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Design, synthesis and structure–activity relationship studies of novel phenoxyacetamide-based free fatty acid receptor 1 agonists for the treatment of type 2 diabetes

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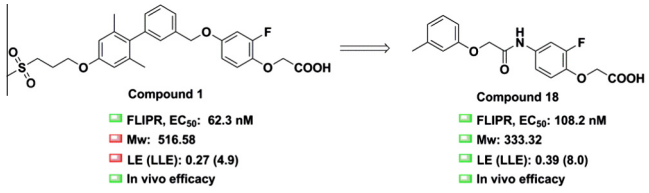
ABSTRACT



The free fatty acid receptor 1 (FFA1) has attracted extensive attention as a novel antidiabetic target in the last decade. Several FFA1 agonists reported in the literature have been suffered from relatively high molecular weight and lipophilicity. We have previously reported the FFA1 agonist **1**. Based on the common amide structural characteristic of SAR1 and NIH screened compound, we here describe the continued structure–activity exploration to decrease the molecular weight and lipophilicity of the compound **1** series by converting various amide linkers. All of these efforts lead to the discovery of the preferable lead compound **18**, a compound with considerable agonistic activity, high LE and LLE values, lower lipophilicity than previously reported agonists, and appreciable efficacy on glucose tolerance in both normal and type 2 diabetic mice.

1. Introduction

The increasing and alarming prevalence of type 2 diabetes mellitus (T2DM) along with the undesirable side effects (such as body weight gain, gastric symptoms, risk of hypoglycemia, etc.) associated with many oral antidiabetic agents has promoted a great development in evaluating novel targets to achieve preferable hypoglycemic drugs.[1–4] The free fatty acid receptor 1 (FFA1, also known as GPR40), the prominent ones of novel antidiabetic targets in the last decade, play a key role in amplifying glucose-stimulated insulin secretion (GSIS) on pancreatic β-cells but does not affect insulin secretion at low blood glucose levels.[5–8] Therefore, this particular mechanism of FFA1 provides the tremendous potential for boosting insulin levels without the risk of hypoglycemia.

Recently, a number of synthetic FFA1 agonists contained acidic moieties have been reported in the literature (Fig. 1),[9–17] and the compounds TAK-875, AMG-837 and LY2881835 were in clinical trials for treatment of T2DM. However, many of these agonists have relatively high molecular weight and lipophilicity (red mark in Fig. 1), which most likely associated with poor water-solubility, high promiscuity, strong metabolic toxicity, and correlated with a high risk of attrition in clinical trials.[18–22] Studies have suggested

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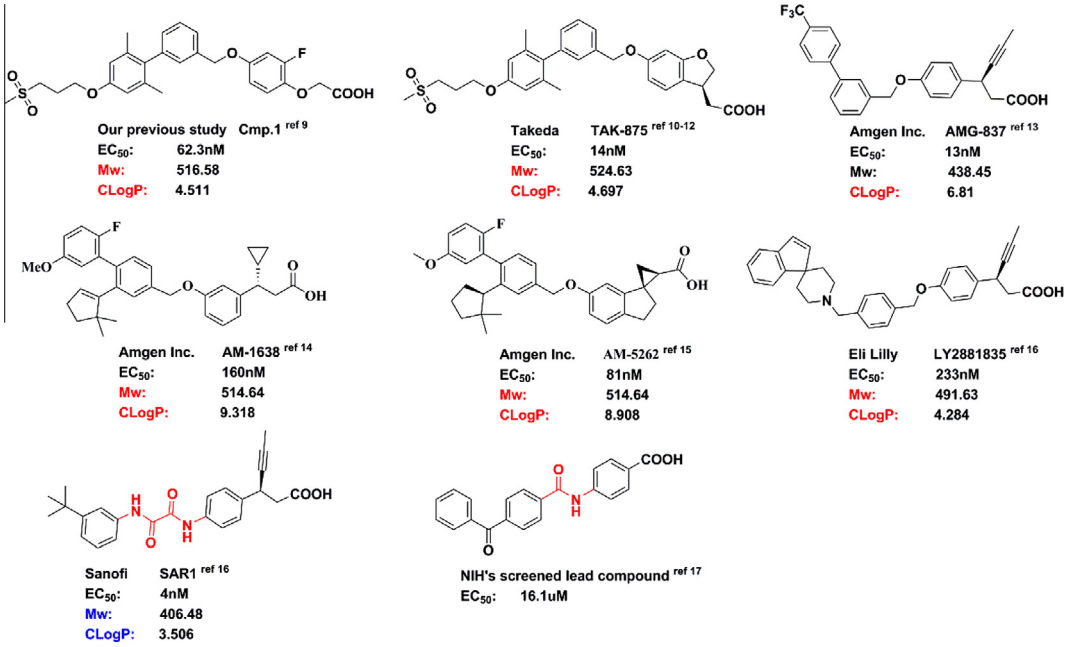


Figure 1. Selected examples of synthetic GPR40 agonists. *c*log*P* values are calculated with ChemDraw Ultra 12.0 using the '*c*log*P*' option.

