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Original article

Discovery, design and synthesis of Y-shaped peroxisome proliferator-activated receptor δ agonists as potent anti-obesity agents *in vivo*

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ABSTRACT

We have discovered and demonstrated the *in vitro* and *in vivo* PPARδ-selective activity of novel Y-shaped agonists. These compounds activated hPPARδ with EC_{50} values between 1 and 523 nM. Surprisingly, compounds **10a**, **11d**, **11e** and **11f** were the most potent and most selective hPPARδ agonists with 10^4-fold selectivity over the other two subtypes, namely, hPPARα and hPPARγ. The PPARδ ligands **10a**, **11e** and **11f** showed good bioavailability and *in vivo* efficacy.

1. Introduction

Peroxisome Proliferator-Activated Receptors (PPARs) comprise three known subtypes: PPARα, PPARγ and PPARδ [1]. These subtypes have differential tissue expression and tissue-specific functions *in vivo*. PPARα is mainly expressed in the heart, kidneys, skeletal muscle and large intestine in humans [2–4] and is involved in the β-oxidation of peroxisomes and mitochondria [5,6]. PPARγ is expressed in skeletal muscle at a low level but is mainly expressed in adipose tissue to induce adipocyte differentiation and to store energy as fat. PPARγ is also involved in the homeostatic regulation of insulin and glucose [7]. PPARδ, which was first found in *Xenopus laevis*, was previously known as PPARβ [8] in humans and has also been known as NUCI [9], PPARδ [10] and FAAR [11].

Previous studies have revealed that PPARδ plays an important role in embryo implantation [12] and that it has physiological functions in the differentiation of neuronal cells in the central nervous system (CNS) [13] and in wound healing, exhibiting anti-inflammatory effects [14,15]. Recent studies have also demonstrated that PPARδ is involved in lipid metabolism and muscle transformation [15–17]. For example, PPARδ activates the expression of key genes involved in β-oxidation in fatty acid catabolism. PPARδ also affects uncoupling proteins (UCPs), which are involved in energy metabolism, and the effect of PPARδ on UCPs has positive effects on anti-obesity [16,18,19]. The activation of PPARδ increases HDL levels, improves type 2 diabetes without weight change [20], and favors the treatment of atherosclerosis by inhibiting the genes associated with atherosclerosis [21]. Therefore, studies on the regulation of lipid metabolism using PPARδ have provided insight into the development of a treatment strategy for obesity and related metabolic diseases.

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Merck has reported on a series of synthetic ligands, such as L-631033, that are weak activators of the PPARs [22]. These ligands resemble fatty acids, with a rigidifying ring located in the middle of the chain, which is reminiscent of certain eicosanoids. The established leukotriene antagonist L-165041 was identified through random screening as an activator of human PPARδ [23–26]. The phenylacetic acid derivatives L-796449 and L-783483 have high affinity (EC_{50} = 7.9 nM) for human PPARδ without subtype specificity. GW2433, reported by GlaxoSmithKline, is a high affinity ligand for human PPARδ and was demonstrated to be a dual activator of PPARδ and PPARα in a cell-based transfection assay [27]. Recently, GW501516 was discovered through structure-based drug design and combinatorial chemistry, and this newly identified compound appeared to be the most effective and selective agonist for PPARδ. According to our results, GW501516 has an extremely high affinity for hPPARδ (EC_{50} = 2.5 nM) and is more than 10^2-fold selective for PPARδ over PPARα and PPARγ. However, GW501516 activated hPPARα and hPPARγ above 1 μM concentration (Fig. S1). These results suggest that GW501516 might induce undesirable effects. Previous research has demonstrated that PPARα is implicated in the development of cardiac hypertrophy caused by decreased glucose utilization and increased lipid accumulation in the diabetic heart [28]. In addition, PPARγ is a suspected regulator causing osteoporosis because of its ability to induce adipogenesis in progenitor cells derived from bone marrow [29]. In addition, the well-known agonists of PPARγ, rosiglitazone and pioglitazone, being insulin-sensitizers, are associated with a significant increase in the risk of cardiovascular disease [30,31]. Thus, more selective PPARδ agonists that do not activate PPARα and PPARγ are needed. In the present study, we developed highly PPARδ-selective agonists with no activity against the PPARα and PPARγ subtypes at concentrations ranging up to 10 μM.

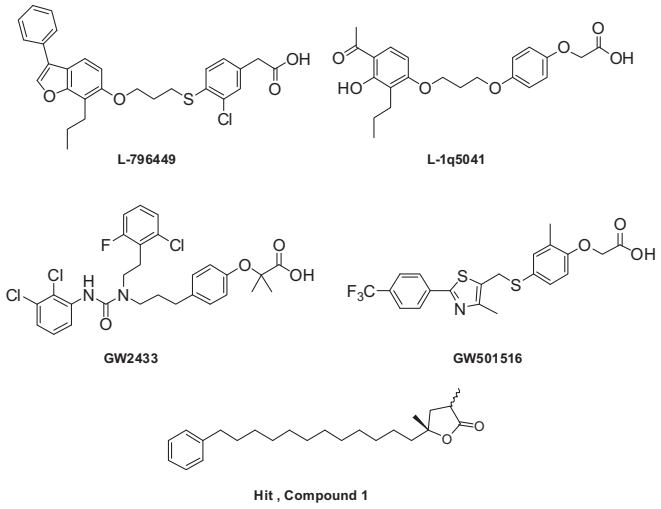


Fig. 1. Chemical structures of PPARδ ligands.

2. Results and discussion

2.1. Chemistry

In this paper, we report the development of novel PPARδ-selective ligands, which led to the preparation of Y-shaped agonists. Using high-throughput screening (HTS) of our marine natural product libraries, we focused on discovering novel classes of PPARδ ligands. In particular, (2*R**,4*R**)-2,4-dimethyl-4-hydroxy-16-phenylhexadecanoic acid 1,4-lactone [32] showed an interesting activity profile (Fig. 1). This finding was not unexpected, as compound **1** had essential pharmacophores for PPARδ agonist activity (an acidic head group and a hydrophobic tail group). The γ-lactone moiety in compound **1** corresponded to an acidic head group that allowed hydrogen bonding with the AF-2 helix in the PPARδ receptor protein. The phenyldecyl group corresponded to the hydrophobic tail common to PPARδ agonists. To understand the activity of compound **1** at a molecular level, docking experiments were performed into the binding pocket of the PPARδ receptor using the automated docking program, AutoDock.

The docking of compound **1** into hPPARδ revealed that an L-shaped conformation allowed the lactone group to form hydrogen bonds with His413, Tyr427, His287 and Thr253. This hydrogen bonding pattern is expected to be essential for the formation of a tightly binding ligand complex. The phenyl group of compound **1** was docked into two hydrophobic cavities in two alternate configurations, similar to the binding mode observed for EPA in the hPPARδ receptor protein (Fig. 2). The human apo-PPARδ LBD contains a bundle of thirteen α helices and four small stranded β strands, and its ligand binding pocket is a large cavity within the protein with a total volume of ~1300 Å. The ligand binding pocket of PPARδ assumes a Y-shape, with each of the arms being approximately 12 Å in length. The co-crystal structure of PPARδ with GW2433 revealed that the strong affinity of this ligand to PPARδ was caused by its ability to adopt a Y-shaped conformation in the hPPARδ binding pocket, which may be important for subtype selectivity. The docking of GW501516 into PPARδ with AutoDock revealed that this ligand occupied only two of three sites with its L-shaped conformation. An overlay of GW501516 and compound **1** in the PPARδ ligand binding pocket is shown in Fig. 2. This overlay suggests that a new Y-shaped compound synthesized by introducing a phenyl alkyl substituent at the α-position to the sulfide could create a ligand that is more potent than GW501516 (Fig. 3).

In our initial design, we synthesized Y-shaped analogs with various alkyl and arylalkyl substituents at the α-position to the sulfide. The synthesis of compounds **10a**–**g** is outlined in Scheme 1. Compounds **10a**–**g** were prepared from 2-methyl-4-iodophenol in 6 steps. Compound **5** was synthesized using our one-pot methodology from the commercially available 2-methyl-iodophenol in 92% yield [33]. The reaction proceeded through *in situ* protection of the hydroxyl group by reaction with isopropyl magnesium bromide, lithium-halogen exchange with *t*-BuLi, and the formation of a lithium thiolate that underwent nucleophilic attack of chloromethyl thiazole **4** in one vessel. Compound **6** was prepared in 90% yield by reacting the phenolic hydroxyl group with TBDMSCl and imidazole.

Compounds **7a**–**g** were synthesized by the alkylation of α-thiooorganolithiums prepared from the lithiation of compound **6**. The lithiation of compound **6** was achieved by treatment with *t*-BuLi (2 eq) or LDA (2 eq) in THF at −78 °C. The corresponding α-thiooorganolithium was alkylated with various electrophiles. Interestingly, the use of 1.2 eq of *t*-BuLi or LDA produced the corresponding alkylated compounds **7a**–**g** in low yield (30–45%). However, using 2 equivalents of *t*-BuLi or LDA afforded compounds **7a**–**g** in high yield (60–87%).

The deprotection of **7a**–**g** by TBAF provided compounds **8a**–**g** in quantitative yield. O-Alkylated compounds **9a**–**g** were obtained by the alkylation of phenol with ethyl bromoacetate (1.2 eq) in the presence of K_2CO_3 (2 eq) in 88–91% yield. Finally, *o*-alkylated compounds **9a**–**g** were hydrolyzed using 2 N LiOH followed by acidification to produce compounds **10a**–**g** in 85–90% yield. The EC_{50} values for compounds **10a**–**g** against human PPAR subtypes listed in Table 1 were determined by an *in vitro* transfection assay.

