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Discovery of HWL-088: A highly potent FFA1/GPR40 agonist bearing a phenoxyacetic acid scaffold

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ABSTRACT

Based on a previously reported phenoxyacetic acid scaffold, compound **7** (HWL-088) has been identified as a superior free fatty acid receptor 1 (FFA1) agonist by comprehensive structure-activity relationship study. Our results indicated that the introduction of *ortho*-fluoro greatly increased the activity of phenoxyacetic acid series, and the unique structure-activity relationship in biphenyl moiety is different from previously reported FFA1 agonists. Moreover, the modeling study was also performed to better understand the binding mode of present series. Compound **7** significantly improved glucose tolerance both in normal and diabetic models, and even exerted greater potential on glucose control than that of TAK-875. These findings provided a novel candidate HWL-088, which is currently in preclinical study to evaluate its potential for the treatment of diabetes.

1. Introduction

Type 2 diabetes mellitus (T2DM) is mainly displayed as sustained hyperglycemia due to insulin insufficient and/or resistance [1,2]. Current insulin secretagogues such as sulfonylureas are widely used [3], but these agents usually cause the side effect of hypoglycemia due to the glucose-independent insulin secretion [4]. Moreover, most of current anti-diabetic drugs are associated with lactic acidosis, cardiovascular risk, and weight gain [5–8]. Hence, there still have many unmet needs to provide new anti-diabetic drugs. The free fatty acid receptor 1 (FFA1) has attracted considerable attention as a potential anti-diabetic target [9]. In pancreatic β-cells, the activation of FFA1 promotes insulin secretion in a glucose dependent manner, which provides a lower risk of hypoglycemia than sulfonylureas [10–12]. Moreover, FFA1 is mainly expressed in pancreas islet, which reduces the possibility of target-induced side effects in other tissues [13].

As described in review [14], many FFA1 agonists containing phenylpropionic acid scaffold have been disclosed (Fig. 1) [15–25]. Among them, several candidates including AMG-837, LY2881835 and TAK-875 have reached in clinical trials [9,14]. More recently, a series of novel FFA1 agonists were also explored based on hybrid of quercetin and oleic acid [26,27]. In our laboratory, several novel scaffolds were also comprehensively explored to extend the chemical space of FFA1 agonists [28–34]. To avoid β-oxidation of phenylpropanoic acid, a phenoxyacetic acid scaffold has been used to replace the phenylpropanoic acid of FFA1 agonists. However, the obtained phenoxyacetic acid analog significantly reduced agonistic activity compared to phenylpropanoic acid analog (Fig. 2A and B) [22,35]. Herein, we describe structural optimization of phenoxyacetic acid series and our efforts toward the discovery of superior agonist (Fig. 2C). All of these efforts ultimately led to the identification of a new superior agonist **7** (HWL-088, EC_{50} = 19 nM), which revealed better *in vitro* and *in vivo* activity than TAK-875, the most advanced candidate of FFA1 agonists.

2. Results and discussion

2.1. Chemistry

The synthetic route of compounds **4–11** and **14–17** is illustrated in Scheme 1. Treatment of methyl bromoacetate with phenol **1a-b** provided **2a-b**, which were converted to intermediates **3a-b** by Baeyer–Villiger oxidation. Hydrolysis of intermediates **3a-b** in the presence of sodium methoxide provided **4a-b**. Treating commercially available **5a** with thionyl chloride yielded intermediate **6a**, which was condensed with **4a-**

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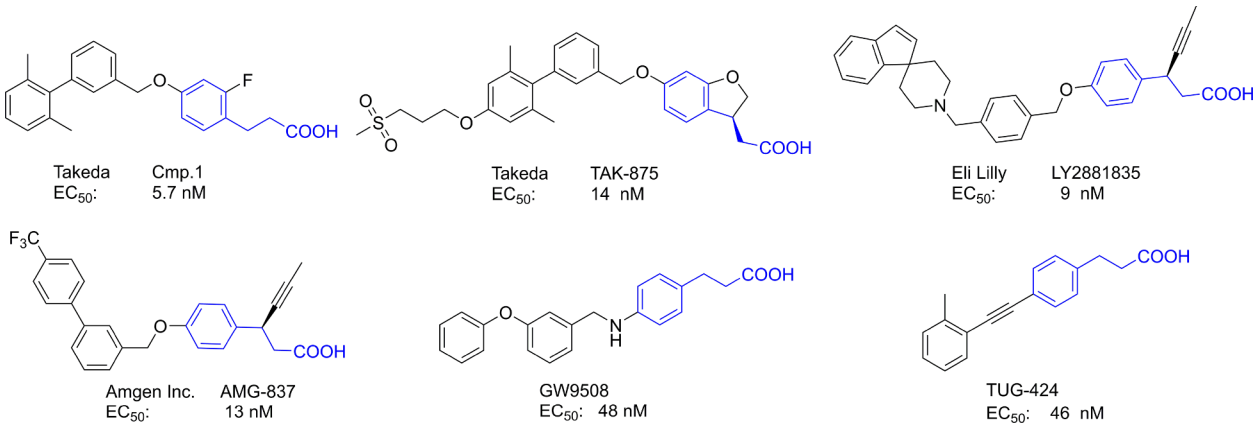


Fig. 1. Typical FFA1 agonists containing phenylpropionic acid scaffold (blue).

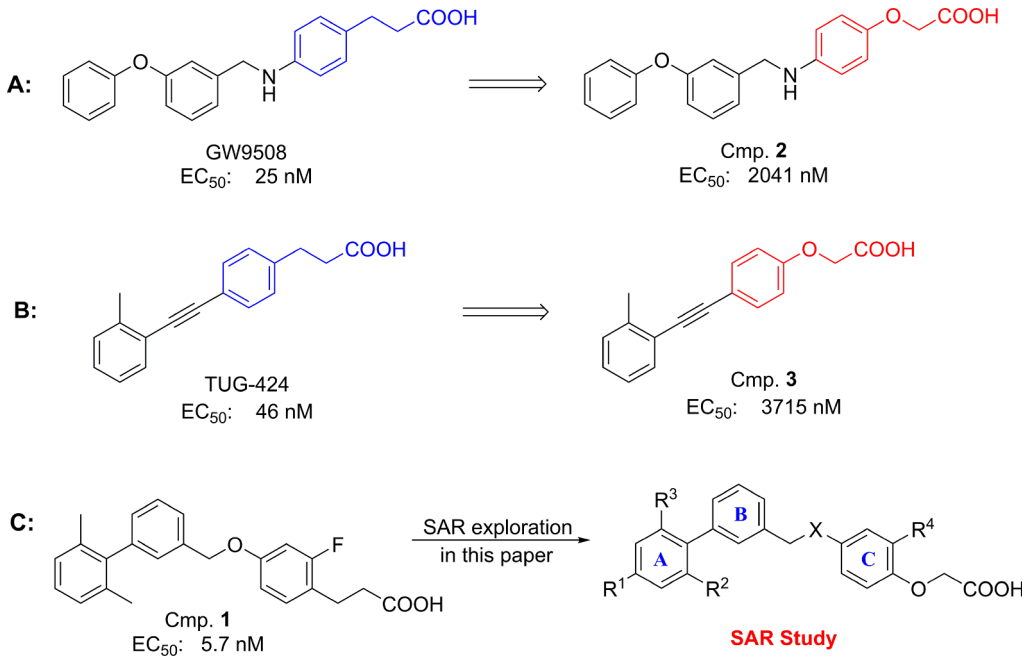


Fig. 2. Reported examples of FFA1 agonists bearing phenoxyacetic acid scaffold (A and B), and the design of structure-activity relationship in this paper (C).