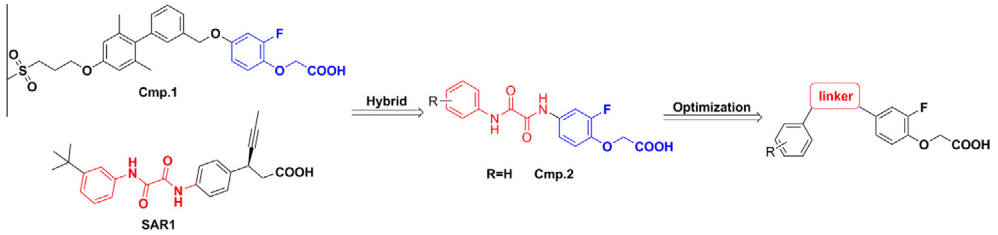


Figure 2. Our strategy to decrease the molecular weight and lipophilicity of compound **1**.

that *c*log*P* values should not exceed 4–5,[18,23] and concepts such as ligand efficiency (LE) and ligand lipophilicity efficiency (LLE) have been recommended to direct the optimization process.[24,25] Inspired by the relatively low molecular weight and lipophilicity of SAR1, we first designed and synthesized compound **2**, which was a hybrid structure containing both the oxalamide moiety of SAR1 and phenoxyacetic acid structure of our previously reported compound **1** (Fig. 2). However, the compound **2** and its analogs appeared to diminish the in vitro agonistic activity, indicating that the interaction mode of compound **2** series was different from SAR1. Subsequently, a series of amide linkers were designed based on the common amide structural characteristic of SAR1 and NIH screened compound. Among them, the most potent amide linker phenoxyacetamide was selected to systematically explore the SAR, which lead to the identification of lead compound **18**, a compound with considerable agonistic activity, high LE and LLE values, lower lipophilicity than previously reported agonists, and appreciable antihyperglycemic effect in both normal and type 2 diabetic mice.

