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Discovery of first-in-class thiazole-based dual FFA1/PPARδ agonists as potential anti-diabetic agents

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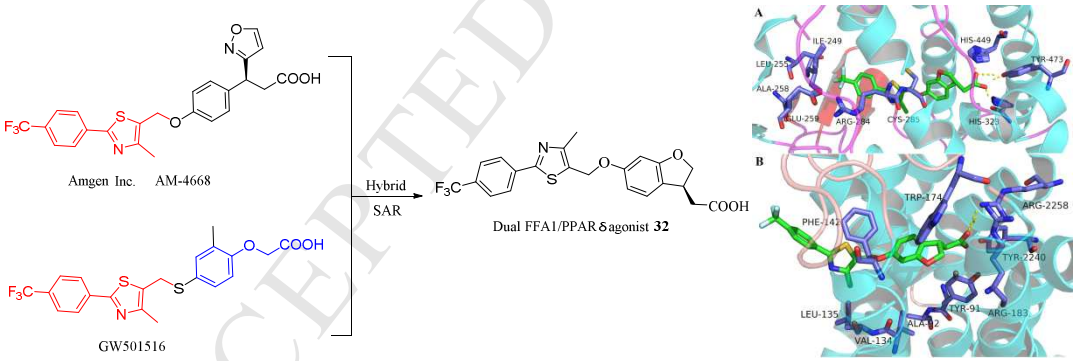
Graphical Abstract



Aiming to develop potent dual FFA1/PPARδ agonists, we have hybrid the FFA1 agonist AM-4668 with PPARδ agonist GW501516 based on their structural similarity, exemplified by the orally bioavailable dual FFA1/PPARδ agonist **32**.

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Discovery of first-in-class thiazole-based dual FFA1/PPARδ agonists as potential anti-diabetic agents

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Abstract

The free fatty acid receptor 1 (FFA1 or GPR40) and peroxisome proliferator-activated receptor δ (PPARδ) have attracted a lot of attention due to their role in promoting insulin secretion and sensibility, respectively, which are two major features of diabetes. Therefore, the dual FFA1/PPARδ agonists would increase insulin secretion and sensibility by FFA1 and PPARδ activation. In this study, we hybrid FFA1 agonist AM-4668 with PPARδ agonist GW501516, leading to the identification of orally bioavailable dual agonist **32**, which revealed high selectivity

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over other PPARs. Moreover, compound **32** exhibited good pharmacokinetic profiles with high plasma concentration, sustained half-life and low clearance *in vivo*. During the hypoglycemic test, a dual agonist **32** enhanced the tolerance of *ob/ob* mice for glucose loading in a dose-dependent manner. Our results suggest that dual FFA1/PPARδ agonist could be a valuable therapy for type 2 diabetes.

Keywords: Dual agonist; FFA1; Hybrid; PPAR; Diabetes.

1. Introduction

Type 2 diabetes is usually caused by insufficient insulin production and resistance to insulin.[1, 2] Agents with pharmacological mechanism on improving the pathogenesis of diabetes are used singly or combined, such as sulfonylureas, metformin and thiazolidinediones. However, there are several potential risks for most of current drugs, including the limited efficacy, hypoglycemia and weight gain.[3-6] Hence, there still have many unmet clinical needs to obtain novel anti-diabetic drugs.[7-9]

In pancreatic β-cells, the free fatty acid receptor 1 (FFA1/GPR40) promotes insulin secretion dependent on blood glucose levels.[10, 11] Based on its physiological mechanism, FFA1 is considered as a novel hypoglycemic target because of its low possibility of hypoglycemia.[12] At present, many groups reported FFA1 agonists with acid moieties (**Figure 1**), and many clinical trials of candidates (e.g. TAK-875) have been completed. Readers want to know more details on the related research progress may refer to the reviews.[13, 14] In this field, we also did some preliminary exploration on FFA1 agonists to extend its chemical space.[15-23]

The peroxisome proliferator-activated receptors (PPARs) have received significant attention due to their function on regulating energy metabolism.[24] There are three subtypes for PPARs: including PPARα, PPARγ, and PPARδ. Among them, PPARα and PPARγ exhibited considerable advantages on improvement of insulin sensibility and lipid disorder.[25-27] The physiological mechanisms of PPARδ include anti-inflammatory, adipocyte differentiation, insulin sensibilization and lipid metabolism.[28-31] Notably, PPARδ activation protects β cells from apoptosis,[32] up-regulating GLP-1 receptor,[33] and increasing mitochondrial function in β cells.[34] Therefore, PPARδ has been considered as a potent target for the treatment of various metabolic disorders.[35]

Based on their complementary mechanisms, the dual FFA1/PPARδ agonists would increase insulin secretion and sensibility by FFA1 and PPARδ activation, respectively. Interestingly, the pharmacophoric features of FFA1 and PPARδ agonists are very similar,[36] and we have explored several hits as FFA1/PPARδ agonists, though the potency is limited to micromole levels.[37] As shown in **Figure 1**, AM-4668 (FFA1 agonist)[38] has the same thiazole moiety (red lable in **Figure 1**) with GW501516 (a selective PPARδ agonist).[39] Thus, it will be a feasible strategy by hybrid AM-4668 with GW501516 to provide dual FFA1/PPARδ agonist. Furthermore, PPARδ agonist GW501516 has the same acid moiety (blue lable in **Figure 1**) with our previous reported FFA1 agonist CP-1.[40, 41] In this study, FFA1 agonists AM-4668 and CP-1 are hybrid with PPARδ agonist GW501516 to maintain the common scaffolds as much as possible (**Figure 2**). After comprehensive exploration, compound **32** was identified as a dual FFA1/PPARδ agonist with high selectivity over other PPARs. Herein, we focus on the description of hybrid strategy, structural optimization, and the hypoglycemic effects in *ob/ob* mice.

2. Results and Discussion

2.1. Chemistry

Preparative process of compounds **1-8** are described in **Scheme 1**. Condensation of raw materials **1a-b** with methyl bromoacetate provided **2a-b**, which were then converted to compounds **3a-b** through Baeyer–Villiger oxidation. Hydrolysis of acetates **3a-b** with sodium methoxide afforded phenols **4a-b**. Cyclization of various thiobenzamides **5a-f** with ethyl 2-chloroacetoacetate generated intermediates **6a-f**, followed by ester reduction using sodium borohydride,[42] and further treated with thionyl chloride under catalytic condition yielded chlorinated intermediates **7a-f**. Condensation of **7a-f** with **4a-b** using classical Williamson ether synthesis with potassium carbonate as base, followed by basic hydrolysis, furnished compounds **1-8**. Compounds **9-14** were synthesized as summarized in **Scheme 2**. Condensation of ethyl 2-chloroacetoacetate with **8a** formed oxazole **9a**, which was further converted to intermediates **10a**. Condensing raw materials **11a** with **12a**, followed by region-selective reaction in the presence of N-bromobutanimide afforded **14a**.[43] The intermediate **10a** or **14a** was connected with **4b** or **15a**, and then hydrolysis using lithium hydroxide, provided target compounds **9-11**. The thiazole ester **16a** or **20a** was prepared by cyclization reaction, and convert to chlorinated intermediate **17a** or **21a**. Condensation of halogenate intermediates with phenols **4a-b**, followed by hydrolysis under basic condition, afforded the carboxylic acids **9-14**.

The compounds **15-20** were synthesized as shown in **Scheme 3**. The condensation of 2-aminoacetophenone with chloroacetyl chloride provided **23a**, which was further converted to **24a** or **25a** by cyclization reaction in the condition of phosphorus oxychloride or Lawesson's reagent, respectively.[44, 45] Similar reaction process furnished **28a** or **29a** from the starting material benzoylhydrazide. Condensation of these chlorinated intermediates with **4a-b**, then

hydrolysis to provide the target compounds **15-20**. **Scheme 4** describes the synthesis of compounds **21-32**. The phenols **30a**,[21] **31a**[22] and **32a**[46] were synthesized *via* reported procedures. The optically pure **35a** was separated from racemate **33a** in the presence of R-phenethylamine. Condensation of phenols (**30a-32a**, **35a**) with **7a-h**, and then ester hydrolysis, provide **21-32**.

2.2. SAR study

The potency of synthesized compounds on FFA1 was measured using FFA1-expressed Chinese hamster ovary cells, and the potency of PPARδ was evaluated in Gal4 receptor cell-based assay. First, we explore the hybrids of FFA1 agonist AM-4668 and PPARδ agonist GW501516 (**Table 1**), and briefly investigated the tolerability of substituents at terminal B ring. Compound **1**, a direct analog of GW501516, exhibited an approximately 130-fold reduced potency of PPARδ compared to positive control GW0742. Replacement of methyl group at A ring with fluorine (compound **2**) hardly affected potency on PPARδ, while the agonistic activity of FFA1 has significant improvement compared to compound **1**. These results indicated that fluorine at A ring might be a better substituent to explore the dual FFA1/PPARδ agonists. Introduction of trifluoromethyl group at the B ring (compounds **3** and **4**) drastically increased potency on PPARδ, suggesting that small hydrophobic substituent in B ring is very important for matching with the hydrophobic pocket of PPARδ. Based on previously reported SAR studies on PPARδ,[39] the 4-position and 3-position substituents at B ring were incorporated to evaluate their potential on dual FFA1/PPARδ agonists in this study. Fluorine and methyl scanning in B ring (compounds **5-8**) indicated that hydrophobic substitution is preferred in 4-position and tolerated in 3-position for the potency on FFA1 and

PPARδ.

Meanwhile, we focused on the exploration of other heterocycles to replace the thiazole scaffold (**Table 2**). Replacement of sulphur (compound **2**) with oxygen (compound **9**) resulted in remarkable reduction of activity on FFA1 and PPARδ, might attributing to these differences of sulphur and oxygen between hydrophobic effect and electronic distribution. Indeed, the potency of oxazole derivatives (**15** and **16**) was inferior to that of the corresponding thiazole analog **17**. The exchange of sulphur and nitrogen atom in compound **2** provided analog **10** appears to diminishing agonistic activities both in FFA1 and PPARδ, implying that there is a strict ligand-receptor interaction in binding pockets. Incorporation of nitrogen-containing linker (compound **11**) improved agonistic activity of FFA1 to some extent, while significantly decreased potency on PPARδ. Compounds **12-14** revealed lower potencies on FFA1 and PPARδ compared to compound **10**, which might be attributed to the reduced hydrophobic interaction due to the absence of methyl group at thiazole moiety. To decrease the lipophilicity of ligand, the thiadiazole and oxdiazole derivatives (**18-20**) were designed. However, none of them (compounds **18-20**) exhibited desired agonistic activity, probably because of the highly hydrophobic pockets in FFA1 and PPARδ.

Based on SAR studies above, thiazole scaffold of compound **3** was selected as our starting point for further optimization. In this part, our efforts were directed to explore whether other acid scaffolds could also retain dual potency on FFA1 and PPARδ (**Table 3**). The sulfone acid (**21** and **22**) and deuterated acid (**23-25**), two reported scaffolds of FFA1 agonists,[21, 22] were incorporated to investigate the potency on FFA1 and PPARδ. The sulfone acid analogs **21** and **22** revealed moderate activity for FFA1, but their potential to activate PPARδ was found to be rather low (> 10 μM). The deuterated acid derivatives **23-25** indicated improved PPARδ activities back

to the level of phenoxyacetic acid analog **4** but, nevertheless, led to reduced potency on FFA1 (compound **4** *vs* **25**). Interestingly, introduction of dihydrobenzofuran carboxylic acid of TAK-875 provided compounds **26**-**32**, which revealed better balance between FFA1 and PPARδ compared to phenoxyacetic acid series. In this series, different substituents at left benzene ring have little effect on agonistic activity of FFA1, while 4-methoxy group (compound **30**) decreased potency on PPARδ. Notably, compound **31** (4-CF_3) exhibited the best balance between FFA1 and PPARδ, which was selected for further evaluation by separating its optimal enantiomer. As expected, the optimal enantiomer **32** (FFA1: 68 nM; PPARδ: 102 nM) revealed marked improvement compared with racemate **31** (FFA1: 113 nM; PPARδ: 175 nM). Moreover, compound **32** exhibited high selectivity for FFA1 and PPARδ over other nuclear receptors of PPAR family (for which EC_{50} > 10 μM, **Table 4**).

