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Synthesis and biological evaluation of phenoxyacetic acid derivatives as novel free fatty acid receptor 1 agonists

Xuekun Wang [a], Tianxiao Zhao [a], Baowei Yang [a,b], Zheng Li [a], Jian Cui [a], Yuxuan Dai [a], Qianqian Qiu [a], Hao Qiang [a], Wenlong Huang [a,*], Hai Qian [a,*]

[a] Centre of Drug Discovery, State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[b] Medical and Health Management Department, Jiangsu Food & Pharmaceutical Science College, 4 Meicheng South Road, Higher Education Area, Huaian, Jiangsu 223003, China

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ABSTRACT

Free fatty acid receptor 1 (FFA1) is a new potential drug target for the treatment of type 2 diabetes because of its role in amplifying glucose-stimulated insulin secretion in pancreatic β-cell. In the present studies, we identified phenoxyacetic acid derivative (**18b**) as a potent FFA1 agonist (EC_{50} = 62.3 nM) based on the structure of phenylpropanoic acid derivative 4p. Moreover, compound **18b** could significantly improve oral glucose tolerance in ICR mice and dose-dependently reduced glucose levels in type 2 diabetic C57BL/6 mice without observation of hypoglycemic side effect. Additionally, compound **18b** exhibited acceptable PK profiles. In summary, compound **18b** with ideal PK profiles exhibited good activity in vitro and in vivo, and might be a promising drug candidate for the treatment of diabetes mellitus.

1. Introduction

Diabetes mellitus is becoming a common and frequently occurring metabolic disease, trending to be a major public health problem. The International Diabetes Federation estimates that 382 million people have diabetes in 2013 and this number is expected to rise to 592 million by 2035.[1] Type 2 diabetes mellitus (T2DM), resulting from insulin deficiency and insulin resistance, accounts for almost 90% of diabetes cases and becomes a major risk factor for vascular complication such as atherosclerosis, stroke, nephropathy, retinopathy, and neuropathy.[2] To date, various oral antidiabetic agents have been used for the treatment of T2DM, such as metformin, rosiglitazone, sitagliptine, acarbose, and glimepiride. However, their limited efficacy and adverse side effects (most notably hypoglycemia and weight gain) has stimulated an intense effort to evaluate new mechanisms to achieve glycemic control.[3–5]

Free fatty acid receptor 1 (FFA1, formerly known as GPR40 receptor) has become an attractive and potential target for the treatment of T2DM. The FFA1 predominantly expressed in pancreatic β-cells is activated by naturally occurring medium- to long-chain free fatty acids (FFAs) such as oleic acid and linoleic acid and mediates FFA-amplified insulin secretion.[6,7] The mechanism of action of FFA1 remains to be fully elucidated; however, several reports have shown that FFA1 is mainly coupled with the G protein α-subunit of the Gq family (Gαq), which activates phospholipase C, resulting in the production of inositol triphosphate and mobilization of intracellular Ca^{2+} from the endoplasmic reticulum. The activation of FFA1 also stimulates Ca^{2+} influx through voltage-gated Ca^{2+} channels, and the resulting increase in intracellular Ca^{2+} concentrations enhances glucose-stimulated insulin secretion (GSIS).[8–10] The insulinotropic effects via FFA1 are dependent on glucose concentration, indicating that a selective FFA1 agonist has a low risk of hypoglycemia.[11,12]

A variety of synthetic FFA1 agonists contained acidic moieties such as phenylpropanoic acid and thiazolidinedione have been reported (Fig. 1).[13,14] However, some phenylpropanoic acid derivatives exhibited high clearance and low oral bioavailability due to susceptibility to β-oxidation at the phenylpropanoic acid moiety.[15,16] To avoid this drawback, the β-position of the carboxylic acid was substituted by small residues (such as AMG-837) or cyclized to aromatic ring (such as TAK-875) in order to reduce the potential of β-oxidation.[14] TAK-875, developed by Takeda based on the structure of 4p, showed significant improvements in glycemic control in patients with type 2 diabetes in clinical studies.[17,18] Unfortunately, Takeda decided to terminate the development of TAK-875 due to its liver toxicity in December 2013.[19]

Our hypothesis is the process of β-oxidation can also be reduced by introduction of a hetero atom to replace the β-carbon without decreasing its efficacy.[20] In the present studies, we synthesized 14 phenoxyacetic acid derivatives, which β-carbon of compound

* Corresponding authors. Tel.: +86 25 83271302; fax: +86 25 83271480.
E-mail addresses: ydhuangwenlong@126.com (W. Huang), qianhai24@163.com (H. Qian).

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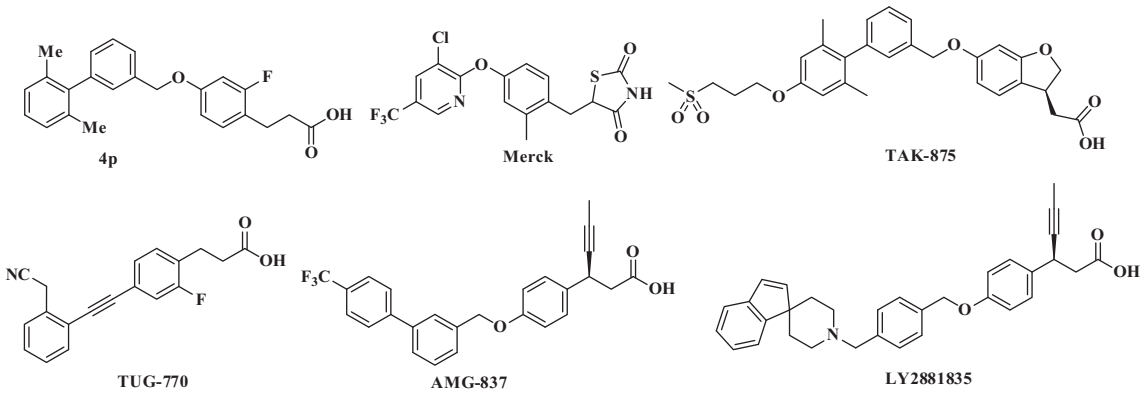


Figure 1. Representative FFA1 agonists.

4p (Fig. 2) was replaced by an oxygen atom, and proved that phenoxyacetic acid derivatives were novel FFA1 agonists by conducting in vitro and in vivo activity tests. Among these compounds, compound **18b**, which showed best in vitro and in vivo efficacy, also presented qualified PK profiles as a drug candidate.

2. Results and discussion

2.1. Chemistry

Phenoxyacetic acid derivatives **10a**–**10f** were synthesized as outlined in Scheme 1. Compounds **1** was synthesized via published procedures.[21] The key intermediate bromide **3** was prepared by the substitution of alcohol **2**, which was derived from the reduction of aldehyde **1** with $NaBH_4$. Acetophenones **6** was synthesized by Friedel–Crafts acylation of acetyl chloride with intermediate **5** prepared by substitution reaction of phenol **4** with methyl chloroacetate.[22] Baeyer–Villiger oxidation of acetophenones **6** with 3-chloroperoxybenzoic acid followed by hydrolysis of acetates **7** with CH_3ONa provided the phenol **8**.[23] Substitution of the key bromide **3** with phenol **8**, followed by hydrolysis of the ester **9**, furnished the desired compounds **10a**–**10f**.