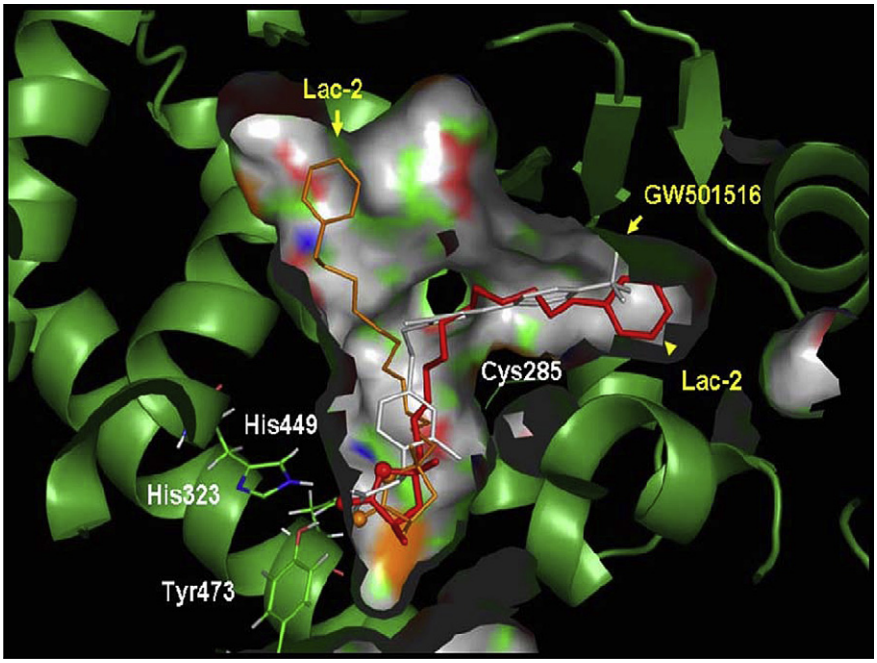


Fig. 2. Overlay of compound **1** (Lac-2, red and orange) and GW501516 (gray) docked into hPPARδ. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Biological evaluation

2.2.1. In vitro evaluation

Remarkably, compounds **10a**–**c** (n = 1, 2, 3) displayed excellent activity for hPPARδ (EC_{50} = 1.6, 2.6 and 6.1 nM, respectively). However, compounds **10d**–**g** (n = 4, 5, 7, and 10), which contain longer phenyl alkyl groups, displayed low activities for hPPARδ. It was evident that the activity linearly decreased with the length of the alkyl side chain. Interestingly, **10e** (n = 5) showed a more potent activity than **10d** (n = 4) despite an increased chain length. These results suggested that the incorporation of a short phenyl alkyl group (n = 1 or 2) at the α-position to the sulfide in GW501516 would enhance the affinity for PPARδ and impart selectivity over other subtypes (PPARα and PPARγ) through filling the Y-shaped binding pocket. Compound **10a** showed excellent potency (1.6 nM) and 10^4-fold selectivity for PPARδ over PPARα and PPARγ. It was clear from these results that the compound having a benzyl group at the α-position to the sulfide had enhanced the agonistic activity for hPPARδ with selectivity over the other subtypes (hPPARα and hPPARγ). In addition, a time-resolved FRET-based binding assay showed that compound **10a** directly binds to hPPARδ with an EC_{50} value of 13 nM (Fig. S2). Based on these results, we synthesized a series of analogs of **10a** to optimize the activity for hPPARδ. Compounds **11a**–**11t** were synthesized from intermediate **6** by

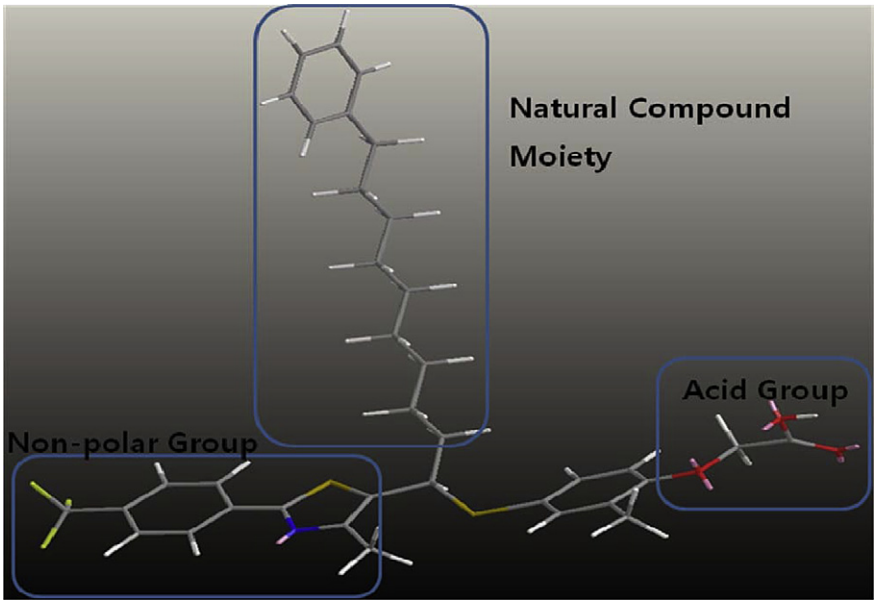
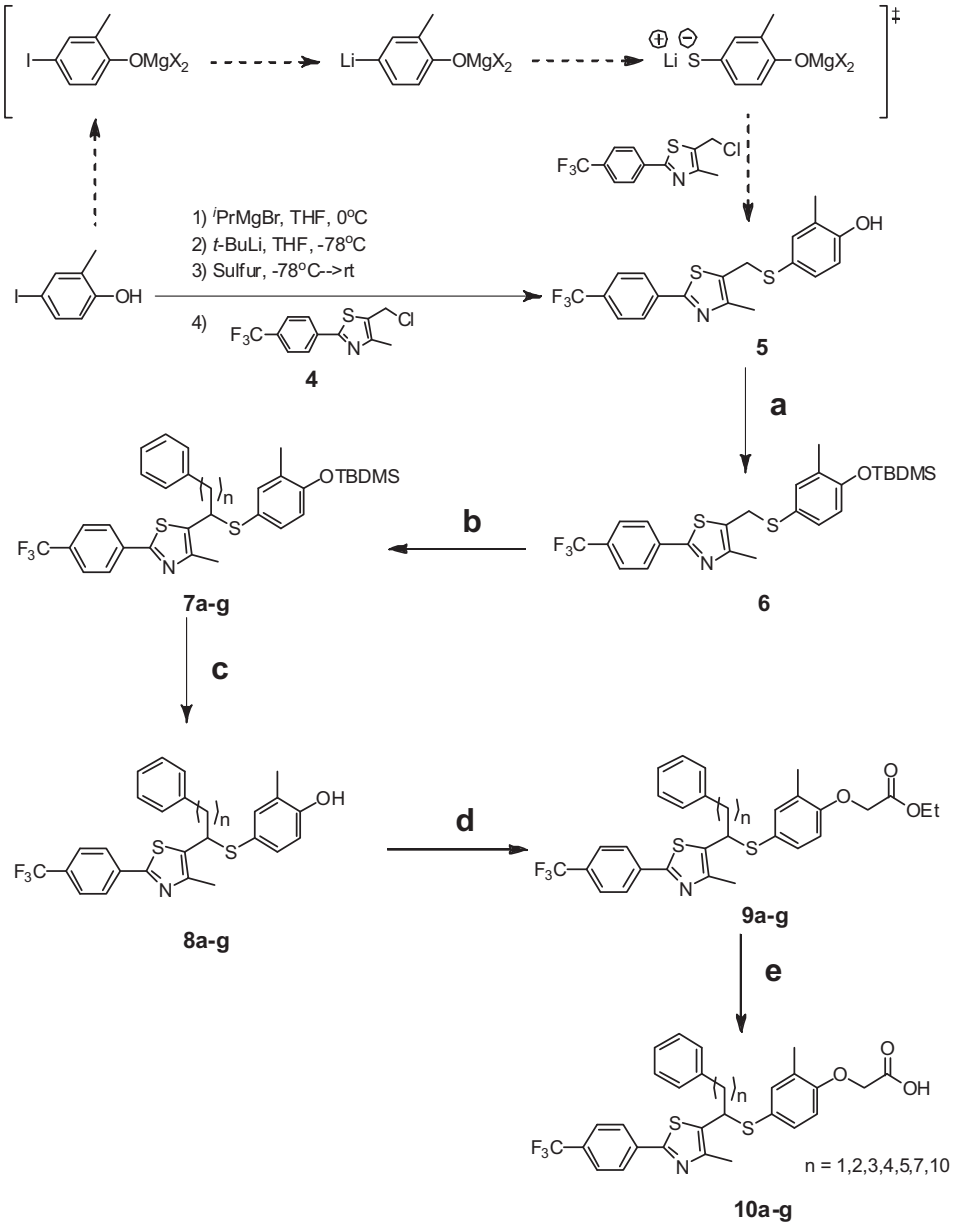


Fig. 3. Design of a Y-shaped agonist based on molecular modeling studies.