2.3. Docking study

Based on co-crystal structure of PPARδ (PDB number: 1GWX) and FFA1 (4PHU), the induced-fit docking study was performed to explore the interactional mode of the dual FFA1/PPARδ agonist **32**. As shown in **Figure 3**, the dual agonist **32** fitted well with these two receptors in binding site. In binding site of PPARδ (**Figure 3A**), carboxylic acid of agonist **32** formed interactions with residues His449, Tyr473 and His323 by hydrogen-bonding network. Moreover, trifluoromethyl group at the left benzene is fitted well with the small hydrophobic pocket surrounded by Ile249, Leu255 and Ala258. Therefore, the un-substituted derivative **26** and compound **30** with bulkier substituent revealed significantly reduced activities on PPARδ. In binding site of FFA1 (**Figure 3B**), carboxylic acid of **32** formed anchor point with Arg2258 and Arg183. Furthermore, an

edge-on interaction was generated between the plane of dihydrobenzofuran and Trp174. The trifluoromethyl group was exposed to the outside of receptor, rationally explained that the left phenyl ring could tolerate various types of substituents while little effect for FFA1 activity.

2.4. Pharmacokinetic study

The pharmacokinetic (PK) profiles of compound **32** were evaluated in fasted rats. As shown in **Table 5**, compound **32** exhibited good PK profiles with high maximum plasma concentration (C_{max} = 9.45 μg/mL), which was 200-fold higher than its EC_{50} values on FFA1 and PPARδ. Moreover, compound **32** showed low metabolic clearance (CL = 16.73 mL/h/kg) and sustained half-life ($T_{1/2}$ = 4.31 h) suitable for twice daily, resulting in high exposure *in vivo* (AUC_{0-24h} = 95.62 μg/mL·h).

2.5. Hypoglycemic effects of 32

To investigate glucose-lowering effect *in vivo*, different doses of compound **32** (10, 30 and 100 mg/kg) were evaluated in *ob*/*ob* mice, a genetic defect model with metabolic disorders such as diabetes-related obese and insulin resistance.[47, 48] As shown in **Figure 4**, the dual agonist **32** (10, 30 and 100 mg/kg) exhibited hypoglycemic effect in a dose-dependent manner, with changes in plasma glucose $AUC_{0-120min}$ of +1.1%, -6.1%, and -10.9%, respectively. Notably, **32** (100 mg/kg) exhibited significantly glucose-lowering effect though it is inferior to the typical agonist TAK-875 (-15.7% $AUC_{0-120min}$).

3. Conclusion

In this paper, FFA1 agonist AM-4668 was hybrid with PPARδ agonist GW501516 to design dual

FFA1/PPARδ agonists. After comprehensive SAR exploration, we identified the orally bioavailable dual FFA1/PPARδ agonist **32** (FFA1: 68 nM; PPARδ: 102 nM), which has high selectivity over other PPARs. The dual agonist **32** exhibited good PK profiles with high plasma exposure, which ensured the enough effective concentration to simultaneously activate FFA1 and PPARδ *in vivo*. Moreover, compound **32** reduced the levels of glucose in a dose-response manner. These interesting findings indicated that the dual agonist **32** is meaningful as a tool compound, which will help us to seek better dual FFA1/PPARδ agonist.

4. Experimental section

4.1. General chemistry

Reagents and solvents were purchased from commercial sources. The progress of reaction was monitored by thin layer chromatography analysis on GF254 plates at 254 and 365 nm. Chromatographic purification was performed on silica gel (200-300 mesh). Melting points were carried out on a RY-1 melting-point apparatus. Nuclear magnetic resonance spectra (^{1}H NMR at 300 MHz and ^{13}C NMR at 75 MHz) were measured by Bruker ACF-300Q instrument, coupling constants (*J* values) are expressed as hertz (Hz), and chemical shifts are expressed as parts per million (ppm) relative to internal standard (tetramethylsilane). Liquid chromatography-tandem mass spectrometry (Waters) was used to determine LC-MS spectra. Elemental analysis was recorded on Heraeus CHN-O-Rapid analyzer within 0.4% of theoretical values. Experimental protocols for TAK-875 were disclosed in the supporting information of previous literature.[46]

General synthetic procedure for intermediates 4a and 4b

To a stirred solution of **1a** or **1b** (1 equiv) in acetone was added potassium carbonate (3 equiv) and

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

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

Methyl 2-(2-fluoro-4-hydroxyphenoxy)acetate (4b). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.44 (s,

1H), 6.92 – 6.85 (m, 1H), 6.63, 6.59 (dd, *J* = 13.1, 2.8 Hz, 1H), 6.51-6.47 (m, 1H), 4.73 (s, 2H), 3.69 (s, 3H). ESI-MS *m/z*: 201.1 $[M+H]^+$.

General synthetic procedure for intermediates 6a-h

A solution of **5a-h** (1 equiv) and ethyl 2-chloroacetoacetate (1.2 equiv) in ethanol (25 mL) was heated to reflux for 6 h, then the mixture was allowed to stand at 0 °C for 10 hrs, and a white needle crystal was precipitate out. The reaction mixture was filtered and the filter cake was washed with ethanol (10 mL), dried to give the title compounds.

Ethyl 4-methyl-2-phenylthiazole-5-carboxylate (6a). Yield 75%; white powder; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.95 – 7.86 (m, 2H), 7.45 – 7.40 (m, 3H), 4.30 (q, *J* = 7.1 Hz, 2H), 2.68 (s, 3H), 1.31 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z*: 248.1 $[M+H]^+$.

Ethyl 4-methyl-2-(4-(trifluoromethyl)phenyl)thiazole-5-carboxylate (6b). Yield 89%; white powder; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.92 (d, *J* = 8.1 Hz, 2H), 7.67 (d, *J* = 8.1 Hz, 2H), 4.29 (q, *J* = 7.1 Hz, 2H), 2.67 (s, 3H), 1.30 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z*: 316.1 $[M+H]^+$.

Ethyl 2-(4-fluorophenyl)-4-methylthiazole-5-carboxylate (6c). Yield 72%; white powder; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.02 (d, *J* = 8.6 Hz, 2H), 7.59 (d, *J* = 8.6 Hz, 2H), 4.30 (q, *J* = 7.1 Hz, 2H), 2.90 (s, 3H), 1.30 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z*: 266.1 $[M+H]^+$.

Ethyl 2-(3-fluorophenyl)-4-methylthiazole-5-carboxylate (6d). Yield 67%; white powder; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.84 – 7.79 (m, 2H), 7.60 – 7.56 (m, 1H), 7.44 – 7.40 (m, 1H), 4.29 (q, *J* = 7.1 Hz, 2H), 2.67 (s, 3H), 1.30 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z*: 266.1 $[M+H]^+$.

Ethyl 4-methyl-2-(*p*-tolyl)thiazole-5-carboxylate (6e). Yield 85%; white powder; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.87 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 4.29 (q, *J* = 7.1 Hz,

2H), 2.67 (s, 3H), 2.36 (s, 3H), 1.30 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z*: 262.1 $[M+H]^+$.

Ethyl 4-methyl-2-(*m*-tolyl)thiazole-5-carboxylate (6f). Yield 68%; white powder; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.86 – 7.81 (m, 1H), 7.76 (d, *J* = 7.2 Hz, 1H), 7.41 – 7.38 (m, 2H), 4.28 (q, *J* = 7.1 Hz, 2H), 2.69 (s, 3H), 2.38 (s, 3H), 1.30 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z*: 262.1 $[M+H]^+$.

Ethyl 2-(4-chlorophenyl)-4-methylthiazole-5-carboxylate (6g). Yield 84%; white powder; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.01 (d, *J* = 8.6 Hz, 2H), 7.59 (d, *J* = 8.6 Hz, 2H), 4.30 (q, *J* = 7.1 Hz, 2H), 2.69 (s, 3H), 1.30 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z*: 282.1 $[M+H]^+$.

Ethyl 2-(4-methoxyphenyl)-4-methylthiazole-5-carboxylate (6h). Yield 67%; white powder; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.95 (d, *J* = 8.7 Hz, 2H), 7.06 (d, *J* = 8.7 Hz, 2H), 4.29 (q, *J* = 7.1 Hz, 2H), 3.83 (s, 3H), 2.67 (s, 3H), 1.30 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z*: 278.1 $[M+H]^+$.

General synthetic procedure for intermediates 7a-h

To a stirred mixture of sodium borohydride (3 equiv), **6a-h** (1 equiv) in reflux tetrahydrofuran (20 mL) was added methanol (1 mL). After refluxing for futher 30 min, the reaction mixture was pouring into water (20 mL), and extracted with ethyl acetate (3 × 10 mL), the organic fractions were combined, washed with saturated brine (2 × 10 mL) prior to drying over anhydrous sodium sulfate. After filtration and concentrate, the residue was dissolved in dichloromethane (20 mL), thionyl chloride (6 equiv) and catalytic DMF were added at room temperature. After stirring at 40 °C for 4 h, the reaction was concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 20:1, v/v) to afford the title compounds.

5-(chloromethyl)-4-methyl-2-phenylthiazole (7a). Yield 83%; yellow semisolid; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.93 – 7.85 (m, 2H), 7.43 – 7.38 (m, 3H), 5.12 (s, 2H), 2.68 (s, 3H). ESI-MS *m/z*: 224.1 $[M+H]^+$.

5-(chloromethyl)-4-methyl-2-(*p*-tolyl)thiazole (7e). Yield 89%; yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.85 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 5.13 (s, 2H), 2.67 (s, 3H), 2.36 (s, 3H). ESI-MS *m/z*: 238.1 $[M+H]^+$.

General synthetic procedure for target compounds 1-8

To a solution of **4a-b** (1 equiv) and **7a-f** (1.1 equiv) in acetonitrile was added potassium carbonate (2 equiv) and catalytic potassium iodide. The mixture was heated at 45 °C for 12 h. Then the mixture was cooled followed by filtration and the filtrate was concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 10:1, v/v) to afford a white solid, which was dissolved in the mixed solvent of THF/MeOH/H_2O (18 mL, 2:3:1), and added lithium hydroxide (1.5 equiv). After stirring at room temperature for 4 h, the volatiles were removed under reduced pressure. The residue was acidified with 1N hydrochloric acid solution, and then filtered and the filter cake was washed with cold water (5 mL), dried in vacuum to afford a white powder. Recrystallization from 75% ethanol provides pure target compounds.

2-(2-methyl-4-((4-methyl-2-phenylthiazol-5-yl)methoxy)phenoxy)acetic acid (1)

Yield 53%; white solid; m.p. 154-156 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.74 (brs, 1H), 7.90 (d, J = 4.0 Hz, 2H), 7.56 – 7.38 (m, 3H), 6.89 (s, 1H), 6.86 – 6.70 (m, 2H), 5.22 (s, 2H), 4.62 (s, 2H), 2.43 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.95, 165.63, 152.19, 151.64, 151.09, 133.46, 130.63, 129.65, 128.10, 127.85, 126.36, 118.48, 112.96, 112.76, 65.79, 62.53,

16.66, 15.54. ESI-MS *m/z*: 367.5 $[M-H]^-$. Anal. calcd. For $C_{20}H_{19}NO_4S$: C, 65.02; H, 5.18; N, 3.79; Found: C, 65.27; H, 5.06; N, 3.73.

2-(2-fluoro-4-((4-methyl-2-phenylthiazol-5-yl)methoxy)phenoxy)acetic acid (2)

Yield 65%; white solid; m.p. 106-108 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.08 – 7.81 (m, 2H), 7.60 – 7.39 (m, 3H), 7.17 – 6.94 (m, 2H), 6.80 (d, J = 8.6 Hz, 1H), 5.27 (s, 2H), 4.68 (s, 2H), 2.44 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.39, 165.85, 152.82, 152.05, 140.85, 133.48, 130.64, 129.62, 127.44, 126.41, 116.66, 111.25, 105.17, 104.88, 66.59, 62.97, 15.51. ESI-MS *m/z*: 371.5 $[M-H]^-$. Anal. calcd. For $C_{19}H_{16}FNO_4S$: C, 61.12; H, 4.32; N, 3.75; Found: C, 61.35; H, 4.21; N, 3.58.

