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Discovery of Novel Pyrrole-Based Scaffold as Potent and Orally Bioavailable Free Fatty Acid Receptor 1 Agonists for the Treatment of Type 2 Diabetes

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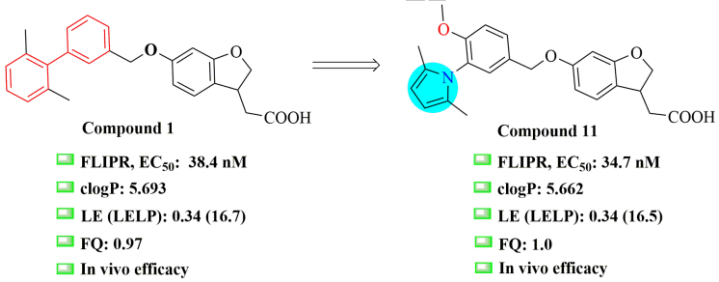
Abstract



The free fatty acid receptor 1 (FFA1) has gained significant interest as a novel antidiabetic target. Most of FFA1 agonists reported in the literature bearing a common biphenyl scaffold, which was crucial for toxicity verified by the researchers of Daiichi Sankyo. Herein, we describe the systematic exploration of non-biphenyl scaffold and further chemical modification of the optimal pyrrole scaffold. All of these efforts led to the identification of compound **11** as a potent and orally bioavailable FFA1 agonist without the risk of hypoglycemia. Further molecular modeling studies promoted the understanding of ligand-binding pocket and might help to design more promising FFA1 agonists.

Keywords: FFA1 agonist, GPR40, ligand efficiency, type 2 diabetes, pyrrole.

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1. Introduction

The increasing and alarming prevalence of type 2 diabetes mellitus (T2DM) along with the undesirable side effects associated with many oral antidiabetic agents (most notably high risk of hypoglycemia, body weight gain and gastric symptoms) has stimulated an intense effort to evaluate novel targets to achieve preferable glycemic control.[1-4] The free fatty acid receptor 1 (FFA1, also known as GPR40), a new drug target attracted considerable attention for the treatment of T2DM, play a key role in amplifying insulin secretion in a glucose concentration-dependent manner.[5-8] Therefore, this glucose-stimulated insulin secretion mechanism of FFA1 provides the enormous potentiality for improving insulin levels without the risk of hypoglycemia.

Recently, a number of synthetic FFA1 agonists have been reported in the literature (**Figure 1**),[9-16] and the candidates AMG-837 and TAK-875 were both in clinical trials for treatment of T2DM. However, many of these agonists bearing a common biphenyl scaffold (red mark in **Figure 1**) have high lipophilicity, which likely correlated with poor water-solubility, metabolic toxicity, and associated with a higher risk of attrition.[17-21] Furthermore, some studies derived from Daiichi Sankyo have shown that the biphenyl moiety of FFA1 agonists was crucial for toxicity.[22] Therefore, it is of profound significance to hunt a novel scaffold by modifying the biphenyl moiety. We have previously reported a series of phenoxyacetic acid derivatives which successfully removed the biphenyl structure by introducing a phenoxyacetamide linker.[23] Herein, we chose compound **1** (**Figure 1**) as a starting structure to explore novel FFA1 agonists, because it has robustly FFA1 agonistic activity and a simple structure to avoid undue increase in molecular size and lipophilicity in the optimization process. Fortunately, the recently reported crystal structure of FFA1 indicates that the terminal benzene ring of biphenyl moiety had no direct interactions with the binding site of FFA1.[24] Based on these results, we envisioned that replacement of the terminal benzene ring of compound **1** with a polar heterocycle was a worthy approach to hunt a new scaffold with desired drug-like properties (**Figure 2**). After systematic exploration of scaffold and application of molecular docking, the promising FFA1 agonist **11** (EC_{50} = 34.7 nM, LE = 0.34) and its binding mode (fit quality = 1.0) were identified. Further pharmacological studies demonstrated that compound **11** has a robustly hypoglycemic effect in both normal and type 2 diabetic mice without hypoglycemia even at the oral dose of 80 mg/kg.

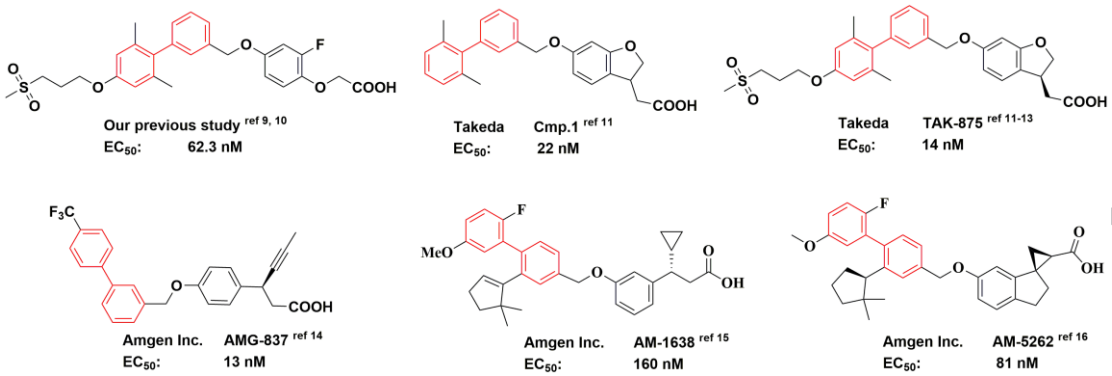


Figure 1: Selected examples of synthetic FFA1 agonists.