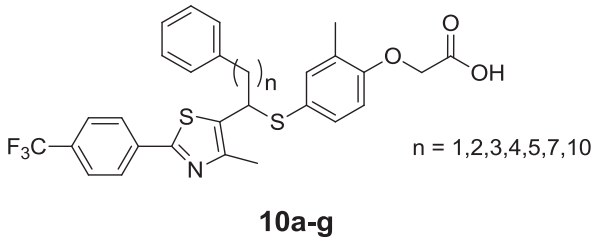
Scheme 1. Reaction conditions: (a) TBDMSCl (1.5 eq), imidazole (2 eq), DMF, r.t.(b) LDA (2 eq), alkyl bromide (1 equiv), THF, −78 °C(c) TBAF (1.1 equiv), THF, r.t.(d) ethyl bromoacetate (1.2 eq), acetone, K_2CO_3, r.t.(e) 2 N LiOH, 0.5 M $NaHSO_4$.

incorporation of various benzyl substituents or alkyl groups at the α-position to the sulfide. The EC_{50} values for compounds **11a**–**11t** were derived from an *in vitro* transfection assay against human PPAR subtypes (Tables 2and 3). Substitution with an electron-donating group, such as a methoxy group, at the *p*-position on the phenyl group resulted in better activity than substitution with an electron-withdrawing group (entries 1, 2 and 3, EC_{50} = 60.0, 9.4 and 4.8 nM, respectively). Compounds with fluoride, chloride, or trifluoromethyl at the *o*-position on the phenyl group also displayed excellent potency for PPARδ and were ultra-selective hPPARδ agonists, showing 10^4-fold selectivity over the other two subtypes (entries 4, 5, 6, and 7, EC_{50} = 1.2, 2.1, 1.2, and 2.8 nM, respectively). The *o*,*p*-fluoride-substituted phenyl group showed better activity for PPARδ than did the *o*,*m*-fluoride-substituted group for PPARδ (entries 8 and 9, EC_{50} = 4.6 and 16.7 nM, respectively).

The *m*,*p*-fluoride-substituted and totally fluoride-substituted compounds had almost the same activity (entries 10 and 11, EC_{50} = 6.7 and 8.1 nM, respectively). Entries 8, 9 and 10 revealed that the substituents at the *o*- and *p*-positions were more important than the substituent at the *m*-position. Meanwhile, substitution of bulky aromatic functional groups on the phenyl group resulted in considerable decreases in the PPARδ activities (entries 13, 14, and 15, EC_{50} = 490.1, ia, 523.0 nM). Interestingly, replacing the phenyl group with an alkyl group resulted in excellent PPARδ activity (entries 16, 17, 18, 19 and 20, EC_{50} = 1.8, 1.0, 2.3, 1.1, 1.1 nM) but only moderate activity for hPPARα.

2.2.1.1. In vivo evaluation. We also conducted an animal experiment using ten-week-old C57BL/6 mice to investigate the anti-obesity effect of compound **10a** *in vivo*. Compound **10a** was the most potent and selective PPARδ agonist and showed good oral

Table 1
In vitro activities of compounds **10a**–**g**.



10a-g

Compound	*n*	Transactivation EC_{50}(nM)[a]		
		HPPARα	hPPARγ	hPPARδ
10a	1	ND	ND	1.6
10b	2	ND	ND	2.6
10c	3	ND	ND	6.1
10d	4	ND	ND	38.8
10e	5	ND	ND	11.4
10f	7	ND	ND	57.2
10g	10	ND	ND	132.0
GW501516		229	>1.2 μM	2.5

[a] Compounds were screened for agonist activity on PPARs in transiently transfected CV-I cells. EC_{50} value is the molar concentration of the test compound that afforded 50% of the maximal reporter activity. 'ND' means that its EC_{50} value is bigger than 10 μM.

bioavailability (88%) in mice (Table S1). To induce obesity, the mice were fed a high-fat diet (HFD) containing 35% fat (w/w). Compound **10a** was subsequently administered at 10 mg/kg of body weight by oral intubation daily. During the experiment, no significant difference in food intake between the vehicle-treated group and the group treated with compound **10a** was observed at any point. After 78 days, the body weight of the vehicle-treated mice had increased by 102%, whereas the body weight of the compound **10a**-treated mice increased by 60%. Moreover, the compound **10a**-treated group was observed to have decreased serum triglyceride (TG) and free fatty acid (FFA) levels (29% and 26%, respectively). To test the anti-obesity effect of compound **10a** at the tissue level, we performed tissue staining using hematoxylin and eosin (Fig. 4). The compound **10a**-treated group did not show fatty liver. Furthermore, lipid accumulation in brown and white adipose tissue was significantly decreased in the **10a**-treated group.

2.2.1.2. In vivo evaluation. To evaluate the *in vivo* activity of the newly synthesized and optimized PPARδ agonists, eight-week-old C57BL/6J mice were fed a high-fat diet containing 35% fat to induce obesity. The control group was orally administered 0.5% carboxymethyl cellulose in water and fed a high-fat diet, and the treated groups were orally administered compounds **11e** or **11f** (10 mg/kg/day) and fed a high-fat diet. There were six animals in each group. The results showed that mice treated with the optimized PPARδ agonists had body weight gains that were 35% (compound **11e**) and 24% (compound **11f**) lower than those of the vehicle group (Fig. 5).

3. Conclusion

We synthesized novel Y-shaped PPARδ ligands by combining a known ligand and a marine natural product with automatic docking experiments. An efficient synthetic route to these analogs was also developed. These compounds activated hPPARδ with EC_{50} values between 1 and 523 nM. Surprisingly, compounds **10a**, **11d**, **11e** and **11f** were the most potent and most selective hPPARδ agonists with 10^4-fold selectivity over the other two subtypes, namely, hPPARα and hPPARγ. The most potent and most selective PPARδ ligands, **10a**, **11e** and **10f**, showed good bioavailability and *in vivo* efficacy (Figs. 4and 5). These results suggest that our novel PPARδ agonists, especially compounds **10a**, **11e** and **11f**, could be promising candidates for anti-obesity drugs without causing adverse effects, such as cardiac hypertrophy and osteoporosis.

4. Experimental

4.1. General

All reactions were performed in oven- and flame-dried glassware under nitrogen atmosphere. Air and moisture sensitive reagents and solvents were transferred *via* syringes or cannula, and they were introduced into the reaction vessel through a rubber septum. Chemicals obtained from commercial sources were used without further purification. Flash column chromatography was carried out on silica gel (230–400 mesh). Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F254. TLC plates were visualized with UV light and 5% ammonium dimolybdate or *p*-anisaldehyde in ethanol with heat. ^{1}H NMR (400 MHz) in $CDCl_3$ was recorded on a Bruker Avance III 400 MHz NMR spectrometer and chemical shifts (δ) were expressed in ppm downfield from the internal tetramethylsilane or with reference to residual $CHCl_3$. The purity of compounds was assessed by HPLC/MS spectra.

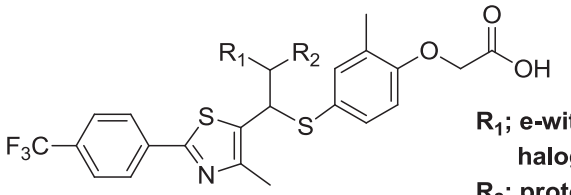
4.2. 2-Methyl-4-((4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)methylthio)phenol, compound *5*

To a stirred solution of 2-methyl-4-iodophenol (500 mg, 2.14 mmol) in anhydrous THF (20 ml) at 0 °C was added 2.0 M isopropyl magnesium chloride solution (1.1 ml, 2.16 mmol). The reaction mixture was stirred at 0 °C for 10 min under N_2 atmosphere and cooled to −78 °C in dryice-acetone bath. A 1.7 M *tert*-butyllithium solution (2.77 ml, 4.70 mmol) was added dropwise to reaction mixture and stirred at same temperature for 30 min. Sulfur power (69 mg, 2.14 mmol) was added in one-portion to reaction mixture and then the reaction mixture was warmed to room temperature for 1 h. After the reaction mixture was cooled to 0 °C again, a solution of **4** (624 mg, 2.14 mmol) in THF (3 ml) was added dropwise to reaction mixture and stirred at room temperature for 1 h. After completion of the reaction, sat. NH_4Cl solution (50 ml) and ethyl acetate (25 ml) were added to reaction mixture. The organic layer was washed by distilled water, dried by $MgSO_4$, and concentrated under reduced pressure to give crude compound. The crude compound was purified by column chromatography on silica gel (*n*-hexnane/ethyl acetate = 5/1) to obtain **5** as white crystal (728 mg, 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, 2H, J = 8.1 Hz), 7.65 (d, 2H, J = 8.3 Hz), 7.19 (d, 1H, J = 1.5 Hz) 7.01 (dd, 1H, J = 8.2, 2.0 Hz), 6.62 (d, 1H, J = 8.2 Hz), 5.86 (brs, 1H), 4.07 (s, 2H), 2.19 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.9, 155.5, 151.7, 137.4, 136.9, 133.5, 131.9 (q, J = 32.6 Hz), 131.7, 126.8, 126.3 (q, J = 3.9 Hz), 125.8, 123.8, 115.7, 33.2, 16.2, 14.8 HRMS: calcd for $C_{19}H_{16}F_3NOS_2$ [M + H] $^+$ 395.0625, found 395.0622.

4.3. 5-((4-(tert-butyldimethylsilyloxy)-3-methylphenylthio)methyl)-4-methyl-2-(4-(trifluoromethyl)phenyl)thiazole, compound *6*

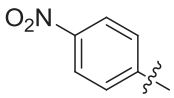
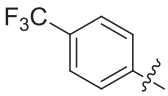
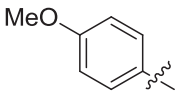
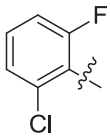
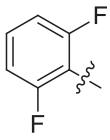
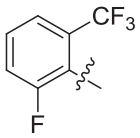
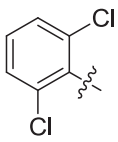
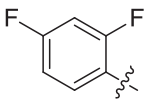
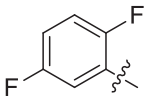
To a stirred solution of **5** (500 mg, 1.26 mmol) in anhydrous DMF (5 ml) were added imidazole (171 mg, 2.52 mmol) and *tert*-butyldimethylsilyl chloride (209 mg, 1.38 mmol). The reaction mixture was stirred at room temperature for 4 h. After completion of the reaction, the reaction mixture was concentrated under reduced

Table 2
In vitro activities of compounds **11a**–**11k**.