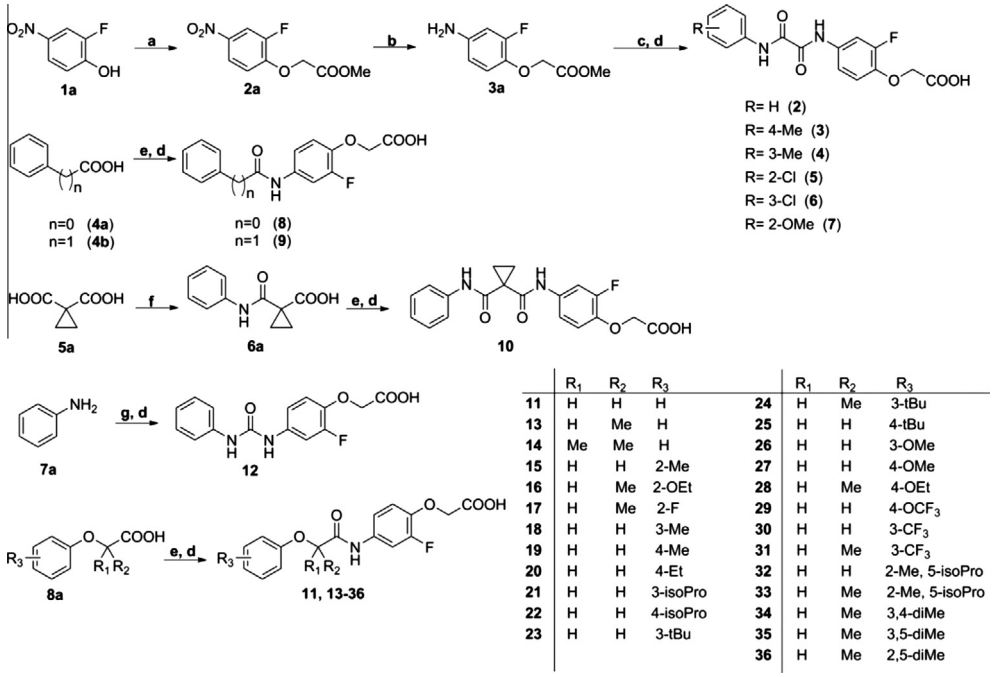
2. Results and discussion

2.1. Chemistry

The synthetic routes of target compounds **2**–**36** are summarized in Scheme 1. The key intermediate **3a** was prepared by the reduction of nitrobenzene **2a**, which was derived from the substitution of commercially available phenol **1a** with methyl chloroacetate in the presence of K_2CO_3. The intermediate **3a** was treated with various substituted anilines and oxalyl chloride, followed by basic hydrolysis, afforded the desired carboxylic acids **2**–**7**. Acylation of the intermediate **3a** with corresponding acyl chloride, formed from commercially available carboxylic acid **4a** or **4b** with oxalyl chloride catalyzed by DMF catalyze, generated the desired esters, which were isolated pure from ethanol. Hydrolysis of esters with lithium hydroxide provided the designed compounds **8** and **9** in high yield. Mono-acylation of the cyclopropane-1,1-dicarboxylic acid with aniline to afford compound **6a**, which was subsequently converted to compounds **10** by acylation with intermediate **3a** and esterolysis. The desired compound **12** was obtained from aniline and compound **3a** in the presence of triphosgene and Et_3N, followed by hydrolysis. The target compounds **11** and **13**–**36** were synthesized from the starting material various substituted phenoxyacetic acid according to the method for the synthesis of compound **8**.

2.2. FFA1 agonistic activity and SAR study

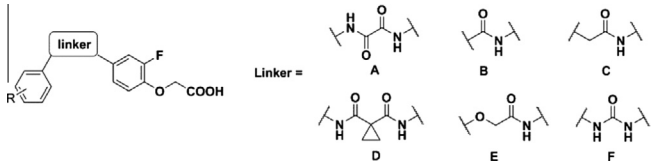
Inspired by the low molecular weight and lipophilicity of SAR1, we first designed and synthesized compound **2**, a hybrid structure containing both the oxalamide moiety of SAR1 and phenoxyacetic acid structure of compound **1**. The compound **2**, however, appeared to diminish the FFA1 agonistic activity compared with the parent compound **1** (Table 1). The introduction of various substituents in compound **2** to give compounds **3**–**7** did not appear to



	R_1	R_2	R_3		R_1	R_2	R_3
11	H	H	H	**24**	H	Me	3-tBu
13	H	Me	H	**25**	H	H	4-tBu
14	Me	Me	H	**26**	H	H	3-OMe
15	H	H	2-Me	**27**	H	H	4-OMe
16	H	Me	2-OEt	**28**	H	Me	4-OEt
17	H	Me	2-F	**29**	H	H	4-OCF_3
18	H	H	3-Me	**30**	H	H	3-CF_3
19	H	H	4-Me	**31**	H	Me	3-CF_3
20	H	H	4-Et	**32**	H	H	2-Me, 5-isoPro
21	H	H	3-isoPro	**33**	H	Me	2-Me, 5-isoPro
22	H	H	4-isoPro	**34**	H	Me	3,4-diMe
23	H	H	3-tBu	**35**	H	Me	3,5-diMe
				36	H	Me	2,5-diMe

Scheme 1. Synthesis of target compounds **2** to **36**. Reagents and conditions: (a) methyl chloroacetate, K_2CO_3, acetonitrile, K_i, reflux, 6 h; (b) H_2, Pd–C, rt, 18 h; (c) various substituted anilines, oxalyl chloride, then **3a**, acetonitrile; (d) LiOH·H_2O, THF/MeOH/H_2O, rt, 4 h; (e) oxalyl chloride, dichloromethane, then **3a**, Et_3N; (f) aniline, $SOCl_2$, Et_3N, THF, 0 °C to rt, 3 h; (g) aniline, triphosgene, Et_3N, then **3a**.

Table 1
In vitro activities and select physicochemical properties of target compounds



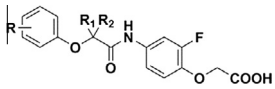
Compd	R	Linker	Act %[a] (300 nM)	Act %[b] (100 nM)	Mw	*c*log*P*[c]
TAK-875			76.01	65.32	524.63	4.697
1			67.32	54.94	516.58	4.511
2	H	A	26.54	9.78	332.29	0.761
3	4-Me	A	29.86	11.35	346.31	1.259
4	3-Me	A	36.27	17.65	346.31	1.259
5	2-Cl	A	27.89	10.23	366.73	1.474
6	3-Cl	A	33.67	13.28	366.73	1.474
7	2-OMe	A	19.85	6.78	362.31	0.679
8	H	B	12.78	3.56	289.26	2.154
9	H	C	39.57	19.87	303.29	2.203
10	H	D	34.17	13.56	372.35	1.824
11	H	E	58.68	29.75	319.29	2.182
12	H	F	23.69	8.47	304.28	2.569

[a] Agonist activities mean values at a screening concentration of 300 nM were obtained from three independent experiments.
[b] Agonist activities mean values at a screening concentration of 100 nM were obtained from three independent experiments.
[c] *c*log*P* values were estimated with ChemDraw Ultra, version 12.0.