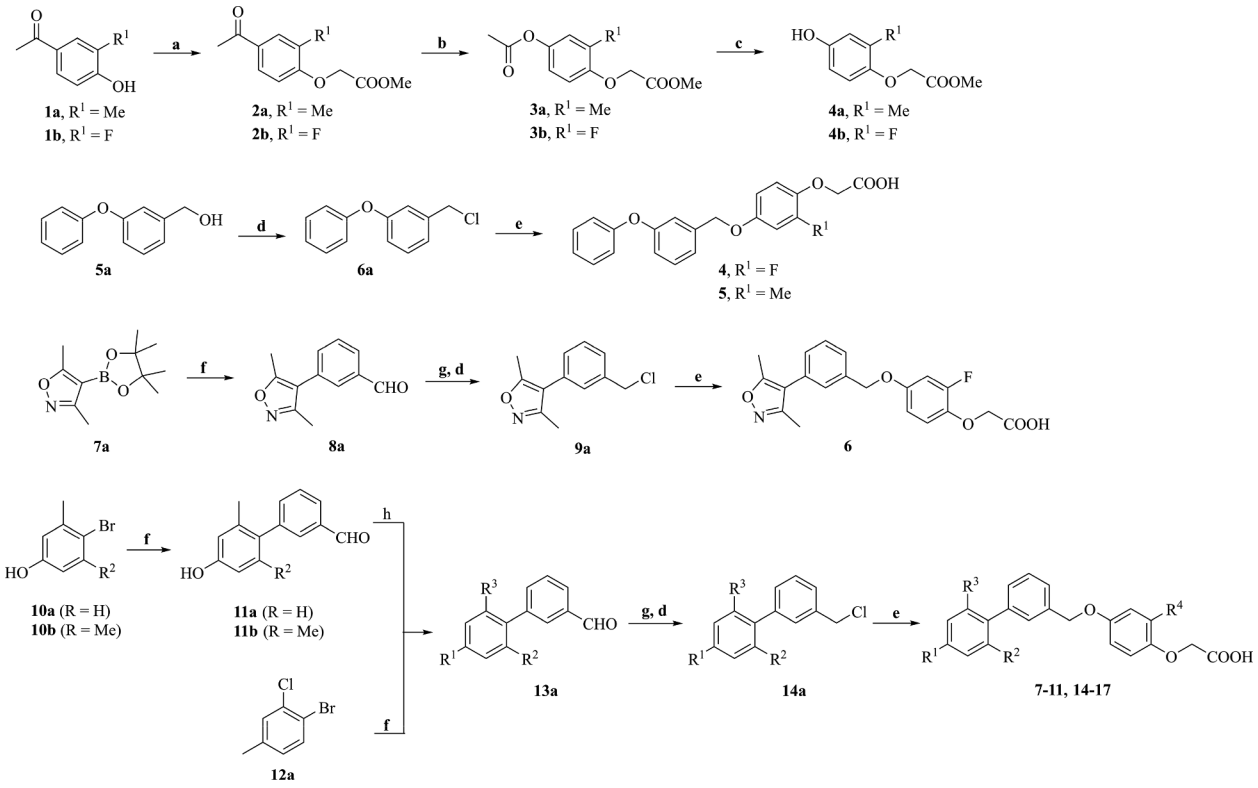
b by Williamson ether synthesis, followed by hydrolysis to furnish target compounds **4** and **5**. The isoxazole **8a** was synthesized using Suzuki coupling by treating (3-formylphenyl) boronic acid with commercially available isoxazole borate **7a**. The intermediate **13a** was synthesized by alkylation of **11a** or **11b**, which were derived from Suzuki coupling reaction of (3-formylphenyl) boronic acid with **10a** or **10b**. Reduction of aldehyde **8a** or **13a** in the presence of sodium borohydride, followed by treating with thionyl chloride yielded intermediate **9a** or **14a**. Condensation of **9a** or **14a** with **4a-b** by Williamson ether synthesis, and then basic hydrolysis provided target compounds **6–11** and **14–17**.

The target compounds **12–13** were synthesized as shown in Scheme 2. Intermediate **17a-b** was produced from reduction of nitro **16a-b**, which was prepared by Williamson ether synthesis of methyl chloroacetate or chloroacetamide with phenols **15a**. Compound **17a** or **17b** was connected with **18a** by reductive amination reaction in the presence of $NaBH_3CN$ to provide target compound **13** and intermediate **19a**. The ester hydrolytic reaction of intermediate **19a** afforded target compound **12**.

2.2. Structure-activity relationship study

All of target compounds were evaluated in a cell-based functional calcium mobilization assay. As shown in Table 1, we firstly explored the importance of *ortho*-fluoro at C ring, a substituent introduced to improve metabolic stability and slightly increase potency on phenylpropionic acid series in previous report [15]. Indeed, the introduction of *ortho*-fluoro (compound **4**) significantly improved potency compared to parent compound **2**, while *ortho*-methyl analog **5** turned out to be more than 3-fold reduced activity compared to analog **4**. Similar results were observed in biphenyl scaffold (such as compound **7** *vs* **8**, and **9** *vs* **10**), which suggested that *ortho*-fluoro is crucial to maintain high potency of phenoxyacetic acid derivative. The 3,5-dimethylisoxazole has been identified as a potent scaffold of FFA1 agonists with high activity [36], however, hybrid of 3,5-dimethylisoxazole scaffold with phenoxyacetic acid (compound **6**) resulted in a lower potency than that of biphenyl scaffold. Moreover, mono-methyl analog (**9**) revealed better potency than dimethyl analog (**11**), indicating that mono-methyl group is preferable to replace in the *ortho*-position of A ring. Further exploration exhibited *ortho*-chlorine was also tolerated in A ring (compound **17**).

Replacement of oxygen atom with amine provided compound **12**, which maintained the potency on FFA1. Introducing an amide (compound **13**), the bioisosteres of acid, resulted in a significant drop of activity. This result indicated that the carboxylic acid is crucial to activity of FFA1, which is consistent with previous report [35]. Next,



Scheme 1. Synthesis of target compounds **4–11** and **14–17**. Reagents and conditions: (a) Methyl bromoacetate, K_2CO_3, acetone, 45 °C, 12 h; (b) *m*-CPBA, *p*-TSA, CH_2Cl_2; (c) CH_3ONa, CH_3OH; (d) $SOCl_2$, CH_2Cl_2, DMF, 40 °C, 4 h; (e) **4a-b**, K_2CO_3, acetonitrile, KI, 45 °C, 12 h, and then LiOH·H_2O, THF/MeOH/H_2O, r.t., 4 h. (f) (3-formylphenyl)boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, ethanol, H_2O, 80 °C, 12 h; (g) $NaBH_4$, CH_3OH, THF, 0 °C, 30 min; (h) R_3Br, K_2CO_3, KI, acetone, reflux, 12 h.