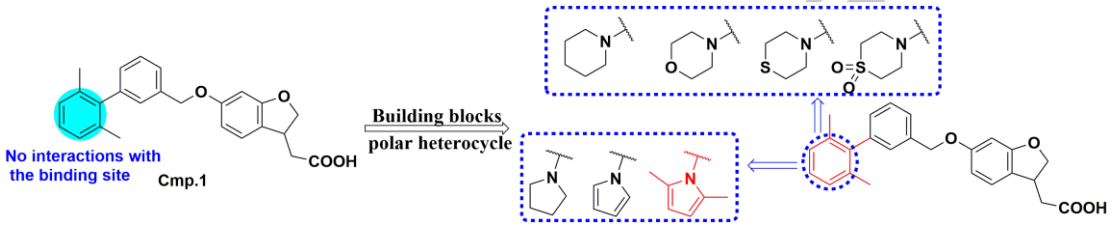


Figure 2: Our strategy to hunt a novel scaffold with decreased lipophilicity by modifying the biphenyl moiety of compound **1**.

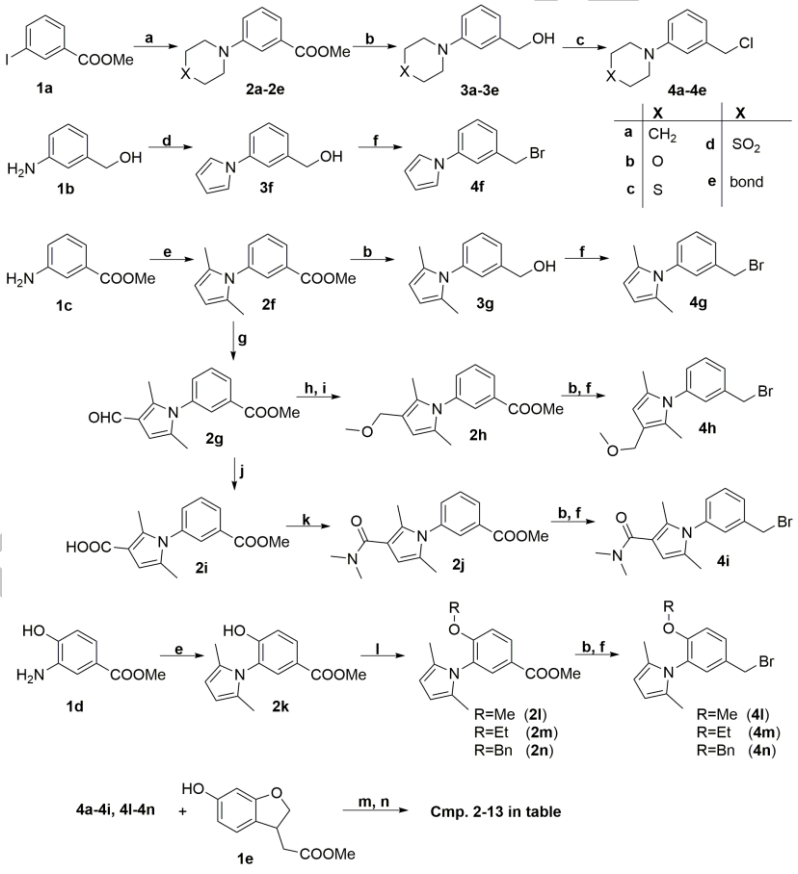
2. Results and Discussion

2.1. Chemistry

The synthetic routes of target compounds **2-13** are summarized in **Scheme 1**. The key intermediates **3a-e** were prepared by the reduction of **2a-e**, which were derived from Ullmann coupling reaction of corresponding amine and methyl 3-iodobenzoate in the presence of CuI.[25] Next, the compounds **3a-e** treated with thionyl chloride catalyzed by DMF catalyzer to generate the chlorinated intermediates **4a-e**. The intermediate **3f** was synthesized upon simple mixing of 3-aminobenzylalcohol and 2, 5-dimethoxytetrahydrofuran in the presence of $FeCl_3 \cdot 7H_2O$ (2 mol%) at 60 °C.[26] It's worth mentioning that our initial effort was to afford the chlorinated intermediate of **3f** by treating with thionyl chloride. However, the **3f** tend to produce a coke tar due to the polymerization reaction of pyrrole in the strongly acidic system of thionyl chloride. Thus, the brominated intermediate **4f** was successfully synthesized by treating PBr_3 with pyridine in a weakly basic system.