have a significant improvement on potency (though a slight improvement was observed in *meta*-substituted compounds **4** and **6**), indicating that the interaction mode of compound **2** series was different from SAR1. Therefore, we focused our redesign efforts on exploring optimal amide linker based on the common amide structural characteristic of SAR1 and NIH screened compound. The compound **8**, directly structural analog of NIH screened compound, led to 2-fold erosion of potency at 300 nM concentration compared with the compound **2**, which likely suggesting that a planar template might be unfavorable. Subsequently, a methylene was attached in the amide linker of compound **8** to reduce the planarity of molecule, and the resulting compound **9** indeed obtained an approximately 3-fold increase in potency over the compound **8**. The encouraging result prompted us to reevaluate the compound **10**, a more flexible analog of compound **2**. The compound **10** revealed a slight improvement on potency compared to the parent compound **2** but still deviate the desired effect. Gratifyingly, the stretched compound **11** (phenoxyacetamide linker), with relatively low molecular weight and lipophilicity, exhibited a marked improvement on potency in comparison with the corresponding compound **9**. This result demonstrated that a more appropriate conformation was induced by topological structure of phenoxyacetamide linker. The urea derivative **12**, designed as a constrained planar analog of compound **9**, turned out almost an order of magnitude less active but still indicates that the receptor binding pocket can accommodate the conformation with the terminal phenyl extended in the plane of urea.

Based on these results above, we therefore selected this linker of the most potent compound **11** as our starting point for further modification. Besides the agonistic activity, the LE and log $D_{7.4}$, as well as LLE were taken advantage of in the evaluation of derivatives. As shown in Table 2, methyl substituent on the phenoxyacetamide linker (**13**) resulted in further improvement, likely attributed to the hydrophobic interaction of methyl group. On the whole, the compound **13** series shared almost the same SAR with the compound **11** series (**11** vs **13**, **23** vs **24**, **30** vs **31** and **32** vs **33**). Interestingly, the introduction of gem-dimethyl group (**14**), however, showed a drastic loss of activity despite increased lipophilicity, suggesting that the limited space in the binding pocket around this area. Then, an initial optimization of the terminal phenyl with methyl group (**15**, **18**, and **19**) demonstrated that substitution is tolerated in all positions and preferred in the *meta*-substituent. Further modification in the *meta*-position suggested that the bulkier substitution also tolerated in this position (**21**, **23** and **26**), whereas the

Table 2
In vitro activities and physicochemical properties of designed compounds



Compd	R	R_1	R_2	Act %[a] (100 nM)	EC_{50}[b] (nM)	*c*log*P*[c]	Log$D_{7.4}$[d]	LE (LLE)[e]
TAK-875				65.32	29.6	4.697	2.43	0.27 (5.1)
1				54.94	62.3	4.511	2.31	0.27 (4.9)
11	H	H	H	29.75	ND	2.182	−1.25	
13	H	Me	H	35.67	135.3	2.491	−0.18	0.38 (7.0)
14	H	Me	Me	3.56	ND	2.801	ND	
15	2-Me	H	H	20.78	ND	2.681	ND	
16	2-OEt	Me	H	8.79	ND	2.759	ND	
17	2-F	Me	H	35.07	135.9	2.574	0.07	0.37 (6.8)
18	3-Me	H	H	45.37	108.2	2.681	−0.99	0.39 (8.0)
19	4-Me	H	H	31.23	148.2	2.681	ND	
20	4-Et	H	H	14.59	ND	3.210	ND	
21	3-Isopro	H	H	43.28	115.6	3.609	0.15	0.36 (6.7)
22	4-Isopro	H	H	11.83	ND	3.609	ND	
23	3-*t*Bu	H	H	40.19	121.7	4.008	0.35	0.34 (6.5)
24	3-*t*Bu	Me	H	32.76	140.3	4.317	0.63	0.33 (6.2)
25	4-*t*Bu	H	H	7.65	ND	4.008	ND	
26	3-OMe	H	H	31.69	147.6	2.271	ND	
27	4-OMe	H	H	5.73	ND	2.271	ND	
28	4-OEt	Me	H	3.56	ND	3.109	ND	
29	4-OCF_3	H	H	6.45	ND	3.380	ND	
30	3-CF_3	H	H	11.35	ND	3.310	ND	
31	3-CF_3	Me	H	12.73	ND	3.619	ND	
32	2-Me, 5-Isopro	H	H	14.56	ND	3.708	0.47	
33	2-Me, 5-Isopro	Me	H	19.38	ND	4.017	ND	
34	3,4-DiMe	Me	H	36.75	132.9	3.439	0.33	0.37 (6.5)
35	3,5-DiMe	Me	H	25.67	ND	3.489	ND	
36	2,5-DiMe	Me	H	18.65	ND	3.489	ND	

ND = Not determined.
[a] Agonist activities mean 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 independent determinations.
[c] *c*log*P* values were estimated with ChemDraw Ultra, version 12.0.
[d] Log$D_{7.4}$ values were determined by shake-flask procedure.
[e] LE values were calculated by $-\Delta g = RT \ln K_d$, presuming $EC_{50} \approx K_d$, and the LLE values were calculated by the formula $pEC_{50} - \log D_{7.4}$.