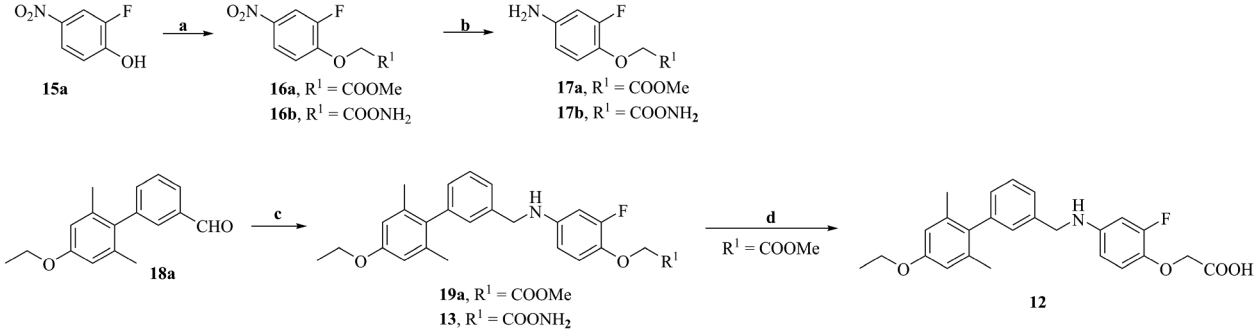
various substituents were explored in *para*-position of A ring based on the optimal compound **7**. For *para*-substituted analogs, the potency of compound **9** (MeO) > **14** (EtO) > **15** (PrO) > **16** (cyclopropyl methoxyl), indicating that the steric effect of *para*-substituent in A ring might be an influence factor on potency.

2.3. Molecular modeling study

To better understand the binding mode of present series, we performed docking studies of compounds **7** and **15** based on X-ray structure of FFA1 [37]. As shown in Fig. 3A, the carboxylic acid of compound **7** formed four hydrogen bonds with key residues Arg183, Arg2258, and Tyr2240. However, only three hydrogen bonds were formed for the propoxy analog **15** (Fig. 3B), which reasonably explained that the potency of compound **15** was inferior to compound **7**. Moreover, the orientation of biphenyl scaffold in compound **7** was completely different from that of compound **15**, which might be attributed to the steric effect of propoxy in compound **15**. Furthermore, an additional ionic bond could be formed between basic residues (Arg183, Arg2258) and carboxylic acid, but amide could not. Therefore, the amide analog **13** revealed a lower potency than carboxylic acid derivatives.

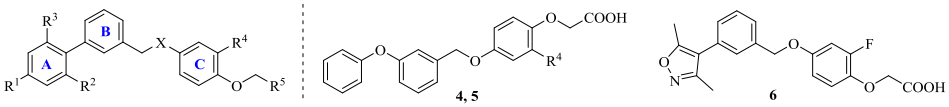
2.4. Oral glucose tolerance test in mice

The target compounds with EC_{50} value less than 50 nM (compounds **7**, **9**, **12** and **14**) were selected to evaluate their oral glucose tolerance test in normal mice. As shown in Fig. 4, all of these tested compounds revealed significant glucose-lowering effects. Gratifyingly, the optimal compound **7** exerted the best effect in the improvement of glucose tolerance, and its glucose $AUC_{0-120min}$ was significantly lower than that of TAK-875. Besides, the plasma glucose curve in compound **7**-treated group tends to stabilize after 60 min rather than decreased continually, suggesting its low risk of hypoglycemia.



Scheme 2. Synthesis of target compounds **12** and **13**. Reagents and conditions: (a) methyl chloroacetate or chloroacetamide, K_2CO_3, acetonitrile, KI, reflux, 6 h; (b) H_2, Pd-C, rt, 18 h; (c) $NaBH_3CN$, CH_3OH, THF, rt, 12 h; (d) LiOH·H_2O, THF/MeOH/H_2O, r.t., 4 h.

Table 1
Structure-activity relationship of target compounds.



Compd.	R^1	R^2	R^3	R^4	R^5	X	EC_{50} (nM)[a]
TAK-875							53.8
1							36.3
4				F	COOH	O	320.7
5				Me	COOH	O	2065.3
6				F	COOH	O	381.6
7 (HWL-088)	H	Me	H	F	COOH	O	19.0
8	H	Me	H	Me	COOH	O	1387.2
9	MeO	Me	H	F	COOH	O	43.6
10	MeO	Me	H	Me	COOH	O	1895.3
11	MeO	Me	Me	F	COOH	O	64.5
12	EtO	Me	Me	F	COOH	NH	34.6
13	EtO	Me	Me	F	$CONH_2$	NH	615.4
14	EtO	Me	H	F	COOH	O	47.5
15	PrO	Me	H	F	COOH	O	73.5
16		Me	H	F	COOH	O	79.8
17	Me	Cl	H	F	COOH	O	56.9

[a] EC_{50} values for FFA1 activities represent the mean values of three independent determinations.

2.5. Glucose-lowering effects in HF/STZ mice

To evaluate anti-diabetic effects, the oral glucose tolerance test of optimal compound **7** was performed in HF/STZ mice, a high-fat fed and streptozotocin-treated diabetic model with insulin resistance and insulin insufficient [38]. As shown in Fig. 5, compound **7** significantly improved the glucose tolerance in HF/STZ diabetic mice, and glucose $AUC_{0-120min}$ was slightly better than that of TAK-875, the most advanced candidate of FFA1 agonists. Our results suggested that compound **7** might be a potential choice for the control of hyperglycemia in T2DM.

3. Conclusion

In conclusion, we identified a superior FFA1 agonist **7** (HWL-088, EC_{50} = 19 nM) based on comprehensive structure-activity relationship study. The important aspects resulted in the discovery of compound **7** were as follows: (1) the introduction of *ortho*-fluoro at acid head greatly increased the activity on FFA1; (2) fine tuning of biphenyl scaffold further explored the optimal substituent with improved potency. Compound **7** exhibited significantly glucose-lowering effects both in normal mice and diabetic model, and even exerted greater potential on the improvement of hyperglycemia than that of TAK-875. This study extended the chemical space of FFA1 agonists bearing phenoxyacetic acid scaffold, and provided us a novel superior agonist HWL-088 for the treatment of T2DM.

4. Experimental section

4.1. General chemistry

All starting materials, reagents and solvents were obtained from commercial sources. Purifications of chromatography were performed by silica gel and detected by thin layer chromatography using UV light at 254 and 365 nm. Melting points were measured on RY-1 melting-point apparatus. NMR spectra were recorded on a Bruker ACF-300Q instrument (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR spectra), chemical shifts are expressed as values (ppm) relative to tetramethylsilane as internal standard, and coupling constants (*J* values) were given in hertz (Hz). LC/MS spectra were recorded on a Waters LC-MS system (ESI). Elemental analyses were performed by the Heraeus CHN-O-Rapid analyzer and were within 0.4% of the theoretical values. TAK-875 was synthesized by published procedures [16].

4.1.1. General synthetic procedure for intermediates *4a* and *4b*

To a stirred solution of **1a** or **1b** (1 equiv) in acetone was added potassium carbonate (3 equiv) and methyl bromoacetate (2 equiv). The mixture was stirred at 45 °C for 12 h, and filtered. The filtrate was concentrated and the residue was dissolved in ethyl acetate. The organic layers was washed with brine (2 × 20 mL), dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to give intermediate **2a** or **2b** as colorless oil, which was used for the next reaction without further purification. To a solution of intermediate **2a** or **2b** (1 equiv) in dichloromethane was added *p*-toluenesulfonic acid (0.1 equiv) and 3-chloroperoxybenzoic acid (2 equiv) at 0 °C. The mixture was stirred at room temperature for 24 h and then poured into saturated sodium bisulfite solution (25 mL) stirred for 20 min. The aqueous layer was separated and extracted with dichloromethane (3 × 10 mL). The combined organic phases were washed with water (15 mL), dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated and the residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 20:1, v/v) to afford intermediates **3a** and **3b** as white solid. To a solution of **3a** or **3b** (1 equiv) in methanol was added sodium methoxide (3 equiv). The mixture was stirred at room temperature for 4–6 h and then quenched with 1 N hydrochloric acid (20 mL). The mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic phases were washed with brine (15 mL), dried and filtered. The filtrate was evaporated and the residue was purified by column chromatography (petroleum ether/ethyl acetate, 10:1, v/v) to afford intermediates **4a** and **4b** as white solid.