Phenoxyacetic acid derivatives **18a**–**18h** were synthesized as outlined in Scheme 2. 4-Acetyl phenol **13** was achieved by Fries rearrangement of acetyl-phenyl ester **12** in the presence of $AlCl_3$, which was prepared by acetylation of phenol **11** with acetyl chloride.[24] Indeed, Fries rearrangement produced two corresponding acetophenones, which be separated by column chromatography for the different polarity induced by hydrogen bond. The phenol **16** was obtained from 4-acetyl phenol **13** via substitution, Baeyer–Villiger oxidation and hydrolysis.[25] Substitution of the key bromide **3** with phenol **16**, followed by hydrolysis of the ester **17**, furnished the desired compounds **18a**–**18h**.

2.2. FFA1 agonist activity and SAR study

The agonist activities of synthesized compounds were evaluated by monitoring Ca^{2+} influx using the FlexStation3 Molecular Devices in Chinese hamster ovary (CHO) cells expressing human FFA1. The agonist activities were depicted in Table 1. The FFA1 agonist TAK-875 used as a positive control. The SARs of synthesized phenoxyacetic acid derivatives was discussed as follows: phenoxyacetic acid derivative (**10a**) showed more potent agonist activity compared to the carbonyl-α-substituted derivative (**10b**, **10c** and **10f**), and introducing substituents on the *ortho*-position of the phenyl ring did not improve the activity of phenoxyisobutyric acid, regardless the substituents' electron-donating or electron-withdrawing properties. These results indicated that their active conformations of phenoxyisobutyric acid are influenced by α-substitution.

In the series of the phenoxyacetic acid derivatives, the size of the substituent affect the activity. Methyl substituted on the *ortho*-position of the phenyl ring (**10d**) slightly reduced agonist activity compared with unsubstituted (**10a**), while larger group ethyl or methoxyl (**10e** and **18g**) lost agonist activities. When the electron-withdrawing chlorine was introduced into the *ortho*-position, the resulting compound **18a** improved agonist activity slightly compared with unsubstituted (**10a**). Replacement of the chlorine with a smaller fluorine gave compound **18b** with an EC_{50} value of 62.3 nM, a superior agonist of FFA1 than **10a**, and approximately as potent as TAK-875 (EC_{50} = 38.6 nM).

To better understand the SAR for FFA1, we performed a molecular modeling study using MOE based upon the recently reported crystal structure of FFA1 (PDB accession code: 4PHU[26]) (Fig. 3). This model allows explanation of some of the SAR that we observed. In the binding-pocket, the presence of the multiple arginine and tyrosine residues (Arg $183^{5.39}$, Arg $258^{7.35}$, Tyr $91^{3.37}$ and Tyr $240^{6.51}$) interacting with the carboxylate suggests a possible redundancy in the charge network, which is supported by the observation that certain carboxylate containing ligands are not affected to the same degree upon single mutation of these arginine residues. For example, the half-maximum effective concentration values of docosahexaenoic acid and AM1638 are only modestly affected by mutation of Arg $183^{5.39}$ or Arg $258^{7.35}$, suggesting that the absence of hydrogen bond interactions with one arginine might lead to little or no effect on the potency of agonists.[26] The 3D

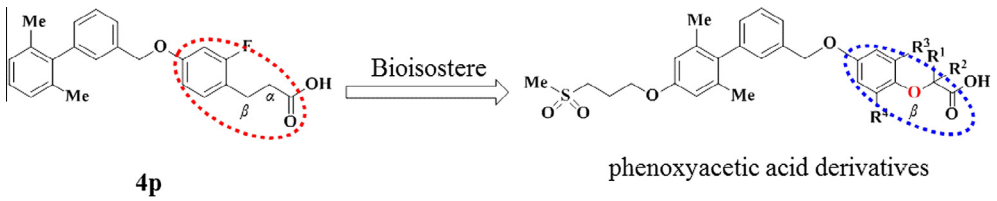
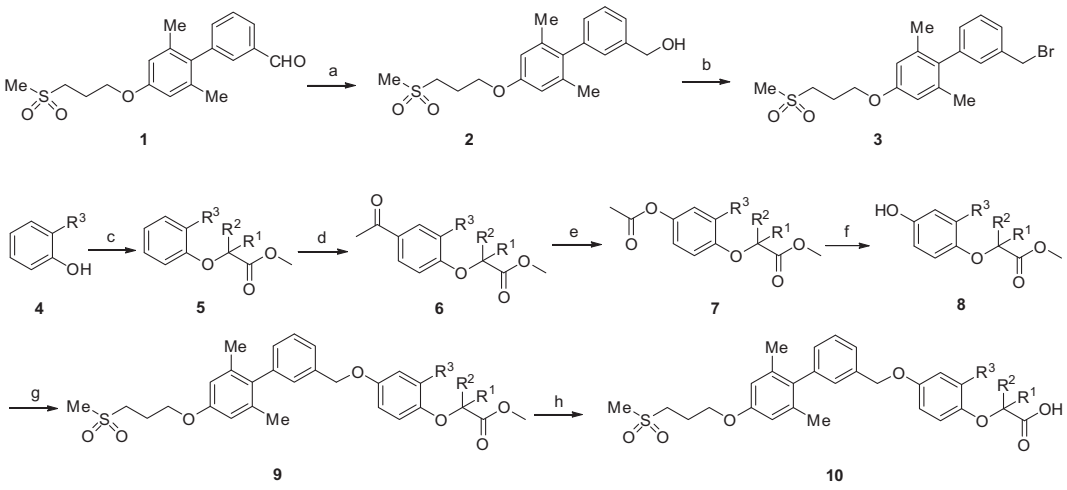
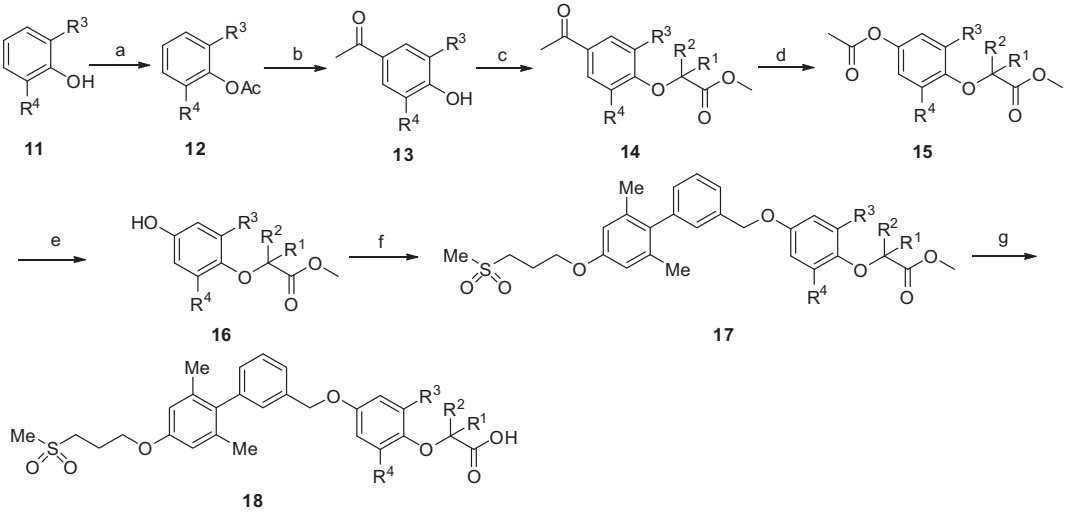


Figure 2. Design of phenoxyacetic acids.