2-(2-methyl-4-((4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)methoxy)phenoxy)acetic acid (3)

Yield 61%; white solid; m.p. 160-162 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.75 (brs, 1H), 8.09 (d, J = 7.3 Hz, 2H), 7.82 (d, J = 7.3 Hz, 2H), 7.06 – 6.64 (m, 3H), 5.25 (s, 2H), 4.61 (s, 2H), 2.45 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.92, 163.64, 152.12, 152.06, 151.12, 136.90, 129.81, 127.86, 126.99, 126.57, 118.43, 112.94, 112.76, 65.77, 62.51, 16.64, 15.52. ESI-MS *m/z*: 435.6 $[M-H]^-$. Anal. calcd. For $C_{21}H_{18}F_3NO_4S$: C, 57.66; H, 4.15; N, 3.20; Found: C, 57.45; H, 4.27; N, 3.12.

2-(2-fluoro-4-((4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)methoxy)phenoxy)acetic acid (4)

Yield 57%; white solid; m.p. 148-150 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.99 (brs, 1H), 8.10 (d, J = 6.7 Hz, 2H), 7.83 (d, J = 6.7 Hz, 2H), 7.13 – 6.77 (m, 3H), 5.30 (s, 2H), 4.69 (s, 2H), 2.46 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.34, 163.89, 152.81, 152.47, 140.86, 136.94, 129.09, 127.05, 126.53, 116.65, 111.23, 105.16, 104.87, 66.48, 62.94, 15.47. ESI-MS *m/z*: 439.8 [M-H]$^-$. Anal. calcd. For $C_{20}H_{15}F_4NO_4S$: C, 54.42; H, 3.43; N, 3.17; Found: C, 54.67; H, 3.55; N, 3.26.

2-(2-fluoro-4-((2-(4-fluorophenyl)-4-methylthiazol-5-yl)methoxy)phenoxy)acetic acid (5)

Yield 59%; white solid; m.p. 142-144 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.72 (brs, 1H), 7.93 (d, J = 7.8 Hz, 2H), 7.29 (d, J = 7.8 Hz, 2H), 7.13 – 6.92 (m, 2H), 6.79 (d, J = 8.7 Hz, 1H), 5.25 (s, 2H), 4.69 (s, 2H), 2.42 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.44, 164.70, 153.87, 152.68, 150.63, 140.70, 130.03, 128.73, 127.49, 116.80, 116.31, 111.06, 105.05, 104.77, 66.18, 62.74, 15.47. ESI-MS *m/z*: 389.6 [M-H]$^-$. Anal. calcd. For $C_{19}H_{15}F_2NO_4S$: C, 58.31; H, 3.86; N, 3.58; Found: C, 58.53; H, 3.79; N, 3.46.

2-(2-fluoro-4-((2-(3-fluorophenyl)-4-methylthiazol-5-yl)methoxy)phenoxy)acetic acid (6)

Yield 52%; white solid; m.p. 128-130 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.77 – 7.63 (m, 2H), 7.61 – 7.46 (m, 1H), 7.36 – 7.21 (m, 1H), 7.10 – 6.90 (m, 2H), 6.79 (d, J = 8.8 Hz, 1H), 5.27 (s, 2H), 4.63 (s, 2H), 2.43 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.46, 164.73, 153.85, 152.69, 150.65, 140.72, 130.06, 127.65, 126.33, 116.81, 115.93, 115.46, 111.03, 105.05, 104.77, 66.18, 62.74, 15.45. ESI-MS *m/z*: 389.7 [M-H]$^-$. Anal. calcd. For $C_{19}H_{15}F_2NO_4S$: C, 58.31; H, 3.86; N, 3.58; Found: C, 58.58; H, 3.74; N, 3.67.

2-(2-fluoro-4-((4-methyl-2-(*p*-tolyl)thiazol-5-yl)methoxy)phenoxy)acetic acid (7)

Yield 69%; white solid; m.p. 152-153 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.79 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 7.11 – 6.97 (m, 2H), 6.81, 6.78 (dd, J = 9.0, 1.4 Hz, 1H), 5.26 (s, 2H), 4.70 (s, 2H), 2.42 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.35, 164.83, 153.81, 152.59, 150.65, 141.85, 140.31, 130.06, 129.45, 129.07, 127.49, 116.85, 110.87, 105.05, 66.16, 62.78, 21.46, 15.45. ESI-MS *m/z*: 386.2 [M-H]$^-$. Anal. calcd. For $C_{20}H_{18}FNO_4S$: C, 62.00; H, 4.68; N, 3.62; Found: C, 62.27; H, 4.56; N, 3.71.

2-(2-fluoro-4-((4-methyl-2-(*m*-tolyl)thiazol-5-yl)methoxy)phenoxy)acetic acid (8)

Yield 54%; white solid; m.p. 138-140 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 13.02 (brs, 1H), 7.79 – 7.60 (m, 2H), 7.45 – 7.19 (m, 2H), 7.14 – 6.94 (m, 2H), 6.80 (d, J = 9.1 Hz, 1H), 5.27 (s, 2H), 4.70 (s, 2H), 2.43 (s, 3H), 2.37 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.47, 165.99, 154.36, 153.64, 151.99, 139.06, 133.34, 131.41, 129.59, 127.25, 126.78, 123.64, 116.25, 111.08, 66.12, 62.77, 21.33, 15.53. ESI-MS *m/z*: 385.6 [M-H]$^-$. Anal. calcd. For $C_{20}H_{18}FNO_4S$: C, 62.00; H, 4.68; N, 3.62; Found: C, 62.15; H, 4.73; N, 3.75.

Ethyl 4-methyl-2-phenyloxazole-5-carboxylate (9a). A solution of benzamide (1.0 g, 8.3 mmol) and ethyl 2-chloroacetoacetate (1.6 g, 9.9 mmol) in ethanol (20 mL) was heated to reflux for 16 h, the solvent was removed under reduced pressure, and the residue was washed with water (25 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 25:1, v/v) to afford the title compound (0.8 g, 42%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.07 – 8.01 (m, 2H), 7.43 – 7.38 (m, 3H), 4.34 (q, J = 7.11 Hz, 2H), 2.57 (s, 3H), 1.35 (t, J = 7.11 Hz, 3H). ESI-MS *m/z*: 232.1 $[M+H]^+$.

2-(2-fluoro-4-((4-methyl-2-phenyloxazol-5-yl)methoxy)phenoxy)acetic acid (9)

The title compound was prepared by the method similar to that described for compound **1** using the intermediate **9a** as starting material. Yield 37%; white solid; m.p. 159-161 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.87 (brs, 1H), 8.12 – 7.97 (m, 2H), 7.56 – 7.42 (m, 3H), 7.19 – 6.96 (m, 2H), 6.85 (d, J = 8.4 Hz, 1H), 5.31 (s, 2H), 4.67 (s, 2H), 2.43 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.42, 158.34, 154.65, 153.16, 141.63, 138.13, 130.52, 129.65, 128.73, 127.56, 125.47, 116.92, 110.97, 103.42, 66.57, 62.95, 15.23. ESI-MS *m/z*: 356.1 $[M-H]^-$. Anal. calcd. For $C_{19}H_{16}FNO_5$: C, 63.86; H, 4.51; N, 3.92; Found: C, 63.58; H, 4.43; N, 3.76.

4-(bromomethyl)-5-methyl-2-phenylthiazole (14a). A solution of thiobenzamide (1.0 g, 7.3 mmol) and 3-chloro-2-butanone (1.2 g, 8.5 mmol) in ethanol (20 mL) was heated at reflux for 6 h. The reaction mixture was concentrated, diluted with 1N sodium bicarbonate solution (15 mL) and ethyl acetate (25 mL), and then washed with water (1 × 15 mL) and brine (1 × 15 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to provide 4,5-dimethyl-2-phenyl-1,3-thiazole (**13a**). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.93, 7.91 (dd, J = 7.6, 1.3 Hz, 2H), 7.45-7.40 (m, 3H), 2.38 (s, 3H), 2.21 (s, 3H). ESI-MS *m/z*: 190.1 $[M+H]^+$.

To a solution of **13a** (1 g, 5.3 mmol) in acetonitrile (8 mL) was added N-bromosuccinimide (0.9 g, 5.3 mmol) and the mixture was stirred at room temperature for 2 h. To the mixture, water

(10 mL) was added dropwise at room temperature to give colourless to pale yellow precipitation. The mixture was stirred at 0–5 °C for 1 h. The precipitation was filtered and washed with cold water (8 mL) to afford the title compound **14a** (0.7 g, 50%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.93, 7.91 (dd, J = 7.6, 1.3 Hz, 2H), 7.45-7.40 (m, 3H), 4.56 (s, 2H), 2.36 (s, 3H). ESI-MS *m/z*: 268.1 $[M+H]^+$.

2-(2-fluoro-4-((5-methyl-2-phenylthiazol-4-yl)methoxy)phenoxy)acetic acid (10)

The title compound was prepared by the method similar to that described for compound **1** using the intermediate **14a** as starting material. Yield 75%; white solid; m.p. 119-121 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.98 – 7.83 (m, 2H), 7.57 – 7.45 (m, 3H), 7.13 – 6.87 (m, 2H), 6.76 (d, J = 8.8 Hz, 1H), 5.28 (s, 2H), 4.65 (s, 2H), 2.43 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.25, 168.12, 154.37, 153.62, 152.09, 143.37, 141.87, 130.96, 129.23, 128.74, 123.13, 116.93, 110.94, 103.42, 68.73, 62.58, 12.35. ESI-MS *m/z*: 372.1 $[M-H]^-$. Anal. calcd. For $C_{19}H_{16}FNO_4S$: C, 61.12; H, 4.32; N, 3.75; Found: C, 61.31; H, 4.24; N, 3.58.

2-(2-fluoro-4-(((5-methyl-2-phenylthiazol-4-yl)methyl)amino)phenoxy)acetic acid (11)

To a solution of **14a** (0.20 g, 0.75 mmol) and **15a** (0.15 g, 0.75 mmol) in ethanol (20 mL) was added sodium bicarbonate (0.19 g, 2.25 mmol) at room temperature. The reaction mixture was stirred at room temperature for 24 h. Then the reaction mixture was filtrated and the filtrate was concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 8:1, v/v) to afford a white solid (0.18 g, 64%), which was dissolved in the mixed solvent of THF/MeOH/H_2O (18 mL, 2:3:1), and added lithium hydroxide (0.02 g, 0.73

mmol). After stirring at room temperature for 4 h, the volatiles were removed under reduced pressure. The residue was acidified with 1N hydrochloric acid solution (pH = 5 – 6), then filtered and the filter cake was washed with cold water (5 mL), dried to afford a white powder. Recrystallization from 75% ethanol gave the title compound **11** (0.12 g, 67%) as white solid, m.p. 136-138 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.95 – 7.81 (m, 2H), 7.54 – 7.43 (m, 4H), 7.06 – 6.82 (m, 1H), 6.62 (d, J = 14.1 Hz, 1H), 6.44 (s, 1H), 4.54 (s, 2H), 4.25 (s, 2H), 2.49 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.60, 164.53, 152.95, 152.07, 143.56, 141.59, 137.43, 130.93, 129.08, 128.76, 123.15, 116.69, 111.47, 103.58, 67.12, 42.85, 14.35. ESI-MS *m/z*: 371.1 $[M-H]^-$. Anal. calcd. For $C_{19}H_{17}FN_2O_3S$: C, 61.28; H, 4.60; N, 7.52; Found: C, 61.57; H, 4.48; N, 7.36.

Ethyl 2-phenylthiazole-4-carboxylate (16a). A solution of ethyl bromopyruvate (0.68 g, 3.5 mmol) and thiobenzamide (0.40 g, 2.9 mmol) in ethanol (20 mL) was heated at reflux for 4 h. The solvent was removed, and the residue was added water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 20:1, v/v) to afford the title compound (0.52 g, 76%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.96 (s, 1H), 7.89 (d, J = 8.0 Hz, 2H), 7.31 (m, 3H), 4.39 (q, J = 7.1 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H). ESI-MS *m/z*: 234.1 $[M+H]^+$.