4.1.1.1. Methyl 2-(4-hydroxy-2-methylphenoxy)acetate ***(4a)***. ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 9.35 (s, 1H), 7.06–7.01 (m, 1H), 6.84–6.63 (m, 2H), 4.75 (s, 2H), 3.67 (s, 3H), 2.18 (s, 3H). ESI-MS *m/z*: 197.1 $[M+H]^+$.

4.1.1.2. Methyl 2-(2-fluoro-4-hydroxyphenoxy)acetate ***(4b)***. ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 9.44 (s, 1H), 6.92–6.85 (m, 1H), 6.63, 6.59 (dd, *J* = 13.1, 2.8 Hz, 1H), 6.51–6.47 (m, 1H), 4.73 (s, 2H), 3.69 (s, 3H). ESI-MS *m/z*: 201.1 $[M+H]^+$.

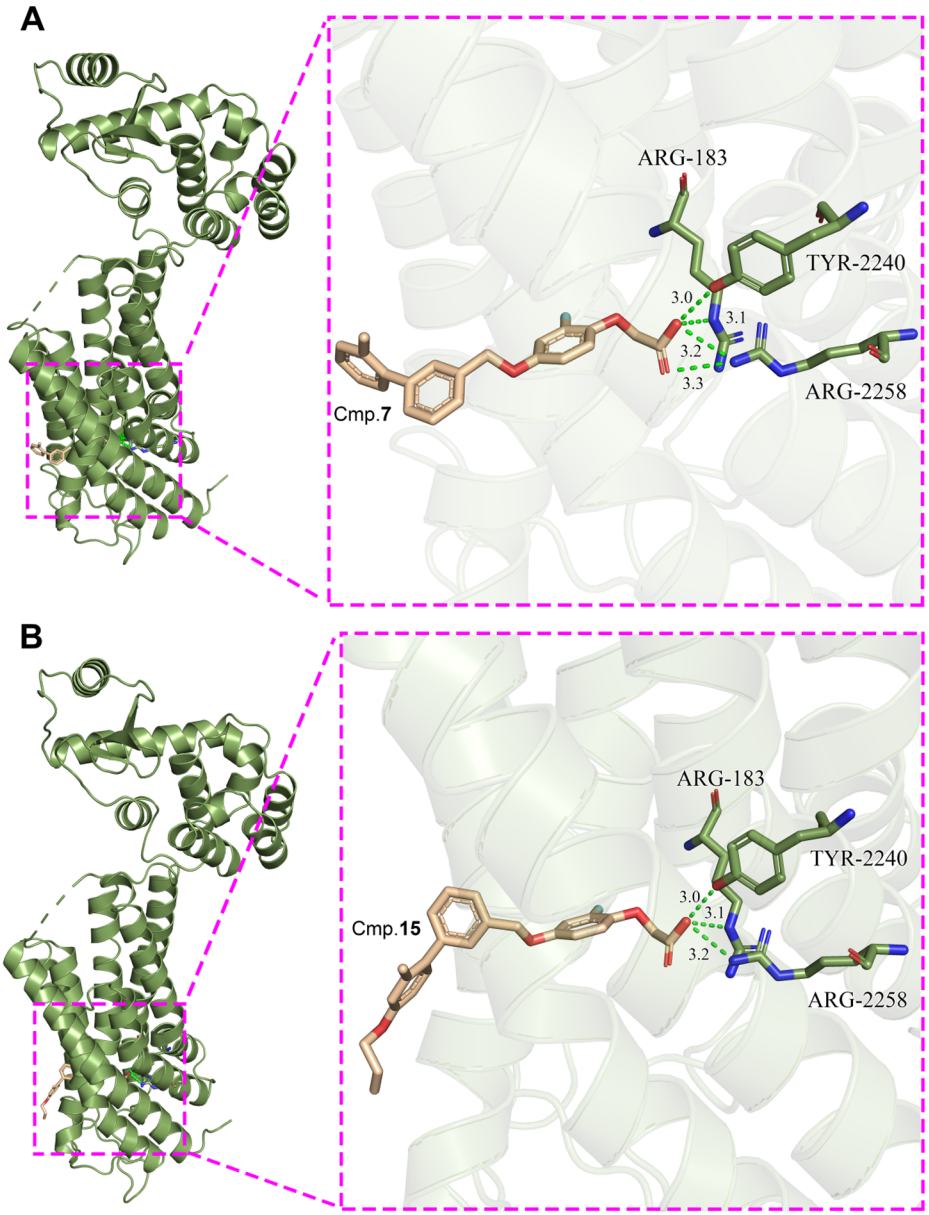


Fig. 3. The docking study of compound **7** (A) and **15** (B) in the reported complex of FFA1 (PDB code: 4PHU). Key residues are labeled in black. Hydrogen bonds are represented by green dashed lines.

*4.1.1.3. General procedure for **11a-b**.* The bromobenzene (1 equiv) and (3-formylphenyl)boronic acid (1 equiv) were dissolved in a mixture of 1 M sodium carbonate solution (15 mL), EtOH (5 mL) and toluene (15 mL). After nitrogen substitution, $Pd(PPh_3)_4$ (0.05 equiv) was added. The reaction mixture was stirred at 80 °C under nitrogen atmosphere for 12 h. The reaction mixture was cooled, and water (15 mL) was added. The mixture was diluted with ethyl acetate (15 mL), and the insoluble material was filtered off through Celite. The organic layer of the filtrate was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by column chromatography using a mixture of petroleum ether/ethyl acetate (10:1, v/v) as eluent to afford the desired product as a solid.

*4.1.1.4. 4′-Hydroxy-2′-methyl-3-biphenylcarbaldehyde **(11a)**.* Yield: 92%; white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.04 (s, 1H), 7.87 (d, J = 7.6 Hz, 1H), 7.66 (t, J = 1.7 Hz, 1H), 7.59–7.53 (m, 2H), 7.42 (d, J = 7.4 Hz, 1H), 6.89 (d, J = 1.5 Hz, 1H), 6.76–6.72 (m, 1H), 4.67 (s, 1H), 1.98 (s, 3H).

*4.1.1.5. 4′-Hydroxy-2′,6′-dimethyl-3-biphenylcarbaldehyde **(11b)**.* Yield: 83%; white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.05 (s, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.68 (t, J = 1.5 Hz, 1H), 7.61–7.55 (m, 2H), 6.62 (s, 2H), 4.69 (s, 1H), 1.97 (s, 6H).

*4.1.2. General synthetic procedure for **13a***

To a solution of **11a-b** (1 equiv) and alkyl halide (1.2 equiv) in acetone was added K_2CO_3 (2 equiv) and a catalytic amount of KI at room temperature. The reaction mixture was heated to reflux with stirring overnight. Then reaction mixture was cooled followed by filtration and the filtrate was concentrated under vacuum. The residue was purified by column chromatography using a mixture of petroleum ether/ethyl acetate (10:1, v/v) as eluent to afford a white solid.

