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Discovery of novel free fatty acid receptor 1 agonists bearing triazole core via click chemistry

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ABSTRACT

The free fatty acid receptor 1 (FFA1/GPR40) is a novel antidiabetic target based on particular mechanism in enhancing glucose-stimulated insulin secretion. Most of reported FFA1 agonists, however, have been suffered from relatively high lipophilicity and molecular weight. Aiming to develop potent agonists with improved physicochemical property, 25 compounds containing triazole scaffold and various carboxylic acid fragments were synthesized via the click chemistry. Among them, the optimal lead compound **26** with relatively low lipophicity ($\mathrm{Log}D_{7.4} = 1.95$) and molecular weight (Mw = 391.78) exhibited a considerable FFA1 agonistic activity (36.15%). In addition, compound **26** revealed a significant improvement in the glucose tolerance with a 21.4% and 14.2% reduction of glucose AUC_{0-2h} in normal ICR mice and type 2 diabetic C57BL/6 mice, respectively. All of these results demonstrated that compound **26** was considered to be a promising lead compound suitable for further optimization.

1. Introduction

The worldwide epidemic of type 2 diabetes mellitus (T2DM), a metabolic syndrome characterized by impaired glucose homeostasis, has become a serious health problem.[1,2] Besides the lifestyle intervention, the most common treatments are associated with side effects such as risk of hypoglycemia, gastric symptoms and weight gain.[3–6] Hence, there is an urgent need for improved treatment with increased safety and durability.[7,8] The free fatty acid receptor 1 (FFA1, previously known as GPR40), the prominent ones of antidiabetic targets in the last decade, is predominantly expressed in pancreatic β-cells and amplify glucose-stimulated insulin secretion.[9–12] This glucose concentration-dependent mechanism provides the potential for improving the insulin levels without the risk of hypoglycemia.

Recently, a variety of FFA1 agonists have been reported in the literature (Fig. 1),[13–21] and the TAK-875 and AMG-837 were both in clinical trials. Unfortunately, Takeda decided to terminate the development of TAK-875 due to concerns about liver toxicity.[22,23] It is widely believed that the hepatotoxicity is compound related since the expression of FFA1 is not found in the human liver.[10,24–26] Most current FFA1 agonists have somewhat high lipophilicity and molecular weight (red mark in Fig. 1), likely correlated with poor water-solubility, high toxicity, metabolic instability and associated with high risk of attrition in clinical test.[27–31] At present, many classic strategies to decrease lipophilicity have been extensively adopted by introducing hydrophilic group and scaffold hopping with polar skeleton.[28,32–34] With these perspectives in mind, our previous study identified a series of polar skeletons such as thiazole,[35] pyrrole,[36] oxime ether[37] and phenoxyacetamide linker[38] to improve the lipophilicity of TAK-875. The applications of click chemistry are increasingly found in every aspects of drug discovery, ranging from hit-to-lead finding by using combinatorial chemistry and target labeling, to proteomics or DNA research.[39–41] In this study, the hydrophilic triazole ring was introduced via click chemistry as a bioisostere of benzene ring to decrease the lipophilicity (Fig. 2). After systematic exploration of SAR, the compound **26** was discovered as an orally bioavailable lead compound with a considerable 21.4% and 14.2% decrease of blood glucose levels in normal ICR mice and type 2 diabetic C57BL/6 mice, respectively.