The common intermediates **2f** and **2k** were obtained from the intermolecular cyclization between corresponding amine and acetonylacetone by using Paal-Knorr pyrrole synthesis.

Subsequently, the intermediate **2g** was achieved from **2f** by Vilsmeier-Haack-Arnold reaction. Reduction of the **2g** with $NaBH_4$ and then methylation provided the intermediate **2h**. Oxidation of the **2g** with $NaClO_2$ in the presence of NaH_2PO_4 generated the desired carboxylic acid **2i**, which was followed by amidation using DMAP and EDCI to yield the desired intermediate **2j**. Alkylation of **2k** with corresponding haloalkane afforded the intermediates **2l-n**. Reduction and bromination of the intermediates **2h, 2j** and **2l-n** provide the brominated intermediates **4h-i** and **4l-n**. The dihydrobenzofuran intermediate **1e** was synthesized *via* published procedures.[11] Condensation of the obtained intermediates **4a-i** or **4l-n** with **1e** by using Williamson ether synthesis, followed by basic hydrolysis, afforded the target compounds **2-13**.



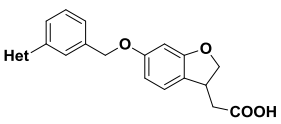
Scheme 1. Synthesis of target compounds **2** to **13**. Reagents and conditions: (a) corresponding amine, K_2CO_3, CuI, L-proline, 60 °C, 8 h; (b) $NaBH_4$, MeOH, THF, reflux, 2 h; (c) $SOCl_2$, CH_2Cl_2, DMF, 40 °C, 4 h; (d) 2,5-dimethoxytetrahydrofuran, $FeCl_3{\cdot}7H_2O$ (2 mol%), H_2O, 60 °C, 3 h; (e) acetonylacetone, *p*-toluenesulfonic acid, toluene, reflux, 3 h; (f) PBr_3, pyridine, CH_2Cl_2, 0 °C, 1 h; (g) $POCl_3$, DMF, toluene, 70 °C, 4 h; (h) $NaBH_4$, THF, rt, 2 h; (i) NaH, MeI, THF, 0 °C to rt, 8 h; (j) NaH_2PO_4, $NaClO_2$, 2-methylbut-2-ene, t-BuOH, THF, rt, 3 h; (k) dimethylamine in THF, DMAP, EDCI, DMF, 0 °C to rt, 8 h; (l) RBr or MeI, K_2CO_3, acetone, KI, 45 °C, 12 h; (m)

K_2CO_3, acetone, KI, 60 °C, 8 h; (n) LiOH·H_2O, THF/MeOH/H_2O, rt, 4 h.

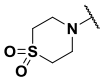
2.2. FFA1 agonistic activity and SAR study

Lovering et al. pointed out that an increase of saturation is a practical strategy to improve the drug-like properties of compound.[27, 28] Moreover, Ishikawa and Hashimoto were profoundly reviewed an alternative tactic for improving physicochemical property by means of disruption of molecular planarity.[29] Inspired by these new perspectives, we first designed and synthesized piperidine derivative **2**, a more saturated analogue of compound **1** (**Table 1**). The compound **2** revealed a significant improvement on lipophilicity (clogP = 3.993) compared to the parent compound **1** (clogP = 5.693) but unfortunately also markedly reduced potency. A slight improvement in potency over the compound **2** was achieved only by replacing piperidine of compound **2** with bioisosteres in compound **3** and **4**. We speculated that the increased affinity was attributed to the solvent effect of heteroatom which stabilized the binding conformation of ligand. However, the compound **5** (clogP = 1.645), a sulphone analogue of compound **4** designed to further improve the lipophilicity as TAK-875, showed a drastic loss of activity despite increased solvent effect, likely suggesting that the limited space in the binding pocket around this area. On the basis of the above analysis, we removed a carbon atom in compound **2** to reduce the steric effect with ligand-binding pocket, and the obtained compound **6** showed a significant improvement in potency compared with the compound **2**. The pyrrole derivative **7**, designed as a constrained planar analogue of compound **6**, appeared to diminish the *in vitro* agonistic activity. Gratifyingly, borrowing elements of known FFA1 agonists such as the *ortho*-dimethyl moiety of TAK-875, the obtained compound **8** exhibited a marked improvement on potency in comparison with the corresponding compound **7**. This result demonstrated that a more appropriate twisted conformation was induced by 2, 5-dimethyl pyrrole.