4.1.2.1. 4′-ethoxy-2′,6′-dimethyl-[1,1′-biphenyl]-3-carbaldehyde. Yield: 78%; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.06 (s, 1H), 7.98 (d, J = 7.6 Hz, 1H), 7.69 (t, J = 1.7 Hz, 1H), 7.63–7.57 (m, 2H), 6.63 (s, 2H), 4.02 (q, J = 6.7 Hz, 2H), 1.96 (s, 6H), 1.31 (t, J = 6.7 Hz, 3H).

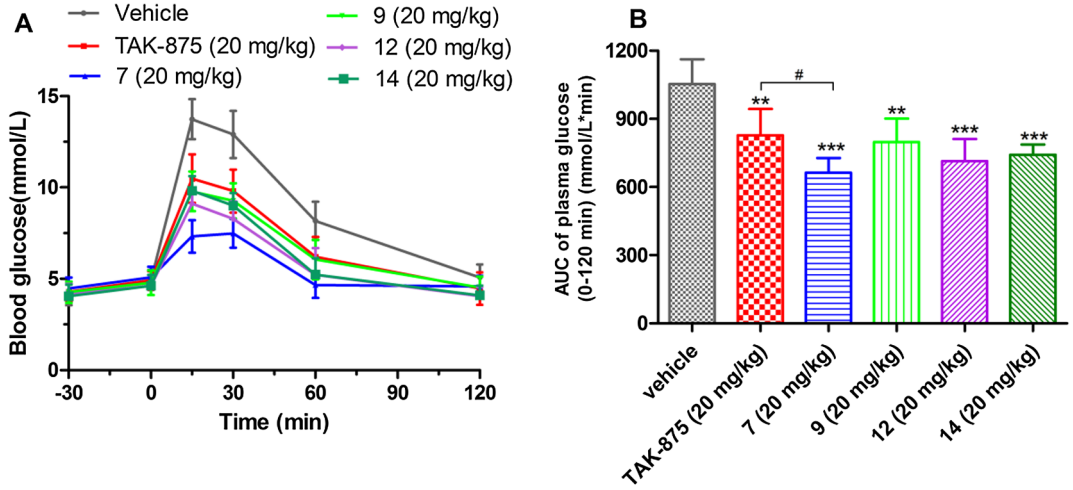


Fig. 4. Oral glucose tolerance test of selected compounds in fasting male ICR mice. (A) represented time-dependent changes of blood glucose levels after oral administration of selected compounds, followed by 3 g/kg oral glucose challenge, respectively. (B) represented the $AUC_{0-120min}$ of blood glucose levels shown in (A). Values are mean ± SD (n = 6 per group). $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$ compared to vehicle mice by using a one-way ANOVA with Tukey's multiple-comparison post hoc test. $^{\#}p \leq 0.05$ compared to TAK-875 treated mice by using a one-way ANOVA with Tukey's multiple-comparison post hoc test.

*4.1.3. General synthetic procedure for **14a***

To a solution of **13a** (1 equiv) in MeOH (10 mL) and THF (20 mL) was added portionwise sodium borohydride (3 equiv) at 0 °C and the mixture was stirred at 0 °C for 1 h. The reaction mixture was pouring into ice water (10 mL), and extracted with ethyl acetate (3 × 15 mL), the organic fractions were combined, washed with saturated brine (2 × 15 mL) prior to drying over anhydrous sodium sulfate. After filtration and concentrate using a rotary evaporator, the residue was used in next step without further purification. To a solution of the obtained solid (1 equiv) in dichloromethane (20 mL) was slowly added thionyl chloride (6 equiv) and a catalytic amount of DMF at room temperature. After stirring at 40 °C for 4 h, the reaction was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a mixture of petroleum ether/ethyl acetate (20:1, v/v) as eluent to afford the desired product.

4.1.3.1. 3′-(chloromethyl)-4-ethoxy-2,6-dimethyl-1,1′-biphenyl. Yield: 59%; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.44–7.39 (m, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.14 (s, 1H), 7.05–7.10 (m, 1H), 6.82 (s, 2H), 4.52 (s, 2H), 4.02 (q, J = 6.7 Hz, 2H), 1.96 (s, 6H), 1.31 (t, J = 6.7 Hz, 3H).

*4.1.3.2. 3-(3,5-dimethylisoxazol-4-yl)benzaldehyde **(8a)***. To a mixture of 3,5-dimethyl-4- (4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) isoxazole (0.50 g, 2.2 mmol), 3-bromobenzaldehyde (0.50 g, 2.7 mmol), $Pd(PPh_3)_4$ (0.13 g, 0.12 mmol) and sodium carbonate (0.71 g, 6.7 mmol) in toluene/ethanol/H_2O (35 mL, 3/1/3) was refluxed under nitrogen atmosphere for 24 h. Then the mixture was diluted with saturated ammonium chloride solution and ethyl acetate, and the insoluble material was filtered through Celite. The organic layer of the filtrate was washed with water (25 mL) and brine (25 mL), dried over anhydrous sodium sulfate, and evaporated in vacuo. The residue was purified by column chromatography using a mixture of petroleum ether/ethyl acetate (10 : 1, v/v) as eluent to afford the desired product **8a** (0.37 g, 77%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.08 (s, 1H), 7.99–7.85 (m, 2H), 7.81–7.63 (m, 2H), 2.43 (s, 3H), 2.25 (s, 3H).

*4.1.3.3. 4-(3-(chloromethyl)phenyl)-3,5-dimethylisoxazole **(9a)***. To a solution of **8a** (0.3 g, 1.49 mmol) in MeOH (10 mL) and THF (20 mL) was added portionwise sodium borohydride (0.17 g, 4.47 mmol) at 0 °C and the mixture was stirred at 0 °C for 1 h. The reaction mixture was pouring into ice water (10 mL), and extracted with ethyl acetate (3 × 15 mL), the organic fractions were combined, washed with saturated brine (2 × 15 mL) prior to drying over anhydrous sodium

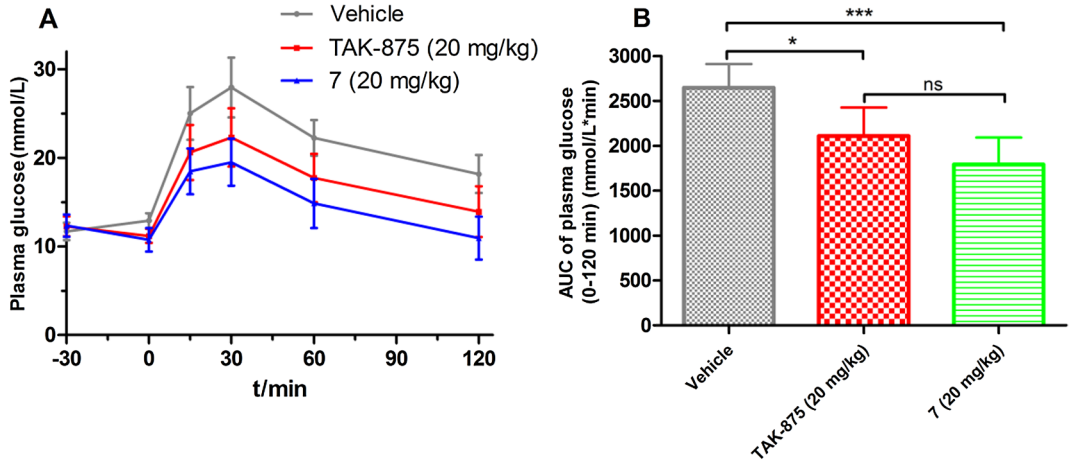


Fig. 5. Effects of compound **7** on blood glucose levels (A) and corresponding $AUC_{0-120min}$ of glucose (B) in diabetic HF/STZ mice. Overnight-fasted mice were orally given compound **7** (20 mg/kg) 30 min before glucose load (2 g/kg). Values are mean ± SD (n = 6 per group). $^{*}p \leq 0.05$ and $^{***}p \leq 0.001$ were analyzed using a one-way ANOVA with Tukey's multiple-comparison post hoc test.