2-(2-fluoro-4-((2-phenylthiazol-4-yl)methoxy)phenoxy)acetic acid (12)

The title compound was prepared by the method similar to that described for compound **1** using the intermediate **16a** and **4b** as starting material. Yield 46%; white solid; m.p. 150-152 °C; ^{1}H

NMR (300 MHz, DMSO-d_6) δ: 12.74 (brs, 1H), 8.01 – 7.90 (m, 2H), 7.77 (s, 1H), 7.58 – 7.43 (m, 3H), 7.11 – 6.99 (m, 2H), 6.89 – 6.76 (m, 1H), 5.18 (s, 2H), 4.70 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.15, 168.95, 159.31, 154.55, 153.12, 143.25, 141.67, 130.96, 129.63, 128.59, 116.93, 115.62, 110.92, 103.46, 66.67, 62.74. ESI-MS *m/z*: 357.6 $[M-H]^-$. Anal. calcd. For $C_{18}H_{14}FNO_4S$: C, 60.16; H, 3.93; N, 3.90; Found: C, 60.39; H, 3.78; N, 3.82.

2-(2-methyl-4-((2-phenylthiazol-4-yl)methoxy)phenoxy)acetic acid (13)

The title compound was prepared by the method similar to that described for compound **1** using the intermediate **16a** and **4a** as starting material. Yield 53%; white solid; m.p. 134-136 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.74 (brs, 1H), 8.12 – 7.86 (m, 2H), 7.71 (s, 1H), 7.61 – 7.43 (m, 3H), 6.91 (s, 1H), 6.87 – 6.70 (m, 2H), 5.15 (s, 2H), 4.61 (s, 2H), 2.20 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.81, 167.76, 153.91, 152.78, 150.98, 133.47, 130.75, 129.70, 127.95, 126.61, 118.48, 118.24, 113.10, 112.69, 66.47, 66.12, 16.59. ESI-MS *m/z*: 353.7 $[M-H]^-$. Anal. calcd. For $C_{19}H_{17}NO_4S$: C, 64.21; H, 4.82; N, 3.94; Found: C, 64.47; H, 4.68; N, 3.75.

Ethyl 4-phenylthiazole-2-carboxylate (20a). A solution of ethyl thiooxamate (0.40 g, 3.0 mmol) and 2-Bromoacetophenone (0.60 g, 3.0 mmol) in ethanol (15 mL) was heated to reflux for 6 h. The mixture was concentrated, then diluted with ethyl acetate (20 mL), and washed with 1N sodium bicarbonate solution (2 × 15 mL) and brine (15 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 20:1, v/v) to afford the title compound (0.52 g, 74%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.55 (s, 1H),

8.03 (d, *J* = 1.2 Hz, 1H), 8.01 (d, *J* = 7.2 Hz, 1H), 7.52-7.39 (m, 3H), 4.42 (q, *J* = 7.1 Hz, 2H), 1.37 (t, *J* = 7.1 Hz, 3H). ESI-MS *m/z*: 234.1 $[M+H]^+$.

2-(2-fluoro-4-((4-phenylthiazol-2-yl)methoxy)phenoxy)acetic acid (14)

The title compound was prepared by the method similar to that described for compound **1** using the intermediate **20a** as starting material. Yield 48%; white solid; m.p. 174-176 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.11 (s, 1H), 8.03 – 7.90 (m, 2H), 7.56 – 7.30 (m, 3H), 7.17 – 7.00 (m, 2H), 6.86 (d, *J* = 8.9 Hz, 1H), 5.46 (s, 2H), 4.66 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.41, 166.72, 154.75, 152.42, 134.42, 129.27, 128.59, 126.51, 116.63, 115.52, 111.04, 105.10, 104.81, 68.02, 66.65. ESI-MS *m/z*: 357.6 $[M-H]^-$. Anal. calcd. For $C_{18}H_{14}FNO_4S$: C, 60.16; H, 3.93; N, 3.90; Found: C, 60.27; H, 3.83; N, 3.87.

2-chloro-N-(2-oxo-2-phenylethyl)acetamide (23a). The choroacetylchloride (1.71 mL, 22.7 mmol) dissolved in dichloromethane (15 mL) was added dropwise to a solution of 2-aminoacetophenonehydrochloride (3 g, 17.5 mmol) and triethylamine (7.27 mL, 52.4 mmol) in dichloromethane (30 mL), keeping at 0 °C for 2 h, and then stirred at room temperature for 16 h. The reaction solution was washed with 1N hydrochloric acid (2 × 15 mL), saturated sodium carbonate solution (2 × 15 mL), and brine (1 × 15 mL) in sequence. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 5:1, v/v) to afford the title compound (2.3 g, 62%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.58 (s, 1H), 7.99 (d, *J* = 7.2 Hz, 2H), 7.71-7.53 (m, 3H), 4.69 (d, *J* = 5.5 Hz, 2H), 4.21 (s, 2H). ESI-MS

m/z: 212.1 $[M+H]^+$.

2-(chloromethyl)-5-phenyloxazole (24a). To a solution of **23a** (1.0 g, 4.8 mmol) in acetonitrile (20 mL) was added phosphorus oxychloride (0.86 mL, 9.4 mmol) at ambient temperature. After addition was complete, the solution was heated to reflux for 4 h. The reaction mixture was concentrated, then diluted with ethyl acetate (25 mL), and washed with water (2 × 15 mL), 1N sodium bicarbonate solution (2 × 15 mL) and brine (25 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 20:1, v/v) to afford the title compound (0.71 g, 77%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.21 (s, 1H), 7.70, 7.69 (dd, J = 7.1, 0.9 Hz, 2H), 7.48-7.36 (m, 3H), 5.13 (s, 2H). ESI-MS *m/z*: 194.1 $[M+H]^+$.

2-(chloromethyl)-5-phenylthiazole (25a). To a solution of **23a** (1 g, 4.7 mmol) in tetrahydrofuran (20 mL) was added Lawesson's reagent (1.14 g, 2.8 mmol), and the mixture was heated to reflux for 4 h. The reaction mixture was concentrated, then diluted with ethyl acetate (30 mL), and washed with 1N sodium bicarbonate solution (2 × 20 mL) and brine (20 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 30:1, v/v) to afford the title compound (0.75 g, 75%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.23 (s, 1H), 7.70, 7.69 (dd, J = 7.1, 0.9 Hz, 2H), 7.49-7.37 (m, 3H), 5.14 (s, 2H). ESI-MS *m/z*: 210.0 $[M+H]^+$.

2-(2-fluoro-4-((5-phenyloxazol-2-yl)methoxy)phenoxy)acetic acid (15)

The title compound was prepared by the method similar to that described for compound **1** using the intermediate **24a** and **4b** as starting material. Yield 42%; white solid; m.p. 156-158 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.80 – 7.62 (m, 3H), 7.57 – 7.34 (m, 3H), 7.16 – 6.96 (m, 2H), 6.89 – 6.79 (m, 1H), 5.25 (s, 2H), 4.70 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.41, 159.21, 154.26, 152.00, 150.13, 129.63, 129.30, 127.61, 124.55, 123.29, 116.29, 110.82, 104.99, 104.70, 66.15, 62.99. ESI-MS *m/z*: 341.5 [M-H]$^-$. Anal. calcd. For $C_{18}H_{14}FNO_5$: C, 62.97; H, 4.11; N, 4.08; Found: C, 62.83; H, 4.23; N, 4.15.

2-(2-methyl-4-((5-phenyloxazol-2-yl)methoxy)phenoxy)acetic acid (16)

The title compound was prepared by the method similar to that described for compound **1** using the intermediate **24a** and **4a** as starting material. Yield 47%; white solid; m.p. 154-156 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.80 – 7.65 (m, 3H), 7.56 – 7.30 (m, 3H), 6.95 – 6.70 (m, 3H), 5.19 (s, 2H), 4.63 (s, 2H), 2.18 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.87, 159.68, 152.08, 151.88, 151.22, 129.62, 129.25, 127.87, 127.68, 124.52, 123.24, 118.34, 112.74, 65.78, 62.83, 16.66. ESI-MS *m/z*: 338.1 [M-H]$^-$. Anal. calcd. For $C_{19}H_{17}NO_5$: C, 67.25; H, 5.05; N, 4.13; Found: C, 67.46; H, 5.12; N, 4.25.

2-(2-fluoro-4-((5-phenylthiazol-2-yl)methoxy)phenoxy)acetic acid (17)

The title compound was prepared by the method similar to that described for compound **1** using the intermediate **25a** and **4b** as starting material. Yield 57%; white solid; m.p. 123-125 °C; ^{1}H

NMR (300 MHz, DMSO-d_6) δ: 7.83 – 7.67 (m, 2H), 7.52 – 7.38 (m, 3H), 7.16 – 7.05 (m, 1H), 7.04 (s, 1H), 6.95 – 6.76 (m, 2H), 5.27 (s, 2H), 4.72 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.41, 168.95, 154.56, 153.13, 141.86, 141.32, 132.97, 132.46, 129.31, 128.76, 126.47, 116.27, 110.85, 103.76, 67.85, 66.43. ESI-MS *m/z*: 358.1 $[M-H]^-$. Anal. calcd. For $C_{18}H_{14}FNO_4S$: C, 60.16; H, 3.93; N, 3.90; Found: C, 60.34; H, 3.78; N, 3.85.

N'-(2-chloroacetyl)benzohydrazide (27a). To the slurry of benzoylhydrazide (3 g, 22.0 mmol) in acetonitrile (20 mL) were added simultaneously choroacetylchloride (2 mL, 26.4 mmol) and 50% sodium hydroxide (26.4 mmol) while maintaining temperature below 0˚C. After 1h, the resulting slurry was filtered and the solid was washed with water (2 × 5 mL). The filter cake was dried to provide the title compound (3.2 g, 68%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.50 (s, 1H), 10.38 (s, 1H), 7.80-7.90 (m, 2H), 7.56-7.63 (m, 1H), 7.40-7.55 (m, 2H), 4.21 (s, 2H). ESI-MS *m/z*: 213.1 $[M+H]^+$.

2-(chloromethyl)-5-phenyl-1, 3, 4-thiadiazole (28a). The title compound was obtained from **27a** according to the method described for the synthesis of the compound **25a** in 63% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.04, 8.02 (dd, J = 7.6, 1.3 Hz, 2H), 7.76-7.57 (m, 3H), 5.26 (s, 2H). ESI-MS *m/z*: 211.0 $[M+H]^+$.

2-(2-fluoro-4-((5-phenyl-1,3,4-thiadiazol-2-yl)methoxy)phenoxy)acetic acid (18)

The title compound was prepared by the method similar to that described for compound **1** using the intermediate **28a** and **4b** as starting material. Yield 65%; white solid; m.p. 172-174 °C; ^{1}H

NMR (300 MHz, DMSO-d_6) δ: 8.03 – 7.90 (m, 2H), 7.67 – 7.48 (m, 3H), 7.18 – 6.99 (m, 2H), 6.86 (d, J = 8.7 Hz, 1H), 5.58 (s, 2H), 4.67 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.30, 169.66, 166.78, 152.24, 152.11, 131.93, 129.91, 129.85, 128.18, 116.65, 111.11, 105.21, 66.55, 65.42. ESI-MS *m/z*: 358.6 $[M-H]^-$. Anal. calcd. For $C_{17}H_{13}FN_2O_4S$: C, 56.66; H, 3.64; N, 7.77; Found: C, 56.43; H, 3.57; N, 7.56.

