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Discovery of novel dual functional agent as PPARγ agonist and 11β-HSD1 inhibitor for the treatment of diabetes

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ABSTRACT

PPARγ and 11β-HSD1 are attractive therapeutic targets for type 2 diabetes. However, PPARγ agonists induce adipogenesis, which causes the side effect of weight gain, whereas 11β-HSD1 inhibitors prevent adipogenesis and may be beneficial for the treatment of obesity in diabetic patients. For the first time, we designed, synthesized a series of α-aryloxy-α-methylhydrocinnamic acids as dual functional agents which activate PPARγ and inhibit 11β-HSD1 simultaneously. The compound **11e** exhibited the most potent inhibitory activity compared to that of the lead compound **2**, with PPARγ (EC_{50} = 6.76 μM) and 11β-HSD1 (IC_{50} = 0.76 μM) in vitro. Molecular modeling study for compound **11e** was also presented. Compound **11e** showed excellent efficacy for lowering glucose, triglycerides, body fat, in well established mice and rats models of diabetes and obesity and had a favorable ADME profile.

1. Introduction

Type 2 diabetes has rapidly reached an epidemic proportion and become a considerable public health problem.[1] Insulin resistance plays a key role in the pathogenesis and development of type 2 diabetes.[2] Over the upcoming decades, the most challenging issue in diabetic patients will be to re-establish their tissues' insulin sensitivity in order to correct glucose and lipid disturbance. Peroxisome proliferators-activated receptor γ (PPARγ) and 11β-hydroxysteroid dehydrogenase type 1(11β-HSD1) are two novel molecular therapeutic targets for type 2 diabetes by increasing insulin sensitivity and improving glycemic control.[3]

Peroxisome proliferators-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily, which involved in the regulation of glucose and lipid metabolism.[4] Of the three PPAR isoforms (PPARα,δ/β,γ) discovered so far, PPARγ is the most extensively studied, and has been firstly proved as an attractive target for the treatment of type 2 diabetes. PPARγ is predominantly expressed in white and brown adipose tissue, with lower expression in muscle, liver and other tissues.[5] Activation of PPARγ by its ligands or agonists initiates heterodimerization with 9-*cis*-retinoic acid receptor (RXR), and then bind to peroxisome proliferator response elements (PPREs), regulating the transcription of genes that are involved in glucose and lipid homeostasis.[6] Among the PPARγ agonists, a class of thiazolidinedione (TZD) derivatives, pioglitazone and rosiglitazone are currently used for the treatment of type 2 diabetes.[7] However, the TZD drugs possess undesirable side effects, such as weight gain, edema, significant cardiac hypertrophy, and the risk of heart failure.[8]

11β-HSD1 is a microsomal enzyme, which is highly expressed in adipose tissue, liver, gonad and brain. It catalyzes the regeneration of the active glucocorticoids from its inactive forms, thus amplifies local glucocorticoid action independently of the level of glucocorticoids in the circulating plasma.[9] Since glucocorticoids antagonize the action of insulin and induce insulin resistance when in excess, the pharmacological inhibition of 11β-HSD1 has been suggested to be a very attractive therapy for insulin resistance and other disorders in type 2 diabetic patients.[10] Up to date, more than 25 pharmaceutical companies and academic laboratories have explored 11β-HSD1 inhibitors.[11] Among them, several compounds showed in vivo anti-diabetic efficacy in rodent models. Moreover, Incyte's compound INCB 13739 has shown reducing fasting glucose and glucose production, and increasing glucose disposal in phase IIa clinical trial, which encouraged the development of 11β-HSD1 inhibitors as anti-diabetic drugs.[12]

Since PPARγ agonists induce adipogenesis, which cause the side effect of weight gain and increases body fat mass, a considerable effort has been made to search novel compounds with good

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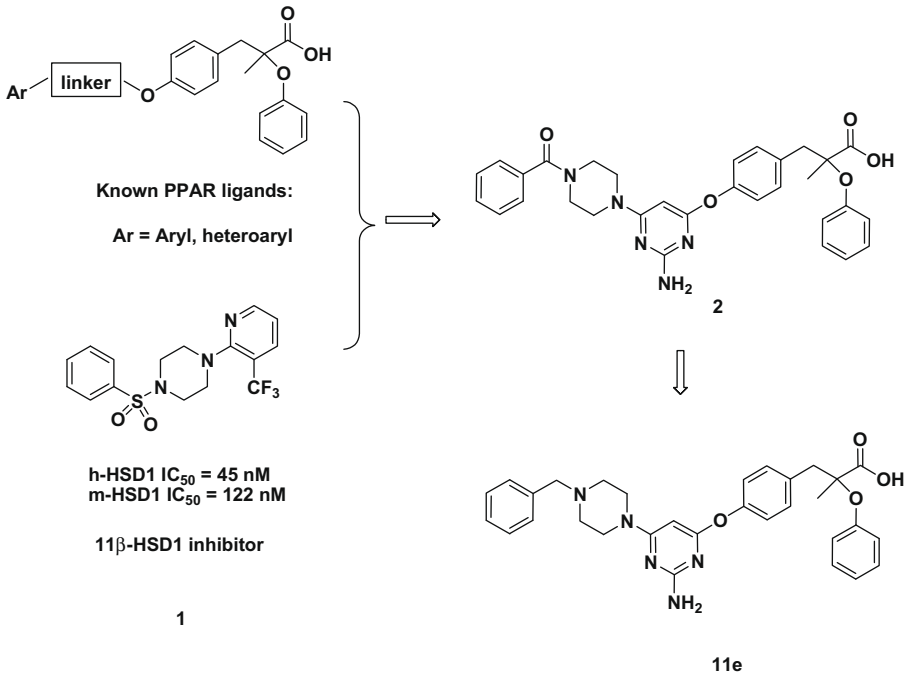


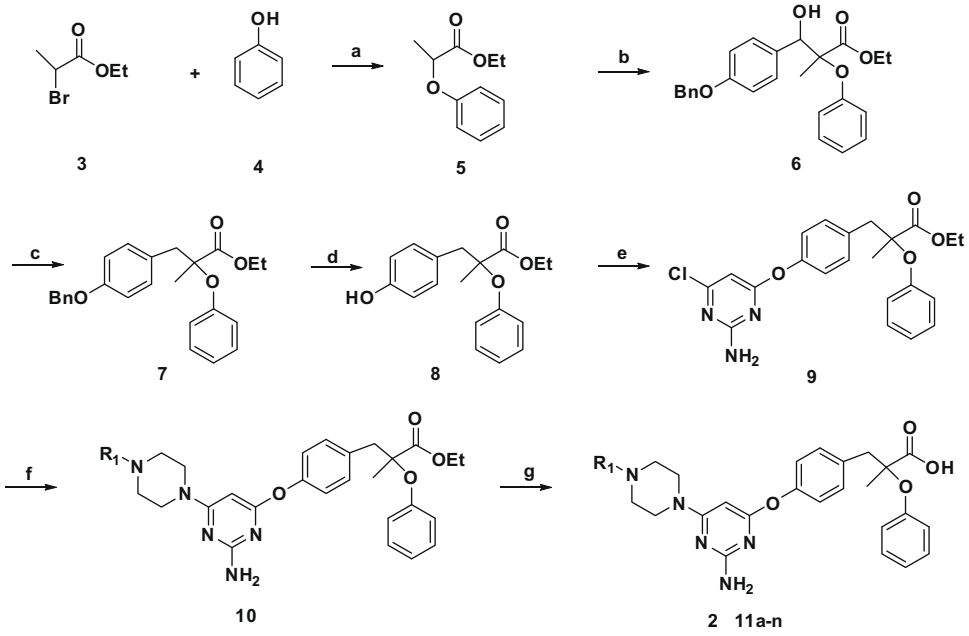
Figure 1. Design of α-aryloxy-α-methylhydrocinnamic acids.

anti-diabetic efficacy, but weak adipogenic effects. It has been proved that the increase in 11β-HSD1 mRNA and activity is essential for the induction of adipogenesis by regulating the local level of glucocorticoids in adipose tissue.[13] Meanwhile, suppression of 11β-HSD1 with RNAi attenuates 3T3-L1 adipogenesis,[14] and the pharmacological inhibition of 11β-HSD1 could prevent human adipocyte differentiation in vitro.[15] Therefore, based on the different effects of PPARγ agonists and 11β-HSD1 inhibitors on adipogenesis, we hypothesized that the combination of PPARγ activation and 11β-HSD1 inhibition might counteract the side effect of weight gaining and show better anti-diabetic activity. In the present study, a series of α-aryloxy-α-methylhydrocinnamic acids were designed and synthesized as dual functional agents, which activate PPARγ and inhibit 11β-HSD1 activity simultaneously. The adipogenic and anti-diabetic effects of these compounds were evaluated both in vitro and in vivo.