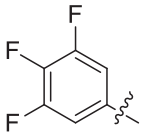
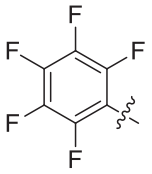
11

R_1; e-withdrawing, -donating groups, halogen fused aromatic rings.
R_2; proton

Entry	Compound	R_1	R_2	Transactivation EC_{50}(nM)[a]		
				hPPARα	hPPARδ	hPPARλ
1	**11a**		H	ND[a]	60.0	ND
2	**11b**		H	ND	9.4	ND
3	**11c**		H	ND	4.8	ND
4	**11d**		H	ND	1.2	ND
5	**11e**		H	ND	2.1	ND
6	**11f**		H	ND	1.2	ND
7	**11g**		H	ND	2.8	ND
8	**11h**		H	ND	4.6	ND
9	**11i**		H	ND	16.7	ND

(*continued on next page*)

Table 2 (*continued*)

Entry	Compound	R_1	R_2	Transactivation EC_{50}(nM)[a]		
				hPPARα	hPPARδ	hPPARλ
10	**11j**		H	ND	6.7	ND
11	**11k**		H	ND	8.1	ND

[a] 'ND' means that its EC_{50} value is bigger than 10 μM.

pressure and diluted with ethyl acetate (20 ml) and distilled water (40 ml). The organic layer was dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give crude compound. The crude compound was purified by column chromatography on silica gel (*n*-hexnane/ethyl acetate = 10/1) to obtain **6** as white crystal (610 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, 2H, *J* = 8.1 Hz), 7.65 (d, 2H, *J* = 8.2 Hz), 7.19 (d, 1H, *J* = 1.9 Hz) 7.07 (m, 1H), 6.69 (d, 1H, *J* = 8.3 Hz), 4.11 (s, 2H), 2.21 (s, 3H), 2.11 (s, 3H), 1.01 (s, 9H), 0.21 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.2, 154.7, 151.5, 137.1, 136.6, 133.3, 132.4, 131.7, 131.2, 131.0, 130.2, 129.2, 126.6, 126.1, 126.0_6, 126.0_1, 125.9, 124.9, 119.4, 32.8, 25.9, 18.5, 16.9, 15.0, −4.0 HRMS: calcd. for $C_{19}H_{16}F_3NOS_2$ [M + H] $^+$ 395.0625, found 395.0622.

4.4. 5-(1-(4-(tert-butyldimethylsilyloxy)-3-methylphenylthio)-2-phenylethyl)-4-methyl-2-(4-(trifluoromethyl)phenyl)thiazole, compound ***7a***

A stirred solution of 6 (300 mg, 0.59 mmol) in anhydrous THF (5 ml) was cooled to −78 °C in dryice-acetone bath under N_2 atmosphere. 2.0 M lithiumdiisopropylamide (619 μl, 1.24 mmol) was added dropwise to reaction mixture at −78 °C. The reaction mixture was stirred at −78 °C for 30 min. A solution of benzyl bromide (77 μl, 0.65 mmol) in THF (1 ml) was added dropwise at −78 °C. After the mixture was stirred at same temperature for further 30 min, it was quenched by adding sat. NH_4Cl solution. The mixture was diluted by ethyl acetate and the organic layer was dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give crude compound. The crude compound was purified by column chromatography on silica gel (*n*-hexnane/ethylacetate = 10/1) to obtain 7a as colorless oil (265 mg, 75%) ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, 2H, *J* = 8.1 Hz), 7.65 (d, 2H, *J* = 8.2 Hz), 7.03–7.26 (m, 7H) 6.63 (d, 1H, *J* = 8.3 Hz), 4.51 (dd, 1H, *J* = 9.8, 5.3 Hz), 3.37 (dd, 1H, *J* = 9.8, 5.3 Hz), 3.05 (dd, 1H, *J* = 13.6, 9.9 Hz), 2.10 (s, 3H), 1.83 (s, 3H), 0.98 (s, 9H), 0.18 (s, 6H) ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.5, 155.1, 151.8, 138.4, 137.7, 136.5, 133.5, 130.3, 129.3, 128.9, 127.2, 126.8, 126.7, 126.2, 126.1, 124.5, 119.4, 49.2, 44.4, 26.1, 18.7, 17.1, 15.1, -3.8_1, -3.8_4.

4.5. Ethyl 2-(2-methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)-2-phenylethylthio)phenoxy)acetate, compound ***9a***

To a stirred solution of **7a** (200 mg, 0.33 mmol) in THF (5 ml) was added 1 M tetrabutylammonium fluoride (660 μl, 0.66 mmol) at room temperature. The reaction mixture was sirred for 1 h. After completion of the reaction, ethyl acetate (3 ml) and distilled water (5 ml) was added to the reaction mixture. The organic layer was separated, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude compound. This crude compound was dissolved in acetone (5 ml) at room temperature. Potassium carbonate (0.76 mmol) and ethyl bromoacetate (55 μl, 0.50 mmol) were added to the reaction mixture in that order. The reaction mixture was stirred for further 6 h at room temperature. After completion of reaction, the reaction mixture was diluted with ethyl acetate, filtered, dried with $MgSO_4$, and concentrated under reduced pressure to give the crude compound. The crude compound was purified by column chromatography on silica gel (*n*-hexnane/ethylacetate = 5/1) to obtain **9a** as pale yellowish oil (177 mg, 94%) ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.98 (d, 2H, *J* = 8.1 Hz), 7.65 (d, 2H, *J* = 8.3 Hz), 7.06–7.27 (m, 7H), 6.55 (d, 1H, *J* = 8.4 Hz), 4.59 (s, 2H), 4.53 (dd, 1H, *J* = 9.7, 5.3 Hz), 4.22 (q, 2H, *J* = 7.1 Hz), 3.37 (dd, 1H, *J* = 13.7, 5.3 Hz), 3.17 (m, 1H) 2.20 (s, 3H), 1.83 (s, 3H), 1.26 (t, 3H, *J* = 7.2 Hz) ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.1, 163.6, 156.9, 151.8, 138.3, 137.4, 137.3, 136.4, 133.4, 129.3, 128.9, 128.6, 127.2, 126.8, 126.3, 126.2, 126.1, 125.1, 111.8, 65.9, 61.7, 49.1, 44.4, 16.5, 15.1, 14.5.

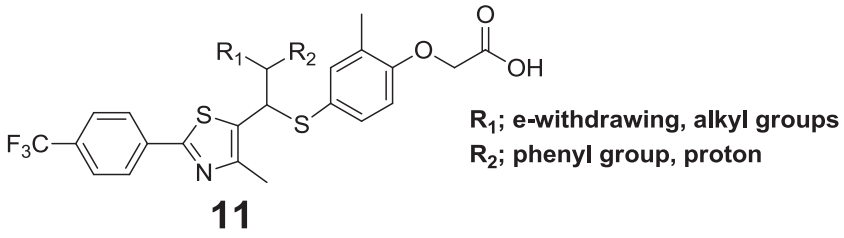
4.6. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)-2-phenylethylthio) phenoxy)acetic acid, compound ***10a***

To a stirred solution of **9a** (150 mg, 0.262 mmol) in ethanol (5 ml) was added 1 N NaOH (0.27 ml). The reaction mixture was stirred for 1 h. After completion of the reaction, 1 N HCl was added to reaction mixture until pH 3 was reached. The acidified mixture was concentrated under reduced pressure and diluted with ethylacetate and sat. NaCl solution. The organic layer was separated, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give **10a** as white solid (134 mg, 94%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.96 (d, 2H, *J* = 8.1 Hz), 7.66 (d, 2H, *J* = 8.2 Hz), 7.01–7.26 (m, 7H), 6.55 (d, 1H, *J* = 8.5 Hz), 4.62 (s, 2H), 4.23 (dd, 1H, *J* = 9.7, 5.4 Hz), 3.38 (dd, 1H, *J* = 13.8, 5.4 Hz), 3.08 (dd, 1H, *J* = 13.8, 9.7), 2.18 (s, 3H), 1.77 (s, 3H) ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.9, 163.7, 156.8, 151.9, 143.2, 137.7, 137.3, 137.2, 133.7, 128.6, 128.4, 126.5, 126.1, 125.8, 111.5, 65.8, 61.6, 47.6, 37.7, 36.2, 31.7, 29.7, 29.5, 29.4, 27.8, 16.3, 15.2, 14.3.

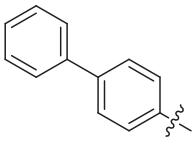
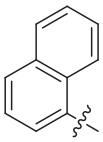
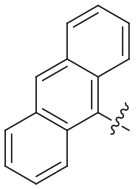
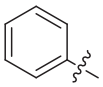
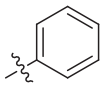
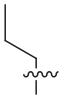
4.7. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)-3-phenylpropylthio)phenoxy)acetic acid, compound ***10b***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.98 (d, 2H, *J* = 8.1 Hz), 7.68 (d, 2H, *J* = 8.3 Hz), 7.26 (m, 3H), 7.12 (m, 4H), 6.55 (d, 1H, *J* = 8.5 Hz), 4.64 (s,

Table 3
In vitro activities of compounds **11l**–**11t**.