2-(2-methyl-4-((5-phenyl-1,3,4-thiadiazol-2-yl)methoxy)phenoxy)acetic acid (19)

The title compound was prepared by the method similar to that described for compound **1** using the intermediate **28a** and **4a** as starting material. Yield 47%; white solid; m.p. 135-137 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.11 – 7.94 (m, 2H), 7.69 – 7.48 (m, 3H), 7.04 – 6.70 (m, 3H), 5.55 (s, 2H), 4.63 (s, 2H), 2.19 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.89, 169.46, 167.62, 151.65, 151.44, 131.95, 129.95, 129.86, 128.18, 128.03, 118.42, 112.87, 112.79, 65.81, 65.16, 16.65. ESI-MS *m/z*: 354.6 $[M-H]^-$. Anal. calcd. For $C_{18}H_{16}N_2O_4S$: C, 60.66; H, 4.53; N, 7.86; Found: C, 60.89; H, 4.65; N, 7.73.

2-(chloromethyl)-5-phenyl-1, 3, 4-oxadiazole (29a). The title compound was obtained from **27a** according to the method described for the synthesis of the compound **24a** in 75% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.03, 8.01 (dd, J = 7.6, 1.3 Hz, 2H), 7.65-7.60 (m, 3H), 5.16 (s, 2H). ESI-MS *m/z*: 195.1 $[M+H]^+$.

2-(2-fluoro-4-((5-phenyl-1,3,4-oxadiazol-2-yl)methoxy)phenoxy)acetic acid (20)

The title compound was prepared by the method similar to that described for compound **1** using the intermediate **29a** and **4b** as starting material. Yield 53%; white solid; m.p. 186-188 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.08 – 7.95 (m, 2H), 7.69 – 7.46 (m, 3H), 7.16 – 6.97 (m, 2H), 6.84 – 6.73 (m, 1H), 5.43 (s, 2H), 4.58 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 170.30, 165.43, 163.15, 154.52, 153.16, 141.85, 129.35, 128.16, 127.47, 123.45, 116.92, 110.96, 103.42, 66.18, 62.93. ESI-MS *m/z*: 343.1 $[M-H]^-$. Anal. calcd. For $C_{17}H_{13}FN_2O_5$: C, 59.31; H, 3.81; N, 8.14; Found: C, 59.56; H, 3.65; N, 8.27.

4.2. FLIPR Assay

Human FFA1-expressed CHO cells were plated and incubated in the condition of 5% CO_2 for 16 h at 37 °C. After, removed the medium and washed, then, loading cells with buffer (containing 2.5 μg/mL Fluo 4-AM, 0.1% fatty acid-free bovine serum albumin and 2.5 mmol/L probenecid) for 1 h at 37 °C. During the test, γ-linolenic acid (Sigma) or compounds with different concentrations were added and the signals of intracellular calcium flux after addition were measured using FLIPR (Molecular Devices). The potency of compound on hFFA1 was calculated as the equation of [(A−B)/(C−B)]×100%. Among them, the raise of calcium level in compound-treated cells was expressed as A, the increase of calcium level in vehicle-treated cells was expressed as B, and the increase of calcium level in 10 μM γ-linolenic acid-treated cells was expressed as C. EC_{50} values were obtained from GraphPad 5.00 (San Diego, USA).

4.3. Evaluation for PPARα, PPARγ and PPARδ

Detailed descriptions on transfection and cell-based evaluation for PPARα, PPARγ and PPARδ were given in our previously reported literature.[37] Briefly, HepG2 or HEK293 cells were

transfected with pBIND-PPARα, PPARδ or PPARγ according to the manufacturer's protocol. After transfection, positive controls or compounds with different concentrations were added and incubated for 18 h, then lysed with lysis buffer, and added Luciferase Assay Reagent II. The luciferase signals of firefly and renilla were measured using Dual Luciferase Reporter Assay System (Promega). EC_{50} values were obtained from GraphPad 5.00 (San Diego, USA).

4.4. Docking studies

The induced-fit docking study was performed on MOE 2014.0901 (Montreal, Canada) based on structure of PPARδ (PDB number: 1GWX) and FFA1 (4PHU). Before docking study, removing water and other ligands from co-crystal structure, and then performed by Protonate 3D to add hydrogen at co-crystal complex. After that, Gaussian Contact surface was analyzed around ligand to identify property of binding site, and then isolated. In the binding pocket, compound **32** was docked by using the induced-fit mode and set as the London dG scoring. Moreover, the Forcefield Refinement was performed and set as London dG scoring for energy minimization. Docking image was drawn by Pymol-1.5.0.3 (DeLano Scientific LLC).

4.5. Animals

Male SD rats (180-200 g) were obtained from Guangdong Medical Laboratory Animal Center (Guangzhou, China), and male *ob*/*ob* mice (six weeks old) were obtained from Model Animal Research Center of Nanjing University (Nanjing, China). Before experimental operations, rats and mice were acclimatized for one week. Under circadian rhythm of 12 h light/black, animal room was maintained relative humidity 50 ± 10% at 23 ± 2 ℃ throughout experimental period. The

standard food and water were allowed ad libitum access for animals unless otherwise stated, and 0.5% Carboxy Methyl Cellulose solution was used as vehicle for drug administration. The ethical committee of Guangdong Pharmaceutical University has approved all experimental procedures involved in animal, and these experimental procedures were performed based on Laboratory Animal Management Regulations in China and adhered to the Guide for the Care and Use of Laboratory Animals (NIH publication, 2011).

4.6. Pharmacokinetic studies

SD rats acclimatized for seven days were fasted for 12 h, and weighted (n = 4). After oral administration of compound **32** (3 mg/kg), collect blood samples at 5, 15, 30, 45 min and 1, 2, 4, 6, 8, 12, 18, 24 h. By centrifuging, the plasma samples were separated, and acetonitrile containing internal standard was used to precipitate proteins. The supernatant was diluted and determined by LC-MS/MS (Waters) to obtain the plasma concentration of compound **32**. PK parameters were analyzed by DAS 2.1.1.

4.7. Hypoglycemic effects of 32

ob/*ob* mice were fasted for 12 h, weighted, and randomized into 5 groups (n = 6). Mice were orally administrated with single doses of vehicle, TAK-875 (40 mg/kg), or compound **32** (10, 30, 100 mg/kg) and then orally dosed with 3 g/kg glucose solution after 30 min. Blood were collected before drug administration (-30 min), before glucose loading (0 min), and at 15, 30, 60 and 120 min after glucose challenge. The levels of glucose were measured by glucometer (SanNuo ChangSha, China). Data were performed using GraphPad 5.00 (San Diego, USA).

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References

[1] R.A. DeFronzo, From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus, Diabetes, 58 (2009) 773-795.

[2] G. Danaei, M.M. Finucane, Y. Lu, G.M. Singh, M.J. Cowan, C.J. Paciorek, J.K. Lin, F. Farzadfar, Y.-H. Khang, G.A. Stevens, M. Rao, M.K. Ali, L.M. Riley, C.A. Robinson, M. Ezzati, C. Global Burden Metab Risk Factors, National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants, Lancet, 378 (2011) 31-40.

[3] O.J. Phung, J.M. Scholle, M. Talwar, C.I. Coleman, Effect of noninsulin antidiabetic drugs added to metformin therapy on glycemic control, weight gain, and hypoglycemia in type 2 diabetes, JAMA, 303 (2010) 1410-1418.

[4] M.A. Avery, C.S. Mizuno, A.G. Chittiboyina, T.W. Kurtz, H.A. Pershadsingh, Type 2 diabetes and oral antihyperglycemic drugs, Curr Med Chem, 15 (2008) 61-74.

[5] S.A. Stein, E.M. Lamos, S.N. Davis, A review of the efficacy and safety of oral antidiabetic drugs, Expert Opin Drug Saf, 12 (2013) 153-175.

[6] H.E. Lebovitz, Type 2 diabetes mellitus—current therapies and the emergence of surgical options, Nat. Rev. Endocrinol., 7 (2011) 408-419.

[7] A.A. Tahrani, C.J. Bailey, S. Del Prato, A.H. Barnett, Management of type 2 diabetes: new and future developments in treatment, Lancet, 378 (2011) 182-197.

[8] X. Xu, G. Wang, T. Zhou, L. Chen, J. Chen, X. Shen, Novel approaches to drug discovery for the treatment of type 2 diabetes, Expert Opin Drug Dis, 9 (2014) 1047-1058.

[9] C.J. Bailey, A.A. Tahrani, A.H. Barnett, Future glucose-lowering drugs for type 2 diabetes, Lancet Diabetes Endo, 4 (2016) 350-359.

[10] 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, Free fatty acids regulate insulin secretion from pancreatic β cells through GPR40, Nature, 422 (2003) 173-176.

[11] 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, The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids, J Biol Chem, 278 (2003) 11303-11311.

[12] G. Milligan, B. Shimpukade, T. Ulven, B.D. Hudson, Complex Pharmacology of Free Fatty Acid Receptors, Chem Rev 117 (2017) 67-110.

[13] Z. Li, Q.Q. Qiu, X.Q. Geng, J.Y. Yang, W.L. Huang, H. Qian, Free fatty acid receptor agonists for the treatment of type 2 diabetes: drugs in preclinical to phase II clinical development, Expert Opin Inv Drug, 25 (2016) 871-890.

[14] Z. Li, X. Xu, W. Huang, H. Qian, Free Fatty Acid Receptor 1 (FFAR1) as an Emerging Therapeutic Target for Type 2 Diabetes Mellitus: Recent Progress and Prevailing Challenges, Med Res Rev 38 (2018) 381–425.

[15] Z. Li, Q.Q. Qiu, X. Xu, X.K. Wang, L. Jiao, X. Su, M.B. Pan, W.L. Huang, H. Qian, Design, synthesis and Structure-activity relationship studies of new thiazole-based free fatty acid receptor 1 agonists for the treatment of type 2 diabetes, Eur J Med Chem, 113 (2016) 246-257.

[16] Z. Li, M.B. Pan, X. Su, Y.X. Dai, M.A. Fu, X.G. Cai, W. Shi, W.L. Huang, H. Qian, Discovery of novel pyrrole-based scaffold as potent and orally bioavailable free fatty acid receptor 1 agonists for the treatment of type 2 diabetes, Bioorgan Med Chem, 24 (2016) 1981-1987.

[17] Z. Li, J. Yang, X. Wang, H. Li, C. Liu, N. Wang, W. Huang, H. Qian, Discovery of novel free fatty acid receptor 1 agonists bearing triazole core via click chemistry, Bioorgan Med Chem, 24 (2016) 5449-5454.

[18] Z. Li, J.Y. Yang, W.J. Gu, G.S. Cao, X.T. Fu, X.D. Sun, Y. Zhang, H. Jin, W.L. Huang, H. Qian, Discovery of a novel oxime ether scaffold as potent and orally bioavailable free fatty acid receptor 1 agonists, RSC Adv, 6 (2016) 46356-46365.

[19] Z. Li, X. Wang, X. Xu, J. Yang, Q. Qiu, H. Qiang, W. Huang, H. Qian, Design, synthesis and structure-activity relationship studies of novel phenoxyacetamide-based free fatty acid receptor 1 agonists for the treatment of type 2 diabetes, Bioorgan Med Chem, 23 (2015) 6666-6672.

[20] J. Yang, Z. Li, H. Li, C. Liu, N. Wang, W. Shi, C. Liao, X. Cai, W. Huang, H. Qian, Design, synthesis and structure-activity relationship studies of novel free fatty acid receptor 1 agonists bearing amide linker, Bioorgan Med Chem, 25 (2017) 2445-2450.

[21] Z. Li, C. Liu, X. Xu, Q. Qiu, X. Su, Y. Dai, J. Yang, H. Li, W. Shi, C. Liao, M. Pan, W. Huang, H. Qian, Discovery of phenylsulfonyl acetic acid derivatives with improved efficacy and safety as potent free fatty acid receptor 1 agonists for the treatment of type 2 diabetes, Eur J Med Chem, 138 (2017) 458-479.

[22] Z. Li, C. Liu, X. Xu, W. Shi, H. Li, Y. Dai, X. Cai, W. Huang, H. Qian, Design, Synthesis, and Biological Evaluation of Deuterated Phenylpropionic Acid Derivatives as Potent and Long-Acting Free Fatty Acid Receptor 1 Agonists, Bioorg Chem 76 (2018) 303–313.