2. Chemistry

2.1. Design and synthetic procedures of analogues of α-aryloxy-α-methylhydrocinnamic acids

A series of α-aryloxy-α-methylhydrocinnamic acids have been synthesized according to the design concept of combination of known structure of PPARγ agonist and 11β-HSD1 inhibitor shown in Figure 1. The compounds were synthesized via key intermediate



Scheme 1. Synthesis of the compounds. Reagents and conditions: (a) K_2CO_3, acetone; (b) LDA, THF, −78 °C, then *p*-benzoloxy benzaldehyde; (c) BF_3–Et_2O, $(C_2H_5)_3SiH$, CH_2Cl_2, 0 °C; (d) Pt/H_2, ethanol; (e) Cs_2CO_3, DMF, 4,6-dichloro-pyrimidin-2-ylamine, 80 °C; (f) Cs_2CO_3, DMF, 1-R_1-piperazine, 80 °C; (g) KOH, THF/CH_3OH/H_2O = 3:1:1, 25 °C.

8, which was prepared from *p*-benzoloxy benzaldehyde and α-phenoxy ester as previously reported.[16] Coupling with the 2-amino-4,6-dichloropyrimidine using Cs_2CO_3 in DMF and treating with phenyl(piperazin-1-yl)methanone in the same condition followed by hydrolysis to afford compound **2** and the substitutions **11a–n** (Scheme 1).

3. Results

3.1. In vitro activities

The potency of the α-aryloxy-α-methylhydrocinnamic acids on the activation of human PPARγ and inhibition of mouse 11β-HSD1 was determined using the transactivation assay[17] and scintillation proximity assay.[18] The results were shown in Table 1. The initial compound **2** only exhibited moderate inhibitory activities against 11β-HSD1 and lost the PPARγ transactivation activity. However, some compounds (**11d–i, 11l–m**) optimized from compound **2** by change of the benzyl group of lipophilic tail showed dual effects on the activation of PPARγ and inhibition of 11β-HSD1. The result indicates that increasing the hydrophobicity of the lipophilic tail may improve the activities of PPARγ agonist and 11β-HSD1 inhibition synchronously.

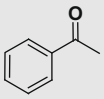
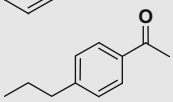
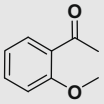
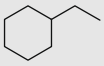
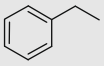
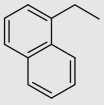
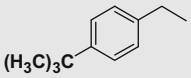
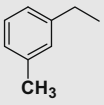
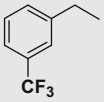
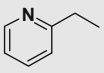
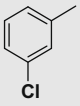
We further evaluated the adipogenic potency of those dual activate compounds on 3T3-L1 cells. 3T3-L1 preadipocytes were grown to differentiate in the absence or presence of 0.1 or 1 μM of different compounds. Rosiglitazone was used as positive control. The triglyceride content of the cells was assessed as an index of adipogensis (Table 2). As expected, 0.1 and 1 μM rosiglitzone significantly increased the triglyceride content by 22.1% and 34.1% after 5 days treatment compared with vehicle control which suggested the strong adipogenic potency of PPARγ agonist. However, almost all of the tested α-aryloxy-α-methylhydrocinnamic acids did not increase the triglyceride content of the cells, except that compound **11i** showed a weak enhancement of triglyceride content at the dose of 1 μM. It is suggested that those dual active α-aryloxy-α-methylhydrocinnamic acids had no obvious adipogenic effect. Among them compound **11e** was chosen for further evaluation.

Another important issue is the mode of inhibition of **11e** to 11β-HSD1.As shown in Figure 2A, compound **11e** inhibited 11β-HSD1 reductase activity in a dose-dependent fashion with an apparent Ki of 1 μM. Figure 2B shows the results of a representative experiment in which the effects of **11e** on the kinetic characteristics of 11β-HSD1 reductase were examined. The two Lineweaver–Burk plots[19] demonstrate that the two lines, one in the presence of **11e** and the other in its absence, intercept on the *y*-axis but diverge on the *x*-axis. This reveals that the K_m but not V_{max} is altered by **11e**, indicating that **11e** is a competitive inhibitor of l lβ-HSD1 reductase.

3.2. Molecular modeling of a typical compound with PPARγ and 11β-HSD1

To gain insight into the interaction between the designed compounds and targets, the 3D binding models of compound **11e** to PPARγ were generated based on the docking simulation. As shown in Figure 3, the binding mode for compound **11e** is in good agreement with the agonist GI262570 in the crystal structure, especially the carboxyl head group. There is a strong electric field within the space where the head group locates, which forms strong electrostatic interactions to fix the head group of the agonists. More specifically, it forms hydrogen bonds with Ser289, His323, Tyr449, and Tyr473. On the other hand, respectable hydrophobic interactions have been found between the tail of agonist and residues in the

Table 1
In vitro PPARγ activity and 11β-HSD1 inhibition of α-aryloxy-α-methylhydrocinnamic acids

Compound	R_1	PPAR γ EC_{50}[a] (μM)	11β-HSD1 IC_{50}[a] (μM)
2		⩾50	3.29 ± 0.13
11a		⩾50	0.49 ± 0.02
11b		⩾50	⩾10
11c		⩾50	⩾10
11d		5.54 ± 1.60	1.32 ± 0.05
11e		6.62 ± 0.41	0.71 ± 0.23
11f		3.50 ± 2.18	2.34 ± 0.38
11g		6.36 ± 0.17	0.85 ± 0.07
11h		4.46±2.48	2.37 ± 0.08
11i		4.79 ± 0.76	1.04 ± 0.15
11j		⩾50	⩾10
11k		5.01 ± 1.02	⩾10
11l		4.94 ± 0.07	1.92 ± 0.24
11m		4.47 ± 1.46	1.52 ± 0.22
11n		6.13 ± 0.44	⩾10
Rosiglitazone		0.05	
Glycyrrhizic acid			0.015 ± 0.002

[a] Data represent the mean values from at least three independent experiments.

Table 2
Increasing of TG/protein concentration after treated with test compound by fold of control (%, mean ± SE)

Compounds	Concentration (μmol/L)	
	0.1	1.0
Rosiglitazone	22.05 ± 4.20	34.10 ± 3.48
11d	−30.21 ± 1.73	−15.43 ± 12.12
11e	−14.98 ± 7.06	−20.50 ± 4.36
11f	0.45 ± 7.59	4.47 ± 4.87
11g	−1.99 ± 3.93	−1.15 ± 5.17
11i	−0.87 ± 0.34	18.41 ± 3.82
11h	−1.04 ± 2.51	−2.33 ± 2.18
11l	−40.18 ± 8.95	−5.99 ± 7.01
11m	−19.32 ± 6.11	−37.99 ± 9.60

pocket such as Ile326, Leu330, Tyr327, Leu465, Phe363, Leu469, and Glu286. Conclusively, it can be assumed that this series of analogues interact with PPARγ through a network of H-bonds involved the head acid group and numerous hydrophobic interactions by the tail. Similarly, the interaction model of compound **11e** with 11β-HSD1 was also generated, as shown in Figure 3. The headgroup of this compound points outward whereas its tail binds deep inside the pocket. The two nitrogen atoms in the pyrimidine ring and piperazine ring form two important H-bonds with Ser170 (Fig. 3), which are also found to be essential to the interaction between 11β-HSD1 and natural inhibitor CBO (CARBENOXOLONE) and between 11β-HSD1 and synthetic inhibitor NN1([(1R,2S,3S, 5S,7S)-5-(METHYLSULFONYL)-2- ADAMANTYL]PROPANAMIDE).[20] Additionally, the head group also forms several H-bonds with Tyr177 and Thr265 (Fig. 3). So it was noteworthy that the results of our docking studies were in good agreement with the experimental data, and it was also confirmed the acid head group can be adopted as a common unit by both PPARγ receptor and 11β-HSD1 enzyme.