	R_1	R_2	R_3		R_1	R_2	R_3
10a	H	H	H	**10d**	H	H	CH_3
10b	CH_3	H	H	**10e**	H	H	CH_2CH_3
10c	CH_2CH_3	H	H	**10f**	CH_3	CH_3	H

Scheme 1. General synthesis of **10a–f**. Reagents and conditions: (a) $NaBH_4$, CH_3OH, THF, 0 °C; (b) PBr_3, CH_2Cl_2, 0 °C; (c) K_2CO_3, DMF; (d) $AlCl_3$, CH_2Cl_2, (e) *m*-CPBA, *p*-TSA, CH_2Cl_2; (f) CH_3ONa, CH_3OH; (g) K_2CO_3, acetone, 60 °C; (h) 2 M NaOH aq, MeOH, THF, rt.



	R_1	R_2	R_3	R_4		R_1	R_2	R_3	R_4
18a	H	H	Cl	H	**18e**	CH_3	CH_3	CH_2CH_3	H
18b	H	H	F	H	**18f**	CH_3	CH_3	CH_3	CH_3
18c	CH_3	CH_3	Cl	H	**18g**	H	H	OCH_3	H
18d	CH_3	CH_3	F	H	**18h**	CH_3	CH_3	OCH_3	H

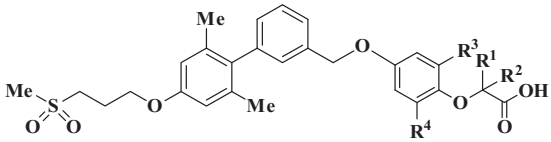
Scheme 2. General synthesis of **18a–f**. Reagents and conditions: (a) AcCl, Py, CH_2Cl_2, 0 °C; (b) $AlCl_3$, CH_2Cl_2, 100 °C; (c) K_2CO_3, DMF, 0 °C; (d) *m*-CPBA, *p*-TSA, DCM; (e) CH_3ONa, CH_3OH; (f) K_2CO_3, acetone, 60 °C; (g) 2 M NaOH aq, MeOH, THF, rt.

binding mode of compound **18b** generated based on docking simulation indicated the carboxyl group of **18b** formed three hydrogen bonds with Arg $183^{5.39}$, Tyr $91^{3.37}$ and Tyr $240^{6.51}$, and the activity is only modestly affected compared with TAK-875 which formed four hydrogen bonds with Arg $183^{5.39}$, Arg $258^{7.35}$, Tyr $91^{3.37}$ and Tyr $240^{6.51}$ (Fig. 3A). While the carbonyl group of α-substituted derivatives (**10b** and **10c**), phenoxyisobutyric acid derivatives (**10f**) or large groups *ortho*-substituted derivatives (**10e**) could only form one or two hydrogen bonds, because the conformation of carboxyl groups are restricted, which leads to the potency decrease, such as **10f**, could form two hydrogen bonds with Arg $183^{5.39}$ and Tyr $91^{3.37}$ (Fig. 3B).

2.3. Hypoglycemic effect of 10a, 10b, 10d, 18a, and 18b in ICR mice

Based on the in vitro potency, compounds with good activity in vitro were selected for acute efficacy evaluation in ICR mice by oral glucose tolerance test (OGTT). Oral administration of compounds **10a**, **10b**, **10d**, **18a**, and **18b** (20 mg/kg) and TAK-875 (20 mg/kg) 30 min prior to glucose challenge significantly improved glucose intolerance. Among these compounds, compound **18b** exhibited excellent hypoglycemic effect, which was similar to the hypoglycemic effect of TAK-875. Compound **18b** and TAK-875 reduced area under the curve from 0 to

Table 1
In vitro activities of phenoxyacetic acids



Compound	R^1	R^2	R^3	R^4	Agonist activities (100 nM)[a]	hFFA1 EC_{50} (nM)[b]	Clog*P*[c]
10a	H	H	H	H	6.04±0.23	121.1	3.89
10b	CH_3	H	H	H	4.04±0.44	389.3	4.54
10c	CH_2CH_3	H	H	H	3.04±0.44	ND	5.03
10d	H	H	CH_3	H	5.64±0.37	153.3	4.38
10e	H	H	CH_2CH_3	H	3.19±0.44	ND	4.80
10f	CH_3	CH_3	H	H	2.45±0.42	ND	4.78
18a	H	H	Cl	H	6.18±0.58	92.6	4.45
18b	H	H	F	H	6.42±0.04	62.3	4.05
18c	CH_3	CH_3	Cl	H	3.04±0.13	ND	5.34
18d	CH_3	CH_3	F	H	3.06±0.05	ND	4.94
18e	CH_3	CH_3	CH_2CH_3	H	1.48±0.41	ND	5.68
18f	CH_3	CH_3	CH_3	CH_3	1.60±0.18	ND	5.75
18g	H	H	OCH_3	H	2.02±0.23	ND	3.77
18h	CH_3	CH_3	OCH_3	H	1.81±0.47	ND	4.65
TAk-875					6.89±0.18	38.6	4.02

ND = Not determined.
[a] Agonist activities values at a screening concentration of 100 nM were obtained from three independent experiments.
[b] EC_{50} values for FFA1 activities represent the mean of three determinations.
[c] Clog*P* values were calculated by the BioByte's algorithm as implemented in ChemBioDraw Ultra 12.0.

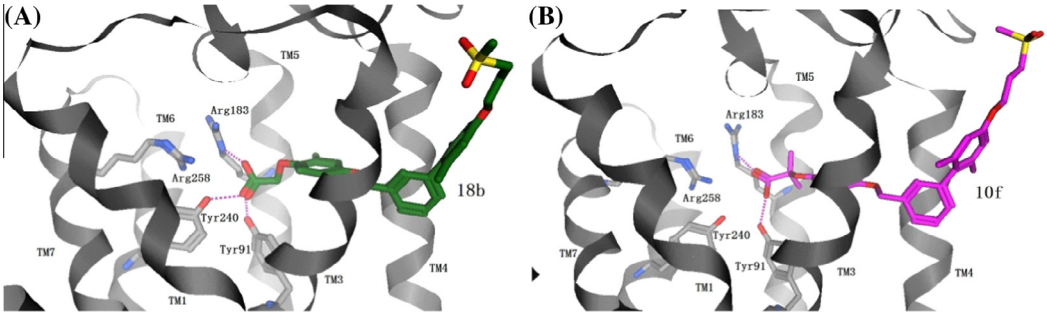


Figure 3. The interaction mode of compounds **18b** and **10f** within the binding site of FFA1. (A) Binding interaction of **18b**, *h*FFA1 is represented as grey ribbon and **18b** as sticks colored by atom type, with carbon in green, sulphur in yellow and oxygen in red. Hydrogen bonds are represented by pink dashes. (B) Binding interactions of **10f**, **10f** as sticks colored by atom type, with carbon in pink. Hydrogen bonds are represented by pink dashes.

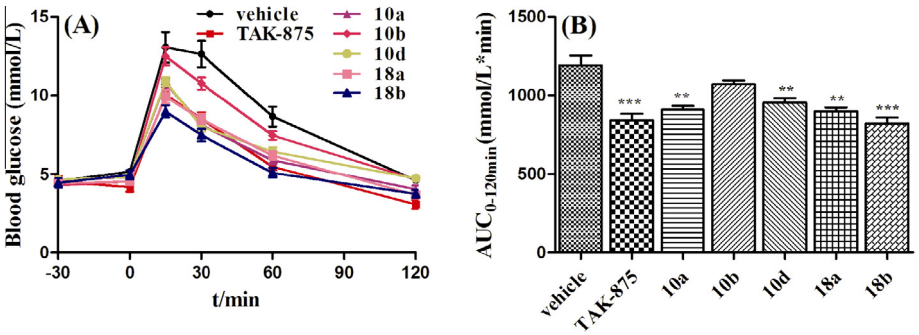


Figure 4. Effect of compounds during an OGTT in male ICR mice. (A) show time-dependent changes of blood glucose after oral administration of compounds, followed by 2 g/kg oral glucose challenge. Date in (B) represent $AUC_{0-120\ min}$ of blood glucose levels. Values are mean ± SEM ($n = 6$). ** $P \leqslant 0.01$ compared to vehicle-treated ICR mice by Student's *t* test; *** $P \leqslant 0.001$ compared to vehicle-treated ICR mice by Student's *t* test.