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



Compd.	Het	Act%(300nM)[a]	Act%(100nM)[b]	Mw	clogP[c]

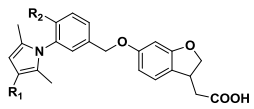
TAK-875		76.01	65.32	524.63	4.697
1		74.37	62.58	388.17	5.693
2		50.32	34.65	367.45	3.993
3		55.73	41.17	369.42	2.611
4		52.18	38.25	385.48	3.444
5		25.86	8.28	417.48	1.645
6		56.26	43.68	353.42	3.434
7		41.87	28.56	349.39	4.306
8		71.34	59.86	377.44	5.556

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

Based on these results above, we therefore selected 2, 5-dimethyl pyrrole scaffold of the most potent compound **8** as our starting point for further modification. Besides the agonistic activity, the ligand efficiency dependent lipophilicity (clogP / LE, LELP),[30, 31] a metric incorporated affinity and lipophilicity, was taken advantage of in the drug-like evaluation of derivatives. Moreover, the fit quality (LE / LE_Scale, FQ),[32, 33] with score close to 1 indicative of near perfect binding, was also introduced to assess the binding characteristics with FFA1. As shown in **Table 2**, incorporation of substituent at the 3-position of pyrrole (**9** and **10)** turned out a drastic loss of activity, indicating that the introduction of a substituent at 3-pyrrole is unfavorable for FFA1 agonistic activity. Next, our optimized efforts were directed to evaluate the R_2 group, the substituent at the *para*-position of the central phenyl ring. Among them, the methoxy analogue **11** revealed a strongest potency (34.7 nM), desired LELP value (16.5) and perfect FQ score (1.0) despite slightly increased lipophilicity. Furthermore, as the Van der Waals radius of substituent increases the activity decrease (compound **11** > **12** > **13**), implying that the steric bulkiness of substituent might interrupt the proper interaction with the receptor. Meanwhile, the order of

potency is reversed relative to the TAK-875 series,[13] suggesting that the previous SAR information can not directly convert to our pyrrole series. Among all of the tested compounds, the most potent agonist **11**, with excellent LE (LELP) value and perfect FQ score, was selected for further investigation.

Table 2: *In vitro* activities and physicochemical properties of designed compounds



Compd.	R_1	R_2	Act% (100nM)[a]	EC_{50} (nM)[b]	clogP[c]	LE (LELP)[d]	FQ[e]
TAK-875			65.32	29.6	4.697	0.27 (17.3)	0.94
1			62.58	38.4	5.693	0.34 (16.7)	0.97
8	H	H	59.86	46.9	5.556	0.35 (15.8)	0.98
9		H	31.69	ND	5.304		
10		H	25.72	ND	4.207		
11	H	MeO	63.76	34.7	5.662	0.34 (16.5)	1.0
12	H	EtO	54.62	61.2	6.191	0.31 (19.9)	0.94
13	H	BnO	40.75	ND	7.430		

ND = Not determined. [a] Agonist activities mean values at 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] clogP values were estimated with ChemDraw Ultra, version 12.0. [d] LE values were calculated by $-\Delta g = RT \ln KD$, presuming $EC_{50} \approx KD$, and the LELP values were calculated by the formula clogP / LE. [e] FQ (fit quality) = LE / LE_Scale, and LE_Scale = $0.0715 + 7.5328/HAC + 25.7079/HAC^2 - 361.4722/HAC^3$, HAC: heavy atom count.

2.3. Docking study of compound 11 with FFA1

To understand the interaction mode and SAR of pyrrole series in-depth, a molecular docking study based on the crystal structure of FFA1 (PDB accession code: 4PHU) was performed by using the Molecular Operating Environment (MOE).[24] As shown in **Figure 3**, the compound **11** docked very well to the same binding site for TAK-875, and the modeling study can explain the SAR

reasonably. The head acid moiety was highly coordinated by Tyr91, Arg183 and Arg2258 forming anchor point. Meanwhile, the residue Trp174 is oriented nearly orthogonal to the plane of the dihydroisobenzofuran ring of compound **11** where it forms an edge-on interaction. Moreover, the 2, 5-dimethyl pyrrole scaffold is roughly perpendicular to the central phenyl ring, which fully explained the activity difference between 2, 5-dimethyl pyrrole (compound **8**) and non-substituted pyrrole (compound **7**). Interestingly, the methoxy group on the central phenyl moiety has hydrophobic interaction with receptor, and the additional interaction render compound **11** showed best agonistic activities in this series. As mentioned above, compound **11** was occupied the binding pockets effectively, and it was confirmed as the values of LE and FQ (compound **11**: 0.34 and 1.0, TAK-875: 0.27 and 0.94, respectively).