3.3. Pharmacokinetic

Compound **11e** has an excellent ADME profile (Table 3). The orally administered **11e** was found to be rapidly absorbed from gastrointestinal tract. The mean peak concentration (C_{max}) was 3.79 μg mL^{-1} achieved at 1.0 h after oral administration. It has very good oral bioavailability of 100% in rats. The elimination half-lives ($T_{1/2}$) of **11e** by oral and intravenous administration were 6.3 and 9.0 h respectively, while the clearance (CL) was 2.8 and 3.3 mL min^{-1} kg^{-1} respectively.

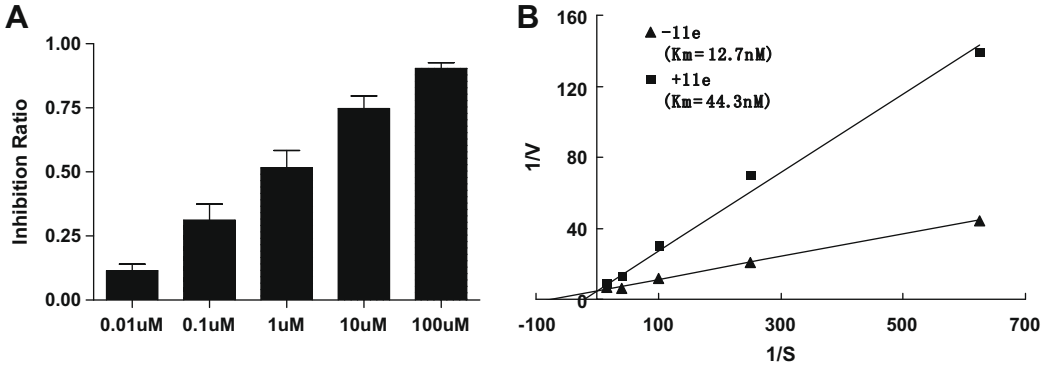


Figure 2. Effect of **11e** on the kinetic characteristics of 11β-HSD1 reductase. (A) Inhibition effect of **11e** on 11β-HSD1 reductase with different concentration. (B) The 11β-HSD1 reductase activity in mice liver microsomes was determined with increasing concentrations of cortisone (1.6–62.5 nM) in the absence and presence of 1 μM **11e**. Shown here is a representative Lineweaver–Burk double reciprocal plot, demonstrating that **11e** is a competitive inhibitor.

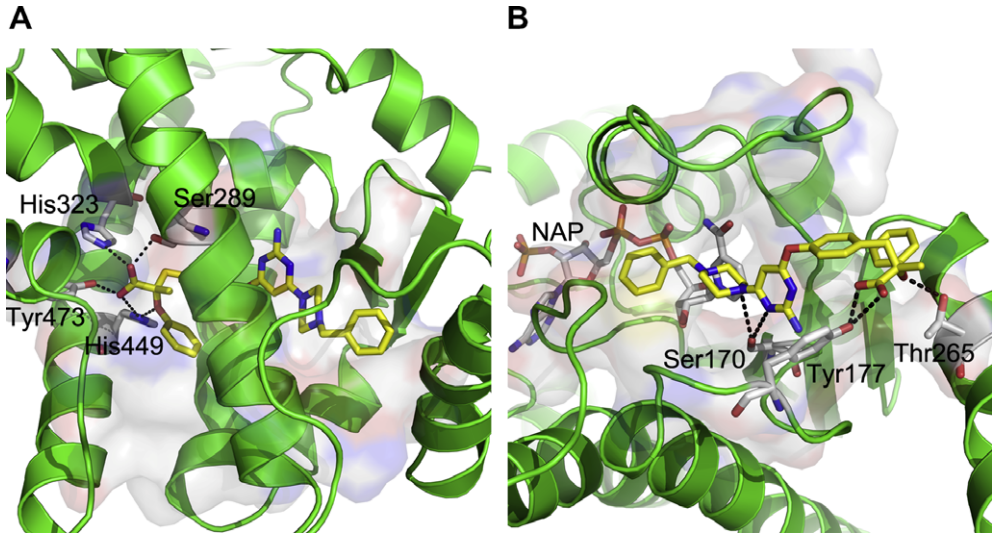


Figure 3. The binding models of the compound **11e** with PPARγ (A) and 11β-HSD1 (B) derived from the docking simulations. The residues which form H-bonds with the compound **11e** are shown in sticks. The binding pocket is represented by watermarked surface. Both images were generated using the PYMOL program (http://www.pymol.org/).

Table 3
Pharmacokinetic profile of compound **11e** in Wistar rats[a]

Pharmacokinetic parameter	Oral	Intra-arterial
Dose dose (mg kg^{-1})	10.0	10.0
AUC_{∞} (μg h mL^{-1})	5.92 ± 0.47	5.15 ± 1.63
C_{max} (μg mL^{-1})	3.79 ± 2.67	
T_{max} (h)	1.0 ± 0.4	
$T_{1/2}$ (h)	6.3 ± 2.7	9.0 ± 2.1
CL (mL min^{-1} kg^{-1})	2.8 ± 0.2	3.3 ± 1.2
V_{ss} (L kg^{-1})		0.8 ± 0.2
V_z (L kg^{-1})	1.6 ± 0.7	2.5 ± 0.7
F (%)	100	

[a] $n = 4$. See Section 6 for details.

3.4. Hypoglycemic and hypolipidemic effects of compound 11e in KKAy mice and MSG rats

KKAy mice are characterized by severe insulin resistance and hyperglycemia which has been widely used as a diabetic mice model, whereas MSG rats are a well established model of obesity induced by damage of feeding center. Here, we evaluated the effects of **11e**, a compound with the best potency in the activation of PPARγ and inhibition of 11βHSD-1 in vitro, on the glucose and lipid metabolism on those two animal models. In KKAy mice, 4 weeks oral administration with 20 mg/kg **11e** reduced blood glucose by 34% compared with that of vehicle control mice, while 10 mg/kg rosiglitazone treatment decreased blood glucose level by 39%. This suggested the hypoglycemic effect of **11e** at a dose of 20 mg/kg was comparable with that of rosiglitazone at the dose of 10 mg/kg in KKAy mice (Fig. 4A). Similarly, 10 mg/kg of compound **11e** showed same hypoglycemic effect with 5 mg/kg of rosiglitazone in MSG rats (Fig. 4B). Moreover, 6 weeks treatment with compound **11e** reduced serum triglyceride level by 39.5% and 21% compared with vehicle control in KKAy mice and MSG rats respectively, although the effects were not as strong as rosiglitazone (Fig. 4C and D). Therefore, compound **11e** showed potent hypoglycemic effects and reduced triglyceride level in both KKAy mice and MSG rats.

3.5. Compound 11e treatment reduced body weight and fat mass in MSG rats

To evaluate the adipogenic effect of compound **11e** in vivo, the body weight of MSG rats was measured weekly and the fat mass and liver triglyceride content were assessed after 6 weeks treatment with 10 mg/kg compound **11e** or 5 mg/kg rosiglitazone. As shown in Figure 5A, compound **11e** significantly decreased the body weight of MSG rats from the third week of the treatment ($P < 0.05$), whereas rosiglitazone showed no effect on the body weight compared with that of controls. As expected, rosiglitazone treatment significantly increased subcutaneous (12.4% vs control rats) and visceral fat weight (16.0% vs control rats), whereas compound **11e** treatment showed opposite effects (Fig. 5B and C). Moreover, compound **11e** treatment significantly decreased the total fat mass (12.5% vs control rats), whereas rosiglitazone showed a tendency to increase the total fat mass of the MSG rats (Fig. 5D). Furthermore, compound **11e** treatment reduced the triglyceride content in liver of MSG rats, whereas rosiglitazone did not show any effect (Fig. 5E).

4. Discussion

Due to the promising therapeutic effect on metabolic syndrome and type 2 diabetes, numerous novel compounds had been developed as the PPARγ agonists or 11β-HSD1 inhibitors.[21] However, to our knowledge, there is no report so far that a compound can act as both PPARγ agonist and 11β-HSD1 inhibitor, which might exhibit better efficacy and avoid the side effect of weight gaining caused by activation of PPARγ.

