Bioorganic & Medicinal Chemistry 23 (2015) 7158–7164

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological activity of phenoxyacetic acid derivatives as novel free fatty acid receptor 1 agonists

Zheng Li [a], Xuekun Wang [a,b], Xue Xu [c], Jianyong Yang [a], Wenting Xia [a], Xianhao Zhou [a], Wenlong Huang [a,d,*], Hai Qian [a,d,*]

[a] Center of Drug Discovery, State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, PR China
[b] Department of Biomedical Engineering, College of Engineering and Applied Sciences, Nanjing University, Nanjing 210093, PR China
[c] Key Laboratory of Drug Quality Control and Pharmacovigilance, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, PR China
[d] Jiangsu Key Laboratory of Drug Discovery for Metabolic Disease, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, PR China

ARTICLE INFO

Article history:
Received 21 September 2015
Revised 7 October 2015
Accepted 8 October 2015
Available online 8 October 2015

Keywords:
FFA1 agonist
Phenoxyacetic acid
Ligand efficiency
Type 2 diabetes
Hypoglycemia

ABSTRACT

The free fatty acid receptor 1 (FFA1) is a novel antidiabetic target for the treatment of type 2 diabetes based on particular mechanism in amplifying glucose-stimulated insulin secretion. We have previously identified a series of phenoxyacetic acid derivatives. Herein, we describe the further chemical modification of this series directed by ligand efficiency and ligand lipophilicity efficiency. All of these efforts lead to the discovery of the promising candidate **16**, an excellent FFA1 agonist with robust agonistic activity (43.6 nM), desired LE and LLE values. Moreover, compound **16** revealed a great potential for improving the hyperglycemia levels in both normal and type 2 diabetic mice without the risk of hypoglycemia even at the high dose of 40 mg/kg.

1. Introduction

Type 2 diabetes mellitus (T2DM) is becoming a common and frequently occurring metabolic disease characterized by impaired glucose homeostasis due to insulin deficiency and tissue resistance to glucose uptake and utilization.[1,2] Insulin secretagogues, such as meglitinides and sulfonylureas, are commonly used for the treatment of T2DM.[3] However, these drugs enhance insulin secretion independent of blood glucose levels, leading to the relatively high risk of hypoglycemia.[4] Novel insulin secretagogues with potent antidiabetic effects and a low risk of hypoglycemia are thus desirable. The free fatty acid receptor 1 (FFA1, also known as GPR40) is highly expressed in pancreatic β-cells and responds to medium- to long-chain free fatty acids resulting in amplification of glucose-stimulated insulin secretion.[5–7] Therefore, this particular mechanism of FFA1 provides the tremendous potential for boosting insulin levels with decreased risk of hypoglycemia compared to classic insulin secretagogues.

Recently, a variety of synthetic FFA1 agonists have been reported in the literature (Fig. 1),[8–15] of which the compounds TAK-875, AMG-837 and LY2881835 have reached clinical trials for the treatment of T2DM. Owing to the structural similarity to fatty acids, most of the reported agonists have relatively high lipophilicity that are found to negatively influence the ADMET properties and correlate with attrition.[16–18] Therefore, the concepts such as ligand efficiency (LE) and ligand lipophilicity efficiency (LLE) have been extensively used to avoid undue increase in molecular size and lipophilicity in the optimization process.[19,20] We have previously reported a series of phenoxyacetic acid derivatives which successfully improved β-oxidation of phenylpropanoic acid moiety.[21,22] Herein, we report the further optimization of this compound series aiming to expand the structure–activity relationship (SAR) studies directed by LE and LLE (Fig. 2). After systematic exploration of SAR and application of molecular modeling, the promising candidate **16** and its interaction mode were identified. In subsequent in vivo pharmacological studies, compound **16** showed a robustly hypoglycemic effect in both normal and type 2 diabetic mice without the risk of hypoglycemia even at the high dose of 40 mg/kg.