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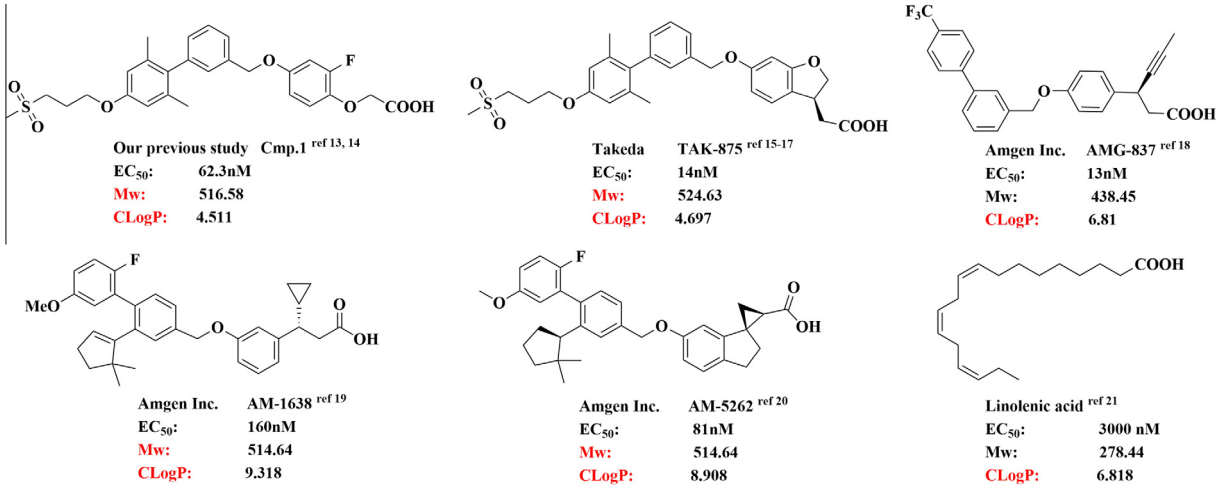


Figure 1. Selected examples of FFA1 agonists.

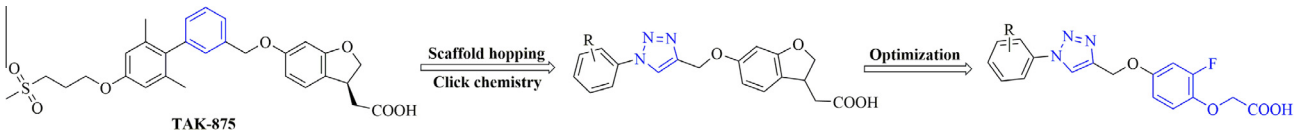


Figure 2. Our strategy to decrease the molecular weight and lipophilicity of TAK-875.

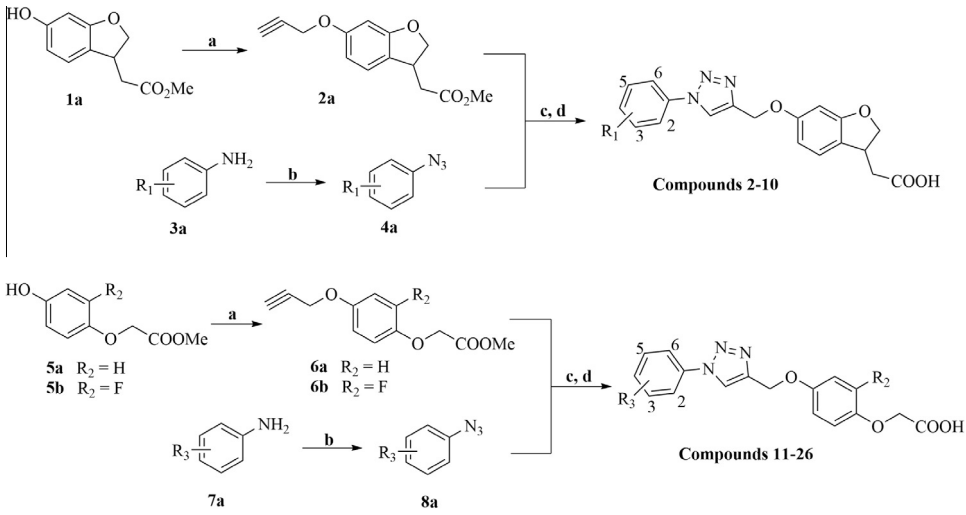
2. Results and discussion

2.1. Chemistry

The synthetic routes of target compounds **2**–**26** are summarized in Scheme 1. The intermediates **1a**, **5a** and **5b** were synthesized via published procedures.[13,15] The alkynyl compounds **2a**, **6a** and **6b** were derived from Williamson ether synthesis of starting material (**1a**, **5a** or **5b**) and propargyl bromide in the presence of K_2CO_3. Azidobenzenes **4a** or **8a** were obtained by treating commercially available anilines **3a** or **7a** with $NaNO_2$ followed by NaN_3 in 50% acetic acid. The intermediates **2a**, **6a** or **6b** reacted smoothly with azidobenzenes **4a** or **8a** at room temperature in the presence of catalytic amount of sodium ascorbate and copper sulfate in 80% methanol, followed by basic hydrolysis, afforded the target carboxylic acids **2**–**26**.