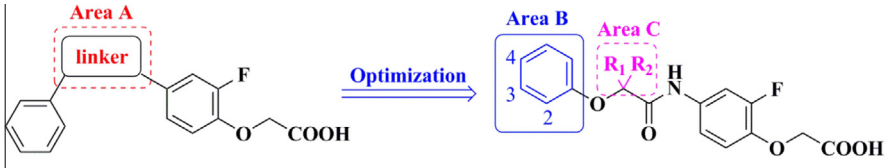
electron-withdrawing substituent such as 3-CF_3 (**30** and **31**) indicated that the electron-withdrawing effect in the area might reduce agonistic activity of FFA1. To complete this SAR study, various substitutions in the *ortho*-position and *para*-position of terminal phenyl were evaluated. For the *ortho*-substituted compounds, the agonistic activity of **13** (2-H) > **17** (2-F) > **15** (2-Me) > **16** (2-OEt) suggested that the steric effect in the *ortho*-position might influence agonistic activity of FFA1. The *para*-substituted compounds, however, revealed dual effects of the steric and electrical property. The agonistic activity of **19** (4-Me) > **20** (4-Et) > **22** (4-isopro) > **25** (4-*t*Bu), showed a good correlation with the Van der Waals radius of substitution. This result demonstrated that the large substitution in this position may be undesirable. Meanwhile, strong electron-donating group such as 4-OMe (**27**), 4-OEt (**28**) and 4-OCF_3 (**29**) occupied in the *para*-position turned out a significant decrease of activity, implying that the electrical effect in the *para*-position was also crucial to agonistic activity.

With this beneficial experience for *meta*-substituent resulted in increased potency, a series of di-substituted compounds containing *meta*-substituents were synthesized and evaluated. Unfortunately, all of the synthetically di-substituted compounds **32**–**36** showed a lower potency than the corresponding mono-substituted analogs. Interestingly, although methyl substituent in the *meta*-position exhibited the best activity, the *meta*-disubstituted compound **35** revealed a markedly lower agonistic activity than the corresponding monomethyl analog **18**. These results implied that the polysubstituted compounds may introduce an unfavorable steric interaction with the ligand-binding pocket of FFA1. Among all of the tested compounds, the compound **18**, a most potent agonist in this series, had a significant advantage compared to TAK-875 and compound **1** in terms of LE and LLE values. After comprehensive investigation of SAR, a clear SAR picture of our chemical scaffold was developed and summarized in Figure 3.

2.3. Effect of compound 18 on glucose tolerance

Based on these results above, the most potent compound **18** (20, 50 and 80 mg/kg) was selected to evaluate the in vivo hypoglycemic effects in normal ICR mice by oral glucose tolerance test (OGTT). As shown in Figure 4**A**, compound **18** exhibited a dose-dependent response in blood glucose levels. Moreover, the compound **18** showed a significant improvement in glucose tolerance at 80 mg/kg dose, similar to the hypoglycemic effect of TAK-875 (20 mg/kg), the most advanced compound once in phase III studies.

To further assess hypoglycemic effects in the diabetic state, STZ-induced type 2 diabetic C57BL/6 mice were used to evaluate the OGTT of compound **18**, an orally bioavailable FFA1 agonist. As shown in Figure 4**B**, the compound **18** was significantly improved the hyperglycemia state of type 2 diabetic mice. These results demonstrated that compound **18**, with a significant advantage in terms of physicochemical property, has a great potential for improving the hyperglycemia state in both normal and type 2 diabetic mice.



Area A: The central linkers are crucial to generate appropriate conformation induced by the specific effect of topological structure. Planar template linkers are unfavorable in this area. Among various amide linker, the flexibled phenoxyacetamide linker exhibits a best potency.

Area B: This region has a direct hydrophobic interaction with the ligand-binding pocket of FFA1, even a slight modification in this area may also lead to significant changes of agonistic activity. The concrete influence of different substituents are described in the following sections.

2-position: The steric effect of substituents in the ortho-position is sensitive to agonistic activity. Increasing the steric bulk of ortho-substituents result in dramaticlly reduced potency (H > F > Me > OEt).

3-position: The preferred site in all positions. The bulkier substitutions are tolerated in the meta-position, but the electron-withdrawing effect in the area reduce the agonistic activity of FFA1. The polysubstituted compounds reveal a markedly lower agonistic activity than the corresponding mono-substituted analogue.

4-position: Both the steric and electronic nature of substiuents have a significant effect on the activity. As the Van der Waals radius of substitutions increase the potency decrease (Me > Et > i-Pr > t-Bu), and strong electron-donating groups turn out a significant decrease of activity.