To find such dual functional agents, the concept of structural combination of PPARγ agonist and 11β-HSD1 inhibitor was explored. At first, the 3-(4-hydroxyphenyl)-2-methyl-2-phenoxypropanoic acid and the phenoxyalky linker were considered as the 'conserved unit' of classic PPARγ agonists[16] and the 'main part' of compound **1** has been reported by Amgen as a potency 11β-HSD1 inhibitor.[22] In medicinal chemistry, bioisosteric replacement

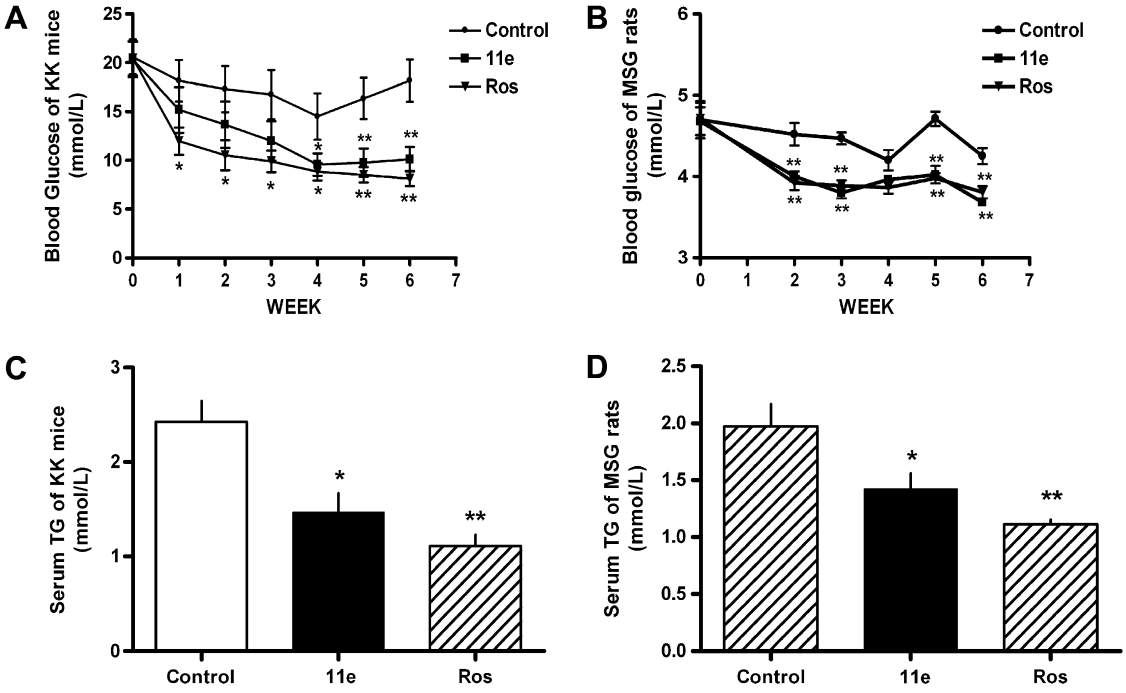


Figure 4. Effects of tested compound on blood glucose and serum lipid levels in KKAy mice and MSG rats. (A) Blood glucose level of KKAy mice after 6 weeks' treatment. (B) Blood glucose level of MSG rats after 6 weeks' treatment. (C) Serum TG levels of KKAy mice after 6 weeks' treatment. (D) Serum TG levels of MSG rats after 6 weeks' treatment.

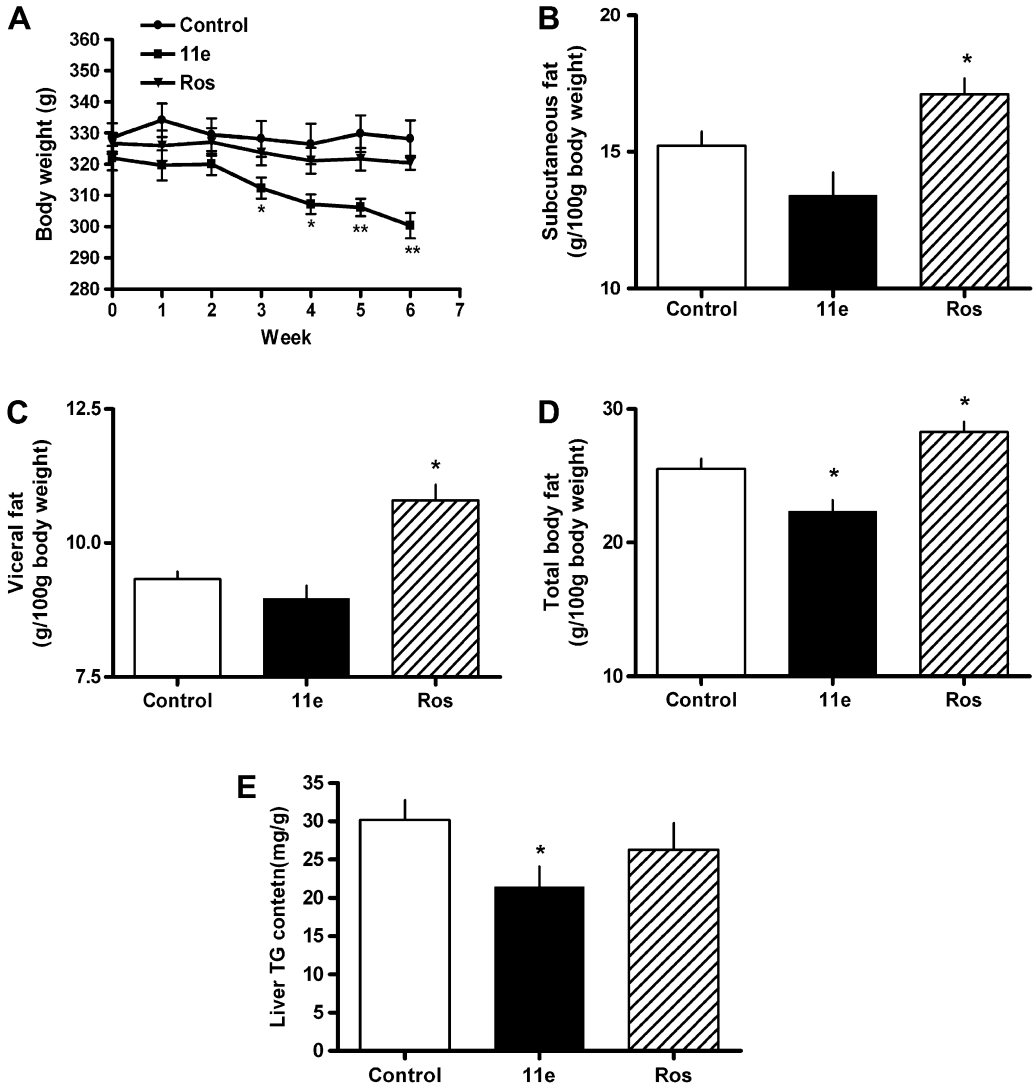


Figure 5. Decreased body weight and fat mass in MSG rats. (A) Body weight observation during treatment. (B) Subcutaneous fat mass after 6 weeks' treating. (C) Viceral fat mass after 6 weeks' treating. (D) Total body fat after 6 weeks' treating. (E) Liver TG content after 6 weeks' treating.

is a frequently used strategy in the lead optimization in order to enhance the desired biological or physical properties of a compound without making significant changes in chemical structure. Generally, amide is one of the bioisosteric replacements for sulfonamide.[23] Similarly, pyrimidine ring is used as a pyridine bioisostere, where a hydrogen bond with side chains in the binding pocket is probably formed. Thus we designed and synthesized the lead compound **2**. First of all, substituents on the benzoyl group were explored (**11a**, **11b**). Then the change of benzoyl group to benzyl group or phenyl group was also investigated in order to increase the hydrophobic of the lipophilic tail (**11e–j**, **11l–m**). Finally, bioisosteric replacements for phenyl group were also examined to study the probably hydrogen bond with side chains in the binding pocket (**11c**, **11j–k**, **11n**). Their in vitro activities were confirmed by transactivation or SPA assay. The compounds **11f–g** kept both the activities of PPARγ agonist and 11β-HSD1 inhibition, which was certified that the hydrophobic pocket of PPARγ appears to be large enough for the optimization.[24] Moreover, our modeling analysis showed that the acid head group of these compounds can be adopted as a common unit to form H-bond interaction with both PPARγ receptor and 11β-HSD1 enzyme. So it implied that the carefully combined structures of PPARγ agonist and 11β-HSD1 inhibitor might be a right outset to find dual functional agents.