11

Entry	Compound	R_1	R_2	Transactiviation EC_{50}(nM)[a]		
				hPPARα	hPPARδ	hPPARλ
12	**11l**		H	ND	4.4	ND
13	**11m**		H	ND	490.1	ND
14	**11n**		H	ND	ia	ND
15	**11o**			ND	523.0	ND
16	**11p**	CH_3	H	135.5	1.8	ND
17	**11q**		H	35.0	1.0	ND
18	**11r**		H	432.4	2.3	ND
19	**11s**		H	591.7	1.1	ND
20	**11t**		H	861.3	1.1	ND

[a] 'ND' means that its EC_{50} value is bigger than 10 μM.

2H), 4.20 (dd, 1H, J = 8.9, 6.1 Hz), 2.73 (m, 2H), 2.38 (m, 1H), 2.20 (m, 1H), 2.17 (s, 3H), 1.89 (s, 3H).

4.8. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)-4-phenylbutylthio)phenoxy)acetic acid, compound ***10c***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.94 (d, 2H, J = 8.2 Hz), 7.66 (d, 2H, J = 8.2 Hz), 7.18 (m, 6H), 6.97 (m, 1H), 6.52 (d, 1H, J = 8.4 Hz), 5.80 (brs, 1H), 4.60 (s, 2H), 4.23 (dd, 1H, J = 8.3, 6.1 Hz), 2.64 (m, 2 Hz), 2.16 (s, 3H), 2.07 (m, 1H), 1.93 (s, 3H), 1.88 (m, 1H), 1.76 (m, 2H) ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.2, 163.9, 156.6, 151.1, 141.7, 137.9, 137.3, 136.7, 133.9, 128.6, 128.5, 128.3, 126.7, 126.2, 124.9, 111.4, 65.4, 47.5, 37.2, 35.6, 29.6, 16.2, 14.8.

4.9. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)-5-phenylpentylthio)phenoxy)acetic acid, compound ***10d***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, 2H, J = 8.2 Hz), 7.66 (d, 2H, J = 8.2 Hz), 7.20 (m, 6H), 6.99 (m, 1H), 6.53 (d, 1H, J = 8.4 Hz), 5.39

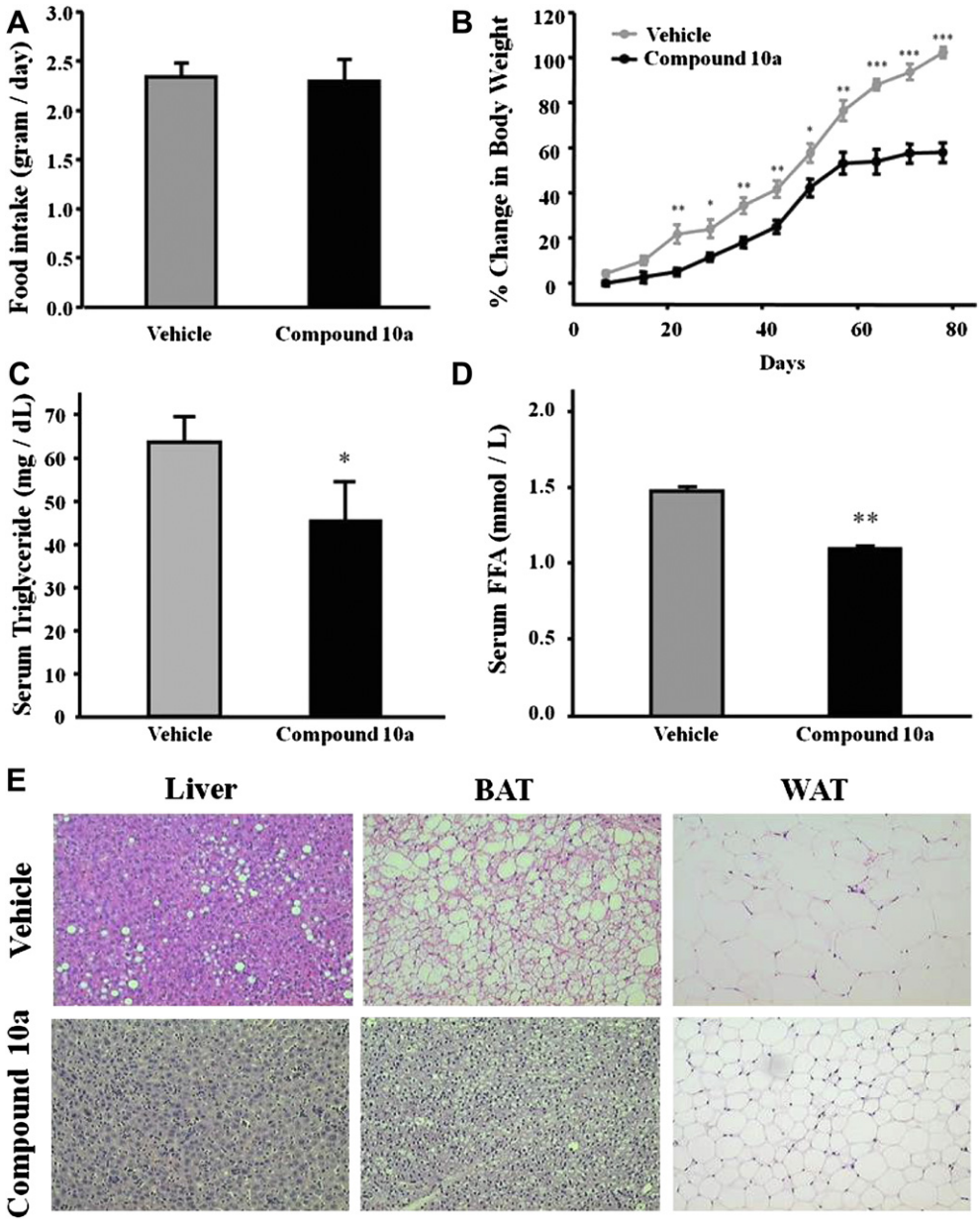


Fig. 4. Anti-obesity effect of compound **10a**. (A) Food intake of vehicle and compound **10a**-treated mice, measured over experimental days, did not show any significant difference between the two groups. (B) The increase in body weight was reduced by administration of compound **10a** in high-fat diet-induced obese mice. Compound **10a**-treated group decreased triglyceride (C) and free fatty acid (D) In serum. ($n = 6$). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (E) Also, H&E staining results showed that compound **10a**-treated mice decreased fat mass in liver, brown adipose tissue (BAT), and white adipose tissue (WAT).

(brs, 1H), 4.61 (s, 2H), 4.23 (dd, 1H, $J = 8.8$, 6.1 Hz), 2.59 (m, 2H), 2.17 (s, 3H), 1.88 (m, 1H), 1.94 (s, 3H), 1.91 (m, 1H), 1.65 (m, 2H), 1.46 (m, 2H) ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.2, 163.9, 156.6, 151.1, 142.4, 137.8, 137.4, 133.8, 128.5, 128.3, 126.7, 126.1, 126.0, 111.7, 65.4, 47.5, 37.6, 35.8, 31.2, 27.5, 16.2, 14.8.

*4.10. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)-6-phenylhexylthio)phenoxy)acetic acid, compound **10e***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, 2H, $J = 8.4$ Hz), 7.66 (d, 2H, $J = 8.5$ Hz), 7.20 (m, 6H), 6.98 (m, 1H), 6.54 (d, 1H, $J = 8.5$ Hz), 4.59 (s, 2H), 4.22 (dd, 1H, $J = 9.0$, 6.0 Hz), 2.58 (m, 2H), 2.17 (s, 3H), 2.03 (m, 1H), 1.93 (s, 3H), 1.89 (m, 1H), 1.61 (m, 2H), 1.42 (m, 4H) ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.4, 163.9, 156.6, 151.1, 142.6, 137.9, 137.6, 136.7, 133.8, 128.6, 128.5, 128.3, 126.8, 126.1, 125.9, 124.9, 111.5, 65.3, 47.5, 37.6, 35.9, 31.3, 28.9, 27.7, 16.2, 14.7.

*4.11. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)-8-phenyloctylthio)phenoxy)acetic acid, compound **10f***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, 2H, $J = 8.1$ Hz), 7.71 (s, 1H), 7.66 (d, 2H, $J = 8.3$ Hz), 7.20 (m, 6H), 6.98 (m, 1H), 6.54 (d, 1H, $J = 8.5$ Hz), 4.62 (s, 2H), 4.23 (dd, 1H, $J = 9.0$, 6.0 Hz), 2.58 (m, 2H), 2.17 (s, 3H), 2.03 (m, 1H), 1.95 (s, 3H), 1.89 (m, 1H), 1.61 (m, 2H), 1.28 (m, 8H) ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.4, 163.9, 156.6, 151.0, 142.9, 137.9, 137.7, 136.7, 133.8, 131.9, 131.5, 128.6, 128.4, 128.2, 126.7, 126.2, 126.1, 125.8, 125.0, 111.5, 65.3, 47.6, 37.7, 36.1, 31.6, 29.4, 29.3_5, 29.3_0, 27.9, 16.2, 14.7.

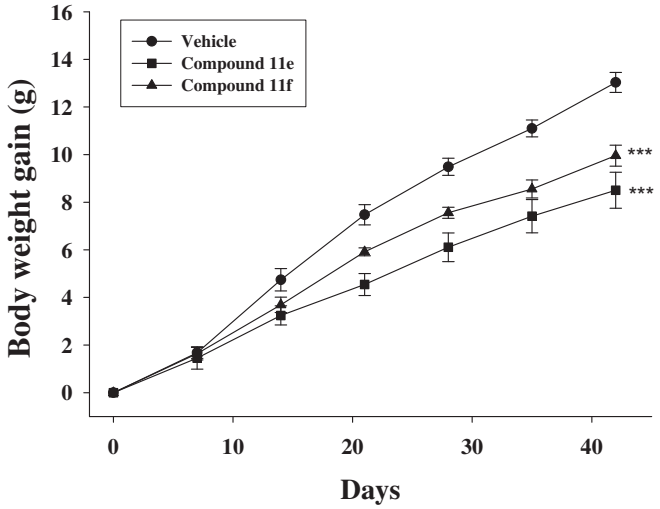


Fig. 5. Anti-obesity effect of compounds **11e** and **11f** in a high-fat diet-induced obese model. Mice were fed with an HFD containing 35% fat (w/w). Compounds **11e** and **11f** were administered to the mice at a dosage of 10 mg/kg with oral intubation daily ($n = 6$). $^{***}P < 0.001$.