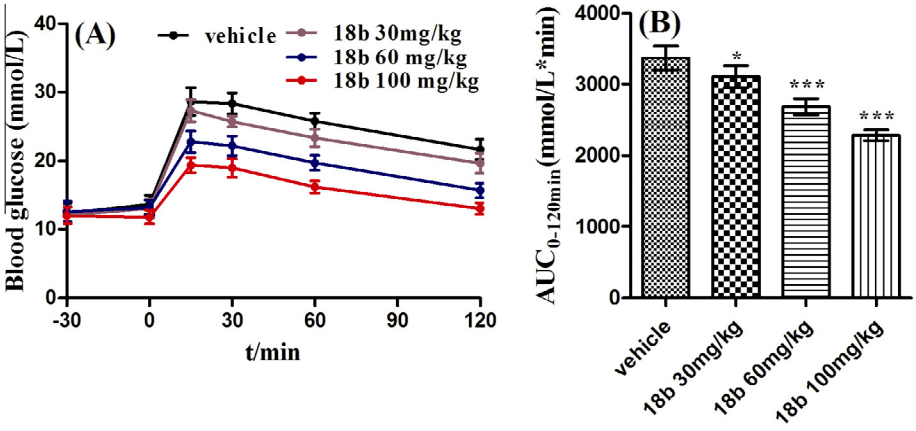


Figure 5. Effect of **18b** during an OGTT in type 2 diabetic C57BL/6 mice. (A) show time-dependent changes of blood glucose after oral administration of **18b**, followed by 1 g/kg oral glucose challenge. Date in (B) represent $AUC_{0-120\ min}$ of blood glucose levels. Values are mean ± SD ($n = 6$). * $P \leqslant 0.05$ compared to vehicle-treated type 2 diabetic C57BL/6 mice by Student's *t* test. *** $P \leqslant 0.001$ compared to vehicle-treated type 2 diabetic C57BL/6 mice by Student's *t* test.

Table 2
Pharmacokinetic profiles of **18b** in rats[a]

iv			po				
Cl (L/h/kg)	V_d (L/kg)	$T_{1/2}$ (h)	$T_{1/2}$ (h)	C_{max} (μg/mL)	T_{max} (h)	AUC(0–8 h) (μg·h /mL)	F (%)
0.11	0.26	4.15	3.27	1.17	1.0	5.34	63.0

[a] Administered at a dose of 1 mg/kg, iv; 1 mg/kg, po. Data are average of three rats.

120 min $(AUC)_{0-120\ min}$ to 31.0% and 29.3% (**18b**, 821.7 ± 95.8; TAK-875, 841.4 ± 105.9; vehicle control, 1190.9 ± 175.5), respectively, (Fig. 4).

2.4. Antihyperglycemic effect of 18b in type 2 diabetic C57BL/6 mice

In order to further confirm the hypoglycemic effect of **18b**, the antihyperglycemic effect of **18b** was evaluated in type 2 diabetic C57BL/6 mice. A single dose of **18b** (30–100 mg/kg) was orally administrated in diabetic C57BL/6 mice prior to glucose challenge. The results indicated that **18b** significantly reduced blood glucose at a dose of 60 mg/kg and oral administration of **18b** dose-dependent reduced blood glucose in type 2 diabetic C57BL/6 mice (Fig. 5).

2.5. Pharmacokinetic profiles of 18b in SD rats

Potent compound **18b** was further evaluated for oral pharmacokinetic profiles in fasted rats. Compound **18b** presented highly desirable PK profiles, namely, rapid absorption (T_{max} = 1.0 h), high maximum concentration (C_{max} = 1166.45 μg/L), high plasma exposure ($AUC_{0-8\ h}$ = 5342.38 μg h/L) after oral administration of **18b** (1 mg/kg) and low plasma clearance (Cl = 0.11 L/h/kg), sustained plasma half-lives ($T_{1/2}$ = 4.15 h) after intravenous administration of **18b** (1 mg/kg) (Table 2). The results illustrated that the process of β-oxidation was interrupted by the introduction of an oxygen atom to replace the β-carbon.

3. Conclusion

In summary, we identified phenoxyacetic acid derivatives as novel FFA1 agonists. Among them compound **18b** with ideal *C*log*P* and excellent potency in vitro displayed a good PK profiles, which illustrated that the process of β-oxidation was interrupted by the introduction of an oxygen atom to replace the β-carbon. Moreover, compound **18b** could significantly improve oral glucose tolerance in ICR mice at a dose of 20 mg/kg and dose-dependently reduce postprandial glucose levels in type 2 diabetic C57BL/6 mice. No hypoglycemic event was observed during the experiments. All the results showed that compound **18b** was worth for further investigation as a drug for treatment of diabetes mellitus.

4. Experimental section

4.1. Chemistry

Reagents and solvents were obtained from commercial sources and used without further purification. All compounds were routinely checked by TLC and ^{1}H NMR. TLCs and preparative TLC were performed on silica gel GF/UV 254. Purifications by column chromatography were carried out over silica gel (200–300 mesh) and visualized under UV light at 254 and 365 nm. NMR spectra were recorded on a BRUKERACF300 instrument (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR spectra, chemical shifts are expressed as values relative to TMS as internal standard). MS spectra were recorded on a Waters liquid chromatography-mass spectrometer system (ESI). Melting points of individual compounds were determined on a MelTEMP II melting point apparatus and uncorrected. Element analysis was performed on an Eager 300 instrument. Compound **1** was synthesized via published procedures.[21]

4.1.1. Preparation of (2′,6′-dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methanol (2)

To a solution of aldehyde **1** (5.0 g, 14.43 mmol) in THF/CH_3OH was added $NaBH_4$ (0.54 g, 14.43 mmol) in small portions at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then quenched with HCl (1 N). The mixture was extracted with ethyl acetate, and the combined organic phases were washed with brine, dried over Na_2SO_4 and filtered. The filtrate was evaporated under vacuum to give alcohol **12** (4.7 g, 93.5%) as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.37 (t, J = 7.5 Hz, 1H), 7.27 (d, J = 7.0 Hz,

1H), 7.02 (s, 1H), 6.95 (d, J = 7.2 Hz, 1H), 6.69 (s, 2H), 5.18 (t, J = 5.6 Hz, 1H), 4.52 (d, J = 5.7 Hz, 2H)), 4.07 (t, J = 6.0 Hz, 2H), 3.27 (t, J = 7.4 Hz, 2H), 3.02 (s, 3H), 2.15-2.08 (m, 2 H), 1.92 (s, 6H).

4.1.2. Preparation of 3′-(bromomethyl)-2,6-dimethyl-4-(3-(methylsulfonyl)propoxy)-1,1′-biphenyl (3)

To a solution of alcohol **2** (4.5 g, 12.91 mmol) in CH_2Cl_2 was added dropwise PBr_3 (1.75 g, 6.46 mmol) in CH_2Cl_2 at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and then quenched with water. The mixture was extracted with CH_2Cl_2, and the combined organic phases were washed with brine, dried over Na_2SO_4 and filtered. The filtrate was evaporated under vacuum and the residue was purified by column chromatography (EtOAc/hexane, 1:1, v/v) to give bromide **13** (4.6 g, 86.6%) as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.43–7.41 (m, 2H), 7.19 (s, 1H), 7.07–7.04 (m, 1H), 6.72 (s, 2H), 4.74 (s, 2H)), 4.09 (t, J = 6.2 Hz, 2H), 3.28 (t, J = 8.0 Hz, 2H), 3.03 (s, 3H), 2.19–2.10 (m, 2H), 1.94 (s, 6H).