2. Results and discussion

2.1. Chemistry

The synthetic routes of target compounds **1**–**16** are detailed in Scheme 1. Compound **34a** was synthesized via our published

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

http://dx.doi.org/10.1016/j.bmc.2015.10.011
0968-0896/

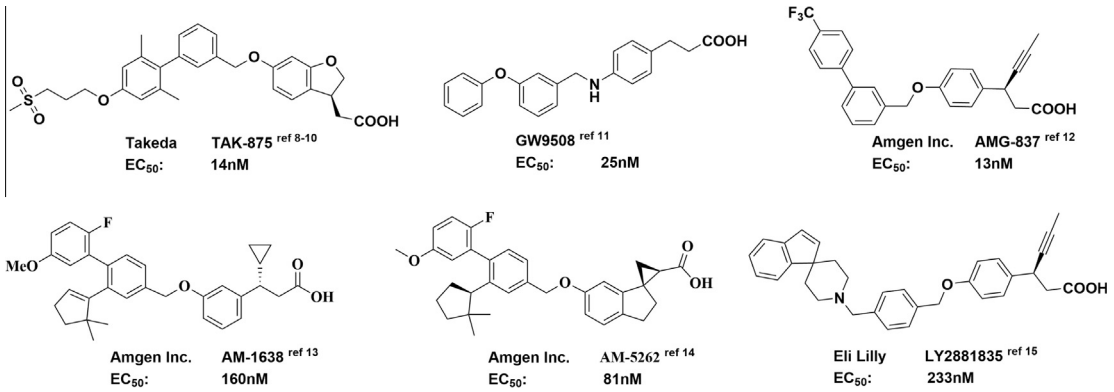


Figure 1. Selected examples of synthetic GPR40 agonists.

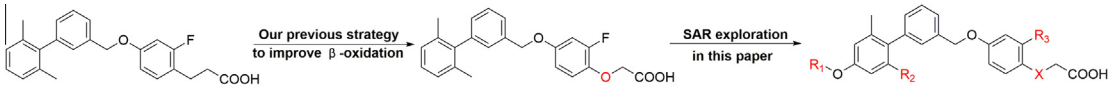
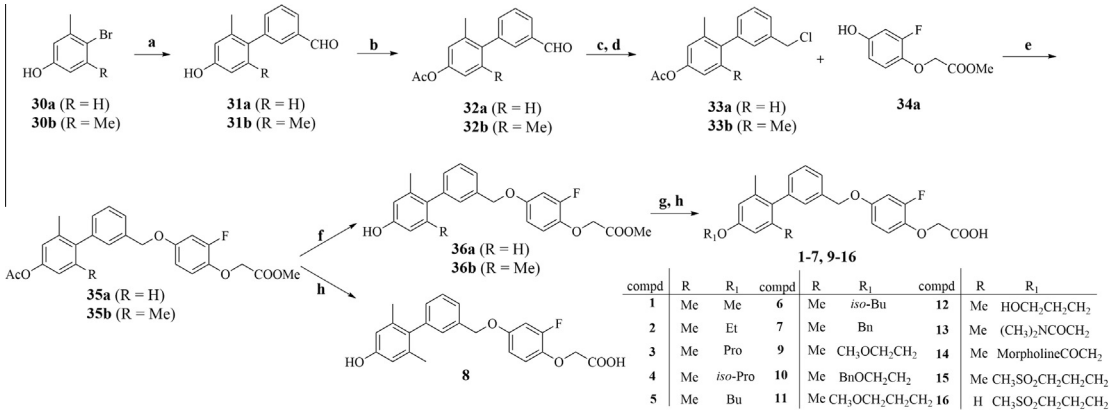