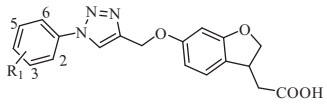
2.2. FFA1 agonistic activity and SAR study

The synthetic compounds were screened on human FFA1 by the fluorometric imaging plate reader (FLIPR) assay. Besides overall activity, the lipophilicity ($c\log P$ and $\log D_{7.4}$) and molecular weight were applied to evaluate the analogues. As shown in Table 1, the compound **2**, initially designed to decrease the lipophilicity ($c\log P = 2.70$), leads to a significant decrease in potency compared with TAK-875 ($c\log P = 4.69$). We speculated that the diminished affinity was associated with the need of enough lipophilicity for ligands to enter the binding pocket via the lipid bilayer.[42] A slight



Scheme 1. Synthesis of target compounds **2**–**26**. Reagents and conditions: (a) Propargyl bromide, K_2CO_3, DMF, rt, 12 h; (b) $NaNO_2$, 50% acetic acid, NaN_3, 0 °C, 2 h; (c) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, CH_3OH, rt, 24 h; (d) $LiOH{\cdot}H_2O$, THF/MeOH/H_2O, rt, 4 h.

Table 1
In vitro agonistic activities and selected parameters of target compounds



Compd.	R_1	Act% (100 nM)[a]	Mw	$c\log P$[b]	$\log D_{7.4}$[c]
TAK-875		65.32	524.63	4.697	2.43
2	H	0.76	351.36	2.702	ND
3	2-F	6.69	369.35	3.017	ND
4	2-Cl	9.34	385.80	3.587	ND
5	3-Cl	22.84	385.80	3.587	1.09
6	4-Cl	24.69	385.80	3.587	ND
7	3-OMe	19.36	381.39	3.182	ND
8	4-OBn	3.67	457.49	4.698	ND
9	2, 6-diCl	21.69	420.25	4.356	1.96
10	2-Me-5-Cl	33.82	399.83	4.337	2.08

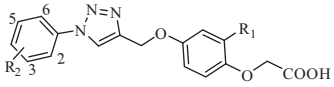
[a] Agonist activities mean values at a screening concentration of 100 nM were obtained from three independent experiments.
[b] $c\log P$ values were estimated with ChemDraw Ultra, version 12.0.
[c] $\log D_{7.4}$ values were determined by shake-flask procedure. ND = Not determined.

improvement in potency was obtained by adding an additional halogen in compound **2** to achieve compound **3** (2-F) and compound **4** (2-Cl). From the results of chlorine scan in the terminal benzene ring (compounds **4**–**6**), the *meta*-position and *para*-position seemed to be better than the *ortho*-position. Besides electron-withdrawing group, the electron-donating methoxy group (compound **7**) was also tolerated in *meta*-position. The bulkier benzyloxy group on the *para*-position (**8**), however, showed a markedly reduced potency, indicating that the introduction of steric group at the *para*-position of terminal phenyl is unfavorable. Borrowing elements of *ortho*-disubstituted FFA1 agonists, the obtained compound **9** exhibited a significantly enhanced affinity in comparison with the parent compound **4**. Interestingly, re-position the chlorine from 6-position to 5-position obtained the compound **10**, which revealed a marked improvement on potency compared to the compounds **5** and **9**, respectively.

These promising results prompted a comprehensive evaluation on our previously reported phenoxyacetic acid scaffold (Table 2).[13,14] The compound **11**, a hybrid structure containing both phenoxyacetic acid scaffold and the triazole moiety of compound **2**, turned out a drastic loss of activity as compound **2**. With the beneficial experience to increased potency using *meta*-substituent and *para*-substituent, the compounds **12**–**15** were synthesized and evaluated. The results indicated that the phenoxyacetic acid series (compound **14**) are slightly inferior to the dihydrobenzofuran series (compound **6**).