4.1.3. The following procedures described the synthesis of compound 10a

These procedures can also be applied to the synthesis of compounds **10b**–**10f**.

4.1.3.1. Methyl 2-phenoxyacetate (5). To a stirred solution of phenol (**4**) (1.0 g, 10.67 mmol) in DMF was added K_2CO_3 (2.95 g, 21.35 mmol) and methyl chloroacetate (1.16 g, 10.67 mmol). The reaction mixture was stirred at room temperature for 24 h, and filtered. The filtrate was concentrated under vacuum and the resultant residue was redissolved in ethyl acetate. The organic layers was washed with NaOH and brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated under vacuum to give **5** (1.48, 83.5%) as a colorless oil, which was used for the next reaction without further purification.

4.1.3.2. Methyl 2-(4-acetylphenoxy)acetate (6). To a solution of **5** (1.4 g, 8.42 mmol) in CH_2Cl_2 was added $AlCl_3$ (2.25 g, 16.85 mmol) in small portions at −5 °C. Subsequently, a solution of AcCl (0.99 g, 12.64 mmol) in CH_2Cl_2 was added dropwise at a rate to ensure that the temperature did not exceed 0 °C. After 2 h, the reaction was quenched by pouring over ice. The resulting suspension was diluted with H_2O and extracted with CH_2Cl_2. The combined organic phases were washed with HCl, H_2O, NaOH, dried over Na_2SO_4 and filtered. The filtrate was evaporated under vacuum and the residue was purified by column chromatography (EtOAc/hexane, 1:10, v/v) to give a white solid (1.3 g, 74.1%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.94 (d, J = 8.9 Hz, 2H), 6.94 (d, J = 8.9 Hz, 2H), 4.71 (s, 2H), 3.82 (s, 3H), 2.56 (s, 3H).

4.1.3.3. Methyl 2-(4-acetoxyphenoxy)acetate (7). To a solution of **6** (1.0 g, 4.80 mmol) in CH_2Cl_2 was added 3-chloroperoxybenzoic acid (75%, 2.21 g, 9.61 mmol) in small portions at 0 °C. The reaction mixture was stirred for 24 h at room temperature and then poured into NaOH (1 N). The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic phases were washed with water, dried over Na_2SO_4 and filtered. The filtrate was evaporated under vacuum and the residue was purified by column chromatography (EtOAc/hexane, 1:10, v/v) to give a white solid (0.72 g, 66.9%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.95 (d, J = 9.0 Hz, 2H), 6.83 (d, J = 9.0 Hz, 1H), 4.55 (s, 2H), 3.74 (s, 2H), 2.21 (s, 3H).

4.1.3.4. Methyl 2-(4-hydroxyphenoxy)acetate (8). To a solution of **7** (0.6 g, 2.68 mmol) in CH_3OH was added CH_3ONa (0.58 g, 10.70 mmol) in small portions. The reaction mixture was stirred for 4–6 h at room temperature and then quenched with HCl (1 N). The mixture was extracted with ethyl acetate, and the combined organic phases were washed with brine, dried and filtered. The filtrate was evaporated under vacuum and the residue was purified by column chromatography (EtOAc/hexane, 1:4, v/v) to give a white solid (0.41 g, 84.1%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.84–6.75 (m, 4H), 4.60 3.82 (s, 3H).

4.1.3.5. Methyl 2-(4-((2′,6′-dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)phenoxy)acetate (9). To a solution of **3** (0.2 g, 0.49 mmol) and **8** (0.09 g, 0.49 mmol) in acetone was added K_2CO_3 (0.13 g, 0.97 mmol) and KI (cat.) at room temperature. The reaction mixture was refluxed with stirring overnight, cooled and filtered. The filtrate was concentrated under vacuum to give **9** (0.2, 80.3%) as a white solid, which was used for the next reaction without further purification.

4.1.3.6. 2-(4-((2′,6′-Dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)phenoxy)acetic acid (10a). To a solution **9** (0.18 g, 0.35 mmol) in THF/CH_3OH/H_2O was added NaOH (2 N, 0.35 mL, 0.70 mmol) at room temperature. The reaction mixture was stirred for 2 h and acidified with HCl (1 N). The mixture was extracted with ethyl acetate, and the combined organic phases were washed with brine, dried over Na_2SO_4 and filtered. The filtrate was evaporated under vacuum and the residue was purified by column chromatography (MeOH/CH_2Cl_2, 20:1, v/v) to give **10a** as a colorless solid (0.16 g, 91.4%). mp: 106–108 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.47–7.37 (m, 2H), 7.14 (s, 1H), 7.05 (d, J = 7.1 Hz, 1H), 6.92 (d, J = 9.0 Hz, 2H), 6.82 (d, J = 9.0 Hz, 2H), 6.71 (s, 2H), 5.08 (s, 2H), 4.52 (s, 2H), 4.09 (t, J = 6.0 Hz, 2H), 3.27 (t, J = 7.5 Hz, 2H), 3.03 (s, 3H), 2.19–2.10 (m, 2H), 1.91 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.7, 156.9, 152.5, 152.1, 140.2, 137.5, 136.6, 133.9, 128.7, 128.5, 125.8, 115.7, 115.2, 113.2, 69.5, 65.4, 50.5, 22.0, 20.7; MS (ESI), m/z: 497.0 $(M-H)^-$. Anal. Calcd for $C_{27}H_{30}O_7S$: C, 65.04; H, 6.06. Found: C, 65.45; H, 6.13.

4.1.4. 2-(4-((2′,6′-Dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)phenoxy)propanoic acid (10b)

Compound **10b** (0.14 g, 20.9%) as a colorless solid. mp: 102–104 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.47–7.37 (m, 2H), 7.14 (s, 1H), 7.05 (d, J = 7.2 Hz, 1H), 6.92 (d, J = 9.1 Hz, 2H), 6.79 (d, J = 9.1 Hz, 2H), 6.71 (s, 2H), 5.08 (s, 2H), 4.69 (q, J = 6.8 Hz, 1H), 4.08 (t, J = 6.1 Hz, 2H), 3.28 (t, J = 7.6 Hz, 2H), 2.17–2.12 (m, 2H), 1.91 (s, 6H), 1.46 (d, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.8, 157.4, 153.0, 152.1, 140.7, 140.0, 137.1, 134.4, 129.2, 129.0, 126.4, 116.3, 116.2, 113.8, 72.6, 70.0, 65.9, 51.0, 22.5, 21.2, 18.8; MS (ESI), m/z: 511.0 $(M-H)^-$. Anal. Calcd for $C_{28}H_{32}O_7S$: C, 65.60; H, 6.26. Found: C, 65.84; H, 6.57.

4.1.5. 2-(4-((2′,6′-Dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)phenoxy)butanoic acid (10c)

Compound **10c** (0.13 g, 15.7%) as a colorless solid. mp: 112–114 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.44–7.38 (m, 2H), 7.14 (s, 1H), 7.05 (d, J = 7.1 Hz, 1H), 6.92 (d, J = 9.1 Hz, 2H), 6.80 (d, J = 9.1 Hz, 2H), 6.71 (s, 2H), 5.08 (s, 2H), 4.51 (t, J = 6.7 Hz, 1H), 4.09 (t, J = 6.1 Hz, 2H), 3.28 (t, J = 7.7 Hz, 2H), 2.17–2.12 (m, 2H), 1.94–1.84 (m, 8H), 0.98 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.1, 157.4, 153.1, 152.4, 140.7, 138.0, 137.1, 134.4, 129.2, 129.0, 126.3, 116.4, 116.2, 113.8, 77.7, 70.0, 51.0, 25.9, 22.5, 21.2, 10.0, 9.8; MS (ESI), m/z: 525.3 $(M-H)^-$. Anal. Calcd for $C_{29}H_{34}O_7S$: C, 66.14; H, 6.51. Found: C, 66.78; H, 6.64.