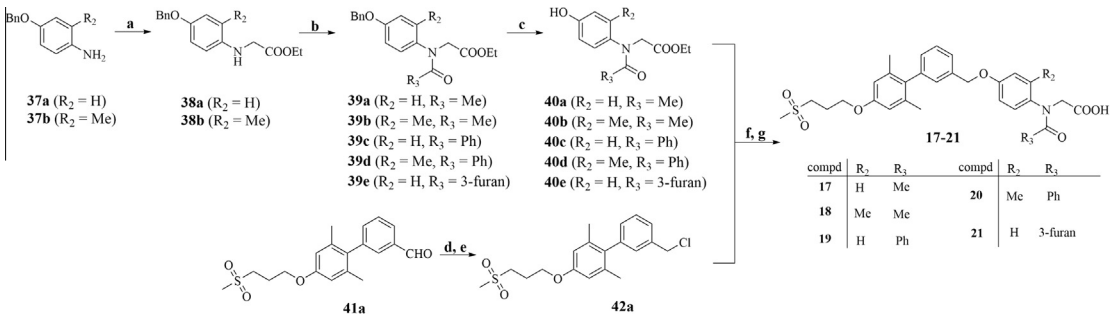
Figure 2. Optimization of phenoxyacetic acid derivatives.



compd	R	R_1	compd	R	R_1	compd	R	R_1
1	Me	Me	6	Me	*iso*-Bu	12	Me	$HOCH_2CH_2CH_2$
2	Me	Et	7	Me	Bn	13	Me	$(CH_3)_2NCOCH_2$
3	Me	Pro	9	Me	$CH_3OCH_2CH_2$	14	Me	MorpholineCOCH$_2$
4	Me	*iso*-Pro	10	Me	$BnOCH_2CH_2$	15	Me	$CH_3SO_2CH_2CH_2CH_2$
5	Me	Bu	11	Me	$CH_3OCH_2CH_2CH_2$	16	H	$CH_3SO_2CH_2CH_2CH_2$

Scheme 1. Synthesis of target compounds **1**–**16**. Reagents and conditions: (a) (3-formylphenyl)boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene, ethanol, H_2O, 80 °C, 12 h; (b) acetyl chloride, NEt_3, CH_2Cl_2, 0 °C, 2 h; (c) $NaBH_4$, CH_3OH, THF, 0 °C, 30 min; (d) $SOCl_2$, CH_2Cl_2, DMF, 40 °C, 4 h; (e) K_2CO_3, acetone, KI, reflux, 12 h; (f) CH_3ONa, MeOH, rt, 2 h; (g) R_1Br or R_1OTs, K_2CO_3, acetone, reflux, 12 h; (h) LiOH·H_2O, THF/MeOH/H_2O, rt, 4 h.

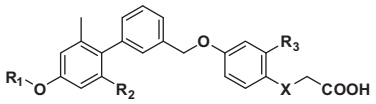
procedures.[21] The key intermediate **32** was prepared by the acetylation of intermediate **31**, which was derived from the Suzuki coupling of commercially available bromobenzene **30** with (3-formylphenyl)boronic acid in the presence of $Pd(PPh_3)_4$. The intermediate **32** was reduced with $NaBH_4$ in the presence of methanol, followed by treating with thionyl chloride catalyzed by DMF catalyzer, generated the chlorinated intermediate **33**. Condensation of the obtained chlorinated intermediate **33** with **34a** by using Williamson ether synthesis to afford the common intermediate **35**. The basic hydrolysis of intermediate **35** gives the phenol intermediate **36** and the desired target molecular **8**. After stirring at reflux temperature for 12 h, the phenol intermediate **36**

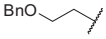
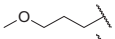
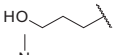
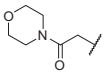
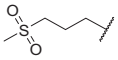
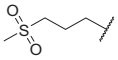
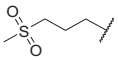
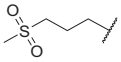
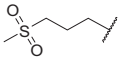
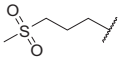
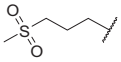


compd	R_2	R_3	compd	R_2	R_3
17	H	Me	20	Me	Ph
18	Me	Me	21	H	3-furan
19	H	Ph			

Scheme 2. Synthesis of target compounds **17**–**21**. Reagents and conditions: (a) ethyl chloroacetate, $NaHCO_3$, ethanol, rt, 24 h; (b) R_3COCl, NEt_3, CH_2Cl_2, 0 °C–rt, 16 h; (c) H_2, Pd–C, rt, 24 h; (d) $NaBH_4$, CH_3OH, THF, 0 °C, 30 min; (e) $SOCl_2$, CH_2Cl_2, DMF, 40 °C, 4 h; (f) K_2CO_3, acetone, KI, reflux, 12 h; (g) LiOH·H_2O, THF/MeOH/H_2O, rt, 4 h.