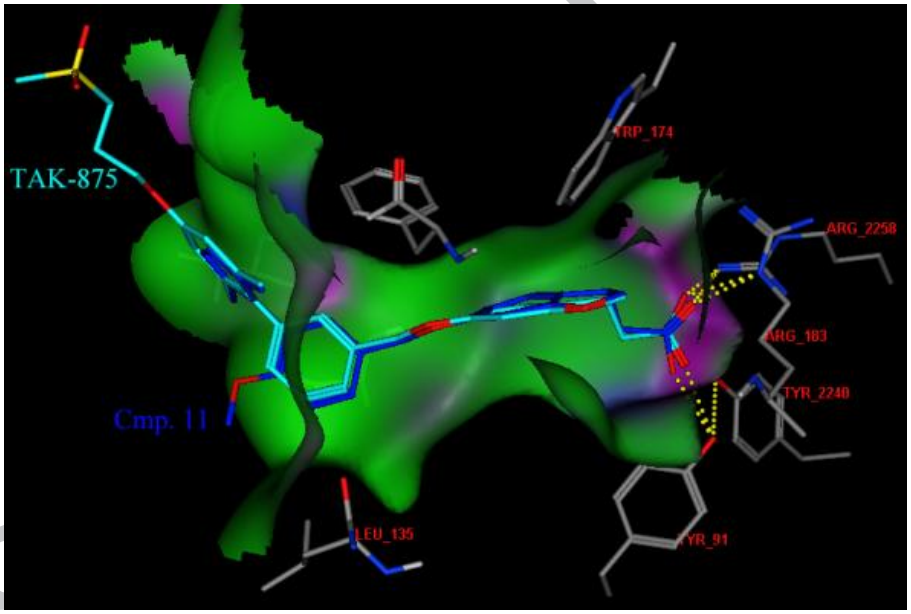


Figure 3: Overlay of TAK-875 and compound **11** in crystal structure of FFA1. Key residues are labeled in red, and hydrogen bonding interactions are represented by yellow dashed lines.

2.4. Effect of compound 11 on glucose tolerance

On the basis of its excellent *in vitro* potency, compound **11** (10, 20 and 40 mg/kg) was selected for further *in vivo* pharmacological evaluation in ICR mice by oral glucose tolerance test (OGTT). As shown in **Figure 4A**, single oral doses of compound **11** robustly reduced the plasma glucose excursion during an OGTT in a dose-dependent manner from 10 to 40 mg/kg. Notably, the compound **11** revealed a significant blood glucose-lowering effect at 40 mg/kg dose, similar to the

hypoglycemic effect of positive control TAK-875 (20 mg/kg), the most advanced candidate once in phase III studies. Furthermore, the blood glucose curve of compound **11** tended to flat after 60 min at the dose of 40 mg/kg. This phenomenon could be at least in part rationalized by the low risk of hypoglycemia showed in compound **11**.

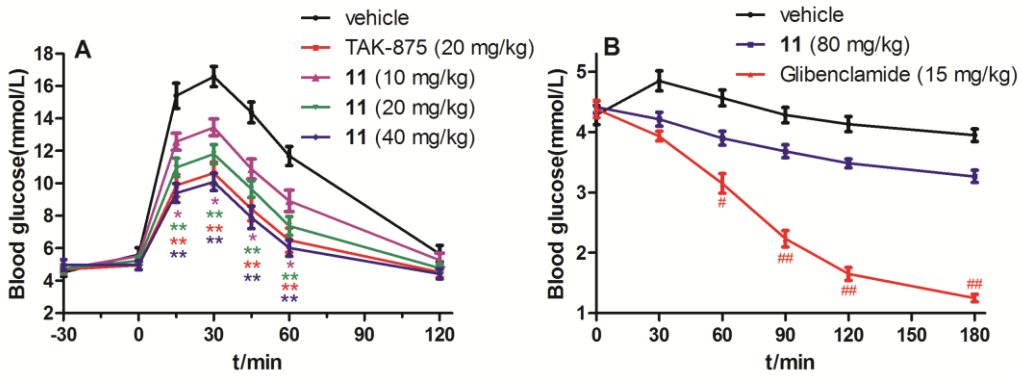


Figure 4: (A) Effect of compound **11** on plasma glucose levels during an OGTT in ICR mice, and glucose load (3 g/kg) at 0 min. (B) Effects of compound **11** and glibenclamide on fasting blood glucose level in normal mice. Data are mean ± SEM for six animals. *P≤0.05 and **P≤0.01 compared to vehicle mice by Student's t test. $^{\#}$P≤0.05 and $^{\#\#}$P≤0.01 compared to compound **11** treated mice by Student's t test.

2.5. Effects of compound 11 on the risk of hypoglycemia

Obtaining a positive result in *in vivo* pharmacological study, the risk of hypoglycemia was subsequently assessed in fasting normal mice by oral administrating a high dose of compound **11** in comparison with glibenclamide (a well-known sulfonylurea) to further confirm the above speculation. As shown in **Figure 4B**, the glibenclamide (15 mg/kg) treated group reduced the plasma sugar levels far below the normal fasting glucose levels. In contrast, compound **11**, even at an oral dose of 80 mg/kg, only slightly alter blood glucose levels in fasting mice with normal glucose homeostasis, and the change of plasma glucose levels was much smaller compared to that of glibenclamide. Thus, our results demonstrated that compound **11** may pose a low risk of hypoglycemia, a serious adverse effect common to sulfonylureas.