We have previously observed a robust improvement of potency in the phenoxyacetic acid series after introduction of 2-fluoro just as compound **1**.[13] The same effect was observed on the introduction of 2-fluoro at compounds **16**, **19** and **20**, which exhibited a marked improvement on potency in comparison with the corresponding compounds **11**, **14** and **15**. As the dihydrobenzofuran series, chlorine scan in the terminal benzene ring (compounds **17**–**19**) revealed that the agonistic activity of *para*-position > *meta*-position > *ortho*-position. The same effect was also observed upon methoxy substituted analogues (compounds **23** and **24**). Moreover,

Table 2
Agonistic activities and selected index of designed compounds



Compd.	R_1	R_2	Act% (100 nM)[a]	Mw	$c\log P$[b]	$\log D_{7.4}$[c]
TAK-875			65.32	524.63	4.697	2.43
11	H	H	0.64	325.32	2.753	ND
12	H	3-Me	11.85	339.35	3.253	ND
13	H	4-Me	16.37	339.35	3.253	0.89
14	H	4-Cl	19.42	359.77	3.638	ND
15	H	4-F	13.79	343.31	3.068	ND
16	F	H	5.83	343.31	2.768	-0.23
17	F	2-Cl	11.35	377.76	3.653	ND
18	F	3-Cl	22.71	377.76	3.653	ND
19	F	4-Cl	26.15	377.76	3.653	1.37
20	F	4-F	20.39	361.30	3.083	0.92
21	F	4-*t*Bu	4.53	399.42	4.594	ND
22	F	4-OEt	7.62	387.37	3.525	ND
23	F	2-OMe	14.61	373.34	2.996	ND
24	F	3-OMe	24.38	373.34	2.996	0.52
25	F	2,6-diCl	29.67	412.20	4.422	2.16
26	F	2-Me-5-Cl	36.15	391.78	4.152	1.95

[a] Agonist activities mean values at a screening concentration of 100 nM were obtained from three independent experiments.
[b] $c\log P$ values were estimated with ChemDraw Ultra, version 12.0.
[c] $\log D_{7.4}$ values were determined by shake-flask procedure. ND = Not determined.

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the bulkier groups on the *para*-position (**21** and **22**) may introduce unfavorable steric effect in the binding pocket. Unlike the phenoxyacetic acid series, the 2-fluoro analogues (compounds **16**–**19** and **24**–**26**) revealed a better activity than the corresponding dihydrobenzofuran series (compounds **2**, **4**–**7** and **9**–**10**). Among them, the optimal lead compound **26** showed a moderate lipophicity (Log$D_{7.4}$ = 1.95) and molecular weight (Mw = 391.78), which is still enough space for avoiding undue increase on lipophilicity and molecular size in the further optimization.

2.3. Molecular modeling study

To further understand the interaction mode of the triazole series, a docking study of compound **26** based on the X-ray structure of FFA1 (PDB accession code: 4PHU) was performed.[42] As shown in Figure 3, the compound **26** docked well to the same binding pocket for TAK-875. Just as TAK-875, an edge-on interaction was also formed between the residue Trp174 and the fluorobenzene ring of compound **26**. The head acid moiety, however, just form two hydrogen bonds with Tyr91 and Arg2258 (Fig. 3B), which explained the reason why the agonistic activity of compound **26** is inferior to TAK-875 (three hydrogen bonds). Moreover, the chlorine of terminal phenyl in compound **26** has a hydrophobic interaction with the residues Leu158 and Pro80. It was likely suggested that the hydrophobic interaction was crucial for the superior agonistic activity of compound **26** rather than other un-substituted compounds (such as **2**, **11** and **16**).

2.4. Effect of optimized compounds on OGTT

In order to select an orally bioavailable lead compound, the optimized compounds **6**, **10**, **19**, **24**, **25** and **26** (50 mg/kg) were selected for pharmacological evaluation in normal ICR mice by oral glucose tolerance test (OGTT). The time-dependent changes of plasma glucose levels and the area under the curve (AUC_{0-2h}) are shown in Figure 4. The hypoglycemic activities of the selected compounds were in accordance with the FFA1 agonistic activities. Among them, compounds **10**, **25** and **26** revealed a significant improvement in the glucose tolerance with a 16.8%, 17.7% and 21.4% reduction in glucose AUC_{0-2h}, respectively. The compound **26**, an orally bioavailable lead compound with the strongest agonistic activity and minimal lipophicity as well as molecular weight in the above three compounds, was selected for further pharmacological evaluation.