sulfate. After filtration and concentrate using a rotary evaporator, the residue was used in next step without further purification. To a solution of the obtained solid in dichloromethane (20 mL) was slowly added thionyl chloride (1.06 g, 8.94 mmol) and a catalytic amount of DMF at room temperature. After stirring at 40 °C for 4 h, the reaction was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a mixture of petroleum ether/ethyl acetate (20:1, v/v) as eluent to afford the **9a** (0.27 g, 82%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.57–7.43 (m, 3H), 7.36, 7.34 (dt, J = 6.9, 1.8 Hz, 1H), 4.82 (s, 2H), 2.41 (s, 3H), 2.23 (s, 3H).

*4.1.3.4. 1-(chloromethyl)-3-phenoxybenzene **(6a)***. The title compound was prepared as described for compound **9a** as white solid. Yield 82%; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.43–7.35 (m, 3H), 7.31–7.15 (m, 5H), 7.04–6.92 (m, 1H), 4.61 (s, 2H).

*4.1.4. General synthetic procedure for target compounds **4–11** and **14–17***

To a solution of chlorine intermediates (1 equiv) and intermediates **4a-b** (0.8 equiv) in acetone was added K_2CO_3 (2 equiv) and a catalytic amount of KI at room temperature. The reaction mixture was heated to reflux with stirring overnight. Then the reaction mixture was cooled to room temprature followed by filtration and the filtrate was concentrated under vacuum. The residue was purified by silica gel column chromatography using a mixture of petroleum ether/ethyl acetate (4:1, v/v) as eluent to afford a white solid. To a solution of the obtained solid (1 equiv) in 2:3:1 THF/MeOH/H_2O (18 mL) was added LiOH·H_2O (3 equiv). After stirring at room temperature for 4 h, the volatiles were removed under reduced pressure. The residue was acidified with 1 N hydrochloric acid solution, and then filtered and the filter cake was washed with 5 mL of water, dried in vacuum to afford a white powder. The white powder was purified by column chromatography using a mixture of petroleum ether/ethyl acetate (2:1–1:2, v/v) as eluent to afford the target compounds as white solid.

*4.1.4.1. 2-(2-fluoro-4-((3-phenoxybenzyl)oxy)phenoxy)acetic acid **(4)***. Yield: 61%; m.p. 120–122 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.52–7.35 (m, 3H), 7.27–7.11 (m, 2H), 7.10–6.91 (m, 6H), 6.81–6.68 (m, 1H), 5.05 (s, 2H), 4.67 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.51, 157.26, 156.89, 153.11, 141.67, 139.64, 130.61, 130.56, 124.06, 122.98, 119.21, 118.33, 117.98, 116.27, 110.66, 104.44, 69.78, 66.23. ESI-MS m/z: 367.1 $[M-H]^-$. Anal. calcd. For $C_{21}H_{17}FO_5$: C, 68.47; H, 4.65; Found: C, 68.63; H, 4.57.

*4.1.4.2. 2-(2-methyl-4-((3-phenoxybenzyl)oxy)phenoxy)acetic acid **(5)***. Yield: 47%; m.p. 107–109 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.51–7.33 (m, 3H), 7.27–7.10 (m, 2H), 7.09–6.90 (m, 4H), 6.87–6.78 (m, 1H), 6.75–6.66 (m, 2H), 5.01 (s, 2H), 4.53 (s, 2H), 2.16 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.18, 157.23, 157.05, 152.83, 150.96, 140.87, 128.67, 128.26, 121.85, 120.67, 118.63, 117.25, 116.85, 112.43, 112.05, 65.87, 62.59, 15.63. ESI-MS m/z: 363.1 $[M-H]^-$. Anal. calcd. For $C_{22}H_{20}O_5$: C, 72.51; H, 5.53; Found: C, 72.77; H, 5.65.

*4.1.4.3. 2-(4-((3-(3,5-dimethylisoxazol-4-yl)benzyl)oxy)-2-fluorophenoxy)acetic acid **(6)***. Yield: 43%; m.p. 86–88 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.76–7.61 (m, 1H), 7.49–7.43 (m, 2H), 7.34 (d, J = 7.2 Hz, 1H), 7.08–6.99 (m, 2H), 6.78 (d, J = 9.0 Hz, 1H), 5.13 (s, 2H), 4.67 (s, 2H), 2.39 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.57, 165.66, 158.55, 153.20, 138.03, 131.97, 130.46, 129.48, 129.13, 128.80, 127.31, 116.36, 116.18, 110.66, 104.73, 69.97, 66.27, 11.75, 10.90. ESI-MS m/z: 370.1 $[M-H]^-$. Anal. calcd. For $C_{20}H_{18}FNO_5$: C, 64.69; H, 4.89; N, 3.77; Found: C, 64.53; H, 4.76; N, 3.62.

*4.1.4.4. 2-(2-fluoro-4-((2′-methyl-[1,1′-biphenyl]-3-yl)methoxy)phenoxy)acetic acid **(7)***. Yield: 59%; m.p. 125–126 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.57–7.37 (m, 3H), 7.34–7.13 (m, 5H), 7.11–6.91 (m, 2H), 6.78 (d, J = 8.6 Hz, 1H), 5.13 (s, 2H), 4.66 (s, 2H), 2.21 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.39, 154.10, 153.51, 141.91, 140.43, 137.35, 135.15, 130.79, 129.90, 128.88, 128.83, 128.67, 127.83, 126.64, 126.37, 116.74, 110.80, 104.82, 70.41, 66.64, 20.50. ESI-MS m/z: 365.1 $[M-H]^-$. Anal. calcd. For $C_{22}H_{19}FO_4$: C, 72.12; H, 5.23; Found: C, 72.37; H, 5.15.

*4.1.4.5. 2-(2-methyl-4-((2′-methyl-[1,1′-biphenyl]-3-yl)methoxy)phenoxy)acetic acid **(8)***. Yield: 57%; m.p. 96–98 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.50–7.37 (m, 3H), 7.34–7.01 (m, 5H), 6.96–6.45 (m, 3H), 5.09 (s, 2H), 4.58 (s, 2H), 2.21 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.92, 152.92, 150.92, 141.85, 141.59, 137.95, 135.15, 130.79, 129.90, 128.77, 128.70, 128.53, 127.91, 127.81, 126.50, 126.38, 118.27, 113.11, 112.75, 70.10, 66.23, 20.50, 16.57. ESI-MS m/z: 361.1 $[M-H]^-$. Anal. calcd. For $C_{23}H_{22}O_4$: C, 76.22; H, 6.12; Found: C, 76.45; H, 6.28.