2.6. Hypoglycemic effects of compound 11 explored in type 2 diabetic mice

To assess antihyperglycemic effects in the diabetic state, the orally bioavailable agonist **11** was evaluated by the OGTT in STZ-induced type 2 diabetic C57BL/6 mice, a model with impaired insulin secretion.[34, 35] As shown in **Figure 5**, the plasma glucose levels was significantly decreased in compound **11** (20 mg/kg) treated group, which presented potency-similarity with TAK-875 (20 mg/kg) treated mice. This result indicated that compound **11** has an outstanding efficacy for improving the diabetic state in type 2 diabetic mice.

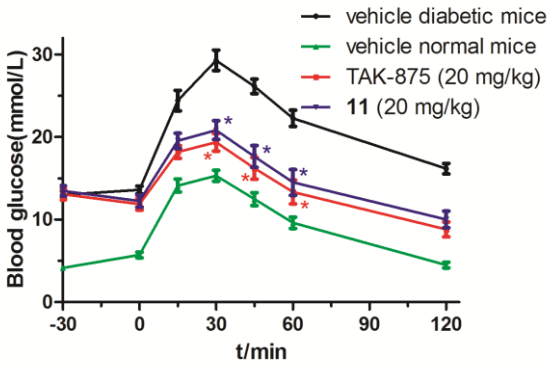


Figure 5: Effect of compound **11** on plasma glucose levels during an OGTT in fasting type 2 diabetic C57BL/6 mice, and glucose load (2 g/kg) at 0 min. Data are mean ± SEM for six animals. *P≤0.05 compared to vehicle diabetic mice by Student's t test.

3. Conclusion

With the aim of developing potent FFA1 agonists with novel drug-like scaffold, we have identified a series of non-biphenyl scaffold agonists by comprehensive evaluating 7 polar heterocycle. Subsequently, systematic exploration of SAR in the optimal pyrrole scaffold, leading to the discovery of compound **11**, an excellent FFA1 agonist with robustly agonistic activity (EC_{50} = 34.7 nM), desired LE (LELP) value and perfect FQ score (FQ = 1). Moreover, compound **11** revealed a great potential for reducing the blood glucose levels in ICR mice and type 2 diabetic C57BL/6 mice without the risk of hypoglycemia even at a high dose of 80 mg/kg. All of these researches manifested that compound **11** was meaningful for further investigation, and the information obtained from this study might help to design more competitive new chemical entities that are structurally related.

4. Experimental section

4.1. Chemistry

Column chromatographic purification was carried out on silica gel (200-300 mesh) and monitored by thin layer chromatography performed on GF/UV 254 plates by using UV light at 254 and 365 nm. Melting points were determined on a RY-1 melting-point apparatus, which was not calibrated. The nuclear magnetic resonance (NMR) spectra were recorded at room temperature on a Bruker ACF-300Q instrument (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR spectra). Chemical shifts are given in parts per million (ppm) with tetramethylsilane as internal standard, and coupling constants (*J* values) were given in hertz (Hz). Elemental analyses were carried out by the Heraeus CHN-O-Rapid analyzer. The LC/MS spectra were determined on a Waters liquid chromatography -mass spectrometer system (ESI). All starting materials, reagents and solvents were purchased from commercial sources and used without further purification. TAK-875 and compound **1** were synthesized *via* published procedures.[11]

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. Molecular modeling

The docking simulation of compound **11** was performed by using MOE (version 2008.10, The Chemical Computing Group, Montreal, Canada). The crystal structure of FFA1 (PDB code: 4PHU) was obtained from the Protein Data Bank. Prior to the procedure of docking, the crystal structure of FFA1 was prepared with Protonate 3D and a Gaussian Contact surface was draw around the binding pocket of ligand. Then, the active site was isolated and the backbone was removed. The ligand poses were filtered using Pharmacophore Query Editor. The structures of compound **11** was docked in the active site with Pharmacophore method and then ranked with the London dG scoring function. For the energy minimization of ligand in the pocket, MOE Forcefield Refinement was used and ranked with the London dG scoring function.

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 plated into 96-well

plates at a density of 15K cells/well and incubated overnight in 5% CO_2 at 37 °C. Then, the medium was removed and washed with Hank's Balanced Salt Solution (100 μL). Subsequently, cells were incubated in loading buffer (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 60 min 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 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). EC_{50} values of selected compounds were 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 obtained from Comparative Medicine Centre of Yangzhou University (Jiangsu, China). Mice were acclimatized for 7 days before experiments. The feeding room was keep on a constant 12 h light/black cycle (lights on from 7:30 to 19:30) with controlled temperature (23 ± 1 °C) and humidity (55 ± 5%) throughout the experimental period. Mice were allowed ad libitum access to standard pellets and water unless otherwise stated, and 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 performed using GraphPad software (GraphPad InStat version 5.00, San Diego, CA, USA). Unpaired comparisons were analyzed using the two-tailed Student's t-test.