*4.12. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)-11-phenylundecylthio)phenoxy)acetic acid, compound **10g***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, 2H, $J = 8.1$ Hz), 7.66 (d, 2H, $J = 8.3$ Hz), 7.18 (m, 6H), 6.99 (m, 1H), 6.54 (d, 1H, $J = 8.5$ Hz), 4.91 (brs, 1H), 4.61 (s, 2H), 4.24 (dd, 1H, $J = 9.0$, 5.9 Hz), 2.58 (m, 2H), 2.17 (s, 3H), 2.03 (m, 1H), 1.95 (s, 3H), 1.85 (m, 1H), 1.59 (m, 2H), 1.28 (m, 14H).

*4.13. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)-2-(4-nitrophenyl)ethylthio)phenoxy)acetic acid, compound **11a***

^{1}H NMR (600 MHz, $CDCl_3$): 8.83 (1H, s, br), 7.91 (2H, d, 7.8 Hz), 7.62 (2H, d, 7.8 Hz), 7.09 (1H, s), 7.01 (1H, d, 7.8 Hz), 6.97 (2H, d, 8.4 Hz), 6.74 (2H, d, 9 Hz), 6.46 (1H, d, 8.4 Hz), 4.48 (1H, q, 5.4 Hz), 4.42 (2H, s), 3.71 (3H, s), 3.30 (1H, q, 5.4 Hz), 3.01 (1H, q, 9.6 Hz), 2.09 (3H, s), 1.78 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$): 163.85, 158.61, 156.49, 151.36, 137.01, 136.58, 136.50, 133.10, 131.66, 131.44, 130.10, 129.96, 128.98, 126.77, 126.06, 124.96, 123.15, 114.24, 55.31, 48.94, 43.12, 30.47, 16.17, 14.60. HRFABMS (+mode): Calcd. for $C_{28}H_{23}F_3N_2O_5S_2$[M + H]$^+$ 588.6178, found 509.10 79.

*4.14. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)-2-(4-(trifluoromethyl)phenyl)ethylthio)phenoxy) acetic acid, compound **11b***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, 2H, $J = 8.1$ Hz), 7.67 (d, 2H, $J = 8.2$ Hz), 7.49 (d, 2H, $J = 8.0$ Hz), 7.21 (d, 2H, $J = 8.0$ Hz), 7.16 (d, 2H, $J = 1.5$ Hz), 7.05 (dd, 1H, $J = 10.3$, 1.9 Hz), 6.56 (d, 1H, $J = 8.5$ Hz), 5.34 (s, 1H), 4.63 (s, 2H), 4.52 (dd, 1H, $J = 9.4$, 5.7 Hz), 3.30 (m, 2H), 2.18 (s, 3H), 1.79 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): 175.00, 163.88, 156.60, 151.53, 141.95, 137.02, 136.60, 135.56, 133.19, 131.59, 129.57, 129.36, 128.34, 126.55, 126.07, 125.64, 124.94, 123.13, 112.51, 48.42, 43.70, 29.92, 16.06, 14.77. HRFABMS (+mode): Calcd. for $C_{29}H_{23}F_6NO_3S_2$[M + H]$^+$ 611.6182, found 612.1102.

*4.15. 2-(4-(2-(4-Methoxyphenyl)-1-(4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)ethylthio)-2-methylphenoxy) acetic acid, compound **11c***

^{1}H NMR (600 MHz, $CDCl_3$): 8.83 (1H, s, br), 7.91 (2H, d, 7.8 Hz), 7.62 (2H, d, 7.8 Hz), 7.09 (1H, s), 7.01 (1H, d, 7.8 Hz), 6.97 (2H, d, 8.4 Hz), 6.74 (2H, d, 9 Hz), 6.46 (1H, d, 8.4 Hz), 4.48 (1H, q, 5.4 Hz), 4.42 (2H, s), 3.71 (3H, s), 3.30 (1H, q, 5.4 Hz), 3.01 (1H, q, 9.6 Hz), 2.09 (3H, s), 1.78 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$): 163.85, 158.61, 156.49, 151.36, 137.01, 136.58, 136.50, 133.10, 131.66, 131.44, 130.10, 129.96, 128.98, 126.77, 126.06, 124.96, 123.15, 114.24, 55.31, 48.94, 43.12, 30.47, 16.17, 14.60. HRFABMS (+mode): Calcd. for $C_{29}H_{26}F_3NO_4S_2$ [M + H]$^+$ 573.6462, found 574.1334.

*4.16. 2-(4-(2-(2-Chloro-6-fluorophenyl)-1-(4-methyl-2-(4-(trifluoromethyl) phenyl)thiazol-5-yl)ethylthio)-2-methylphenoxy) acetic acid, compound **11d***

To a stirred solution of **12e**–**ester** (156 mg, 0.25 mmol) in THF/H_2O (3:2, 5 ml) was added 2 N LiOH (0.27 ml) at room temperature and stirred for 2 h. After completion of the reaction, 0.5 M $NaHSO_4$ was added to reaction mixture until pH 2 was reached. The acidified mixture was concentrated under reduced pressure and diluted with ethylacetate and sat. NaCl solution. The organic layer was separated, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give **12e** as white solid (140 mg, 94%). ^{1}H NMR (600 MHz, $CDCl_3$): 7.96 (2H, d, 8.4 Hz), 7.66 (2H, d, 7.8 Hz), 7.17 (1H, s), 7.15 (2H, t, 8.4 Hz), 7.09(1H, dd, 1.2 Hz, 9.6 Hz), 6.92 (1H, m), 6.77 (1H, s, br), 6.55 (1H, d, 8.4 Hz), 4.79 (1H, q, 6.6 Hz), 4.60 (2H, s), 3.45 (1H, q, 7.2 Hz), 3.35 (1H, q, 9 Hz), 2.17 (3H, s), 1.85 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$): 172.76, 164.21, 162.60, 160.95, 156.44, 151.17, 136.98, 136.20, 135.55, 132.99, 129.13, 128.39, 126.76, 126.13, 125.57, 125.13, 125.00, 124.15, 114.24, 111.63, 65.29, 45.96, 34.57, 29.92, 16.26, 14.54. HRFABMS (+mode): Calcd. for $C_{28}H_{22}ClF_4NO_3S_2$ [M + H]$^+$ 596.0558, found 596.0744(M^++1).

*4.17. 2-(4-(2-(2,6-Difluorophenyl)-1-(4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)ethylthio)-2-methylphenoxy) acetic acid, compound **11e***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (d, 2H, $J = 8.1$ Hz), 7.65 (d, 2H, $J = 8.4$ Hz), 7.13 (m, 3H), 6.81 (m, 2H), 6.55 (d, 1H, $J = 8.4$ Hz), 6.01 (brs, 1H), 4.71 (dd, 1H, $J = 8.9$, 6.8 Hz), 4.63 (s, 2H), 3.35 (dd, 1H, $J = 13.9$, 6.7 Hz), 3.23 (dd, 1H, $J = 13.9$, 9.1 Hz), 2.18 (s, 3H), 1.90 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.6, 164.2, 156.4, 151.0, 136.9, 136.8, 136.5, 136.3, 134.3, 132.9, 128.9, 128.7, 128.4, 126.8, 126.1, 125.4, 111.6, 65.3, 46.0, 38.9, 16.3, 14.5. HRFABMS (+mode): Calcd. for $C_{28}H_{22}F_5NO_3S_2$ [M + H]$^+$ 579.6012, found 580.1040.

*4.18. 2-(4-(2-(2-Fluoro-6-(trifluoromethyl)phenyl)-1-(4-methyl-2-(4-(trifluoromethyl) phenyl)thiazol-5-yl)ethylthio)-2-methylphenoxy)acetic acid, compound **11f***

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.44 (br, 1H), 7.96 (d, 2H, $J = 8.1$ Hz), 7.66 (d, 2H, $J = 8.3$ Hz), 7.47 (d, 1H, $J = 7.8$ Hz) 7.34 (m, 1H), 7.19 (t, 1H, $J = 9.1$ Hz), 7.13 (d, 1H, $J = 1.7$ Hz), 7.05 (dd, 1H, $J = 8.4$, 2.2 Hz), 6.54 (d, 1H, $J = 8.4$ Hz), 4.70 (dd, 1H, $J = 8.0$, 8.0 Hz), 4.61 (s, 2H), 3.48 (dd, 1H, $J = 14.3$, 7.2 Hz), 3.37 (dd, 1H, $J = 14.2$, 8.3 Hz), 2.17 (s, 3H), 1.80 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.9, 164.4, 160.5, 156.5, 150.9, 137.0, 136.6, 133.1, 131.9, 131.5, 129.2, 129.0, 128.4, 126.8, 126.2, 126.1, 125.0, 124.2, 122.6, 119.7, 119.3, 111.6, 65.2, 47.4, 33.7, 16.2, 14.4. HRFABMS (+mode): Calcd. for $C_{29}H_{22}F_7NO_3S_2$ [M + H]$^+$ 629.6087, found 630.1008.

