2O_2$: C, 61.04; H, 4.10. Found: C, 60.91; H, 3.98.

Ca Influx Activity of CHO Cells Expressing Human GPR40/FFA1 (FLIPR Assay). CHO dhfr cells stably expressing human GPR40/FFA1 (accession no. NM_005303) were plated and incubated overnight in 5% CO_2 at 37 °C. Then, cells were incubated in loading buffer (recording medium containing 2.5 μg/mL fluorescent calcium indicator Fluo 4 AM (Molecular Devices), 2.5 mmol/L probenecid (Dojindo), and 0.1% fatty acid-free BSA (Sigma)) for 60 min at 37 °C. Various concentrations of test compounds or γ-linolenic acid (Sigma) were added into the cells and increase of the intracellular Ca^{2+} concentration after addition was monitored by FLIPR Tetra system

(Molecular Devices) for 90 s. The agonistic activities of test compounds and γ-linolenic acid on human GPR40/FFA1 were expressed as [(A − B)/(C − B)] × 100 (increase of the intracellular Ca^{2+} concentration (A) in test compounds-treated cells, (B) in vehicle-treated cells, and (C) in 10 μM γ-linolenic acid-treated cells). EC_{50} values and 95% confidence intervals of each compound were obtained with Prism 5 software (GraphPad).

Preparation of CHO Membrane for GPR40/FFA1 Receptor Binding Assay. Cell lines stably expressing human GPR40/FFA1 and rat GPR40/FFA1 were used for the experiments. Each cell was cultured in Minimum Essential Medium Alpha (MEM-Alpha, Invitrogen) supplemented with 10% dialyzed Fetal-Bovine-Serum (dialyzed FBS, Thermo Trace Ltd.), 100 unit/mL penicillin, and 100 unit/mL streptomycin in 5% CO_2/95% air atmosphere at 37 °C. Cells were harvested at confluence in Dulbecco's Phosphate-Buffered-Saline (D-PBS, Invitrogen) containing 1 mM EDTA and centrifuged. Cells were homogenized in ice-cold membrane preparation buffer (50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.5 mM PMSF (Wako), 20 μg/mL leupeptin, 0.1 μg/mL pepstatin A, 100 μg/mL Phosphoramidon, Peptide Institute, Inc.) and centrifuged (700*g*, 10 min, 4 °C). The supernatant was filtered through 40 μm Cell Strainer (BD Falcon) and ultracentrifuged (100000*g*, 1 H 4 °C) with Optima L-100 XP Ultracentrifuge (Beckman Coulter). The precipitation was suspended in the same buffer, and the protein concentration was determined with the BCA Protein assay reagent (Pierce) following membrane solubilization with 0.1% SDS and 0.1 M NaOH aqueous solution. The membrane suspension was stored at −80 °C until receptor binding assay.

GPR40/FFA1 Receptor Binding Assay. The frozen cell membranes were resuspended in ice-cold assay buffer (25 mmol/L Tris-HCl (pH7.5), 5 mmol/L EDTA, 0.5 mmol/L PMSF, 20 μg/mL leupeptin, 0.1 μg/mL pepstatin A, 0.05% CHAPS (Wako), and 0.2% fatty-acid-free BSA (Sigma)) and used for receptor binding assay. To determine the K_d values of 3-[4-({2′,6′-dimethyl-6-[(4-[^{3}H])-phenylmethoxy]biphenyl-3-yl}methoxy)phenyl] propanoic acid (Amersham Biosciences) for human and rat GPR40/FFA1, binding assays were performed in the presence of various concentrations of the labeled ligand. After incubation at room temperature for 90 min, the membranes were harvested using GF/C filter plates (MILLIPORE) and washed with ice-cold 50 mmol/L Tris-HCl (pH7.5) using a FilterMate Harvester (PerkinElmer). The membrane-associated radioactivities were counted using a TopCount liquid scintillation counter (PerkinElmer). Nonspecific binding was defined as binding in the presence of 10 μmol/L of the unlabeled ligand. To determine the binding affinities of test compounds to human and rat GPR40/FFA1, binding assays were performed in the presence of both various concentrations of test compounds and 2 nmol/L or 6 nmol/L of the labeled ligand. The 50% inhibitory concentrations (IC_{50} values) of test compounds for the labeled ligand were calculated using nonlinear regression analysis in GraphPad Prism 3.0 (GraphPad Software). K_i values were converted as $K_i = IC_{50}/\{1 + (\text{the concentration of the labeled ligand})/K_d\}$.

Caspase-3/7 Activity Assay. HepG2 cells were cultured at 37 °C, 5% CO_2 in DMEM supplemented with 10% fetal bovine serum, 50 IU/mL penicillin, and 50 μg/mL streptomycin. Cells were seeded at 2 × 10^4cells/well in a 96-well white plate (Costar) and cultured with test compounds in DMEM supplemented with 0.5% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 50 IU/mL penicillin, and 50 μg/mL streptomycin for 1 day. Caspase-3/7 activity was measured by using Caspase-GloTM 3/7 assay Kit (Promega) according to the manufacturer's instruction. Caspase-3/7 activity was calculated (n = 3) to the following. Caspase-3/7 activity (%) = (RLU of compound − RLU of 1% DMSO)/(RLU of 30 μM staurosporine − RLU of 1% DMSO) × 100.

Pharmacokinetic Analysis in Rat Cassette Dosing. Test compounds were suspended in 0.5% methylcellulose aqueous solution and administered as a cassette dosing to fasted rats. After oral administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant was diluted and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

Oral Glucose Tolerance Test (OGTT). The care and use of the animals and the experimental protocols used in this research were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company Limited. Female Wistar fatty WF rats and Male GK rats were obtained from Takeda Rabics Limited (Hikari, Japan). They were fed the commercial diet CE-2 (Clea Japan Co.) and tap water ad libitum. Female WF (12−17 weeks old) and male GK (41 weeks old) rats were fasted overnight and orally given vehicle (0.5% methylcellulose aqueous solution) or compounds (suspended in vehicle). All animals received an oral glucose load (1 g/kg) 1 or 4 h after drug administration. Blood samples were collected from tail vein before drug administration (pre) and just before glucose load (time 0) and 10, 30, 60, and 120 min after glucose load. Plasma glucose and plasma insulin levels were measured by Autoanalyzer 7080 (Hitachi, Japan) and radioimmunoassay (Millipore, USA), respectively. Statistical differences were analyzed by the Student's *t* test or the Aspin−Welch test. In the dose-dependent study, statistical significances versus vehicle control were assessed by the one-tailed Williams test or the Shirley−Williams test.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedures and spectroscopic data of final compounds and intermediates not described in the manuscript. Methods and instrumentation used to obtain the X-ray crystal structure of compound **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

GPR40, G protein-coupled receptor 40; FFA1, free fatty acid receptor 1; GPCR, G protein-coupled receptor; FFA, free fatty acid; GSIS, glucose-stimulated insulin secretion; OGTT, oral glucose tolerance test; PK, pharmacokinetic; FLIPR, fluorometric imaging plate reader; BSA, bovine serum albumin; ADME-Tox, absorption, distribution, metabolism, excretion, and toxicology; C_{1h}, plasma concentration at 1 h after administration; C_{4h}, plasma concentration at 4 h after administration; C_{max}, maximum plasma concentration; T_{max}, time of maximum plasma concentration; AUC, area under curve; GK, Goto-Kakizaki

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(26) X-ray crystallographic data for compound **16** has been deposited with the Cambridge Crystallographic Data Center as CCDC 837682. The crystallographic data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223−336033 or e-mail: deposit@ccdc.cam.ac.uk).

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