Table 1
In vitro activities and physicochemical properties of target compounds



Compd	R_1	R_2	R_3	X	Act%[a] (100 nM)	EC_{50}[b] (nM)	Log$D_{7.4}$[c]	LE (LLE)[d]
TAK-875					65.32	29.6	2.43	0.27 (5.1)
1	Me	Me	F	O	58.37	49.7	3.20	0.33 (4.1)
2	Et	Me	F	O	53.12	65.9	3.48	0.31 (3.7)
3	Pro	Me	F	O	47.85	105.8	3.64	0.29 (3.3)
4	*iso*-Pro	Me	F	O	36.92	ND	ND	
5	Bu	Me	F	O	41.57	ND	ND	
6	*iso*-Bu	Me	F	O	35.17	ND	ND	
7	Bn	Me	F	O	26.38	ND	ND	
8	H	Me	F	O	44.26	110.3	2.37	0.32 (4.5)
9		Me	F	O	48.73	103.6	3.15	0.29 (3.8)
10		Me	F	O	23.62	ND	ND	
11		Me	F	O	37.95	ND	ND	
12		Me	F	O	44.37	109.5	2.73	0.28 (4.2)
13		Me	F	O	13.49	ND	ND	
14		Me	F	O	18.35	ND	ND	
15		Me	F	O	54.94	62.3	1.81	0.28 (5.3)
16		H	F	O	61.28	43.6	1.63	0.29 (5.6)
17		Me	H		36.29	ND	ND	
18		Me	Me		18.37	ND	ND	
19		Me	H		23.46	ND	ND	
20		Me	Me		2.57	ND	ND	
21		Me	H		28.85	ND	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] Log$D_{7.4}$ values were determined by shake-flask procedure.
[d] LE values were calculated by$-\Delta g = RT \ln KD$, presuming $KD \approx EC_{50}$, and the LLE values were calculated by the formula $pEC_{50} - \text{Log}D_{7.4}$.

was alkylated by various alkylation reagents in acetone under the action of K_2CO_3, followed by basic hydrolysis, afforded the target compounds **1**–**7** and **9**–**16**.

The synthesis of the designed target compounds **17**–**21** are depicted in Scheme 2. The compound **41a** was synthesized via published procedures.[8] The compound **41a** was reduced with $NaBH_4$, followed by treating with thionyl chloride catalyzed by DMF catalyzer, generated the chlorinated intermediate **42a**. Mono-alkylation of the aniline **37** with ethyl chloroacetate to afford compound **38**, which was subsequently converted to intermediate **39** by acylation with various acyl chloride. After stirring at ambient temperature for 24 h, the obtained intermediate **39** was debenzylated by Pd–C under the hydrogen atmosphere to afford intermediate **40**. The intermediate **40** was connected with **42a** by using Williamson ether synthesis in the presence of K_2CO_3, followed by basic hydrolysis with lithium hydroxide, afforded the target compounds **17**–**21**.

2.2. FFA1 agonistic activity and SAR study

The synthetic compounds were investigated by a fluorometric imaging plate reader (FLIPR) assay in Chinese hamster ovary (CHO) cells expressing human FFA1. Besides overall activity, the Log$D_{7.4}$ and LE, as well as LLE were taken advantage of in the assessment of optimized analogs. From previous SAR studies on other chemical series, it was revealed that incorporation of the various substituents into the *para*-position of the biphenyl ring was tolerated.[9,10] With these observations in mind, we firstly explored the tolerability of the functionalities in our series by using various alkyl groups (compounds **1**–**6**). As shown in Table 1,