4.19. 2-(4-(2-(2,6-Dichlorophenyl)-1-(4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)ethylthio)-2-methylphenoxy) acetic acid, compound ***11g***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, 2H, J = 8.1 Hz), 7.66 (d, 2H, J = 8.3 Hz), 7.08–7.28 (m, 5H), 6.55 (d, 1H, J = 8.5 Hz), 4.85 (dd, 1H, J = 8.4, 6.8 Hz), 4.61 (s, 2H), 3.59 (dd, 1H, J = 13.7, 6.8 Hz), 3.47 (dd, 1H, J = 13.7, 8.52 Hz), 2.18 (s, 3H), 1.80 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.6, 164.2, 156.4, 151.0, 136.9, 136.8, 136.5, 136.3, 134.3, 132.9, 128.9, 128.7, 128.4, 126.8, 126.1, 125.4, 111.6, 65.3, 46.0, 38.9, 16.3, 14.5. HRFABMS (+mode): Calcd. for $C_{28}H_{22}Cl_2F_3NO_3S_2$ $[M + H]^+$612.5104, found 612.0449.

4.20. 2-(4-(2-(2,4-Difluorophenyl)-1-(4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)ethylthio)-2-methylphenoxy) acetic acid, compound ***11h***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (d, 2H, J = 8.1 Hz), 7.66 (d, 2H, J = 8.3 Hz), 7.38 (brs, 1H), 7.16 (m, 1H), 7.03 (m, 2H), 6.74 (m, 2H), 6.56 (d, 1H, J = 8.5 Hz), 4.65 (s, 2H), 4.59 (m, 1H), 3.36 (dd, 1H, J = 14.0, 6.1 Hz), 0.3.06 (dd, 1H, J = 14.0, 9.3 Hz), 2.18 (s, 3H), 1.87 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.8, 164.1, 156.6, 151.5, 137.2, 136.6, 135.9, 133.2, 132.1, 132.0, 131.6, 128.5, 126.8, 126.2, 126.1, 124.8, 121.0, 120.9, 111.6, 111.3, 104.1, 103.7, 65.2, 47.2, 37.1, 16.3, 14.6. HRFABMS (+mode): Calcd. for $C_{28}H_{22}F_5NO_3S_2$ $[M + H]^+$ 579.6012, found 580.1040.

4.21. 2-(4-(2-(2,5-Difluorophenyl)-1-(4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)ethylthio)-2-methylphenoxy) acetic acid, compound ***11i***

^{1}H NMR (600 MHz, $CDCl_3$): 7.81 (2H, d, 7.8 Hz), 7.59 (2H, d, 8.4 Hz), 7.46 (2H, d, 8.4 Hz), 7.16 (2H, d, 8.4 Hz), 7.01(1H, s), 6.93 (1H, d, 7.8 Hz), 6.35 (1H, d, 7.8 Hz), 4.49 (1H, q, 5.4 Hz), 4.20 (2H, s), 3.36 (1H, q, 6 Hz), 3.11 (1H, q, 9.6 Hz), 2.08 (3H, s), 1.74 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$): 163.88, 156.61, 151.53, 141.96, 137.03, 136.60, 135.56, 133.20, 131.81, 131.59, 129.57, 129.46, 128.34, 126.55, 126.07, 125.62, 125.15, 124.94, 123.34, 123.13, 112.48, 48.42, 43.70, 29.92, 16.06, 14.77. HRFABMS (+mode): Calcd. for $C_{28}H_{22}F_5NO_3S_2$ $[M + H]^+$ 579.6012, found 580.1040.

4.22. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)-2-(3,4,5-trifluorophenyl)ethylthio)phenoxy)acetic acid, compound ***11j***

^{1}H NMR (600 MHz, $CDCl_3$): 7.87 (2H, d, 8.4 Hz), 7.58 (2H, d, 8.4 Hz), 7.01(1H, s), 6.95 (1H, d, 8.4 Hz), 6.90 (1H, m), 6.83 (1H, m), 6.73 (1H, m), 6.35 (1H, d, 7.8 Hz), 4.61 (1H, q, 6 Hz), 4.15 (2H, s), 3.32 (1H, q, 5.4 Hz), 3.01 (1H, q, 9.6 Hz), 1.94 (3H, s), 1.86 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$): 175.26, 163.79, 159.33, 158.07, 157.71, 156.52, 151.46, 136.68, 135.53, 132.92, 131.71, 128.32, 126.54, 126.03, 125.10, 123.15, 117.86, 116.54, 115.46, 112.72, 46.58, 37.66, 16.03, 14.80. HRFABMS (+mode): Calcd. for $C_{28}H_{21}F_6NO_3S_2$ $[M + H]^+$ 597.5917, found 580.5914.

4.23. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)-2-(perfluorophenyl)ethylthio)phenoxy)acetic acid, compound ***11k***

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (brs, 1H), 7.96 (d, 2H, J = 8.1 Hz), 7.67 (d, 2H, J = 8.3 Hz), 7.14 (d, 1H, J = 1.7 Hz), 7.07 (dd, 1H, J = 8.4, 2.2 Hz), 6.57 (d, 1H, J = 8.5 Hz), 4.66 (m, 1H), 4.63 (s, 2H), 3.35 (dd, 1H, J = 14.2, 7.5 Hz), 3.26 (dd, 1H, J = 14.2, 8.3 Hz), 2.19 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.2, 164.7, 156.9, 151.7, 137.4, 136.6, 135.1, 133.5, 132.3, 131.9, 128.9, 127.0, 126.4, 126.3, 126.0, 124.5, 111.8, 65.4, 46.3, 30.9, 16.4, 14.9. HRFABMS (+mode): Calculated for $C_{28}H_{19}F_8NO_3S_2$ 633.5726, found 634.0757(M^++1).

4.24. 2-(4-(2-(Biphenyl-4-yl)-1-(4-methyl-2-(4-(trifluoromethyl) phenyl)thiazol-5-yl)ethylthio)-2-methylphenoxy)acetic acid, compound ***11l***

^{1}H NMR (600 MHz, $CDCl_3$): 8.55 (1H, s, br), 7.85 (2H, d, 8.4 Hz), 7.54 (2H, d, 7.8 Hz), 7.48 (2H, d, 7.8 Hz), 7.41 (2H, d, 7.8 Hz), 7.37 (2H, t, 7.8 Hz), 7.29 (1H, t, 7.2 Hz), 7.08 (2H, d, 8.4 Hz), 7.02 (1H, s), 6.96 (1H, d, 8.4 Hz), 6.34 (1H, d, 7.2 Hz), 4.54 (1H, q, 5.4 Hz), 4.16 (2H, s), 3.36 (1H, q, 5.4 Hz), 3.06 (1H, q, 9.6 Hz), 1.94 (3H, s), 1.77 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$): 163.59, 156.45, 151.51, 140.68, 139.91, 136.94, 136.81, 136.74, 136.09, 132.99, 131.57, 131.35, 129.55, 128.95, 128.29, 127.50, 127.31, 127.08, 126.78, 126.51, 126.02, 125.36, 124.97, 112.58, 48.55, 43.70, 16.08, 14.87. HRFABMS (+mode): Calculated for $C_{34}H_{28}F_3NO_3S_2$ $[M + H]^+$ 619.7162, found 620.1541.

4.25. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)-2-(naphthalen-1-yl)ethylthio)phenoxy)acetic acid, compound ***11m***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.98 (2H, d, J = 8.0 Hz), 7.76 (1H, m), 7.71 (2H, d, J = 8.1 Hz), 7.67 (2H, d, J = 8.1 Hz), 7.55 (1H, m), 7.43–7.41 (2H, m), 7.22 (1H, dd, J = 8.2, 1.5 Hz), 7.16 (1H, d, J = 1.5 Hz), 7.1 (1H, d, J = 8.2 Hz), 6.62 (1H, d, J = 8.2 Hz), 4.67–4.64 (1H, m), 4.49 (2H, s), 3.69–3.65 (1H, m), 3.26–3.22 (1H, m), 2.18 (3H, s), 1.79 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.8, 163.8, 157.3, 151.3, 137.1, 136.6, 136.4, 135.4, 133.5, 133.4, 132.4, 131.5 (q, J = 32.5 Hz), 128.2, 128.1, 127.8, 127.7, 127.6, 127.0, 126.7, 126.5, 126.2, 126.0 (q, J = 3.9 Hz), 125.8, 124.9, 123.9, 123.0, 112.0, 67.1, 44.0, 29.7, 16.0, 14.5. HRFABMS (+mode): Calcd. for $C_{32}H_{26}F_3NO_3S_2$ $[M + H]^+$ 593.6789, found 594.1384.

4.26. 2-(4-(2-(Anthracen-9-yl)-1-(4-methyl-2-(4-(trifluoromethyl) phenyl)thiazol-5-yl)ethylthio)-2-methylphenoxy)acetic acid, compound ***11n***

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.37 (1H, S), 8.0–7.93 (6H, m), 7.67 (2H, d, J = 8.0 Hz), 7.42–7.37 (4H, m), 7.17 (1H, d, J = 1.5 Hz), 7.13 (1H, d, J = 8.2 Hz), 6.56 (1H, d, J = 8.2 Hz), 4.75–4.79 (1H, m), 4.62 (2H, s), 4.29–4.27 (1H, m), 4.13–4.11 (1H, m), 2.17 (3H, s), 1.16 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.8, 164.7, 158.4, 151.6, 137.5, 137.4, 137. 3, 133.8, 132.4, 131.0, 130.7, 129.9, 129.0, 127.8, 127.3, 126.7 (q, J = 3.9 Hz), 125.7, 124.8, 124.4, 112.7, 64.1, 36.5, 30.4, 16.5, 14.2. FABMS (+mode): Calcd. for $C_{36}H_{28}F_3NO_3S_2$ $[M + H]^+$ 643.50, found 644.0 (M^+ + 1), 666.0 (M^+ + Na).