4.3.2.1. Effect of compound 11 on OGTT explored in male ICR mice

Ten-week-old normal male ICR mice were fasted overnight (12 h), weighted, bled *via* the tail vein, and randomized into 5 groups (n = 6). A single doses of TAK-875 (10 $mL \cdot kg^{-1}$; 20 $mg \cdot kg^{-1}$), vehicle or compound **11** (10 $mg \cdot kg^{-1}$, 20 $mg \cdot kg^{-1}$, 40 $mg \cdot kg^{-1}$) were orally administered 30 min before dosed orally with 30% glucose aqueous solution (3 $g \cdot kg^{-1}$). Blood samples were collected 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 plasma glucose levels were measured by blood glucose test strips (SanNuo ChangSha, China).

4.3.2.2. Effects of compound 11 on the risk of hypoglycemia

Normal male ICR mice 10 weeks old were fasted overnight (12 h) and randomized into 3 groups (n = 6). A single doses of compound **11** (80 mg·kg^{-1}), glibenclamide (15 mg·kg^{-1}), or vehicle was oral administered, and blood samples were collected from tail tip immediately before drug administration (0 min) and at 30, 60, 90, 120 and 180 min after administration. The blood glucose levels were measured as described above.

4.3.2.3. Hypoglycemic effects of compound 11 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 Mediscience 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 in the experiment as type 2 diabetic mice model.[34, 35]

Type 2 diabetic C57BL/6 mice were fasted overnight (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 (10 mL·kg^{-1}; 20 mg·kg^{-1}), vehicle or compound **11** (10 mL·kg^{-1}; 20 mg·kg^{-1}) were orally administered 30 min before dosed orally with 20% glucose aqueous solution (2 g·kg^{-1}). Blood samples were collected 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 blood glucose test strips (SanNuo ChangSha, China).

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References

[1] O.J. Phung, J.M. Scholle, M. Talwar, C.I. Coleman. *JAMA*. **2010**, 303, 1410-1418.

[2] M.A. Avery, C.S. Mizuno, A.G. Chittiboyina, T.W. Kurtz, H.A. Pershadsingh. *Curr Med Chem.* **2008**, 15, 61-74.

[3] S.A. Stein, E.M. Lamos, S.N. Davis. *Expert Opin Drug Saf*. **2013**, 12, 153-175.

[4] H.E. Lebovitz. *Nat. Rev. Endocrinol*. **2011**, 7, 408-419.

[5] Y. Itoh, Y. Kawamata, M. Harada, M. Kobayashi, R. Fujii, S. Fukusumi, K. Ogi, M. Hosoya, Y. Tanaka, H. Uejima, H. Tanaka, M. Maruyama, R. Satoh, S. Okubo, H. Kizawa, H. Komatsu, F. Matsumura, Y. Noguchi, T. Shinobara, S. Hinuma, Y. Fujisawa, M. Fujino. *Nature*. **2003**, 422, 173-176.

[6] C.P. Briscoe, M. Tadayyon, J.L. Andrews, W.G. Benson, J.K. Chambers, M.M. Eilert, C. Ellis, N.A. Elshourbagy, A.S. Goetz, D.T. Minnick, P.R. Murdock, H.R. Sauls, U. Shabon, L.D. Spinage, J.C. Strum, P.G. Szekeres, K.B. Tan, J.M. Way, D.M. Ignar, S. Wilson, A.I. Muir. *J Biol Chem.* **2003**, 278, 11303-11311.

[7] L.A. Stoddart, N.J. Smith, G. Milligan. *Pharmacol. Rev.* **2008**, 60, 405-417.

[8] P. Wellendorph, L.D. Johansen, H. Bräuner-Osborne. *Mol. Pharmacol.* **2009**, 76, 453-465.

[9] X. Wang, T. Zhao, B. Yang, Z. Li, J. Cui, Y. Dai, Q. Qiu, H. Qiang, W. Huang, H. Qian. *Bioorgan Med Chem.* **2015**, 23, 132-140.

[10] Z. Li, X. Wang, X. Xu, J. Yang, W. Xia, X. Zhou, W. Huang, H. Qian. *Bioorgan Med Chem*. **2015**, 23, 7158-7164.

[11] N. Negoro, S. Sasaki, S. Mikami, M. Ito, M. Suzuki, Y. Tsujihata, R. Ito, A. Harada, K. Takeuchi, N. Suzuki, J. Miyazaki, T. Santou, T. Odani, N. Kanzaki, M. Funami, T. Tanaka, A. Kogame, S. Matsunaga, T. Yasuma, Y. Momose. *ACS Med. Chem. Lett.* **2010**, 1, 290-294.

[12] S. Mikami, S. Kitamura, N. Negoro, S. Sasaki, M. Suzuki, Y. Tsujihata, T. Miyazaki, R. Ito, N. Suzuki, J. Miyazaki, T. Santou, N. Kanzaki, M. Funami, T. Tanaka, T. Yasuma, Y. Momose. *J. Med. Chem.* **2012**, 55, 3756-3776.