2.5. Hypoglycemic effects of 26 explored in type 2 diabetic mice

To assess glucose lowering effects of compound **26** in the diabetic state, an OGTT in STZ-induced type 2 diabetic C57BL/6 mice was performed.[43,44] As shown in Figure 5, the hyperglycemia was markedly controlled with a 14.2% decrease of glucose AUC_{0-2h} in compound **26** (50 mg/kg) treated mice despite the reduction was less than that of TAK-875 (20 mg/kg, −32.5% glucose AUC_{0-2h}). The promising results indicated the compound **26** holds potential for further optimization as an orally bioavailable lead compound.

3. Conclusion

With the purpose of hunting potent lead compound with reduced lipophilicity and molecular weight, we have identified a new series of triazole-based FFA1 agonists via click chemistry. Systematic exploration of SAR in the triazole scaffold leads to the identification of lead compound **26**, a potent FFA1 agonist with relatively low lipophicity (Log$D_{7.4}$ = 1.95) and molecular weight (Mw = 391.78) suitable for further optimization. Moreover, compound **26** showed a great potential for decreasing the plasma glucose levels in normal ICR mice (−21.4% of glucose AUC_{0-2h}) and type 2 diabetic C57BL/6 mice (−14.2% of glucose AUC_{0-2h}). Although the potency of lead compound **26** was inferior to TAK-875, the information obtained from our SAR and molecular modeling studies might help to design more competitive FFA1 agonists with distinct advantage in physicochemical property. Based on the promising lead compound **26**, a more targeted combinatorial chemical library using click chemistry will also provide more active candidates suitable for clinical development.

4. Experimental section

4.1. Chemistry

Chromatographic purification was performed on silica gel (200–300 mesh) and monitored by thin layer chromatography carried out on GF/UV 254 plates by using UV light (254 and 365 nm). Melting points were determined on a RY-1 melting-point apparatus and were not corrected. The NMR (nuclear magnetic resonance) spectra were recorded on a Bruker ACF-300Q instrument (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR spectra) with tetramethylsilane as an internal standard. Chemical shifts are given in parts per million (ppm), and coupling constants (*J* values) were given in hertz (Hz). Elemental analyses were carried out on the Heraeus CHN-O-Rapid

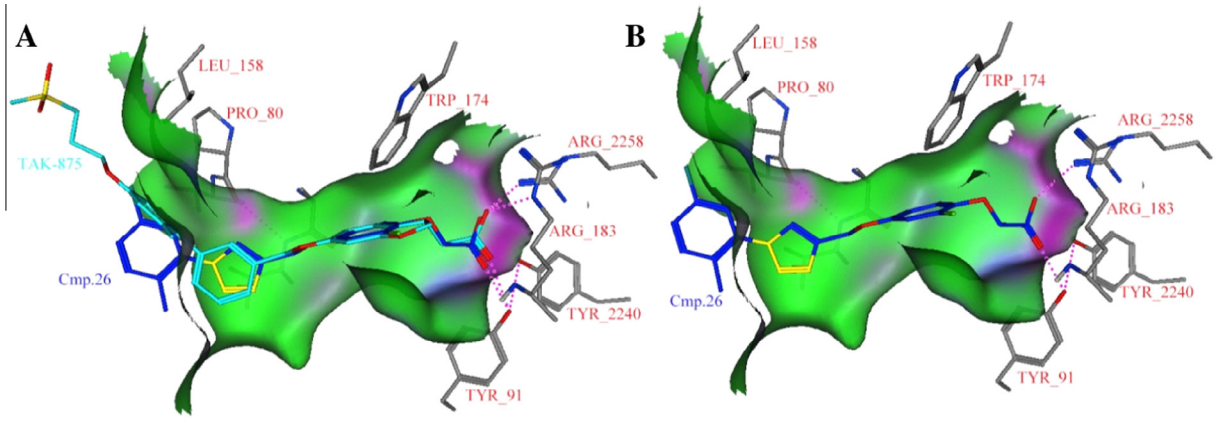

Figure 3. The interaction mode of compound **26** bound to FFA1. Key residues are labeled in red, and hydrogen bonding interactions are represented by purple dashed lines. (A) Overlay of TAK-875 and **26** (blue) bound to FFA1. (B) Compound **26** bound to FFA1.