[23] Z. Li, X. Xu, J. Hou, S. Wang, H. Jiang, L. Zhang, Structure-based optimization of free fatty acid receptor 1 agonists bearing thiazole scaffold, Bioorg Chem, 77 (2018) 429-435.

[24] Z.-H. Zhao, Y.-C. Fan, Q. Zhao, C.-Y. Dou, X.-F. Ji, J. Zhao, S. Gao, X.-Y. Li, K. Wang, Promoter methylation status and expression of PPAR-gamma gene are associated with prognosis of acute-on-chronic hepatitis B liver failure, Clin. Epigenet, 7 (2015) 115.

[25] F.A. Monsalve, R.D. Pyarasani, F. Delgado-Lopez, R. Moore-Carrasco, Peroxisome Proliferator-Activated Receptor Targets for the Treatment of Metabolic Diseases, Mediat. Inflamm., (2013) 1-18.

[26] M. Ahmadian, J.M. Suh, N. Hah, C. Liddle, A.R. Atkins, M. Downes, R.M. Evans, PPAR gamma signaling and metabolism: the good, the bad and the future, Nat. Med., 19 (2013) 557-566.

[27] B. Verges, Clinical interest of PPARs ligands - Particular benefit in type 2 diabetes and metabolic syndrome, Diabetes Metab, 30 (2004) 7-12.

[28] C. Lee, K. Kang, I. Mehl, R. Nofsinger, W. Alaynick, L. Chong, J. Rosenfeld, R. Evans, Peroxisome proliferator-activated receptor delta promotes very low-density lipoprotein-derived fatty acid catabolism in the macrophage, Proc. Natl. Acad. Sci. USA, 103 (2006) 2434-2439.

[29] S. Luquet, C. Gaudel, D. Holst, J. Lopez-Soriano, C. Jehl-Pietri, A. Fredenrich, P.A. Grimaldi, Roles of PPAR delta in lipid absorption and metabolism: a new target for the treatment of type 2 diabetes, Biochim. Biophys. Acta, 1740 (2005) 313-317.

[30] J. Chen, A. Montagner, N.S. Tan, W. Wahli, Insights into the Role of PPAR beta/delta in NAFLD, Int. J. Mol. Sci. , 19 (2018) 1893.

[31] X. Palomer, E. Barroso, J. Pizarro-Delgado, L. Pena, G. Botteri, M. Zarei, D. Aguilar, M. Montori-Grau, M. Vazquez-Carrera, PPAR beta/delta: A Key Therapeutic Target in Metabolic

Disorders, Int. J. Mol. Sci., 19 (2018) 913.

[32] M. Daoudi, N. Hennuyer, M.G. Borland, V. Touche, C. Duhem, B. Gross, R. Caiazzo, J. Kerr-Conte, F. Pattou, J.M. Peters, B. Staels, S. Lestavel, PPAR beta/delta Activation Induces Enteroendocrine L Cell GLP-1 Production, Gastroenterology, 140 (2011) 1564-1574.

[33] Y. Yang, Y. Tong, M. Gong, Y. Lu, C. Wang, M. Zhou, Q. Yang, T. Mao, N. Tong, Activation of PPAR beta/delta protects pancreatic beta cells from palmitate-induced apoptosis by upregulating the expression of GLP-1 receptor, Cell Signal., 26 (2014) 268-278.

[34] T. Tang, M.J. Abbott, M. Ahmadian, A.B. Lopes, Y. Wang, H.S. Sul, Desnutrin/ATGL Activates PPAR delta to Promote Mitochondrial Function for Insulin Secretion in Islet beta Cells, Cell Metab., 18 (2013) 883-895.

[35] S. Takahashi, T. Tanaka, T. Kodama, J. Sakai, Peroxisome proliferator-activated receptor delta (PPAR delta), a novel target site for drug discovery in metabolic syndrome, Pharmacol. Res, 53 (2006) 501-507.

[36] M.A. Helal, K.M. Darwish, M.A. Hammad, Homology modeling and explicit membrane molecular dynamics simulation to delineate the mode of binding of thiazolidinediones into FFAR1 and the mechanism of receptor activation, Bioorg. Med. Chem. Lett, 24 (2014) 5330-5336.

[37] Z. Li, Z. Zhou, F. Deng, Y. Li, D. Zhang, L. Zhang, Design, synthesis, and biological evaluation of novel pan agonists of FFA1, PPARgamma and PPARdelta, Eur J Med Chem, 159 (2018) 267-276.

[38] J. Liu, Y. Wang, Z. Ma, M. Schmitt, L. Zhu, S.P. Brown, P.J. Dransfield, Y. Sun, R. Sharma, Q. Guo, R. Zhuang, J. Zhang, J. Luo, G.R. Tonn, S. Wong, G. Swaminath, J.C. Medina, D.C.H. Lin, J.B. Houze, Optimization of GPR40 Agonists for Type 2 Diabetes, ACS Med. Chem. Lett. , 5

(2014) 517-521.

[39] M.L. Sznaidman, C.D. Haffner, P.R. Maloney, A. Fivush, E. Chao, D. Goreham, M.L. Sierra, C. LeGrumelec, H.E. Xu, V.G. Montana, M.H. Lambert, T.M. Willson, W.R. Oliver, D.D. Sternbach, Novel selective small molecule agonists for peroxisome proliferator-activated receptor delta (PPAR delta) - Synthesis and biological activity, Bioorg. Med. Chem. Lett., 13 (2003) 1517-1521.

[40] X. Wang, T. Zhao, B. Yang, Z. Li, J. Cui, Y. Dai, Q. Qiu, H. Qiang, W. Huang, H. Qian, Synthesis and biological evaluation of phenoxyacetic acid derivatives as novel free fatty acid receptor 1 agonists, Bioorgan Med Chem, 23 (2015) 132-140.

[41] Z. Li, X. Wang, X. Xu, J. Yang, W. Xia, X. Zhou, W. Huang, H. Qian, Design, synthesis and biological activity of phenoxyacetic acid derivatives as novel free fatty acid receptor 1 agonists, Bioorgan Med Chem, 23 (2015) 7158-7164.

[42] N. Boechat, J.C.S. da Costa, J. de Souza Mendonça, P.S.M. de Oliveira, M. Vinícius Nora De Souza, A simple reduction of methyl aromatic esters to alcohols using sodium borohydride–methanol system, Tetrahedron Lett., 45 (2004) 6021-6022.

[43] T. Yamane, H. Mitsudera, T. Shundoh, Highly regioselective direct halogenation: a simple and efficient method for preparing 4-halomethyl-5-methyl-2-aryl-1,3-thiazoles, Tetrahedron Lett., 45 (2004) 69-73.

[44] J. Balsells, L. DiMichele, J. Liu, M. Kubryk, K. Hansen, J.D. Armstrong, Synthesis of [1, 2, 4] triazolo [4, 3-α] piperazines via highly reactive chloromethyloxadiazoles, Org. Lett., 7 (2005) 1039-1042.

[45] P. Kotian, S.W. Mascarella, P. Abraham, A.H. Lewin, J.W. Boja, M.J. Kuhar, F.I. Carroll,

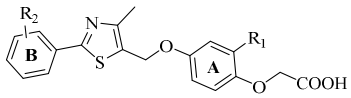
Synthesis, Ligand Binding, and Quantitative Structure-Activity Relationship Study of 3β-(4'-Substituted phenyl)-2β-heterocyclic Tropanes: Evidence for an Electrostatic Interaction at the 2β-Position, J. Med. Chem., 39 (1996) 2753-2763.

[46] 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, Discovery of TAK-875: a potent, selective, and orally bioavailable GPR40 agonist, ACS Med. Chem. Lett., 1 (2010) 290-294.

[47] A.J.F. King, The use of animal models in diabetes research, Brit J Pharmacol, 166 (2012) 877-894.

[48] A. King, J. Bowe, Animal models for diabetes: Understanding the pathogenesis and finding new treatments, Biochem Pharmacol, 99 (2016) 1-10.

Table 1: *In vitro* activities of human FFA1 and PPARδ [a]

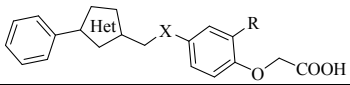


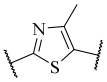
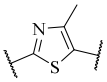
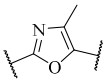
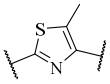
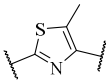
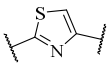
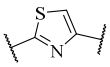
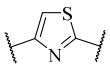
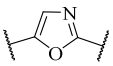
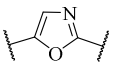
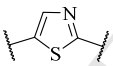
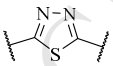
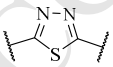
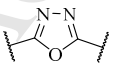
Compd.	R_1	R_2	*h*FFA1 EC_{50} (nM)	*h*PPARδ (LBD)-GAL4 EC_{50} (nM)
TAK-875			45	ND
GW0742			ND [b]	13
1	Me	H	> 10 μM	1650
2	F	H	374	1320
3	Me	4-CF_3	> 10 μM	315
4	F	4-CF_3	149	436
5	F	4-F	112	729
6	F	3-F	185	780
7	F	4-Me	156	564
8	F	3-Me	247	1160

[a] EC_{50} value represent the mean of three determinations, which is the concentration giving 50% of the maximal activity determined for the tested compound.

[b] ND: not determined.

Table 2: *In vitro* activities of designed compounds with various heterocycle scaffolds [a]



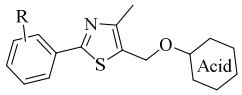
Compd.	Het	X	R	*h*FFA1 EC_{50} (nM)/Act% (10 μM)	*h*PPARδ (LBD)-GAL4 EC_{50} (nM)/Act% (10 μM)
TAK-875				45	ND
GW0742				ND [b]	13
1		O	Me	> 10 μM (13% [c])	1650
2		O	F	374	1320
9		O	F	1350	2660
10		O	F	> 10 μM (37%)	> 10 μM (31%)
11		NH	F	> 10 μM (42%)	> 10 μM (12%)
12		O	F	> 10 μM (23%)	> 10 μM (20%)
13		O	Me	> 10 μM (5%)	> 10 μM (35%)
14		O	F	> 10 μM (15%)	> 10 μM (11%)
15		O	F	> 10 μM (2%)	> 10 μM (8%)
16		O	Me	> 10 μM (2%)	> 10 μM (12%)
17		O	F	> 10 μM (31%)	> 10 μM (23%)
18		O	F	> 10 μM (19%)	> 10 μM (4%)
19		O	Me	> 10 μM (1%)	> 10 μM (9%)
20		O	F	> 10 μM (6%)	> 10 μM (3%)

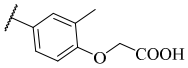
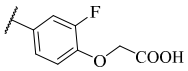
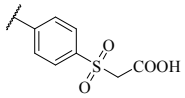
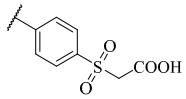
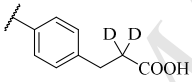
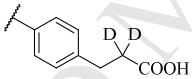
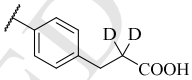
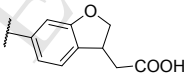
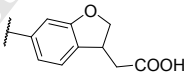
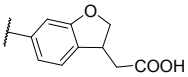
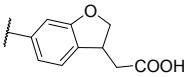
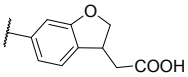
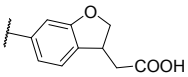
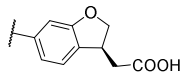
[a] EC_{50} value represent the mean of three determinations, which is the concentration giving 50% of the maximal activity determined for the tested compound.

[b] ND: not determined.

[c] Agonist activities at 10 μM are averages of three independent experiments.

Table 3: *In vitro* activities of target compounds with various acid moieties [a]



Compd.	R	Acid head	*h*FFA1 EC_{50} (nM)	*h*PPARδ (LBD)-GAL4 EC_{50} (nM)
TAK-875			45	ND
GW0742			ND [b]	13
3	4-CF_3		> 10 μM	315
4	4-CF_3		149	436
21	H		370	> 10 μM
22	4-F		253	> 10 μM
23	H		590	972
24	4-F		251	529
25	4-CF_3		325	376
26	H		102	863
27	4-F		79	306
28	4-Cl		105	264
29	4-Me		94	218
30	4-MeO		156	920
31	4-CF_3		113	175
32	4-CF_3		68	102

[a] EC_{50} value represent the mean of three determinations, which is the concentration giving 50% of the maximal activity determined for the tested compound.