Area C: The introduction of gem-dimethyl group shows a drastic loss of activity. Monomethyl and unsubstituted compounds share almost the same SAR.

Figure 3. SAR summary.

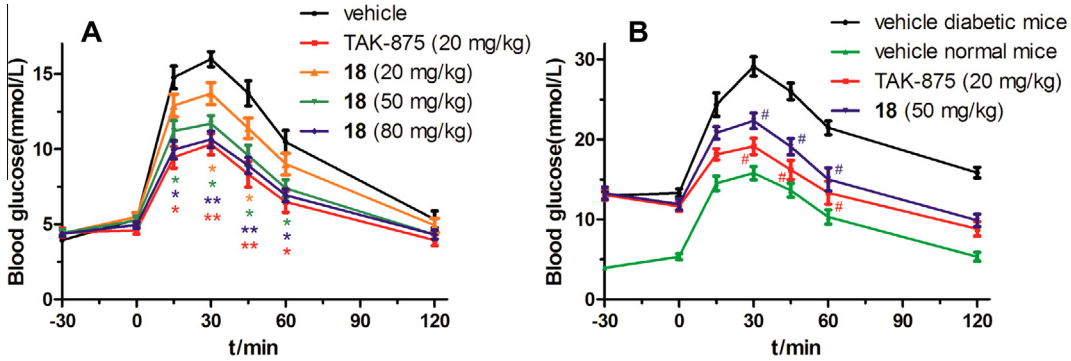


Figure 4. (A) Effect of compound **18** on glucose tolerance in normal ICR mice. (B) OGTT in fasting type 2 diabetic C57BL/6 mice. Values are mean ± SEM ($n = 6$). $^{*}P \leqslant 0.05$ and $^{**}P \leqslant 0.01$ compared to vehicle mice by Student's t test. $^{\#}P \leqslant 0.05$ compared to vehicle diabetic mice by Student's t test.

3. Conclusion

With the aim of developing potent FFA1 agonists with reduced molecular weight and lipophilicity, we have identified a new series of phenoxyacetamide FFA1 agonists by comprehensive evaluating 6 amide linkers based on the common amide structural characteristic of SAR1 and NIH screened compound. Subsequently, systematic exploration of SAR in this series, leading to the identification of lead compound **18**, an excellent FFA1 agonist with considerable agonistic activity, high LE and LLE values, lower lipophilicity than previously reported agonists, and appreciable hypoglycemic effect in both normal and type 2 diabetic mice. Although the agonistic activity of compound **18** was inferior to TAK-875, the information obtained from the SAR studies allowed us to design more active FFA1 agonists with distinct advantage in LE and LLE values.

4. Experimental section

4.1. Chemistry

All starting materials, solvents and reagents were obtained from commercial sources and used without further purification unless otherwise indicated. Column chromatography was carried out on silica gel (200–300 mesh) and monitored by thin layer chromatography performed on GF/UV 254 plates and were visualized by using UV light at 254 and 365 nm. Melting points were measured using a RY-1 melting-point apparatus, which was uncorrected. All of the 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 relative to tetramethylsilane as internal standard, and coupling constants (J values) were given in hertz (Hz). LC/MS spectra were recorded on a Waters liquid chromatography-mass spectrometer system (ESI). Elemental analyses were performed by the Heraeus CHN-O-Rapid analyzer. TAK-875 was synthesized via published procedures.[10]

The physical characteristics, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis data for all intermediates and target molecules, 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. Biological methods

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

CHO cells stably expressing human FFA1 (accession no. NM_005303) were seeded into 96-well plates at a density of 15 K cells/well and incubated 16 h in 5% CO_2 at 37 °C. Then, the culture medium was removed and washed with 100 μL of Hank's Balanced Salt Solution. Subsequently, cells were incubated in loading buffer (containing 2.5 μg/mL fluorescent calcium indicator Fluo 4-AM, 2.5 mmol/L probenecid and 0.1% fatty acid-free BSA) for 1 h at 37 °C. Various concentrations of test compounds or γ-linolenic acid (Sigma) were added into the well and the intracellular calcium flux signals were measured by FLIPR Tetra system (Molecular Devices). The agonistic activities of test compounds on human FFA1 were expressed as $[(A - B)/(C - B)] \times 100$ (increase of the intracellular Ca^{2+} concentration (A) in the test compounds-treated cells and (B) in vehicle-treated cells, and (C) in 10 μM γ-linolenic acid-treated cells). EC_{50} value of selected compound was obtained with Prism 5 software (GraphPad).