4.1.6. 2-(4-((2′,6′-Dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)-2-methylphenoxy)acetic acid (10d)

Compound **10d** (0.13 g, 18.3%) as a colorless solid. mp: 118–120 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.46–7.36 (m, 2H), 7.14

(s, 1H), 7.05 (d, J = 7.3 Hz, 1H), 6.81 (s, 1H), 6.75–6.67 (m, 4H), 5.05 (s, 2H), 4.42 (s, 2H), 4.09 (t, J = 6.1 Hz, 2H), 3.27 (t, J = 7.7 Hz, 2H), 3.03 (s, 3H), 2.18-2.09 (m, 5H), 1.92 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.6, 156.9, 152.2, 150.3, 140.2, 137.6, 136.6, 133.9, 128.6, 128.5, 127.2, 125.7, 117.7, 113.3, 112.3, 112.2, 69.4, 65.5, 65.4, 50.6, 22.0, 20.7, 16.1; MS (ESI), m/z: 511.0 $(M-H)^-$. Anal. Calcd for $C_{28}H_{32}O_7S$: C, 65.60; H, 6.26. Found: C, 65.97; H, 6.62.

4.1.7. 2-(4-((2′,6′-Dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)-2-ethylphenoxy)acetic acid (10e)

Compound **10e** (0.12 g, 12.5%) as a colorless solid. mp: 110–112 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.42–7.37 (m, 2H), 7.15 (s, 1H), 7.05 (d, J = 7.3 Hz, 1H), 6.81 (s, 1H), 6.75 (s, 2H), 6.71 (s, 2H), 5.08 (s, 2H), 4.59 (s, 2H), 4.09 (t, J = 6.1 Hz, 2H), 3.27–3.25 (d, 2H), 3.03 (s, 3H), 2.59–2.54 (t, 2H), 2.17–2.11 (m, 2H), 1.92 (s, 6H), 1.11 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ=170.5, 156.9, 152.4, 149.8, 140.2, 137.6, 136.6, 134.0, 133.2, 128.6, 128.5, 128.4, 125.8, 116.2, 113.3, 112.3, 112.1, 69.4, 65.4, 50.6, 22.8, 22.1, 20.7, 14.1 MS (ESI), m/z: 525.3 $(M-H)^-$. Anal. Calcd for $C_{29}H_{34}O_7S$: C, 66.14; H, 6.51. Found: C, 66.34; H, 6.78.

4.1.8. 2-(4-((2′,6′-Dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)phenoxy)-2-methylpropanoic acid (10f)

Compound **10f** (0.13 g, 18.7%) as a colorless solid. mp: 110–112 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.45–7.38 (m, 2H), 7.14 (s, 1H), 7.05 (d, J = 7.1 Hz, 1H), 6.91 (d, J = 9.1 Hz, 2H), 6.80 (d, J = 9.1 Hz, 2H), 6.70 (s, 2H), 5.09 (s, 2H), 4.09 (t, J = 6.2 Hz, 2H), 3.27 (t, J = 8.0 Hz, 2H), 3.03 (s, 3H), 2.18–2.09 (m, 2H), 1.91 (s, 6H), 1.42 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.1, 156.9, 153.4, 148.9, 140.2, 137.4, 136.6, 133.9, 128.7, 128.5, 125.9, 120.9, 115.4, 113.3, 78.9, 69.4, 65.4, 50.6, 24.9, 22.0, 20.7; MS (ESI), m/z: 525.3 $(M-H)^-$. Anal. Calcd for $C_{29}H_{34}O_7S$: C, 66.14; H, 6.51. Found: C, 66.63; H, 6.71.

4.1.9. The following procedures described the synthesis of compound 18b

These procedures can also be applied to the synthesis of compounds **18a**, **18c**–**18h**.

4.1.9.1. 2-Fluorophenyl acetate (12). To a solution of 2-fluorophenol (**11**) (1.0 g, 8.92 mmol) in CH_2Cl_2 was added pyridine (1.41 g, 17.84 mmol) at 0 °C. Subsequently, a solution of AcCl (1.05 g, 13.38 mmol) in CH_2Cl_2 was added dropwise at a rate to ensure that the temperature did not exceed 0 °C. After 4 h, the reaction was quenched with HCl (1 N). The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic phases were washed with HCl, H_2O, NaOH, dried over Na_2SO_4 and filtered. The filtrate was evaporated under vacuum to give a crude product **12** (1.3 g, 87.3%), which was used for the next reaction without further purification.

4.1.9.2. 1-(3-Fluoro-4-hydroxyphenyl)ethanone (13). To a solution of aluminum chloride (2.08 g, 15.57 mmol) in dichlorobenzene was added a solution of **12** (1.2 g, 7.79 mmol) in dichlorobenzene at room temperature. The reaction was warmed to 100 °C and further stirred for 20 h. After the reaction mixture was allowed to cool to room temperature, added with dichloromethane, and poured into 10% HCl cooled at 0 °C. The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic phases were washed with water, dried over Na_2SO_4. The filtrate was evaporated under vacuum, and the residue was purified by column chromatography (EtOAc/hexane, 1:4, v/v) to give a white solid (0.75 g, 62.5%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73–7.70 (m, 2H), 7.11–7.05 (m, 1H), 2.58 (s, 3H).

4.1.9.3. Methyl 2-(4-acetyl-2-fluorophenoxy)acetate (14). The titled compound was prepared from **13** as described for compound **5**. Compound **14** (0.87 g, 84.7%) as a colorless solid.

4.1.9.4. Methyl 2-(4-acetoxy-2-fluorophenoxy)acetate (15). The titled compound was prepared from **14** as described for compound **7**. Compound **15** (0.62 g, 68.1%) as a colorless solid.

4.1.9.5. Methyl 2-(2-fluoro-4-hydroxyphenoxy)acetate (16). The titled compound was prepared from **15** as described for compound **8**. Compound **16** (0.4 g, 80.7%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.44 (s, 1H), 6.92 (t, J = 9.2 Hz, 2H), 6.61 (dd, J = 13.05, 2.8 Hz, 1H), 6.51–6.47 (m, 1H), 4.73 (s, 2H), 3.69 (s, 3H).

4.1.9.6. Methyl 2-(4-((2′,6′-dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)-2-fluorophenoxy)acetate (17). The titled compound was prepared from **16** as described for compound **9**. Compound **17** (0.21 g, 81.4%) as a colorless solid.

4.1.9.7. 2-(4-((2′,6′-Dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)-2-fluorophenoxy)acetic acid (18b). The titled compound was prepared from **17** as described for compound **10a**. Compound **18b** (0.17 g, 87.3%) as a colorless solid. mp: 106–107 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.47–7.38 (m, 2H), 7.15 (s, 1H), 7.07–6.94 (m, 3H), 6.77–6.71 (m, 3H), 5.11 (s, 2H), 4.65 (s, 2H), 4.09 (t, J = 6.1 Hz, 2H), 3.28 (t, J = 7.6 Hz, 2H), 3.03 (s, 3H), 2.19–2.10 (m, 2H), 1.92 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.0, 156.9, 153.4, 152.8, 152.6, 150.2, 140.3, 139.8, 137.0, 136.6, 133.9, 128.8, 128.6, 128.6, 125.9, 115.9, 113.3, 110.3, 104.3, 104.0, 69.7, 65.8, 65.4, 50.6, 22.0, 20.7; MS (ESI), m/z: 515.3 $(M-H)^-$. Anal. Calcd for $C_{27}H_{29}FO_7S$: C, 62.78; H, 5.66. Found: C, 62.24; H, 5.43.