Due to the opposite role of PPARγ agonists and 11β-HSD1 inhibitors in adipogenesis,[25] we further evaluate the effects of such dual functional compounds on the differentiation of 3T3-L1 cells. In contrast to the enhanced adipogenesis induced by rosiglitazone, almost all of the tested compounds had no obvious effect on the differentiation of 3T3-L1 preadipocytes. This supports the motion that those α-aryloxy-α-methylhydrocinnamic acids as novel dual functional agents of PPARγ agonists and 11β-HSD1 inhibitors did not promote adipogenesis in vitro.

PPARγ agonists, such as rosiglitaozne and pioglitazone enhance insulin sensitivity and improve glycemic control by binding to the PPARγ receptor in adipocytes primarily and then stimulating peripheral glucose uptake and utilization subsequently.[26] 11β-HSD1 inhibitors are also able to increase insulin sensitivity of adipose tissue, but by different action mechanisms that include reducing the local level of active glucocorticoids. Moreover, the inhibition of 11β-HSD1 in liver attenuates gluconeogenic response and increases hepatic insulin sensitivity.[27] Furthermore, the beneficial effects of PPARγ anti-diabetic agents may result, at least in part, from the down-regulation of 11β-HSD1 expression in adipose

tissues.[28] Thus, the combination of PPARγ activation and 11β-HSD1 inhibition might be a useful strategy to generate better therapeutic effect for type 2 diabetes. In the present study, a novel α-aryloxy-α-methylhydrocinnamic acid, **11e**, displayed efficacious hypoglycemic and hypolipidemic activity in both KKAy mice and MSG rats. Although the in vitro efficacy of **11e** on PPARγ activation was about 100 times lower than rosiglitazone, the hypoglycemic and hypolipidemic activity of the two compounds were comparable. Since **11e** had been demonstrated to be a dual functional agent as both of PPARγ agonists and 11β-HSD1 inhibitors, its strong anti-diabetic potency might be explained in part by the inhibition of 11β-HSD1. Thus, our data indicated that the combination of PPARγ activation and 11β-HSD1 inhibition might have a synergistic effect on the improvement of glucose and lipid metabolism in type 2 diabetes.

Although widely used as oral anti-diabetic agents, the side effects of weight gain of PPARγ agonists which in part due to inducing adipogenesis had been extensively characterized.[29] On the other hand, however, 11β-HSD1 inhibitors had been reported to prevent adipogenesis and reduce body weight.[10] Therefore, the neutralized effect on adipogenesis was expected by the combination of PPARγ activation and 11β-HSD1 inhibition. This speculation was confirmed by the results of present study that chronic treatment of **11e** significantly reduced body weight of MSG rats, whereas rosiglitazone did not have any effect. Indeed, the loss of body weight might be caused by the remarkable reduction of total fat mass in **11e** treated rats, whereas rosiglitazone significantly increased total fat mass. Moreover, a clear decrease in subcutaneous fat mass was observed upon **11e** treatment, whereas rosiglitazone had an opposite effect. Therefore, in accordance with the in vitro results, compound **11e**, a novel dual function agent as PPARγ agonist and 11β-HSD1 inhibitor showed decreased body weight and fat mass in diabetic animal models, which reflected its weak adipogenic activity in vivo.

With the in-depth understanding of the molecular basis of PPARγ action, it has been realized that the effects of PPARγ on adipogenesis and insulin sensitivity can be dissociated by inducing differential coactivator recruitment.[30] A considerable effort had been mounted in developing selective PPARγ modulators (SPPARM) that retain efficacious insulin-sensitizing and anti-diabetic action while minimizing potential side effects, such as weight gain and edema. In the past several years, a number of compounds has been reported as selective PPAR modulators with promising therapeutic utility but less weight gain in different animal models, or even in clinical.[31] However, the safer and more effective compounds based on the activation of PPARγ are still being expected. And the combination of activation of PPARγ and inhibition of 11β-HSD1 might be another useful therapeutic strategy for type 2 diabetes other than selective PPARγ modulators.

5. Conclusion

In summary, we identified a series of α-aryloxy-α-methylhydrocinnamic acids as novel dual functional agents with the activity of activation of PPARγ and inhibition of 11β-HSD1. In spite of the much lower in vitro activity on PPARγ activation, **11e**, the representative compound, exhibited comparable hypoglycemic and hypolipidemic efficacy as rosiglitaozne in different animal models. More importantly, this compound does not promote adipogenesis and decreases fat mass and body weight. Therefore, although the exact molecular basis remains to be determined, this series of α-aryloxy-α-methylhydrocinnamic acids, with both of PPARγ agonistic and 11β-HSD1 inhibitory activities, might represent a new class of molecules with novel mechanisms for the treatment of type 2 diabetes.

6. Experimental procedures

6.1. Compounds synthesis

The reagents (chemicals) were purchased from Lancaster, Acros and Shanghai Chemical Reagent Company, and used without further purification. Analytical thin-layer chromatography (TLC) was HSGF 254 (150–200 μm thickness, Yantai Huiyou Company, China). Yields were not optimized. Melting points were measured in capillary tube on a SGW X-4 melting point apparatus without correction. Nuclear magnetic resonance (NMR) spectra were given on a Brucker AMX-400 NMR (IS as TMS). Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Low- and high-resolution mass spectra (LRMS and HRMS) were given with electric, electrospray and matrix-assisted laser desorption ionization (ES, ESI) produced by Finnigan MAT-95, LCQ-DECA spectrometer and IonSpec 4.7 T.

6.2. General procedures for preparations of α-substitution phenoxy carboxylic acid 11a–i, 11l–o described as those for 2

6.2.1. 3-{4-[2-Amino-6-(4-benzoyl-piperazin-1-yl)-pyrimidin-4-yloxy]-phenyl}-2-methyl-2-phenoxy-propionic acid (2)

2-Amino-4, 6-dichloro-pyrimidine (5.46 g, 33.3 mmol), **8** (10 g, 33.3 mmol), and Cs_2CO_3 (10.8 g, 36.6 mmol) were combined in anhydrous *N*,*N*-dimethylformamide (DMF) (50 mL) and stirred at 80 °C; in dry atmosphere for 5 h. The DMF was removed in vacuo, the residue was dissolved in acetone, and **9** (12.21 g, 86.1%) was crystallized form acetone as a white solid: ^{1}H NMR δ 1.22 (t, J = 7.1 Hz, 3H), 1.40 (s, 3H), 3.07 (d, J = 13.5 Hz, 1H), 3.24 (d, J = 13.5 Hz, 1H), 4.03 (q, J = 7.1 Hz, 2H), 5.35 (s, 1H), 6.81–6.93 (m, 3H), 7.14–7.22 (m, 2H), 7.27–7.40 (m, 3H).

A mixture of compound **9** (14.23 g, 33.3 mmol), phenyl(piperazin-1-yl)methanone (6.33 g, 33.3 mmol), and Cs_2CO_3 (10.8 g, 36.6 mmol) in anhydrous *N*,*N*-dimethylformamide (DMF) (50 mL) was stirred at 80 °C; in dry atmosphere overnight. The DMF was removed in vacuo, the residue was dissolved in acetone, and the white products (14.44 g, 74.6%) was crystallized form acetone as a white solid ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.15 (t, 3H, J = 7.2 Hz), 1.35 (s, 3H), 3.12 (d, 1H, J = 13.5 Hz), 3.28 (d, 1H, J = 13.5 Hz), 3.46–3.57 (m, 8H), 4.13 (q, 2H, J = 7.2 Hz), 5.56 (s, 1H), 6.64 (d, 2H, J = 8.1 Hz), 6.90–6.98 (m, 2H), 7.22–7.30 (m, 4H), 7.41–7.60 (m, 5H), 7.95 (d, 1H, J = 7.8 Hz).