*4.1.4.6. 2-(2-fluoro-4-((4′-methoxy-2′-methyl-[1,1′-biphenyl]-3-yl)methoxy)phenoxy)acetic acid **(9)***. Yield: 53%; m.p. 142–143 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.52–7.32 (m, 3H), 7.30–7.20 (m, 1H), 7.12 (d, J = 6.6 Hz, 1H), 7.08–6.91 (m, 2H), 6.89–6.66 (m, 3H), 5.11 (s, 2H), 4.60 (s, 2H), 3.78 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.58, 158.35, 153.37, 150.74, 141.65, 137.38, 136.56, 133.87, 130.85, 129.12, 128.76, 128.75, 126.28, 116.73, 116.58, 112.65, 110.82, 104.76, 70.48, 69.46, 55.85, 20.83. ESI-MS m/z: 395.1 $[M-H]^-$. Anal. calcd. For $C_{23}H_{21}FO_5$: C, 69.69; H, 5.34; Found: C, 69.45; H, 5.48.

*4.1.4.7. 2-(4-((4′-methoxy-2′-methyl-[1,1′-biphenyl]-3-yl)methoxy)-2-methylphenoxy)acetic acid **(10)***. Yield: 61%; m.p. 96–98 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.64 (brs, 1H), 7.51–7.07 (m, 5H), 6.96–6.68 (m, 5H), 5.08 (s, 2H), 4.59 (s, 2H), 3.79 (s, 3H), 2.20 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.99, 167.43, 158.93, 152.80, 150.71, 141.55, 137.81, 136.57, 134.04, 131.05, 128.92, 128.72, 127.75, 126.20, 118.15, 116.15, 112.82, 112.54, 111.89, 69.92, 65.86, 55.51, 20.87, 16.67. ESI-MS m/z: 391.1 $[M-H]^-$. Anal. calcd. For $C_{24}H_{24}O_5$: C, 73.45; H, 6.16; Found: C, 73.71; H, 6.33.

*4.1.4.8. 2-(2-fluoro-4-((4′-methoxy-2′,6′-dimethyl biphenyl-3-yl)methoxy)phenoxy)acetic acid **(11)***. Yield: 65%; m.p. 152–154 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.83 (s, 1H), 7.46–7.37 (m, 2H), 7.15 (s, 1H), 7.05 (d, J = 8.8 Hz, 1H), 7.00–6.94 (m, 2H), 6.75 (d, J = 9.0 Hz, 1H), 6.68 (s, 2H), 5.09 (s, 2H), 4.67 (s, 2H), 3.74 (s, 3H), 1.91 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.1, 157.9, 153.3, 152.7, 140.3, 139.7, 136.8, 133.5, 128.9, 128.6, 128.3, 125.9, 115.8, 112.6, 110.2, 104.1, 69.7, 65.7, 54.8, 20.6; ESI-MS m/z: 409.1 $[M-H]^-$; Anal. calcd. For $C_{24}H_{23}FO_5$: C, 70.23; H, 5.65; Found: C, 70.20; H, 5.66.

*4.1.4.9. 2-(4-((4′-ethoxy-2′-methyl-[1,1′-biphenyl]-3-yl)methoxy)-2-fluorophenoxy)acetic acid **(14)***. Yield: 58%; m.p. 105–106 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.41–7.34 (m, 3H), 7.23 (d, J = 6.2 Hz, 1H), 7.09 (d, J = 7.9 Hz, 1H), 7.03–6.93 (m, 2H), 6.84–6.75 (m, 3H), 5.09 (s, 2H), 4.60 (s, 2H), 4.03 (q, J = 5.7 Hz, 2H), 2.18 (s, 3H), 1.32 (t, J = 5.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.63, 158.26, 153.36, 153.23, 141.70, 140.61, 137.25, 136.54, 133.94, 130.99, 129.05, 128.83, 128.72, 126.24, 116.77, 116.63, 112.41, 110.74, 104.75, 70.45, 67.04, 63.47, 20.76, 15.12. ESI-MS m/z: 409.1 $[M-H]^-$. Anal. calcd. For $C_{24}H_{23}FO_5$: C, 70.23; H, 5.65; Found: C, 70.55; H, 5.83.

*4.1.4.10. 2-(2-fluoro-4-((2′-methyl-4′-propoxy-[1,1′-biphenyl]-3-yl)methoxy)phenoxy)acetic acid **(15)***. Yield: 49%; m.p. 80–82 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.53–7.33 (m, 3H), 7.25 (d, J = 5.1 Hz, 1H), 7.11 (d, J = 7.8 Hz, 1H), 7.06–6.91 (m, 2H), 6.88–6.67 (m, 3H), 5.10 (s, 2H), 4.59 (s, 2H), 3.95 (t, J = 6.8 Hz, 2H), 2.19 (s, 3H), 1.84–1.62 (m, 2H), 1.00 (t, J = 6.4 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ:

170.60, 158.44, 153.30, 150.80, 141.70, 137.27, 136.53, 133.94, 130.99, 129.04, 128.84, 128.71, 126.24, 116.82, 116.62, 112.43, 110.76, 104.73, 70.44, 69.46, 67.08, 22.56, 20.76, 10.81. ESI-MS *m/z*: 423.1 $[M-H]^-$. Anal. calcd. For $C_{25}H_{25}FO_5$: C, 70.74; H, 5.94; Found: C, 70.51; H, 5.78.

4.1.4.11. 2-(4-((4′-(cyclopropylmethoxy)-2′-methyl-[1,1′-biphenyl]-3-yl) methoxy)-2-fluorophenoxy) acetic acid ***(16)***. Yield: 43%; m.p. 116–118 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.49–7.32 (m, 3H), 7.25 (d, J = 7.1 Hz, 1H), 7.10 (d, J = 8.3 Hz, 1H), 7.02–6.91 (m, 2H), 6.89–6.63 (m, 3H), 5.09 (s, 2H), 4.53 (s, 2H), 3.83 (d, J = 7.0 Hz, 2H), 2.18 (s, 3H), 1.27–1.19 (m, 1H), 0.66–0.47 (m, 2H), 0.38–0.25 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.63, 158.47, 153.35, 150.83, 141.78, 137.25, 136.64, 133.87, 130.83, 129.08, 128.87, 128.73, 126.26, 116.88, 116.58, 112.64, 110.73, 104.69, 78.64, 70.48, 67.07, 20.76, 10.63, 2.87. ESI-MS *m/z*: 435.1 $[M-H]^-$. Anal. calcd. For $C_{26}H_{25}FO_5$: C, 71.55; H, 5.77; Found: C, 71.73; H, 5.64.

4.1.4.12. 2-(4-((2′-chloro-4′-methyl-[1,1′-biphenyl]-3-yl)methoxy)-2-fluorophenoxy)acetic acid ***(17)***. Yield: 59%; m.p. 118–120 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.54–7.19 (m, 7H), 7.06–6.89 (m, 2H), 6.77 (d, J = 8.7 Hz, 1H), 5.11 (s, 2H), 4.56 (s, 2H), 2.36 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.55, 158.47, 153.35, 139.58, 139.31, 137.39, 131.63, 131.43, 130.55, 129.18, 128.89, 128.76, 128.64, 127.27, 116.60, 110.77, 104.46, 70.34, 67.17, 20.72. ESI-MS *m/z*: 399.1 $[M-H]^-$. Anal. calcd. For $C_{22}H_{18}ClFO_4$: C, 65.92; H, 4.53; Found: C, 65.76; H, 4.41.

4.1.5. General procedure for ***17a-b***

To a solution of 2-fluoro-4-nitrophenol (1 equiv) in 30 mL acetonitrile was added methyl chloroacetate or chloroacetamide (1.5 equiv) and K_2CO_3 (3 equiv) at room temperature. The solution was heated to reflux for 6 h. The reaction mixture was filtered and evaporated to afford a colorless oil product. To a solution of the obtained product in ethanol was added Pd/C (5%) and the mixture was stirred for 18 h at room temperature in a hydrogen atmosphere. Insoluble matters were removed using Celite, and the filtrate was concentrated in vacuo to give the desired product as a white solid.