4.1.10. 2-(2-Chloro-4-((2′,6′-dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)phenoxy)acetic acid (18a)

Compound **18a** (0.14 g, 15.4%) as a colorless solid. mp: 118–120 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.47–7.37 (m, 2H), 7.16 (s, 1H), 7.11 (d, J = 7.3 Hz, 1H), 6.97–6.90 (m, 2H), 6.71 (s, 2H), 5.12 (s, 2H), 4.68 (s, 2H), 4.09 (t, J = 6.2 Hz, 2H), 3.27 (t, J = 7.6 Hz, 2H), 3.03 (s, 3H), 2.19–2.10 (m, 2H), 1.95 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.9, 156.9, 152.5, 147.6, 140.3, 137.0, 136.6, 133.9, 128.8, 128.6, 125.9, 121.6, 116.8, 114.6, 114.5, 113.3, 69.8, 65.6, 65.4, 50.6, 29.0, 22.0, 20.7; MS (ESI), m/z: 531.2 $(M-H)^-$. Anal. Calcd for $C_{27}H_{29}ClO_7S$: C, 60.84; H, 5.48. Found: C, 61.41; H, 5.72.

4.1.11. 2-(2-Chloro-4-((2′,6′-dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)phenoxy)-2-methylpropanoic acid (18c)

Compound **18c** (0.16 g, 11.6%) as a colorless solid. mp: 112–114 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.49–7.38 (m, 2H), 7.15 (s, 1H), 7.11 (d, J = 2.5 Hz, 1H), 7.06 (d, J = 7.3 Hz, 1H), 6.95–6.93 (m, 2H), 6.71 (s, 2H), 5.13 (s, 2H), 4.09 (t, J = 6.2 Hz, 2H), 3.28–3.19 (m, 2H), 3.03 (s, 3H), 2.17–2.12 (m, 2H), 1.91 (s, 6H), 1.45 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.8, 156.9, 153.5, 145.0, 140.3, 136.9, 136.6, 133.9, 128.9, 128.6, 126.0, 121.2, 116.3, 114.6, 113.3, 80.4, 69.6, 65.4, 50.6, 24.7, 22.0, 20.7; MS (ESI), m/z: 559.2 $(M-H)^-$. Anal. Calcd for $C_{29}H_{33}ClO_7S$: C, 62.08; H, 5.93. Found: C, 62.47; H, 5.78.

4.1.12. 2-(4-((2′,6′-Dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)-2-fluorophenoxy)-2-methylpropanoic acid (18d)

Compound **18d** (0.15 g, 10.9%) as a colorless solid. mp: 124–126 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.53–7.41 (m, 3H),

7.21–7.06 (m, 4H), 6.71 (s, 2H), 5.23 (s, 2H), 4.09 (t, J = 6.1 Hz, 2H), 3.28 (t, J = 7.6 Hz, 2H), 3.03 (s, 3H), 2.19–2.10 (m, 2H), 1.92 (s, 6H), 1.41 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.5, 156.9, 154.3, 152.6, 140.3, 139.7, 136.9, 135.8, 133.9, 128.8, 128.6, 125.8, 115.8, 113.3, 110.2, 104.2, 69.6, 65.7, 50.6, 22.0, 20.7; MS (ESI), m/z: 543.3 (M−H)$^-$. Anal. Calcd for $C_{29}H_{33}FO_7S$: C, 63.95; H, 6.11. Found: C, 64.22; H, 6.61.

4.1.13. 2-(4-((2′,6′-Dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)-2-ethylphenoxy)-2-methylpropanoic acid (18e)

Compound **18e** (0.14 g, 10.4%) as a colorless solid. mp: 112–114 °C; ^{1}H NMR (300 Hz, DMSO-d_6): δ 7.44–7.37 (m, 2H), 7.14 (s, 1H), 7.05 (d, J = 7.2 Hz, 1H), 6.81 (d, J = 2.9 Hz, 1H), 6.75–6.64 (m, 4H), 5.08 (s, 2H), 4.09 (t, J = 6.2 Hz, 2H), 3.32–3.25 (m, 2H), 3.03 (s, 3H), 2.56–2.504 (m, 2H), 2.17–2.12 (m, 2H), 1.91 (s, 6H), 1.44 (s, 6H), 1.09 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.5, 156.9, 152.7, 147.0, 140.2, 137.6, 136.6, 135.9, 133.9, 128.6, 128.5, 125.8, 117.7, 115.9, 113.3, 112.0, 78.4, 69.3, 65.4, 50.5, 25.0, 23.1, 22.0, 20.7, 14.2; MS (ESI), m/z: 553.3 (M−H)$^-$. Anal. Calcd for $C_{31}H_{38}O_7S$: C, 67.12; H, 6.91. Found: C, 67.43; H, 7.53.

4.1.14. 2-(4-((2′,6′-Dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)-2,6-dimethylphenoxy)-2-methylpropanoic acid (18f)

Compound **18f** (0.16 g, 13.2%) as a colorless solid. mp: 116–118 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.47–7.37 (m, 2H), 7.14 (s, 1H), 7.05 (d, J = 7.3 Hz, 1H), 6.71 (s, 2H), 6.64 (s, 2H), 5.07 (s, 2H), 4.09 (t, J = 6.1 Hz, 2H), 3.32–3.19 (m, 2H), 3.03 (s, 3H), 2.19–2.11 (m, 8H), 1.92 (s, 6H), 1.32 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.3, 156.9, 153.7, 146.2, 140.2, 137.5, 136.6, 133.9, 133.1, 128.6, 128.5, 128.5, 125.8, 114.4, 113.3, 79.8, 69.0, 65.4, 50.6, 24.8, 22.0, 20.6, 17.7; MS (ESI), m/z: 553.4 (M−H)$^-$. Anal. Calcd for $C_{31}H_{38}O_7S$: C, 67.12; H, 6.91. Found: C, 67.50; H, 7.44.

4.1.15. 2-(4-((2′,6′-Dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)-2-methoxyphenoxy)acetic acid (18g)

Compound **18g** (0.15 g, 23.4%) as a colorless solid. mp: 122–124 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.43–7.39 (m, 2H), 7.15 (s, 1H), 7.05 (d, 1H), 6.77 (d, J = 8.8 Hz, 1H), 6.69 (s, 2H), 6.65 (d, J = 2.6 Hz, 1H), 6.45 (m, 1H), 5.07 (s, 2H), 4.51 (s, 2H), 4.07 (t, J = 6.1 Hz, 2H), 3.72 (s, 3H), 3.26 (t, J = 7.5 Hz, 2H), 3.01 (s, 3H), 2.15–2.13 (m, 2H), 1.91 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.0, 156.9, 153.4, 152.8, 152.6, 150.2, 140.3, 139.8, 137.0, 136.6, 133.9, 128.8, 128.6, 128.6, 125.9, 115.9, 113.3, 110.3, 104.3, 104.0, 69.7, 65.8, 65.4, 50.6, 22.0, 20.7; MS (ESI), m/z: 527.3 (M−H)$^-$. Anal. Calcd for $C_{28}H_{32}O_8S$: C, 63.62; H, 6.10. Found: C, 63.53; H, 6.24.