A mixture of **10** (19.1 g, 32.8 mmol) and KOH (7.58 g, 135 mmol) in THF/methanol/H_2O (3:1:1, v/v/v, 30 mL) was stirred at room temperature for 12 h. The resulting solution was acidified to pH 6 using 1 N HCl, the white product was precipitated, then filtered through Celite, to afford **2** (17.32 g, 95.5%) as a white solid: mp 117–119 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.33 (s, 3H), 3.13 (d, 1H, J = 13.5 Hz), 3.26 (d, 1H, J = 13.5 Hz), 3.48–3.55 (m, 8H), 5.51 (s, 1H), 6.65 (d, 2H, J = 8.1 Hz), 6.92–6.99 (m, 2H), 7.21–7.26 (m, 4H), 7.42–7.59 (m, 5H), 7.93 (d, 1H, J = 7.8 Hz); ESI-MS: m/e 554 $(M+H)^+$; HRMS (ESI) m/z calcd $C_{31}H_{31}N_5O_5$ $(M+H)^+$ 554.2403, found 554.2411.

6.2.2. 3-{4-{2-Amino-6-[4-(4-propyl-benzoyl)-piperazin-1-yl]-pyrimidin-4-yloxy}-phenyl}-2-methyl-2-phenoxy-propionic acid (11a)

This compound was prepared from **9** and piperazin-1-yl(4-propylphenyl)methanone by means of a procedure similar to that used for **2**, yield 93.4%: mp 101–102 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.88 (t, 3H, J = 7.5 Hz), 1.33 (s, 3H), 1.60 (q, 2H, J = 7.5 Hz), 2.59 (t, 2H, J = 7.5 Hz), 3.17 (d, 1H, J = 13.5 Hz), 3.35 (d, 1H, J = 13.5 Hz),

3.48–3.55 (m, 8H), 5.50 (s, 1H), 6.83 (d, 2H, J = 8.1 Hz), 6.96–7.00 (m, 3H), 7.23–7.36 (m, 7H), 8.35 (d, 1H, J = 7.8 Hz); ESI-MS: m/e 596 $(M-H)^-$; HRMS (ESI) m/z calcd $C_{34}H_{37}N_5O_5$ $(M-H)^-$ 596.2873, found 596.2854.

6.2.3. 3-{4-{2-Amino-6-[4-(2-methoxy-benzoyl)-piperazin-1-yl]-pyrimidin-4-yloxy}-phenyl}-2-methyl-2-phenoxy-propionic acid (11b)

This compound was prepared from **9** and (3-methoxyphenyl)(piperazin-1-yl)methanone by means of a procedure similar to that used for **2**, yield 92.5%: mp 108–109 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.32 (s, 3H), 3.17 (d, 1H, J = 13.5 Hz), 3.35 (d, 1H, J = 13.5 Hz), 3.62 (m, 8H), 3.80 (s, 3H), 5.50 (s, 1H), 6.83 (d, 2H, J = 7.8 Hz), 6.93–7.04 (m, 6H), 7.21–7.25 (m, 4H), 7.37 (m, 1H); ESI-MS: m/e 584 $(M-H)^-$; HRMS (ESI) m/z calcd $C_{34}H_{37}N_5O_5$ $(M-H)^-$ 584.2509, found 584.2487.

6.2.4. 3-(4-(2-Amino-6-(4-(furan-2-carbonyl)piperazin-1-yl)pyrimidin-4-yloxy)phenyl)-2-methyl-2-phenoxypropanoic acid (11c)

This compound was prepared from **9** and furan-2-yl(piperazin-1-yl)methanone by means of a procedure similar to that used for **2**, yield 94.2%: mp 166–168 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 1.36 (s, 3H), 3.08 (m, 4H), 3.19 (d, 1H, J = 13.5 Hz), 3.35 (d, 1H, J = 13.5 Hz), 3.65 (m, 4H), 5.38 (s, 1H), 6.16 (d, 1H, J = 8.7 Hz), 6.83–7.11 (m, 6H), 7.18 (m, 2H), 7.37 (m, 2H), 7.66 (d, 1H, J = 7.8 Hz); ESI-MS: m/e 544 $(M+H)^-$; HRMS (ESI) m/z calcd $C_{29}H_{30}N_5O_6$ $(M-H)^-$ 544.2196, found 544.2209.

6.2.5. 3-{4-[2-Amino-6-(4-cyclohexylmethyl-piperazin-1-yl)-pyrimidin-4-yloxy]-phenyl}-2-methyl-2-phenoxy-propionic acid (11d)

This compound was prepared from **9** and 1-(cyclohexylmethyl)piperazine by means of a procedure similar to that used for **2**, yield 81.9%: mp 180–182 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 0.94 (m, 2H), 1.27–1.3 (m, 4H), 1.35 (s, 3H), 1.69–1.81 (m, 4H), 1.95 (s, 1H), 2.30 (d, J = 6.9 Hz, 2H), 2.56 (t, J = 6.0 Hz, 4H), 3.13 (d, J = 13.8 Hz, 1H), 3.39 (d, J = 13.8 Hz, 1H), 3.52 (t, J = 6.0 Hz, 4H), 5.27 (s, 1H), 6.89–6.94 (m, 3H), 6.98 (d, J = 8.7 Hz, 2H), 7.19 (t, J = 7.2 Hz, 2H), 7.33 (d, J = 8.7 Hz, 2H); ESI-MS: m/e 544 $(M-H)^-$; HRMS (ESI) m/z calcd $C_{31}H_{39}N_5O_4$ $(M-H)^-$ 544.2924, found 544.2911.

6.2.6. 3-{4-[2-Amino-6-(4-benzyl-piperazin-1-yl)-pyrimidin-4-yloxy]-phenyl}-2-methyl-2-phenoxy-propionic acid (11e)

This compound was prepared from **9** and 1-benzylpiperazine by means of a procedure similar to that used for **2**, mp 173–175 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 1.40 (s, 3H), 3.03 (t, J = 5.1 Hz, 4H), 3.18 (d, J = 13.2 Hz, 1H), 3.38 (d, J = 13.2 Hz, 1H), 3.69 (t, J = 5.1 Hz, 4H), 4.09 (s, 2H), 5.35 (s, 1H), 6.88–7.06 (m, 5H), 7.20–7.37 (m, 4H), 7.46–7.55 (m, 5H); ESI-MS: m/e 538 $(M-H)^-$; HRMS (ESI) m/z calcd $C_{31}H_{33}N_5O_4$ $(M-H)^-$ 538.2454, found 538.2459. Anal. Calcd for $C_{31}H_{33}N_5O_4$: C, 69.00; H, 6.16; N, 12.98. Found: C, 68.82; H, 6.05; N, 13.12.

6.2.7. 3-{4-[2-Amino-6-(4-(naphthalen-1-ylmethyl)-piperazin-1-yl)-pyrimidin-4-yloxy]-phenyl}-2-methyl-2-phenoxy-propionic acid (11f)

This compound was prepared from **9** and 1-(naphthalen-1-ylmethyl)piperazine by means of a procedure similar to that used for **2**, yield 89.5%: mp 93–94 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 1.34 (s, 3H), 2.50 (t, J = 5.1 Hz, 4H), 3.09 (d, J = 13.5 Hz, 1H), 3.21 (d, J = 13.5 Hz, 1H), 3.27 (t, J = 5.1 Hz, 4H), 3.93 (s, 2H), 5.47 (s, 1H), 6.82 (d, J = 8.1 Hz, 1H), 6.95–7.01 (m, 2H), 7.23–7.28 (m, 3H), 7.46–7.58 (m, 6H), 7.87–7.96 (m, 3H), 8.28 (d, J = 7.5 Hz, 1H); ESI-MS: m/e 588 $(M-H)^-$; HRMS (ESI) m/z calcd $C_{35}H_{35}N_5O_4$ $(M-H)^-$ 588.2611, found 588.2638.

6.2.8. 3-{4-{2-Amino-6-[4-(4-*tert*-butyl-benzyl)-piperazin-1-yl]-pyrimidin-4-yloxy}-phenyl}-2-methyl-2-phenoxy-propionic acid (11g)

This compound was prepared from **9** and 1-(4-*tert*-butylbenzyl)piperazine by means of a procedure similar to that used for **2**, yield 82.5%: mp 96–97 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.26 (s, 9H), 1.33 (s, 3H), 2.36 (t, J = 5.1 Hz, 4H), 3.18 (m, 2H), 3.26 (s, 2H), 3.44 (t, J = 5.1 Hz, 4H), 5.45 (s, 1H), 6.83 (d, J = 8.4 Hz, 2H), 6.93–6.99 (m, 3H), 7.21 (m, 5H), 7.35 (m, 3H); ESI-MS: m/e 594 $(M-H)^-$; HRMS (ESI) m/z calcd $C_{35}H_{41}N_5O_4$ $(M-H)^-$ 594.3080, found 594.3121.