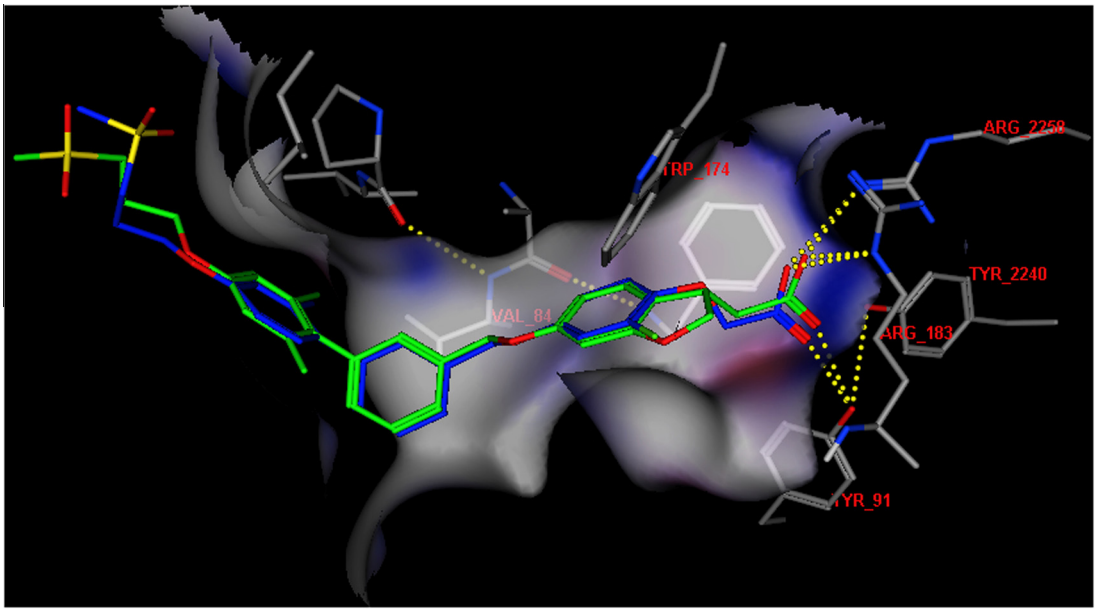


Figure 3. Overlay of TAK-875 (green) and compound **16** (blue) bound to FFA1. Key residues are labeled in red, and hydrogen bonding interactions are represented by yellow dashed lines.

methyl substituted derivative **1**, with relatively high lipophicity ($Log D_{7.4}$ = 3.20), exhibited the strongest agonistic activity in alkyl substituted derivatives. Moreover, more bulky isopropyl (**4**) and isobutyl (**6**) derivative was still tolerated in this area. These results imply that the FFA1 receptors would have a large cavity in the ligand-binding pocket around the tail substituent. Interestingly, the agonistic activity of compound **1** (4-Me) > **2** (4-Et) > **3** (4-Pro) > **5** (4-Bu) > **4** (4-*iso*Pro) > **6** (4-*iso*Bu) > **7** (4-Bn), which revealed a good correlation with Van der Waals radius of corresponding substituents, and this result suggested that the steric effect in the *para*-position of the biphenyl moiety might influence agonistic activity of FFA1. Gratifyingly, the compound **8**, derived from common intermediate **35b**, exhibited a potent agonistic activity and considerable LLE value owing to significantly decreased lipophicity. This result demonstrated that the polar functionality was also tolerated in this region. Therefore, this position was thought to be suitable for modulating the lipophilicity of ligands by introducing hydrophilic substituents. A slight improvement in potency over the compound **5** was obtained only by replacing a carbon atom of compound **5** with oxygen atom in compound **9**. We speculated that the improvement of the activity was associated with solvent effect of oxygen atom which stabilized the lowest energy conformation of ligand. Moreover, the terminal hydroxyl group derivative **12**, with lower lipophilicity, exhibited a significant improvement on potency in comparison with the parent compound **11**, and this result further confirmed the above speculation. The introduction of amide (compounds **13** and **14**), however, showed a drastic drop of activity despite increased solvent effect, likely attributing to the steric effects of dimethylamino and morpholine. Consistent with before-mentioned findings, replacement of the methyl group in compound **9** with benzyl group gave compound **10**, which turned out an approximately 1-fold decrease in potency compared to the compound **9** but still indicates that the receptor binding pocket can accommodate the bulky group. Inspired by the terminal hydrophilic methylsulfonyl fragment of TAK-875, we designed and synthesized compound **15**, a superior agonist revealed a small decrease in agonistic activity relative to compound **1** but more than an order of magnitude reduced lipophilicity, and thereby a significantly increased LLE value compared to compounds **1** and **2**.