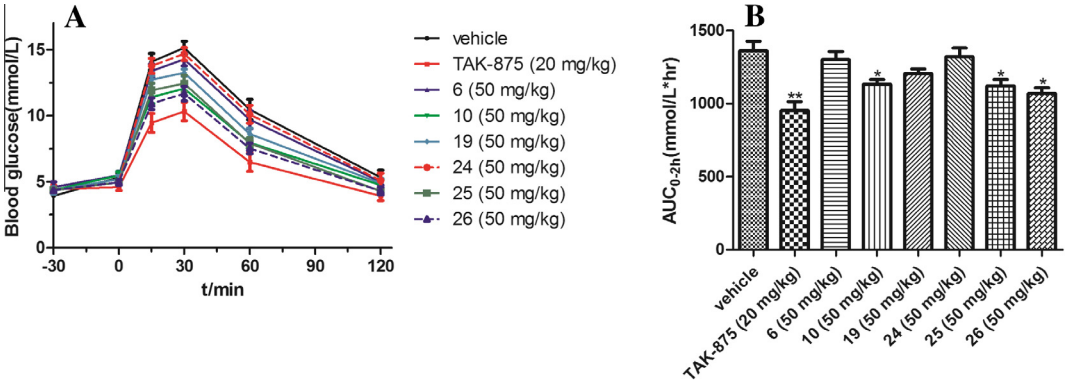

Figure 4. Effects of compounds on blood glucose levels during an OGTT in normal ICR mice. (A) Time dependent changes of plasma glucose levels. (B) The AUC_{0-2h} of blood glucose levels. Values are mean ± SEM ($n = 6$). $^{*}p \leqslant 0.05$, $^{**}p \leqslant 0.01$ compared to vehicle mice by Student's *t*-test.

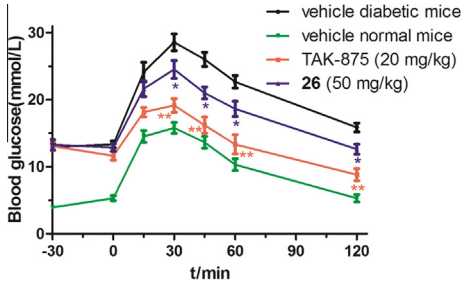

Figure 5. Effects of **26** on blood glucose levels during an OGTT in fasting type 2 diabetic C57BL/6 mice. Values are mean ± SEM ($n = 6$). $^{*}p \leqslant 0.05$ and $^{**}p \leqslant 0.01$ compared to vehicle diabetic mice by Student's t test.

analyzer and have errors within ±0.4% for CHN elements. The LC/MS spectra were run on the Waters liquid chromatography-mass spectrometer system (ESI). All starting materials and reagents were obtained from commercial suppliers and used without further purification. TAK-875 was synthesized via previous reported procedures.[15]

The physical characteristics, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis data for all intermediates and target compounds, were reported in the supporting information.

4.2. Determination of Log$D_{7.4}$

In 10 mL glass vial, 40 μL of 10 mM stock solution in DMSO was added 1980 μL phosphate buffer solution (0.01 M, pH = 7.4) and 1980 μL 1-octanol (Sigma), obtaining 100 μM final concentration of the test compounds. The glass vials were shaken at 700 rpm for 24 h and left for 1 h to allow the phases to separate. The 1-octanol phase was pipetted out and diluted ×10 with a mixture of methanol (containing 0.1% formic acid) and MilliQ H_2O (4:1) prior to analysis on HPLC with 60 μL injections. The buffer phase was analyzed directly in 120 μL injections. Each HPLC analysis was performed in duplicates by the method described above. The Log$D_{7.4}$ values were calculated by dividing the peak area (mAU*min) at 254 nm of the 1-octanol phase by the corresponding peak area of the buffer phase. Peak areas were corrected for systematic errors using two calibration points per compound per solvent. All test compounds were analyzed in three independent experiments.

4.3. Molecular modeling

The molecular docking study of compound **26** was performed by using MOE (version 2008.10, The Chemical Computing Group, Montreal, Canada). The crystal structure of FFA1 (PDB accession code: 4PHU) was retrieved from Protein Data Bank. Prior to docking, water molecules and ligands were deleted except TAK-875. Then, the X-ray crystal structure was prepared with Protonate 3D and a Gaussian Contact surface was draw around the binding site of TAK-875. Then, the active site was isolated and the backbone was removed. The ligand poses were filtered by using Pharmacophore Query Editor. The structure of compound **26** was docked into the binding pocket with Pharmacophore method and then ranked with the London dG scoring function. For the energy minimization of ligand in the active site, MOE Forcefield Refinement was used and ranked with London dG scoring function.