6.2.9. 3-{4-{2-Amino-6-[4-(3-methyl-benzyl)-piperazin-1-yl]-pyrimidin-4-yloxy}-phenyl}-2-methyl-2-phenoxy-propionic acid (11h)

This compound was prepared from **9** and 1-(3-methylbenzyl)piperazine by means of a procedure similar to that used for **2**, yield 87.1%: mp 115–116 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 1.38 (s, 3H), 2.34 (s, 3H), 2.50 (t, J = 5.1 Hz, 4H), 3.17 (d, J = 13.5 Hz, 1H), 3.35 (d, J = 13.5 Hz, 1H), 3.60 (t, J = 5.1 Hz, 4H), 3.94 (s, 2H), 5.32 (s, 1H), 6.9 (m, 4H), 7.00 (d, J = 8.4 Hz, 2H), 7.16–7.25 (m, 5H), 7.33 (d, J = 8.4 Hz, 2H); EI-MS: m/e 553 (M^+); HRMS (EI) m/z calcd $C_{32}H_{35}N_5O_4$ 553.2689, found 553.2670.

6.2.10. 3-{4-{2-Amino-6-[4-(3-trifluoromethyl-benzyl)-piperazin-1-yl]-pyrimidin-4-yloxy}-phenyl}-2-methyl-2-phenoxy-propionic acid (11i)

This compound was prepared from **9** and 1-(3-(trifluoromethyl)benzyl)piperazine by means of a procedure similar to that used for **2**, yield 86.3%: mp 97–98 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 1.38 (s, 3H), 2.53 (t, J = 5.1 Hz,4H), 3.17(d, J = 13.5 Hz, 1H), 3.35 (d, J = 13.5 Hz, 1H), 3.53 (t, J = 5.1 Hz, 4H), 3.70 (s, 2H), 5.25 (s, 1H), 6.9 (m, 3H), 7.00 (d, J = 8.4 Hz, 2H), 7.18 (t, J = 7.8 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 7.55–7.69 (m, 4H); ESI-MS: m/e 606 $(M-H)^-$; HRMS (ESI) m/z calcd $C_{32}H_{32}F_3N_5O_4$ $(M-H)^-$ 606.2328, found 606.2384.

6.2.11. 3-{4-[2-Amino-6-(4-pyridin-2-ylmethyl-piperazin-1-yl)-pyrimidin-4-yloxy]-phenyl}-2-methyl-2-phenoxy-propionic acid (11j)

This compound was prepared from **9** and 1-(pyridin-2-ylmethyl)piperazine by means of a procedure similar to that used for **2**, yield 93.2%: mp 138–139 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 1.38 (s, 3H), 2.61 (t, J = 5.1 Hz, 4H), 3.15 (d, J = 13.5 Hz, 1H), 3.37 (d, J = 13.5 Hz, 1H), 3.56 (t, J = 5.1 Hz, 4H), 3.71 (s, 2H), 5.22 (s, 1H), 6.68–6.94 (m, 3H), 7.00 (d, J = 8.4 Hz, 2H), 7.19 (t, J = 8.1 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 7.37 (s, 1H), 7.54 (d, J = 8.1 Hz, 1H), 7.84 (td, J = 8.1 and 1.5 Hz, 1H), 8.51(d, J = 4.8 Hz, 1H); ESI-MS: m/e 539 $(M-H)^-$; HRMS (ESI) m/z calcd $C_{30}H_{32}N_6O_4$ $(M-H)^-$ 539.2407, found 539.2422.

6.2.12. 3-{4-[2-Amino-6-(4-furan-2-ylmethyl-piperazin-1-yl)-pyrimidin-4-yloxy]-phenyl}-2-methyl-2-phenoxy-propionic acid (11k)

This compound was prepared from **9** and 1-(furan-2-ylmethyl)piperazine by means of a procedure similar to that used for **2**, yield 82.2%: mp 142–143 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 1.38 (s, 3H), 2.59 (t, J = 5.1 Hz, 4H), 3.14 (d, J = 13.8 Hz, 1H), 3.37 (d, J = 13.8 Hz, 1H), 3.54 (t, J = 5.1 Hz, 4H), 3.71 (s, 2H), 5.26 (s, 1H), 6.38 (d, J = 6.3 Hz, 2H), 6.78 (t, J = 5.7 Hz, 2H), 6.96 (s, 1H), 7.02 (d, J = 8.7 Hz, 2H), 7.21 (t, J = 6.3 Hz, 3H), 7.33 (d, J = 8.7 Hz, 2H), 7.49(s, 1H); ESI-MS: m/e 530 $(M+H)^+$; HRMS (ESI) m/z calcd $C_{29}H_{31}N_5O_5$ $(M+H)^+$ 530.2403, found 530.2377.

6.2.13. 3-{4-[2-Amino-6-(4-phenyl-piperazin-1-yl)-pyrimidin-4-yloxy]-phenyl}-2-methyl-2-phenoxy-propionic acid (11l)

This compound was prepared from **9** and 1-phenylpiperazine by means of a procedure similar to that used for **2**, yield 87.2%: mp 150–151 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 1.36 (s, 3H), 3.10–3.19 (m, 5H), 3.37 (d, J = 13.5 Hz, 1H), 3.54 (t, J = 5.1 Hz, 4H), 5.29 (s, 1H), 6.83 (t, J = 7.2 Hz, 1H), 6.89–6.99 (m, 5H), 7.02 (d, J = 8.7 Hz, 2H), 7.21 (q, J = 7.8 Hz, 4H), 7.34 (d, J = 8.1 Hz, 2H); ESI-MS: m/e 524 $(M-H)^-$; HRMS (ESI) m/z calcd $C_{30}H_{31}N_5O_4$ $(M-H)^-$ 524.2298, found 524.2300.

6.2.14. 3-{4-{2-Amino-6-[4-(3-chloro-phenyl)-piperazin-1-yl]-pyrimidin-4-yloxy}-phenyl}-2-methyl-2-phenoxy-propionic acid (11m)

This compound was prepared from **9** and 1-(3-chlorophenyl)piperazine by means of a procedure similar to that used for **2**, yield 82.5%: mp 212–214 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.35 (s, 3H), 3.15 (d, J = 13.5 Hz, 1H), 3.20–3.35(m, 5H), 3.62 (t, J = 5.1 Hz, 4H), 5.53 (s, 1H), 6.83–6.87 (m, 2H), 6.92 (m, 1H), 6.95–7.03 (m, 4H), 7.24–7.29 (m, 5H); ESI-MS: m/e 558 $(M-H)^-$; HRMS (ESI) m/z calcd $C_{30}H_{30}ClN_5O_4$ $(M-H)^-$ 558.1908, found 558.1925.

6.2.15. 3-{4-[2-Amino-6-(4-pyrimidin-2-yl-piperazin-1-yl)-pyrimidin-4-yloxy]-phenyl}-2-methyl-2-phenoxy-propionic acid (11n)

This compound was prepared from **9** and 2-(piperazin-1-yl)pyrimidine by means of a procedure similar to that used for **2**, yield 77.4%: mp 136–137 °C; ^{1}H NMR (300 MHz, CD_3OD): δ 1.40 (s, 3H), 3.10 (d, J = 13.5 Hz, 1H), 3.38 (d, J = 13.5 Hz, 1H), 3.25 (q, J = 13.5 Hz, 2H), 3.60 (t, J = 5.1 Hz, 4H), 3.81 (t, J = 5.1 Hz, 4H), 5.35 (s, 1H), 6.60 (t, J = 4.8 Hz, 1H), 6.90 (d, J = 7.5 Hz, 2H), 6.96 (t, J = 6.6 Hz, 1H), 7.04 (m, 2H), 7.24 (t, J = 7.5 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 8.33 (d, J = 4.8 Hz, 2H); EI-MS: m/e 527 (M^+); HRMS (EI) m/z calcd $C_{32}H_{35}N_5O_4$ 527.2281, found 527.2288.