4.27. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)-2,2-diphenylethylthio)phenoxy)acetic acid, compound ***11o***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (2H, d, J = 8.1 Hz), 7.49–7.46 (4H, m), 7.34 (2H, t, J = 8.1 Hz), 7.24–7.20 (1H, m), 7.17–7.16 (2H, m), 7.12–7.09 (2H, t, J = 8.2 Hz), 7.03–7.0 (1H, m), 6.82 (1H, d, J = 1.6 Hz), 6.76 (1H, dd, J = 8.2, 1.6 Hz), 6.28 (1H, d, J = 8.2 Hz), 5.05 (1H, d, J = 7.5 Hz), 4.32 (1H, d, J = 7.5 Hz), 4.10 (2H, s), 1.85 (3H, s), 1.70 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.1, 163.4, 156.6, 151.4, 141.7, 141.5, 137.2, 136.7, 136.4, 133.5, 131.2 (q, J = 32.5 Hz), 128.7, 128.6, 128.2, 127.7, 127.2, 126.8, 126.2, 125.7 (q, J = 3.9 Hz), 125.5, 124.8, 124.7, 122.9, 112.5, 67.3, 59.3, 51.8, 15.8, 14.6. HRFABMS (+mode): Calcd. for $C_{34}H_{28}F_3NO_3S_2$ $[M + H]^+$ 619.7162, found 620.1541.

*4.28. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)ethylthio)phenoxy)acetic acid, compound **11p***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (2H, d, J = 8.1 Hz), 7.66 (2H, d, J = 8.2 Hz), 7.13 (1H, s), 7.06 (1H, d, J = 8.2 Hz), 6.60 (1H, d, J = 8.3 Hz), 4.47 (2H, s), 4.44 (1H, m), 2.17 (3H, s), 2.10 (3H, s), 1.66 (3H, d, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.2, 163.3, 157.0, 150.1, 138.1, 137.4, 136.7, 133.6, 131.3 (q, J = 32.5 Hz), 128.0, 127.2, 126.4, 125.9 (q, J = 3.9 Hz) 125.0, 124.4, 122.9, 112.0, 66.5, 41.8, 23.5, 15.9, 14.6. HRFABMS (+mode): Calcd. for $C_{22}H_{20}F_3NO_3S_2$ $[M + H]^+$ 467.5243, found 468.0915.

*4.29. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)propylthio)phenoxy)acetic acid, compound **11q***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (2H, d, J = 8.1 Hz), 7.67 (2H, d, J = 8.2 Hz), 7.08 (1H, d, J = 1.5 Hz), 6.98 (1H, dd, J = 8.2, 1.5 Hz), 6.63 (1H, d, J = 8.2 Hz), 4.54 (2H, s), 4.20 (1H, m), 2.16 (3H, s), 2.16–2.02 (1H, m), 2.07 (3H, s), 1.92–1.85 (1H, m), 1.03 (3H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.2, 163.6, 157.2, 150.8, 137.2, 137.1, 136.7, 133.4, 131.4 (q, J = 32.5 Hz), 127.9, 126.4, 125.9 (q, J = 3.9 Hz) 125.0, 124.2, 122.8, 120.7, 112.1, 70.0, 31.9, 29.6, 15.8, 14.6, 12.2. HRFABMS (+mode): Calcd. for $C_{23}H_{22}F_3NO_3S_2$ $[M + H]^+$ 481.5509, found 482.1071.

*4.30. 2-(2-Methyl-4-(1-(4-methyl-2-(4-(trifluoromethyl)phenyl) thiazol-5-yl)butylthio)phenoxy)acetic acid, compound **11r***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (2H, d, J = 8.1 Hz), 7.65 (2H, d, J = 8.2 Hz), 7.12 (1H, d, J = 1.5 Hz), 6.99 (1H, dd, J = 8.2, 1.5 Hz), 6.54 (1H, d, J = 8.2 Hz), 4.62, (2H, s), 4.26 (1H, m), 2.17 (3H, s), 2.05–2.0 (1H, m), 1.96 (3H, s), 1.88–1.83 (1H, m), 1.47–1.42 (2H, m), 0.93 (3H, t, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.6, 163.9, 156.6, 150.9, 137.8, 137.7, 136.6, 133.8, 131.5 (q, J = 32.5 Hz), 128.2, 126.8, 126.1 (q, J = 3.9 Hz) 125.1, 124.9, 123.0, 111.5, 65.3, 47.3, 29.9, 21.1 16.2, 14.6, 13.8. HRFABMS (+mode): Calcd. for $C_{24}H_{24}F_3NO_3S_2$ $[M + H]^+$ 495.5775, found 496.1228.

*4.31. 2-(2-Methyl-4-(3-methyl-1-(4-methyl-2-(4-(trifluoromethyl) phenyl)thiazol-5-yl)butylthio)phenoxy)acetic acid, compound **11s***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (2H, d, J = 8.1 Hz), 7.58 (2H, d, J = 8.2 Hz), 7.03 (1H, d, J = 1.5 Hz), 6.91 (1H, dd, J = 8.2, 1.5 Hz), 6.47 (1H, d, J = 8.2 Hz), 4.54 (2H, s), 4.25 (1H, m), 2.10 (3H, s), 1.86 (3H, s), 1.82–1.61 (3H, m), 0.88 (2H, d, J = 7.0 Hz), 0.83 (2H, d, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.0, 163.4, 156.4, 150.8, 137.6, 137.3, 136.8, 133.5, 131.4 (q, J = 32.5 Hz), 128.2, 127.3, 126.5, 126.0 (q, J = 3.9 Hz) 125.2, 123.0, 120.8, 112.2, 66.6, 46.7, 29.9, 26.2 23.0, 21.9, 16.1, 14.9. HRFABMS (+mode): Calcd. for $C_{25}H_{26}F_3NO_3S_2$ $[M + H]^+$ 509.6040, found 510.1384.

*4.32. 2-(4-(2-Cyclopropyl-1-(4-methyl-2-(4-(trifluoromethyl) phenyl)thiazol-5-yl)ethylthio)-2-methylphenoxy)acetic acid, compound **11t***

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (2H, d, J = 8.0 Hz), 7.70 (2H, d, J = 8.1 Hz), 7.13 (1H, d, J = 1.5 Hz), 7.08 (1H, dd, J = 8.2, 1.5 Hz), 6.62 (1H, d, J = 8.2 Hz), 4.58 (2H, s), 4.42 (1H, m), 2.19 (3H, s), 2.13 (3H, s), 2.0–1.91 (1H, m), 1.85–1.78 (1H, m), 0.93–0.77 (1H, m), 0.48–0.45 (2H, m), 0.13–0.12 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.9, 163.6, 156.9, 151.6, 137.5, 136.8, 136.6, 133.1, 131.3 (q, J = 32.5 Hz), 127.9, 126.5, 126.4, 125.8 (q, J = 3.9 Hz), 125.7, 124.9, 124.1, 122.8, 111.5, 54.3, 47.4, 28.8, 15.7, 14.5, 9.2, 4.6, 4.3, HRFABMS (+mode): Calcd. for $C_{25}H_{24}F_3NO_3S_2$ $[M + H]^+$ 507.5882, found 508.1228.

4.33. Modeling

AutoDock version 3.0.5 was used for the docking calculations. The protein structure used in the calculation was based on the co-crystal structure of hPPARδ-LBD and GW2433 (PDB code 1GWX). The co-crystallized ligand and crystallographic water molecules were removed before docking. Missing heavy atoms and hydrogen atoms were added using the Leap module of the Amber 8 package. The standard ionization state at neutral pH was considered for all ionizable residues because there was no apparent salt bridge between residues with same charge. The affinity grids for docking were centered on the geometric center of GW2433 with a grid spacing of 0.375 Å. By visual inspection, we conformed that this box practically covered the known binding pocket of the receptor. For consistency of partial charges of the receptor, single-point calculations were performed to obtain the electrostatic potential using the HF/6-31G* level of theory. Fitting charges to the electrostatic potential was subsequently performed according to two-step restrained electrostatic potential (RESP) protocol. Equivalent atoms were given equal partial charges. Ligand input files for docking calculations were then prepared using the AutoTors module in AutoDock 3.0.5, and the atomic solvation parameters were determined based on the atom types. The Lamarckian genetic algorithm was selected to search for ligand conformations that matched the binding site. For each compound, the following running parameters were used: random initial position, population size of 50, elitism of 1, mutation rate of 0.02, crossover rate of 0.8, local search rate of 0.06, 4 million energy evaluations and 200 docking trials. The docked conformations from the 200 trials for each ligand were clustered using a tolerance of a 1.0 Å root-mean-square deviation and were sorted in terms of docking energy.

4.34. In vitro transfection assay

The assay was performed as described in previous reports [16]. The monkey kidney cells, CV-1, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% resin-charcoal-stripped fetal bovine serum, 100 U/ml penicillin, and 100 g/ml streptomycin in a humidified incubator (5% CO_2 in air) at 37 °C. CV-1 cells were seeded at 6×10^3 cells per well in 96-well culture plates and then grown to 70% confluence before transfection. The cells were washed with serum-free medium and then transfected with a plasmid mixture containing human PPAR expression vector, β-galactosidase, and TK-PPRE-Luc vector by Superfect reagent (QIAGEN). The 24 h post-transfected cells were washed with serum-free DMEM and incubated with freshly delipidated 5% FBS DMEM supplemented with either compounds or DMSO vehicle for 24 h. After incubation, cell lysates were obtained using cell lysis buffer, and a luciferase activity was determined upon substrate addition using a Microlumat Plus Luminometer (Berthold). The luciferase activity was normalized with β-galactosidase activity using an ONPG buffer. All of the assays were performed in triplicate.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2012.03.055.

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