4.1.5.1. Methyl 2-(4-amino-2-fluorophenoxy)acetate ***(17a)***. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.94–6.90 (m, 1H), 6.79–6.77 (m, 1H), 6.54, 6.50 (dd, J = 2.2, 12.5 Hz, 1H), 5.28 (s, 2H), 4.76(s, 2H), 3.75 (s, 3H).

4.1.6. General procedure for target compounds ***12*** *and* ***13***

To a solution of **18a** (1 equiv) and **17a-b** (1 equiv) in MeOH (10 mL) and THF (20 mL) was added portionwise $NaBH_3CN$ (3 equiv) and the mixture was stirred for 12 h. The reaction mixture was pouring into ice water (10 mL), and extracted with ethyl acetate (3 × 15 mL), washed with saturated brine (2 × 15 mL) prior to drying over anhydrous sodium sulfate. After filtration and concentrate, the residue was purified by column chromatography using a mixture of petroleum ether/ethyl acetate (5:1, v/v) as eluent to afford a white solid.

4.1.6.1. 2-(4-(((4′-ethoxy-2′,6′-dimethyl-[1,1′-biphenyl]-3-yl)methyl) amino)-2-fluorophenoxy)acetic acid ***(12)***. The title compound was obtained from **19a** according to the method of basic hydrolysis described for the synthesis of compound **4**. Yield: 67%; m.p. 102–104 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.43–7.26 (m, 2H), 7.04 (s, 1H), 6.95 (d, J = 7.3 Hz, 1H), 6.81 (t, J = 9.3 Hz, 1H), 6.65 (s, 2H), 6.42, 6.37 (dd, J = 14.1, 2.5 Hz, 1H), 6.29 (d, J = 8.9 Hz, 1H), 4.51 (s, 2H), 4.26 (s, 2H), 4.01 (q, J = 7.0 Hz, 2H), 1.87 (s, 6H), 1.31 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.78, 157.55, 144.89, 140.61, 140.49, 136.93, 134.27, 128.87, 128.77, 128.07, 125.92, 117.63, 113.55, 108.23, 101.25, 66.90, 63.16, 47.14, 21.17, 15.23. ESI-MS *m/z*: 422.2 $[M-H]^-$. Anal. calcd. For $C_{25}H_{26}FNO_4$: C, 70.91; H, 6.19; N, 3.31; Found: C, 70.84; H, 6.08; N, 3.16.

4.1.6.2. 2-(4-(((4′-ethoxy-2′,6′-dimethyl-[1,1′-biphenyl]-3-yl)methyl) amino)-2-fluorophenoxy)Acetamide ***(13)***. Yield: 73%; m.p. 96–97 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.47–7.23 (m, 4H), 7.13–7.03 (m, 1H), 7.01–6.83 (m, 2H), 6.66 (s, 2H), 6.53–6.26 (m, 2H), 6.15 (s, 1H), 4.30 (s, 4H), 4.02 (q, J = 7.0 Hz, 2H), 1.91 (s, 6H), 1.33 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 169.32, 156.35, 152.93, 142.59, 141.36, 137.16, 136.23, 130.78, 129.12, 128.63, 125.93, 126.24, 116.73, 111.47, 110.75, 105.35, 70.23, 67.26, 62.67, 20.73, 14.72. ESI-MS *m/z*: 423.1 $[M+H]^+$. Anal. calcd. For $C_{25}H_{27}FN_2O_3$: C, 71.07; H, 6.44; N, 6.63; Found: C, 71.31; H, 6.28; N, 6.56.

4.2. FLIPR assay

CHO cells stably expressing FFA1 were seeded into 96-well plates and incubated 16 h at 37 °C. After, the culture medium was removed and washed with 100 μL of Hank's Balanced Salt Solution. Then, cells were incubated in loading buffer (containing 2.5 μg/mL Fluo 4-AM, 2.5 mmol/L probenecid and 0.1% fatty acid-free BSA) for 1 h at 37 °C. Various concentrations of compounds or γ-linolenic acid (Sigma) were added and the signals of calcium flux were monitored by FLIPR Tetra system (Molecular Devices). The agonistic activities were calculated as [(A − B)/(C − B)] × 100 (increased concentration of calcium (A) in the compound group and (B) in vehicle group, and (C) in 10 μM γ-linolenic acid group). The EC_{50} value was calculated using GraphPad InStat version 5.00 (GraphPad software, San Diego, CA, USA).

4.3. Molecular docking

Molecular docking simulations were performed using AutoDock vina1.1.2. The Pymol 2.3.1 was employed to analyze the docking results. The crystal structures of FFA1 (PDB code: 4PHU) was obtained from Protein Data Bank. Before the docking process, the structure of protein was treated by adding polar hydrogen atoms, deleting water molecules, adding gasteiger charge, and assigning type of atoms as AD4 using AutoDockTools package. Docking calculations were performed with full flexibility of the ligand inside the binding site. Docking was performed using the bound crystallographic ligand as the centroid of the search space. Other docking parameters were kept to the default values.

4.4. Animals and Statistical analysis

10 weeks old male ICR mice and C57BL/6 mice were purchased from Guangdong Medical Laboratory Animal Center (Guangdong, China) and acclimatized for one week. Animal room was keep constant 12 h light/black cycle at 23 ± 2 °C with relative humidity 50 ± 10%. Animals were allowed ad libitum. All animal experimental protocols were approved by the ethical committee at Guangdong Pharmaceutical University and conducted according to the Laboratory Animal Management Regulations in China and adhered to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication NO. 85-23, revised 2011).

Statistical analyses were carried out by GraphPad InStat 5.00 (San Diego, CA, USA), and analyzed by one-way ANOVA with Tukey's multiple-comparison post hoc test.

4.5. Oral glucose tolerance test in mice

ICR mice were fasted for 12 h, weighted, and randomized into 6 groups (n = 6). Mice were administrated orally with vehicle, TAK-875 (20 mg/kg), or selected compounds (20 mg/kg) and subsequently dosed orally with 3 g/kg glucose solution after 30 min. Blood samples were collected before drug administration (−30 min), before glucose load (0 min), and at 15, 30, 60 and 120 min post-dose. The plasma glucose was measured by blood glucose test strips (SanNuo ChangSha, China).

4.6. Glucose-lowering effects in HF/STZ model

C57BL/6 mice were fed with high-fat diet (45% calories from fat, Mediscience Ltd., Yangzhou, China) ad libitum for 4 weeks and then injected intraperitoneally (i.p.) with STZ (80 mg/kg). The mice were fed with high-fat-diet for another 4 weeks, and diabetes was verified by glucose tolerance. The obtained type 2 diabetic HF/STZ mice were fasted for 12 h, weighted, and randomized into 3 groups (n = 6 per group). Mice were administrated orally with vehicle, TAK-875 (20 mg/kg), or compound **7** (20 mg/kg) and subsequently dosed orally with 2 g/kg glucose solution after 30 min. Blood samples were collected immediately before drug administration (-30 min), before glucose load (0 min), and at 15, 30, 60 and 120 min post-dose. The blood glucose was measured by blood glucose test strips (SanNuo ChangSha, China).

Declaration of Competing Interest

The authors declare no competing financial interest.

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