6.3. Biological evaluation

6.3.1. Transactivation assay for PPAR activators

cDNAs for Human RXR, PPAR were obtained by RTPCR from the human liver or adipose tissues. Amplified cDNAs were cloned into pcDNA3.1 expression vector and the inserts were confirmed by sequencing. U2OS cells were cultured in McCoy's 5A with 10% heat-inactivated fetal bovine serum in a humidified 5% CO_2 atmosphere at 37 °C. Cells were seeded in 96-well plates the day before transfection to give a confluence of 50–80% at transfection. A total of 60 ng of DNA containing 10 ng of hRXR, 10 ng of pCMV Gal, 10 ng of nuclear receptor expression vectors and 30 ng of the corresponding reporters were cotransfected per well using FuGene6 transfection reagent according to the manufacturer's instructions. 24 h after transfection, cells were incubated with 10% charcoal-stripped FBS DMEM and were treated with the individual compound dissolved in DMSO. The final concentration of DMSO in culture medium was 0.1%.

Cells were treated with compound for 24 h, and then collected with Cell Culture Lysis buffer. Luciferase activity was monitored using the luciferase assay kit according to the manufacturer's instructions. Light emission was read in a Labsystems Ascent Fluoroskan reader. To measure galactosidase activity to normalize the luciferase data, 50 L of supernatant from each transfection lysate was transferred to a new microplate. Galactosidase assays were performed in the microwell plates using a kit from Promega and read in a microplate reader. The agonist rates were calculated according to the activation data in 10 M compounds, and the 50% effective concentration (EC_{50}) values were estimated by fitting the activation data to a dose-dependent curve using a logistic derivative equation.

6.3.2. Scintillation proximity assay (SPA) for identification of 11β-HSD1 inhibitors

Inhibition of compounds on mouse 11β-HSD1 enzymatic activities was determined by the scintillation proximity assay (SPA) using microsomes containing 11β-HSD1 according to previous studies.[18] Briefly, the full-length cDNAs of murine 11β-HSD1 were isolated from the cDNA libraries provided by NIH Mammalian Gene Collection and cloned into pcDNA3 expression vector. HEK-293 cells were transfected with the pcDNA3-derived expression plasmid and selected by cultivation in the presence of 700 μg/ml of G418. The microsomal fraction overexpressing 11β-HSD1 was prepared from the HEK-293 cells stable transfected with 11β-HSD1 and used as the enzyme source for SPA. Microsomes containing mouse 11β-HSD1 was incubated with NADPH and [^{3}H]cortisone (Amersham), then the product, [^{3}H]cortisol was specifically captured by a monoclonal antibody coupled to protein A-coated SPA beads (GE). IC_{50} values were calculated by using Prism Version 4 (GRAPHPAD Software, San Diego, CA).

6.3.3. Measurement of differentiation of 3T3-L1 cells

3T3-L1 fibroblasts (American Type Culture Collection) were cultured and induced to differentiate as described before (Christina M, 1993). In short, cells were maintained in Dulbecco's modified Eagles medium (DMEM, Gibco) containing 10% new born bovine serum (Gibco), 100 U/ml penicillin (Gibco), and 100 μg/ml streptomycin (Gibco) in a 10 cm dish (Gorning) at 37 °C in a humidified 95% air, 5% CO_2, atmosphere. Preadipocytes ($0.5v\times 10^4$ cells/well) were cultured to confluency in 24-well plates (corning) for 3 days, then adipocyte differentiation was initiated by treating confluent preadipocytes with 1 μM dexamethasone (Sigma, St. Louis, MO), 0.5 mM isobutylmethylxanthine (IBMX, Sigma) 1.0 μg/ml insulin (Novlin) and test compounds or rosiglitazone in DMEM containing 5% NBS. The medium was changed every 2 days and the differentiation lasted for 5 days. The cells were homogenized by ultrasonic, triglyceride and protein concentrations were measured.

6.3.4. Pharmacokinetic studies

Pharmacokinetic animal experiments were conducted according to protocols approved by the Review Committee of Animal Care and Use at the Shanghai Institute of Materia Medica (Shanghai China).[32] Compound **11e** was administered to Wistar rats (n = 4) by oral gavage and by intravenous injection via the penis vein (n = 4) at a dose of 10 mg/kg (50 mg Compound **11e** was transferred to 25 ml volumetric flask, added 11 mL PEG, 1.25 mL DMSO and 2.5 mL 120 mM NaOH, dilute with water to volume, mix well). Blood samples were centrifuged for cell removal, and precisely 25 μL of plasma supernatant was then transferred to a clean vial, place on dry ice, and subsequently stored in a −70 °C freezer prior to analysis. Plasma samples were prepared as follows. Fifty microliters of acetonitrile and 25 μm of methanol–water (50:50 v/v) was added to 25 μL of plasma to precipitate proteins. Samples were centrifuged at 5000g for 5 min, and supernatant was removed for analysis by high-performance liquid chromatography spectrometric analysis (LC/MS/MS). The LC–MS/MS system consisted of a Thermo Finnigan TSQ Quantum triple-quadrupole mass spectrometer (Thermo Finnigan, San Jose, CA, USA) interfaced via an electrospray ionization probe with a Surveyor series liquid chromatography system, an MS pump and an autosampler (Thermo Finnigan). The XCALIBUR software (Version 1.4) was used to control the LC–MS/MS system, as well as for data acquisition and processing (Thermo Finnigan, San Jose, CA, USA). Chromatographic separations were achieved on a 5-μm Zorbax XDB-C18 column

(4.6 mm × 150 mm I.D; Agilent Technologies, Chadds Ford, PA, USA) maintained at 25 °C. The LC mobile phase was CH_3CN/H_2O/HCOOH (80:20:0.5, v/v) at a flow rate of 0.5 mL/min for an isocratic elution. The mass spectrometer was operated in the positive ion electrospray ionization and multiple-reaction monitoring modes for **11e**. The instrument parameters were optimized for the analyte to maximize generation of the protonated molecules and to efficiently produce the characteristic fragment ions. The precursor-to-product ion transitions *m/z* 540→418 for **11e** were monitored with a scan time of 0.3 s per transition. The pharmacokinetic parameters are summarized in Table 3.

6.3.5. In vivo KKAy mice studies

KKA**y** mice were purchased from Shanghai SLAC Laboratory Animal CO. LTD (Shanghai, China) and maintained under a 12:12 light/dark cycle with free access to water and food. The mice were randomized to different treatment groups according to the experiment designation by body weight and fed glucose levels ($n = 10$). Compound **11e** in 0.25% CMC-Na (20 mg/kg), rosiglitazone in 0.25% CMC-Na (10 mg/kg) or vehicle were administrated orally for 6 weeks. Blood glucose levels were measured twice a week. At the end of the experiment, mice were fasted for 4 h before sacrifice and then blood samples were obtained to measure glucose, TG, and other serum biochemical indicators. All these procedures were approved by the Local Ethical Board.

6.3.6. In vivo MSG rats studies

The neonatal administration of monosodium L-glutamate (**MSG**) in rodents damages the arcuate nuclei of the hypothalamus, thus resulting in a syndrome characterized by stunted growth, hyperadiposity, and hypogonadism during adulthood. Therefore, MSG rats is a well established model of obesity. New born Wister rats were bought from Shanghai SLAC Laboratory Animal CO. LTD (Shanghai, China) and subcutaneous injected with 4 g/kg MSG since the second day of birth for 7 days, weaning on the 21 day. At the age of 11–12 months, MSG rats were randomized to three groups by body weight and serum TG levels. Rats were orally dosed with 10 mg/kg d compound **11e** or 5 mg/kg d rosiglitazone or vehicle for 6 weeks. During dosing, body weight, blood glucose levels and serum TG levels were measured once a week after 10 h fast. After sacrificed, the weight of organs, body fat, viseral and subcutaneous fat were measured blood samples were obtained to measure glucose, TG, and other serum biochemical indicators.

6.4. Molecular modeling of a typical compound with PPARγ and 11β-HSD1

The crystal structure of PPARγ and 11β-HSD1 in complexes with GI262570 (1FM9),[33] and CBO, respectively, recovered from the Brookhaven Protein Data Bank was used as the target for molecular docking. The docking calculations of compound **11e** with PPARγ were performed with AUTODOCK 3.0 program.[34] Up to 10 different docking solutions were obtained for each molecule. All docking conformations in the different receptor conformers were then ranked with AUTODOCK 3.0 scoring function. For each compound, a combination of the free energy score and visual inspection of the binding mode were applied to choose the best conformation for further analysis.

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