Based on these results above, we therefore selected this scaffold of **15** as our starting point for further modification. The monomethyl analog **16**, with relatively lower molecular weight and lipophilicity, exhibited a marked improvement on potency and LLE value in comparison with the parent compound **15**. With these beneficially optimized experiences for phenoxyacetic acid series, our optimized efforts were directed to replace the oxygen atom of phenoxyacetic acid with bioisostere nitrogen atom. Concerns about relatively high dissociation energy between alkaline amino and adjacent carboxylic group led us to introduce amide rather than amino. Among them, the acetamide analog **17** revealed a strongest potency in this series but still deviate the desired potency. Furthermore, as the size of amide substituent increases the potency decrease (compounds **17** > **21** > **19**), suggesting that the limited space in the binding pocket around this area. Incorporation of methyl group in *ortho*-position of acidic head to afford compounds **18** and **20** revealed a markedly lower agonistic activity than the corresponding unsubstituted compounds **17** and **19**. One possible explanation is that the incorporation of bulkier methyl substituent moves the carboxylic acid moiety into an unfavorable conformation. Among all of the tested compounds, the most potent agonist **16**, with excellent lipophilicity and desired LLE value, was selected for further investigation.

2.3. Docking study of compound 16 with hGPR40

To better understand the SAR and interaction mode of compound **16**, a molecular modeling study based on the recently reported X-ray structure of FFA1 (PDB accession code: 4PHU) was performed by using the Molecular Operating Environment (MOE) (Fig. 3).[23] As shown in Figure 3, the interaction mode of compound **16** was nearly perfect overlap with TAK-875, and the carboxylic acid moiety was highly coordinated by Arg183, Arg258 and Tyr91 forming anchor point. Moreover, Trp174 is oriented nearly perpendicularly to the plane of the fluorobenzene ring of compound **16** where it forms an edge-on interaction. These crucial interactions render the acidic head is very sensitive to the structural modifications such as the bioisostere amide derivatives (compounds **17**–**21**). Meanwhile, our model aligns with the SAR

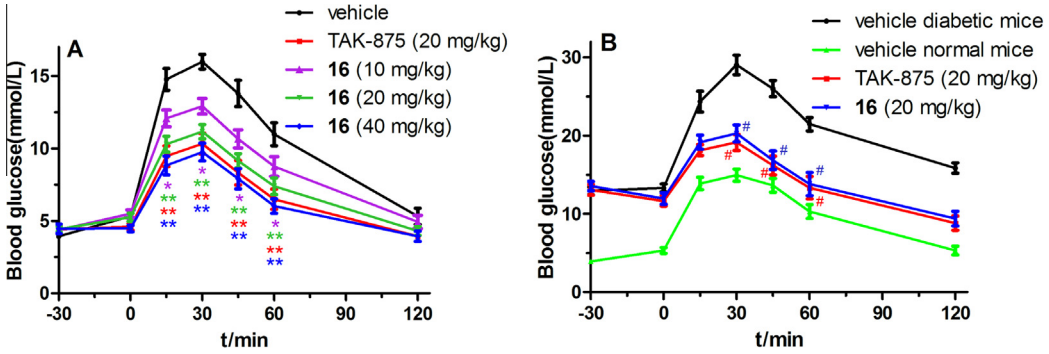


Figure 4. (A) Effect of compound **16** on glucose tolerance in normal ICR mice. (B) OGTT in fasting type 2 diabetic C57BL/6 mice. Values are mean ± SEM ($n = 6$). $^{*}P \leqslant 0.05$ and $^{**}P \leqslant 0.01$ compared to vehicle mice by Student's t test. $^{\#}P \leqslant 0.05$ compared to vehicle diabetic mice by Student's t test.