[b] ND: not determined.

Table 4: Selectivity profile for compound **32**[a]

	*h*FFA1 EC_{50} (nM)	*h*PPARα (LBD)-GAL4 EC_{50} (μM)	*h*PPARγ (LBD)-GAL4 EC_{50} (μM)	*h*PPARδ (LBD)-GAL4 EC_{50} (nM)
Compound **32**	68 nM	> 10 μM	> 10 μM	102 nM

[a] The values represent the mean of three determinations.

Table 5: Pharmacokinetic parameters of compound **32** in fasted SD rats

PK profiles	Dose (po)[a]	CL (mL/h/kg)	C_{max} (μg/mL)	$AUC_{0\text{-}24h}$ (μg/mL·h)	$T_{1/2}$ (h)
Compound **32**	3 mg/kg	16.73 ± 5.26	9.45 ± 2.06	95.62 ± 13.57	4.31 ± 0.96

[a] po = oral administration. Results are expressed as mean ± SD for four male SD rats (fasted for 12 h) in each group. Test compounds were suspended in 0.5% methylcellulose aqueous solution.

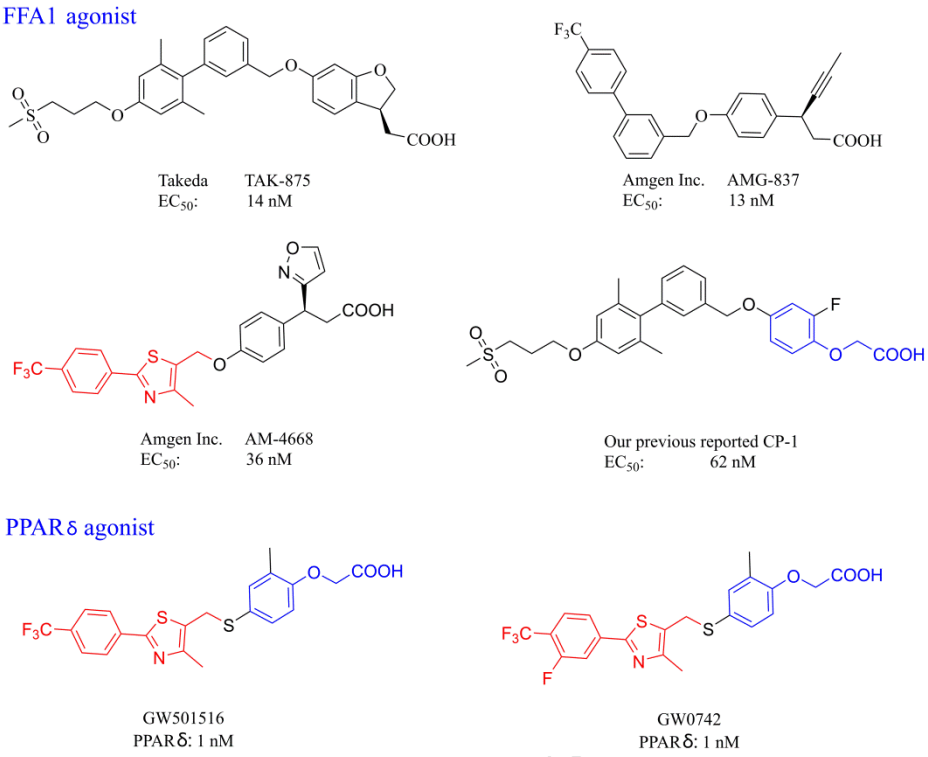


Figure 1: Structure of FFA1 or PPARδ agonists, the common scaffold was labeled.

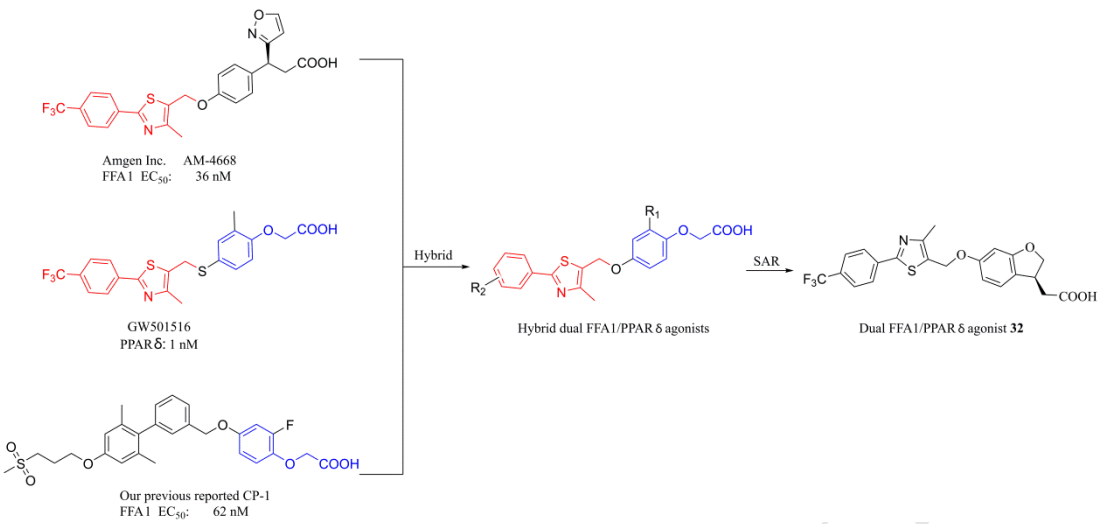


Figure 2: Our strategy to obtain dual FFA1/PPARδ agonists by hybrid the common scaffold of PPARδ and FFA1 agonists.

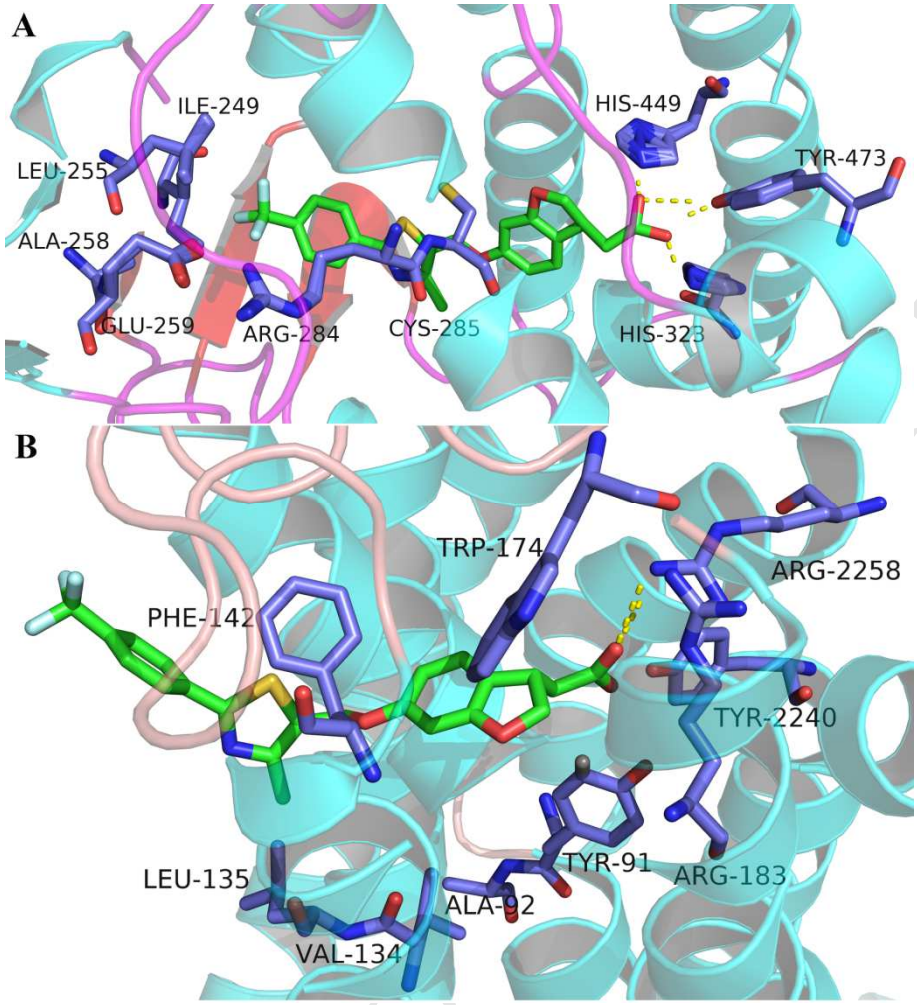


Figure 3: The docking model of compound **32** (green carbon) in crystal structure of PPARδ (1GWX, A) and FFA1 (4PHU, B). Key residues are labeled in black, and the hydrogen-bonding interactions are represented by yellow dashed lines.

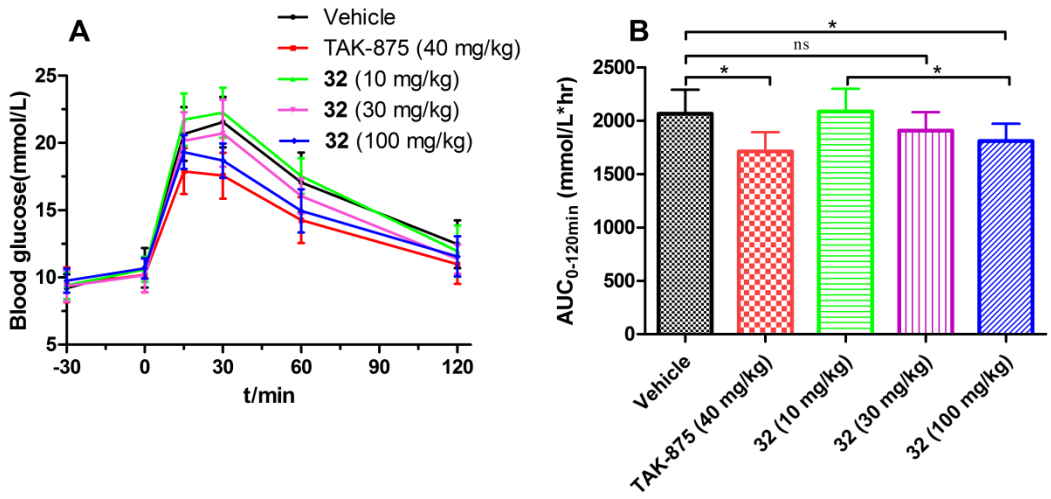
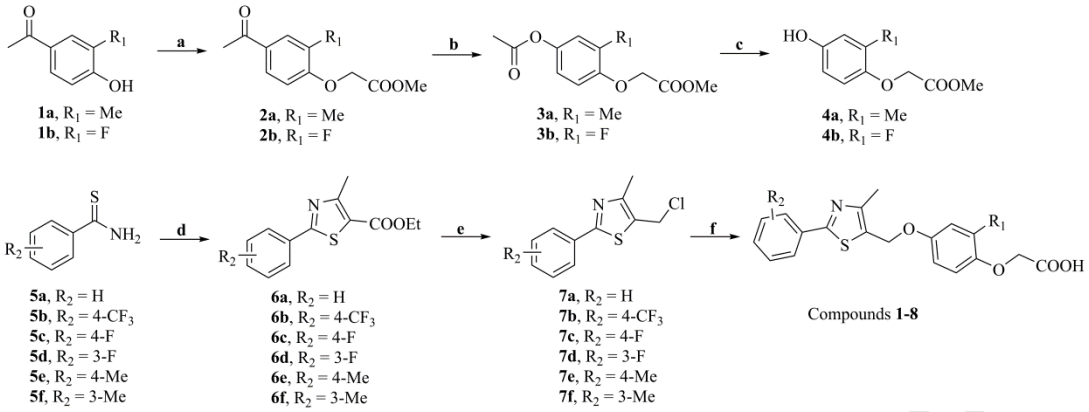


Figure 4: Dose–response relationship of compound **32** explored in *ob/ob* mice. (A) represented time-dependent changes of glucose levels after oral administration of compound **32**, followed by oral glucose load (3 g/kg), respectively. (B) represented $AUC_{0-120min}$ of glucose levels. Results are mean ± SD (n = 6 per group). *p≤0.05 and **p≤0.01 was analyzed using a one-way ANOVA with Tukey's multiple-comparison post hoc test.