4.1.16. 2-(4-((2′,6′-Dimethyl-4′-(3-(methylsulfonyl)propoxy)-[1,1′-biphenyl]-3-yl)methoxy)-2-methoxyphenoxy)-2-methylpropanoic acid (18h)

Compound **18h** (0.14 g, 20.6%) as a colorless solid. mp: 128–130 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.45–7.42 (m, 2H), 7.16 (s, 1H), 7.06 (d, J = 7.1 Hz, 1H), 6.83 (d, J = 8.8 Hz, 1H), 6.71 (s, 2H), 6.65 (d, J = 2.8 Hz, 1H), 6.48 (dd, J = 2.8, 8.8 Hz, 1H), 5.10 (s, 2H), 4.09 (t, J = 6.2 Hz, 2H), 3.70 (s, 3H), 3.32–3.25 (t, 2H), 3.03 (s, 3H), 2.17–2.12 (m, 2H), 1.92 (s, 6H), 1.36 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.1, 156.9, 154.5, 152.9, 140.2, 138.0, 137.3, 136.6, 133.9, 128.7, 128.5, 125.9, 121.8, 113.3, 107.7, 105.0, 101.0, 79.7, 69.4, 65.4, 55.4, 50.6, 24.5, 22.0, 20.7; MS (ESI), m/z: 555.3 (M−H)$^-$. Anal. Calcd for $C_{30}H_{36}O_8S$: C, 64.73; H, 6.52. Found: C, 65.18; H, 6.72.

4.2. Biological methods

4.2.1. Ca^{2+} influx activity of CHO cells expressing human FFA1

CHO cells stably expressing human FFA1 (accession no. NM 005303) were seeded into 96-well plates at a density of 30,000 cells/well and incubated overnight in 5% CO_2 at 37 °C. After, the culture medium in the wells was removed and wells were washed with 100 μL of Hank's Balanced Salt Solution (HBSS). Then, cells were incubated in HBSS containing 2.5 μg/mL fluorescent calcium indicator Fluo-4 AM and 2.5 mmol/L probenecid for 90 min at 37 °C. After the Fluo-4 AM solution was removed and wells were washed with 100 μL of HBSS and the cells were incubated in HBSS containing 2.5 mmol/L probenecid for 10 min at 37 °C. Various concentrations of test compounds were added into the cells, and increase of the intracellular Ca^{2+} concentration after addition was monitored by FlexStation3 Molecular Devices. The agonistic activities of test compounds on human FFA1 were expressed as A/B (increase of the intracellular Ca^{2+} concentration (A) in the test compounds-treated cells and (B) in vehicle-treated cells). EC_{50} value of each compound was obtained with Prism 5 software (GraphPad).

4.2.2. Animals

Male Sprague Dawley (SD) rats weighing 180–200 g, male ICR mice weighing 18–22 g, and male C57BL/6 mice weighing 18–22 g were purchased from Comparative Medicine Centre of Yangzhou University and left to acclimatize for 1 week before the experimental period. The animal room was maintained under a constant 12 h light/black cycle with temperature 23 ± 2 °C and relative humidity 50 ± 10% throughout the experimental period. They were given free access to standard pellets and water. All animal procedures were done in accordance with the applicable institutional and governmental regulations concerning the ethical use of animals.

4.2.2.1. Oral glucose tolerance test in male ICR mice. For glucose tolerance testing, male ICR mice aged 10 weeks were fasted overnight (12 h), weighted, bled via tail tip, and randomized into groups (n = 6). Mice were dosed orally with single doses of vehicle (0.5% methylcellulose aqueous solution), TAK-875 (suspended in vehicle; 1.0 mL/kg; 20 mg/kg) or compounds (suspended in vehicle; 1.0 mL/kg; 20 mg/kg), 30 min prior to oral glucose load (20% aqueous glucose solution, 2 g/kg). The blood glucose measured for the grouping of the animals was used as the data before administration of vehicle, TAK-875 or compounds (time −30). Blood samples were collected just before glucose load (time 0), and 15, 30, 60 and 120 min after glucose load. The blood glucose was measured by blood glucose test strips (SanNuo ChangSha, ChangSha, China).

4.2.2.2. Antihyperglycemic effect of 18b in type 2 diabetic mice. Male C57BL/6 mice after 1 week adaptation were fed with high-fat diet (HFD, 45% calories from fat, from Mediscience Ltd., Yangzhou, China) ad libitum for 4 weeks to induce insulin resistance and then injected intraperitoneally (ip) with low dose of STZ (80 mg/kg, 1.0 mL/kg). The mice were fed with high-fat-diet for another 4 weeks. The mice with fasting blood glucose level 11.1 mmol/L or higher were considered diabetic and selected for further pharmacological studies as type 2 diabetic mice model.[27,28]

Type 2 diabetic C57BL/6 mice were fasted overnight (12 h), weighted, bled via the tail tip, and randomized into four groups (n = 6 per group) based on body weight and blood glucose levels (−30 min). Type 2 diabetic C57BL/6 mice were then dosed orally with single doses of vehicle or **18b** (suspended in vehicle; 1.0 mL/kg; 30–100 mg/kg), 30 min prior to oral glucose load (10%

aqueous glucose solution, 1 g/kg). The blood glucose measured for the grouping of the animals was used as the data before administration of vehicle or **18b** (time −30). Blood samples were collected just before glucose load (time 0), and 15, 30, 60 and 120 min after glucose load. The blood glucose was measured by blood glucose test strips.

4.2.2.3. In vivo pharmacokinetics evaluation of 18b in rats. The pharmacokinetic properties of compound **18b** were determined in fasted male SD rats (n = 3/group, 200–250 g) following single intravenous (1 mg/kg) and oral (1 mg/kg) dosing. Intravenous doses were prepared at a concentration of 0.5 mg/mL in 0.5% methylcellulose aqueous solution and administered in a volume of 2 mL/kg. Oral doses were prepared at a concentration of 1 mg/mL in 0.5% methylcellulose aqueous solution and administered in a volume of 1 mL/kg. At 5, 10, 15, and 30 min and 1, 2, 4, 6, 8, 12, 24, 36 h after intravenous administration or at 5, 15 and 30 min and 1, 2, 4, 6, 8, 12, 24, 36 h after oral administration, blood samples were collected from tail vein into EDTA-containing microcentrifuge tubes. Then centrifuged at 6000 rpm for 10 min to separate the plasma and stored at −20 °C until analysis. Plasma proteins were precipitated with two volumes of acetonitrile containing an internal standard, mixed by vortexing, and centrifuged at 14,000 rpm for 14 min. The supernatant was diluted and centrifuged again, and 10 μL of supernatant was analyzed by Waters LC-PDA-MS/MS to determine plasma drug levels. Pharmacokinetic parameters were determined using the mean data from the rats (n = 3) at each time-point. Statistical analysis of the data was performed using DAS 2.1.1 statistical software program.

4.3. Molecular modeling

Docking simulations were performed using the Molecular Operating Environment (MOE) (The Chemical Computing Group, Montreal, Canada). The crystal structure of FFA1 (PDB ID: 4PHU) was downloaded from the Protein Data Bank (PDB). Prior to ligand docking, hydrogens were added and the crystallized ligand was removed. Subsequently, the structure was prepared with Protonate 3D, and the active site was isolated using MOE Site Finder. The structures were placed in the site with the Triangle Matcher method and then ranked with the London dG scoring function. For the energy minimization in the pocket, MOE Force Field Refinement was used and ranked with the GBVI/WSA dG scoring function.

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