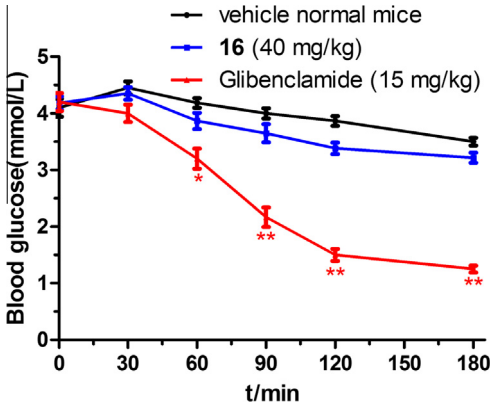


Figure 5. Effects of compound **16** on fasting plasma glucose in normal mice. Values are expressed as mean ± SEM for six animals in each group. $^{*}P \leqslant 0.05$, $^{**}P \leqslant 0.01$ compared to compound **16** treated mice by Student's t-test.

determined above, which identified that the *para*-position of the terminal biphenyl moiety could tolerate a variety of substituents with different polarity and length while minimal effect on FFA1 agonistic activity.

2.4. Effect of compound 16 on glucose tolerance

Based on these results above, the most potent compound **16** (10, 20 and 40 mg/kg) was selected to further investigate the oral glucose tolerance test (OGTT) in normal ICR mice. The time-dependent changes of the plasma glucose levels are shown in Figure 4A. The compound **16** revealed a dose-dependent decrease in blood glucose levels with stronger efficacy at 40 mg/kg compared to TAK-875 at 20 mg/kg. To further evaluate the hypoglycemic effects in the diabetic state, STZ-induced type 2 diabetic C57BL/6 mice were used to assess the efficacy of compound **16**, an orally bioavailable potent agonist. As shown in Figure 4B, the hyperglycemia state was significantly improved in compound **16** treated mice, and the hypoglycemic effect was in close proximity to the positive control TAK-875, the most advanced compound once in phase III studies. These results demonstrated that compound **16**, the most potent agonist among our synthetic compounds, has a great potential for improving the hyperglycemia levels in both normal and type 2 diabetic mice.

2.5. Effects of compound 16 on the risk of hypoglycemia

Obtaining these positive results in pharmacological study, we subsequently assessed the risk of hypoglycemia in fasting normal ICR mice by oral administrating a high dose of compound **16** in comparison with glibenclamide. As shown in Figure 5, the glibenclamide (15 mg/kg) treated group lowered the blood glucose levels far below fasting normal mice. In contrast, compound **16**, even at the high dose of 40 mg/kg, only slightly reduced plasma sugar levels in fasting ICR mice, and the change of blood glucose levels was much smaller than that of glibenclamide. Therefore, these results demonstrated that compound **16** revealed a low risk of hypoglycemia, a serious side effect to sulfonylureas, which are widely used in the treatment of T2DM as one of the first-line drugs.

3. Conclusion

In conclusion, starting from previously identified phenoxyacetic acid derivatives, we have systematically explored the SAR of this series directed by LE and LLE. Among them, the ideal lipophilic compound **16**, with robust in vitro agonistic activity, revealed a great potential for improving the hyperglycemia levels in both normal and type 2 diabetic mice without the risk of hypoglycemia even at the high dose of 40 mg/kg. According to these results, compound **16** will be a promising candidate as a novel FFA1 agonist with a low risk of hypoglycemia, and the information derived from the SAR studies allowed us to design more competitive FFA1 agonists that are structurally related.

4. Experimental section

4.1. Chemistry

All starting materials, reagents and solvents 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 365 and 254 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.[8]

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. Molecular modeling

Docking simulations were performed using MOE (version 2008.10, The Chemical Computing Group, Montreal, Canada). The crystal structure of FFA1 (PDB ID: 4PHU) was downloaded from the Protein Data Bank (PDB). Prior to ligand docking, the structure was prepared with Protonate 3D and a Gaussian Contact surface was draw around the binding site. Subsequently, the active site was isolated and the backbone was removed. The ligand poses was filtered using Pharmacophore Query Editor. The compound structures were placed in the site with Pharmacophore method and then ranked with the London dG scoring function. For the energy minimization in the pocket, MOE Forcefield Refinement was used and ranked with the London dG scoring function.