4.3.2. Animals and statistical analysis of the data

Male ICR mice (18–22 g) and male C57BL/6 mice (18–22 g) were purchased from Comparative Medicine Centre of Yangzhou University (Jiangsu, China), acclimatized for 1 week before experiments. The breeding room was keep on a constant 12 h light/black cycle with temperature at 23 ± 2 °C and relative humidity 50 ± 10% throughout the experimental period. Mice were allowed ad libitum access to standard pellets and water unless otherwise stated, and the vehicle used for drug administration was 0.5% Carboxy 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 performed using specific software (GraphPad InStat version 5.00, GraphPad software, San Diego, CA, USA). Unpaired comparisons were analyzed using the two-tailed Student's t-test, unless otherwise stated.

4.3.2.1. Effect of compound 18 on glucose tolerance explored in male ICR mice. Normal ICR mice 10 weeks old were fasted overnight (12 h), weighted, bled *via* the tail tip, and randomized into 5 groups (n = 6). Mice were administrated orally with a single doses of vehicle, TAK-875 (10 mL kg^{-1}; 20 mg kg^{-1}), or compound **18** (20 mg kg^{-1}, 50 mg kg^{-1}, 80 mg kg^{-1}) and subsequently dosed orally with 30% glucose aqueous solution (3 g kg^{-1}) after half an hour. Blood samples were collected immediately before drug administration (−30 min), before glucose challenge (0 min), and at 15, 30, 45, 60 and 120 min post-dose. The blood glucose was measured by blood glucose test strips (SanNuo ChangSha, China).

4.3.2.2. Hypoglycemic effects of compound 18 explored in type 2 diabetic mice. Male C57BL/6 mice after 1 week adaptation were fed with high-fat diet (45% calories from fat, from Medi-science Ltd, Yangzhou, China) ad libitum for 4 weeks to induce insulin resistance and then injected intraperitoneally (ip) with low dose of STZ (10 mL kg^{-1}; 80 mg kg^{-1}). The mice were fed with high-fat-diet for another 4 weeks, and the development of diabetes was confirmed by measuring blood glucose levels. The mice with fasting blood glucose level 11.1 mmol/L or higher were considered to be diabetic and were used in the experiment as type 2 diabetic mice model.[26,27]

Type 2 diabetic C57BL/6 mice were fasted overnight (12 h), weighted, bled via the tail tip, and randomized into 3 groups (n = 6), another group of normal fasting C57BL/6 mice was added as negative control. Mice were administrated orally with a single doses of vehicle, TAK-875 (10 mL kg^{-1}; 20 mg kg^{-1}), or compound **18** (10 mL kg^{-1}; 50 mg kg^{-1}) and subsequently dosed orally with 20% glucose aqueous solution (2 g kg^{-1}) after half an hour. Blood samples were collected immediately before drug administration (−30 min), before glucose challenge (0 min), and at 15, 30, 45, 60 and 120 min post-dose. The blood glucose was measured by blood glucose test strips (SanNuo ChangSha, China).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.09.010.