[13] N. Negoro, S. Sasaki, M. Ito, S. Kitamura, Y. Tsujihata, R. Ito, M. Suzuki, K. Takeuchi, N. Suzuki, J. Miyazaki, T. Santou, T. Odani, N. Kanzaki, M. Funami, T. Tanaka, T. Yasuma, Y. Momose. *J. Med. Chem.* **2012**, 55, 1538-1552.

[14] J.B. Houze, L. Zhu, Y. Sun, M. Akerman, W. Qiu, A.J. Zhang, R. Sharma, M. Schmitt, Y. Wang, J. Liu, J. Liu, J.C. Medina, J.D. Reagan, J. Luo, G. Tonn, J. Zhang, J.Y.-L. Lu, M. Chen, E. Lopez, K. Nguyen, L. Yang, L. Tang, H. Tian, S.J. Shuttleworth, D.C.H. Lin. *Bioorg. Med. Chem. Lett.* **2012**, 22, 1267-1270.

[15] S.P. Brown, P.J. Dransfield, M. Vimolratana, X. Jiao, L. Zhu, V. Pattaropong, Y. Sun, J. Liu, J. Luo, J. Zhang, S. Wong, R. Zhuang, Q. Guo, F. Li, J.C. Medina, G. Swaminath, D.C.H. Lin, J.B. Houze. *ACS Med. Chem. Lett.* **2012**, 3, 726-730.

[16] Y. Wang, J. Liu, P.J. Dransfield, L. Zhu, Z. Wang, X. Du, X. Jiao, Y. Su, A.-r. Li, S.P. Brown, A. Kasparian, M. Vimolratana, M. Yu, V. Pattaropong, J.B. Houze, G. Swaminath, T. Thanhvien, N. Khanh, Q. Guo, J. Zhang, R. Zhuang, F. Li, L. Miao, M.D. Bartberger, T.L. Correll, D. Chow, S. Wong, J. Luo, D.C.H. Lin, J.C. Medina. *ACS Med. Chem. Lett.* **2013**, 4, 551-555.

[17] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney. *Adv. Drug Deliv. Rev.* **2012**, 64, 4-17.

[18] E. Christiansen, C. Urban, M. Grundmann, M.E. Due-Hansen, E. Hagesaether, J. Schmidt, L. Pardo, S. Ullrich, E. Kostenis, M. Kassack, T. Ulven. *J. Med. Chem.* **2011**, 54, 6691-6703.

[19] M.J. Waring. *Expert Opin Drug Dis*. **2010**, 5, 235-248.

[20] G.M. Keserü, G.M. Makara. *Nat Rev. Drug Discov*. **2009**, 8, 203-212.

[21] P.D. Leeson, R.J. Young. *ACS Med. Chem. Lett.* **2015**, 6, 722-725

[22] R. Takano, M. Yoshida, M. Inoue, T. Honda, R. Nakashima, K. Matsumoto, T. Yano, T. Ogata, N. Watanabe, N. Toda. *Bioorg Med Chem Lett*. **2014**, 24, 2949-2953.

[23] Z. Li, X. Wang, X. Xu, J. Yang, Q. Qiu, H. Qiang, W. Huang, H. Qian. *Bioorgan Med Chem*. **2015**, 23, 6666-6672.

[24] A. Srivastava, J. Yano, Y. Hirozane, G. Kefala, F. Gruswitz, G. Snell, W. Lane, A. Ivetac, K. Aertgeerts, J. Nguyen, A. Jennings, K. Okada. *Nature*. **2014**, 513, 124-127.

[25] D.W. Ma, Q. Cai, H. Zhang. *Org Lett* **2003**, 5, 2453-2455.

[26] N. Azizi, A. Khajeh-Amiri, H. Ghafuri, M. Bolourtchian, M.R. Saidi. *Synlett*. **2009**, 2245-2248.

[27] F. Lovering, J. Bikker, C. Humblet. *J. Med. Chem*. **2009**, 52, 6752-6756.

[28] F. Lovering. *MedChemComm.* **2013**, 4, 515-519.

[29] M. Ishikawa, Y. Hashimoto. *J. Med. Chem.* **2011**, 54, 1539-1554.

[30] G.M. Keserü, G.M. Makara. *Nat. Rev. Drug Discovery*. **2009**, 8, 203-212.

[31] T.T. Wager, R.Y. Chandrasekaran, X. Hou, M.D. Troutman, P.R. Verhoest, A. Villalobos, Y. Will. *ACS Chem. Neurosci*. **2010**, 1, 420-434.

[32] C.H. Reynolds, B.A. Tounge, S.D. Bembenek. *J. Med. Chem*. **2008**, 51, 2432-2438.

[33] S.D. Bembenek, B.A. Tounge, C.H. Reynolds. *Drug Discovery Today*. **2009**, 14, 278-283.

[34] M.S. Winzell, B. Ahrén. *Diabetes*. **2004**, 53, 215-219.

[35] J.A. Koehler, L.L. Baggio, B.J. Lamont, S. Ali, D.J. Drucker. *Diabetes*. **2009**, 58, 2148-2161.