4.3. 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.

4.4. 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 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).

4.4.2. 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.

4.4.2.1. Effect of compound 16 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 **16** (10 mg kg^{-1}, 20 mg kg^{-1}, 40 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).

4.4.2.2. Hypoglycemic effects of compound 16 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.[24,25]

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 **16** (10 mL kg^{-1}; 20 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).

4.4.2.3. Effects of compound 16 on the risk of hypoglycemia. 10 weeks old male normal ICR mice were fasted overnight and randomized into 3 groups ($n = 6$). Compound **16** (40 mg kg^{-1}), glibenclamide (15 mg kg^{-1}), or vehicle was orally administered, and blood was collected from tail vein immediately before administration (0 min) and at 30, 60, 90, 120 and 180 min after administration and measure blood glucose as described above.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (Grants 81172932 and 81273376), the Natural Science Foundation of Jiangsu Province (Grant BK2012356), and the Project Program of State Key Laboratory of Natural Medicines, China Pharmaceutical University (Grant JKGZ201103).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.10.011.

References and notes

1. DeFronzo, R. A. *Diabetes* **2009**, *58*, 773.
2. Danaei, G.; Finucane, M. M.; Lu, Y.; Singh, G. M.; Cowan, M. J.; Paciorek, C. J.; Lin, J. K.; Farzadfar, F.; Khang, Y.-H.; Stevens, G. A.; Rao, M.; Ali, M. K.; Riley, L. M.; Robinson, C. A.; Ezzati, M.C. Global Burden Metab Risk Factors *Lancet* **2011**, *378*, 31.
3. Wajchenberg, B. L. *Endocr. Rev.* **2007**, *28*, 187.
4. Maedler, K.; Carr, R. D.; Bosco, D.; Zuellig, R. A.; Berney, T.; Donath, M. Y. *J. Clin. Endocrinol. Metab.* **2005**, *90*, 501.
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. Edfalk, S.; Steneberg, P.; Edlund, H. *Diabetes* **2008**, *57*, 2280.
8. 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.
9. 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.
10. Negoro, N.; Sasaki, S.; Mikami, S.; Ito, M.; Tsujihata, Y.; Ito, R.; Suzuki, M.; Takeuchi, K.; Suzuki, N.; Miyazaki, J.; Santou, T.; Odani, T.; Kanzaki, N.; Funami, M.; Morohashi, A.; Nonaka, M.; Matsunaga, S.; Yasuma, T.; Momose, Y. *J. Med. Chem.* **2012**, *55*, 3960.
11. McKeown, S. C.; Corbett, D. F.; Goetz, A. S.; Littleton, T. R.; Bigham, E.; Briscoe, C. P.; Peat, A. J.; Watson, S. P.; Hickey, D. M. B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1584.
12. 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.
13. 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.
14. 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.
15. Defossa, E.; Wagner, M. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2991.
16. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **2012**, *64*, 4.
17. Gleeson, M. P. *J. Med. Chem.* **2008**, *51*, 817.
18. Hann, M. M. *MedChemComm* **2011**, *2*, 349.
19. Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discovery Today* **2004**, *9*, 430.
20. Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Disc.* **2007**, *6*, 881.
21. 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.
22. Li, Z.; Wang, X.; Xu, X.; Yang, J.; Qiu, Q.; Qiang, H.; Huang, W.; Qian, H. *Bioorg. Med. Chem.* **2015**, *23*, 6666.
23. Srivastava, A.; Yano, J.; Hirozane, Y.; Kefala, G.; Gruswitz, F.; Snell, G.; Lane, W.; Ivetac, A.; Aertgeerts, K.; Nguyen, J.; Jennings, A.; Okada, K. *Nature* **2014**, *513*, 124.
24. Winzell, M. S.; Ahrén, B. *Diabetes* **2004**, *53*, 215.
25. Koehler, J. A.; Baggio, L. L.; Lamont, B. J.; Ali, S.; Drucker, D. J. *Diabetes* **2009**, *58*, 2148.