References and notes

1. Phung, O. J.; Scholle, J. M.; Talwar, M.; Coleman, C. I. *JAMA* **2010**, *303*, 1410.
2. Avery, M. A.; Mizuno, C. S.; Chittiboyina, A. G.; Kurtz, T. W.; Pershadsingh, H. A. *Curr. Med. Chem.* **2008**, *15*, 61.
3. Stein, S. A.; Lamos, E. M.; Davis, S. N. *Expert Opin. Drug Saf.* **2013**, *12*, 153.
4. Lebovitz, H. E. *Nat. Rev. Endocrinol.* **2011**, *7*, 408.
5. Itoh, Y.; Kawamata, Y.; Harada, M.; Kobayashi, M.; Fujii, R.; Fukusumi, S.; Ogi, K.; Hosoya, M.; Tanaka, Y.; Uejima, H.; Tanaka, H.; Maruyama, M.; Satoh, R.; Okubo, S.; Kizawa, H.; Komatsu, H.; Matsumura, F.; Noguchi, Y.; Shinobara, T.; Hinuma, S.; Fujisawa, Y.; Fujino, M. *Nature* **2003**, *422*, 173.
6. Briscoe, C. P.; Tadayyon, M.; Andrews, J. L.; Benson, W. G.; Chambers, J. K.; Eilert, M. M.; Ellis, C.; Elshourbagy, N. A.; Goetz, A. S.; Minnick, D. T.; Murdock, P. R.; Sauls, H. R.; Shabon, U.; Spinage, L. D.; Strum, J. C.; Szekeres, P. G.; Tan, K. B.; Way, J. M.; Ignar, D. M.; Wilson, S.; Muir, A. I. *J. Biol. Chem.* **2003**, *278*, 11303.
7. Stoddart, L. A.; Smith, N. J.; Milligan, G. *Pharmacol. Rev.* **2008**, *60*, 405.
8. Wellendorph, P.; Johansen, L. D.; Bräuner-Osborne, H. *Mol. Pharmacol.* **2009**, *76*, 453.
9. Wang, X.; Zhao, T.; Yang, B.; Li, Z.; Cui, J.; Dai, Y.; Qiu, Q.; Qiang, H.; Huang, W.; Qian, H. *Bioorg. Med. Chem.* **2015**, *23*, 132.
10. Negoro, N.; Sasaki, S.; Mikami, S.; Ito, M.; Suzuki, M.; Tsujihata, Y.; Ito, R.; Harada, A.; Takeuchi, K.; Suzuki, N.; Miyazaki, J.; Santou, T.; Odani, T.; Kanzaki, N.; Funami, M.; Tanaka, T.; Kogame, A.; Matsunaga, S.; Yasuma, T.; Momose, Y. *ACS Med. Chem. Lett.* **2010**, *1*, 290.
11. Mikami, S.; Kitamura, S.; Negoro, N.; Sasaki, S.; Suzuki, M.; Tsujihata, Y.; Miyazaki, T.; Ito, R.; Suzuki, N.; Miyazaki, J.; Santou, T.; Kanzaki, N.; Funami, M.; Tanaka, T.; Yasuma, T.; Momose, Y. *J. Med. Chem.* **2012**, *55*, 3756.
12. Negoro, N.; Sasaki, S.; Ito, M.; Kitamura, S.; Tsujihata, Y.; Ito, R.; Suzuki, M.; Takeuchi, K.; Suzuki, N.; Miyazaki, J.; Santou, T.; Odani, T.; Kanzaki, N.; Funami, M.; Tanaka, T.; Yasuma, T.; Momose, Y. *J. Med. Chem.* **2012**, *55*, 1538.
13. Houze, J. B.; Zhu, L.; Sun, Y.; Akerman, M.; Qiu, W.; Zhang, A. J.; Sharma, R.; Schmitt, M.; Wang, Y.; Liu, J.; Liu, J.; Medina, J. C.; Reagan, J. D.; Luo, J.; Tonn, G.; Zhang, J.; Lu, J. Y.-L.; Chen, M.; Lopez, E.; Nguyen, K.; Yang, L.; Tang, L.; Tian, H.; Shuttleworth, S. J.; Lin, D. C. H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1267.
14. Brown, S. P.; Dransfield, P. J.; Vimolratana, M.; Jiao, X.; Zhu, L.; Pattaropong, V.; Sun, Y.; Liu, J.; Luo, J.; Zhang, J.; Wong, S.; Zhuang, R.; Guo, Q.; Li, F.; Medina, J. C.; Swaminath, G.; Lin, D. C. H.; Houze, J. B. *ACS Med. Chem. Lett.* **2012**, *3*, 726.
15. Wang, Y.; Liu, J.; Dransfield, P. J.; Zhu, L.; Wang, Z.; Du, X.; Jiao, X.; Su, Y.; Li, A.-R.; Brown, S. P.; Kasparian, A.; Vimolratana, M.; Yu, M.; Pattaropong, V.; Houze, J. B.; Swaminath, G.; Thanhvien, T.; Khanh, N.; Guo, Q.; Zhang, J.; Zhuang, R.; Li, F.; Miao, L.; Bartberger, M. D.; Correll, T. L.; Chow, D.; Wong, S.; Luo, J.; Lin, D. C. H.; Medina, J. C. *ACS Med. Chem. Lett.* **2013**, *4*, 551.
16. Defossa, E.; Wagner, M. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2991.
17. Tikhonova, I. G.; Sum, C. S.; Neumann, S.; Engel, S.; Raaka, B. M.; Costanzi, S.; Gershengorn, M. C. *J. Med. Chem.* **2008**, *51*, 625.
18. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **2012**, *64*, 4.
19. Christiansen, E.; Urban, C.; Grundmann, M.; Due-Hansen, M. E.; Hagesaether, E.; Schmidt, J.; Pardo, L.; Ullrich, S.; Kostenis, E.; Kassack, M.; Ulven, T. *J. Med. Chem.* **2011**, *54*, 6691.
20. Waring, M. J. *Expert Opin. Drug Discov.* **2010**, *5*, 235.
21. Keserü, G. M.; Makara, G. M. *Nat. Rev. Drug Disc.* **2009**, *8*, 203.
22. Leeson, P. D.; Young, R. J. *ACS Med. Chem. Lett.* **2015**, *6*, 722.
23. Gleeson, M. P. *J. Med. Chem.* **2008**, *51*, 817.
24. Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discovery Today* **2004**, *9*, 430.
25. Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Disc.* **2007**, *6*, 881.
26. Winzell, M. S.; Ahrén, B. *Diabetes* **2004**, *53*, 215.
27. Koehler, J. A.; Baggio, L. L.; Lamont, B. J.; Ali, S.; Drucker, D. J. *Diabetes* **2009**, *58*, 2148.