4.4. Biological methods

4.4.1. Ca^{2+} influx activity of CHO cells stably expressing human FFA1 (FLIPR assay)

CHO cells stably expressing human FFA1 (accession no. NM_005303) were plated at a density of 15 K cells/well and incubated 12 h in 5% CO_2 at 37 °C. Subsequently, culture medium was removed and washed with Hank's Balanced Salt Solution (100 μL). Then, cells were incubated in loading buffer (recording medium containing 2.5 μg/mL fluorescent calcium indicator Fluo 4-AM, 0.1% fatty acid-free BSA and 2.5 mmol/L probenecid) for 1 h at 37 °C. Various concentrations of test compounds or γ-linolenic acid (Sigma) were added into the cells, and the intracellular Ca^{2+} flux signals after addition were monitored by FLIPR Tetra system (Molecular Devices) for 90 s. The agonistic activities of test compounds on human FFA1 were expressed as [(A−B)/(C−B)] × 100 (increase of the intracellular calcium concentration (A) in the test compounds-treated cells and (B) in vehicle-treated cells, and (C) in 10 μM γ-linolenic acid-treated cells).

4.4.2. Animals and statistical analysis of the data

Male ICR mice (18–22 g) and male C57BL/6 mice (18–22 g) were obtained from Comparative Medicine Centre of Yangzhou University (Jiangsu, China). Mice were acclimatized for 1 week before experiments, and allowed ad libitum access to standard pellets and water unless otherwise stated. The feeding room was maintained on a constant 12 h light/black cycle with controlled temperature (23 ± 1 °C) and humidity (55 ± 5%). The vehicle was oral administrate 0.5% Caboxy Methyl Cellulose aqueous solution for all animal studies. All animal experiments were performed in compliance with the relevant laws and institutional guidelines, and our experiments have been approved by the institutional committee of China Pharmaceutical University.

Statistical analyses were obtained with GraphPad software (GraphPad InStat version 5.00, San Diego, CA, USA). Unpaired comparisons were analyzed using the two-tailed Student's *t*-test.

4.4.2.1. Effect of compounds on OGTT explored in ICR mice. Ten-week-old male ICR mice were fasted 12 h, weighted, bled via the tail vein, and randomized into eight groups ($n = 6$). A single doses of vehicle, TAK-875 (20 mg·kg^{-1}) or selected compounds (50 mg·kg^{-1}) were orally administered 30 min before oral glucose load (3 g·kg^{-1}). Blood samples were collected from the tail vein immediately before drug administration (−30 min), 0 min (just before glucose load), and at 15, 30, 45, 60 and 120 min after glucose load. The blood glucose levels were measured by using blood glucose test strips (SanNuo ChangSha, China).

4.4.2.2. Hypoglycemic effects of compound 26 explored in type 2 diabetic mice. Male C57BL/6 mice after 7 days adaptation were fed with high-fat diet (45% calories from fat, from Medi-science Ltd., Yangzhou, China) ad libitum for further 4 weeks to induce insulin resistance and then injected intraperitoneally (i.p.) with low dose of STZ (10 mL·kg^{-1}; 80 mg·kg^{-1}). The C57BL/6 mice were fed with high-fat diet for another 4 weeks, and mice with fasting blood glucose level ⩾11.1 mmol/L were used as type 2 diabetic mice model.[43,44]

Type 2 diabetic C57BL/6 mice were fasted 12 h, weighted, bled via the tail vein, and randomized into 3 groups ($n = 6$), another group of normal fasting C57BL/6 mice was added as negative control. A single doses of TAK-875 (20 mg·kg^{-1}), vehicle or compound **26** (50 mg·kg^{-1}) were orally administered 30 min before oral glucose load (2 g·kg^{-1}). Blood samples were collected from the tail vein immediately before drug administration (−30 min), 0 min (just before glucose load), and at 15, 30, 45, 60 and 120 min after glucose load. The blood glucose levels were measured by using blood glucose test strips (SanNuo ChangSha, China).

Conflict of interest

The authors have no conflicts of interest to declare.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.08.068.

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