1a, R_1 = Me
1b, R_1 = F

2a, R_1 = Me
2b, R_1 = F

3a, R_1 = Me
3b, R_1 = F

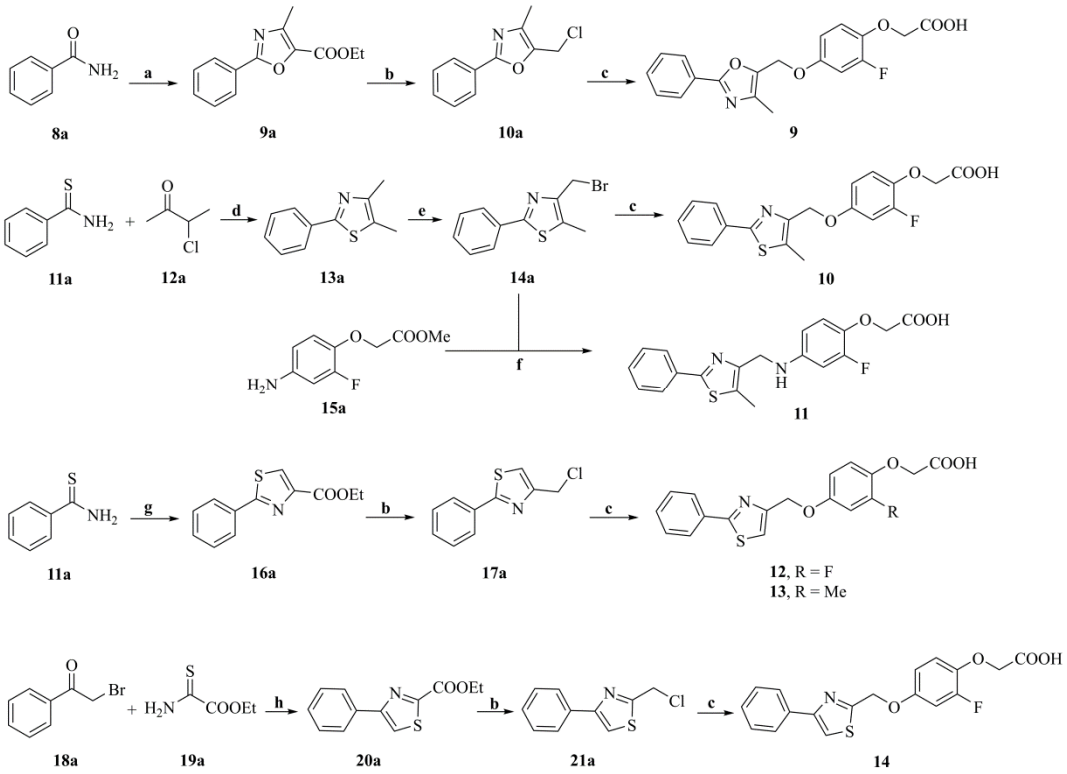
4a, R_1 = Me
4b, R_1 = F

5a, R_2 = H
5b, R_2 = 4-CF_3
5c, R_2 = 4-F
5d, R_2 = 3-F
5e, R_2 = 4-Me
5f, R_2 = 3-Me

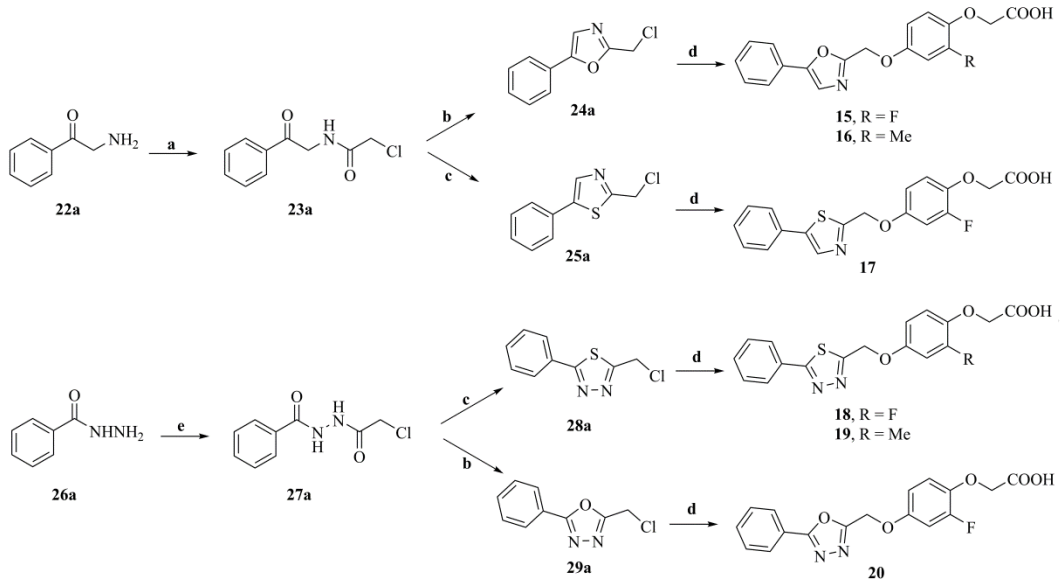
6a, R_2 = H
6b, R_2 = 4-CF_3
6c, R_2 = 4-F
6d, R_2 = 3-F
6e, R_2 = 4-Me
6f, R_2 = 3-Me

7a, R_2 = H
7b, R_2 = 4-CF_3
7c, R_2 = 4-F
7d, R_2 = 3-F
7e, R_2 = 4-Me
7f, R_2 = 3-Me

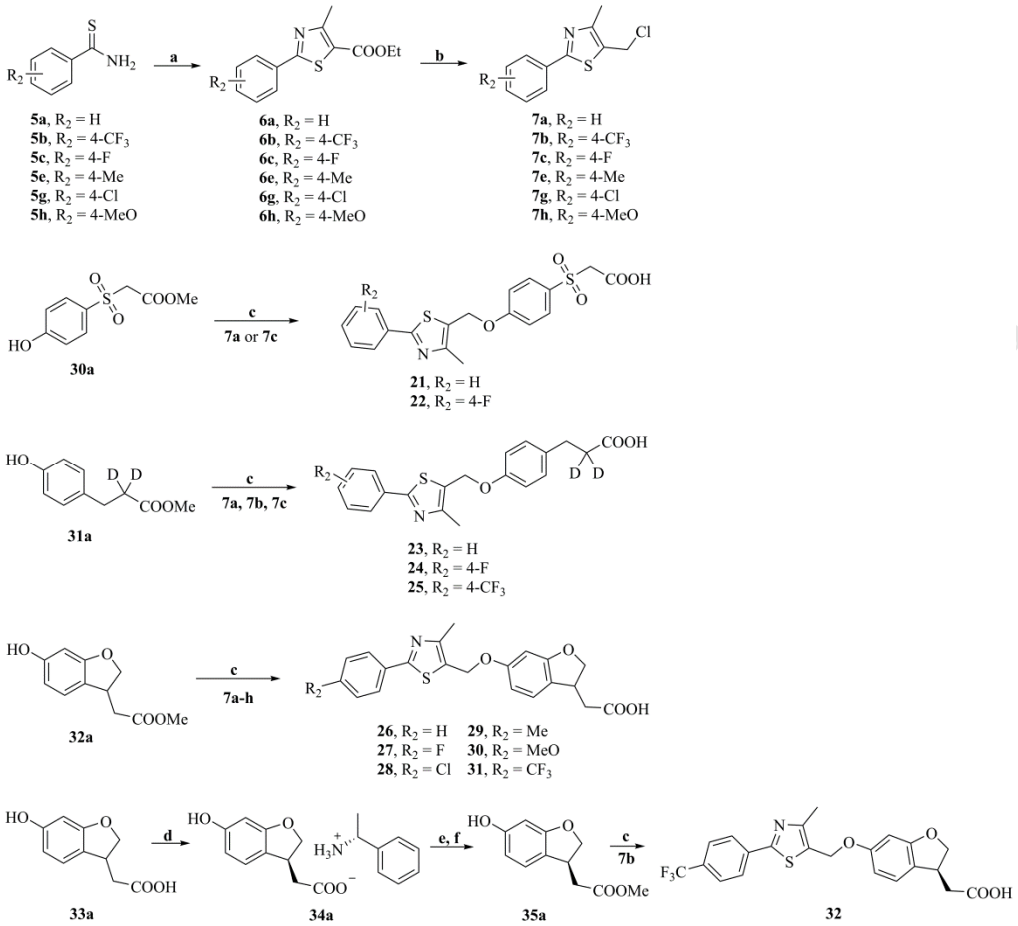
Scheme 1. Synthesis of compounds **1-8**. Reagents and conditions: (a) Methyl bromoacetate, K_2CO_3, acetone, 45 °C, 12 h; (b) *m*-CPBA, *p*-TSA, CH_2Cl_2; (c) CH_3ONa, CH_3OH; (d) Ethyl 2-chloroacetoacetate, EtOH, reflux, 6 h; (e) $NaBH_4$, MeOH, THF, reflux, and then $SOCl_2$, CH_2Cl_2, DMF, 40 °C, 4 h; (f) **4a-b**, K_2CO_3, acetonitrile, KI, 45 °C, 12 h, and then LiOH·H_2O, THF/MeOH/H_2O, r.t., 4 h.



Scheme 2. Synthesis of compounds **9**-**14**. Reagents and conditions: (a) Ethyl 2-chloroacetoacetate, EtOH, reflux, 16 h; (b) $NaBH_4$, MeOH, THF, reflux, and then $SOCl_2$, CH_2Cl_2, DMF, 40 °C, 4 h; (c) **4a** or **4b**, K_2CO_3, acetonitrile, KI, 45 °C, 12 h, and then $LiOH·H_2O$, THF/MeOH/H_2O, r.t., 4 h; (d) 3-chloro-2-butanone, EtOH, reflux, 16 h; (e) NBS, acetonitrile, r.t., 2h; (f) $NaHCO_3$, EtOH, rt, 24 h, and then $LiOH·H_2O$, THF/MeOH/H_2O, r.t., 4 h; (g) Ethyl bromopyruvate, EtOH, reflux, 4 h; (h) EtOH, reflux, 6 h.



Scheme 3. Synthesis of target compounds **15-20**. Reagents and conditions: (a) Choroacetyl chloride, trimethylamine, 0 °C to r.t., 16 h; (b) $POCl_3$, acetonitrile, reflux, 4 h; (c) Lawesson's reagent, THF, reflux, 4 h; (d) **4a** or **4b**, K_2CO_3, acetonitrile, KI, 45 °C, 12 h, and then $LiOH \cdot H_2O$, THF/MeOH/H_2O, r.t., 4 h; (e) Choroacetyl chloride, NaOH, 0 °C, 1 h.



Scheme 4. Synthesis of compounds **21-32**. Reagents and conditions: (a) Ethyl 2-chloroacetoacetate, EtOH, reflux, 6 h; (b) $NaBH_4$, MeOH, THF, reflux, and then $SOCl_2$, CH_2Cl_2, DMF, 40 °C, 4 h; (c) K_2CO_3, acetonitrile, KI, 45 °C, 12 h, and then $LiOH \cdot H_2O$, THF/MeOH/H_2O, r.t., 4 h; (d) R-Phenethylamine, acetone, reflux; (e) 1 M HCl; (f) H_2SO_4, MeOH, reflux.

Highlights

- A therapeutic strategy that simultaneous targets insulin secretion and resistance.
- The first-in-class dual FFA1/PPARδ agonist was identified by the hybrid strategy.
- Compound **32** revealed high activity on FFA1/PPARδ and high selectivity over other PPARs.
- Compound **32** revealed excellent pharmacokinetic profiles.
- Compound **32** suppressed the excursion of glucose levels in a dose-dependent manner.