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Synthesis and Biological Evaluation of Novel Pentacyclic Triterpene Derivatives as Potential PPARγ Agonists

Liying Zhang[1], Jizhe Dong[2], Jun Liu[*,3], Luyong Zhang[3], Lingyi Kong[4], Hequan Yao[4] and Hongbin Sun[*,2,4]

[1]*Institute of Traditional Chinese Medicine, Chengde Medical College, Chengde 067000, People's Republic of China*

[2]*Center of Drug Discovery, College of Pharmacy, China Pharmaceutical University, Nanjing 21009, People's Republic of China*

[3]*Jiangsu Center for Drug Screening, China Pharmaceutical University, Nanjing 210038, People's Republic of China*

[4]*State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 21009, People's Republic of China*

Abstract: Synthesis and biological evaluation of a novel series of substituted pentacyclic triterpene derivatives as potential PPARγ agoinsts and glycogen phosphorylase inhibitors have been described. Compounds **11** and **17** showed potent PPARγ agonistic activity and activated the transcription activity of PPARγ in a dose-dependent manner. On the other hand, eleven compounds exhibited moderate inhibitory activity against rabbit muscle glycogen phosphorylase a (RMGPa), and triterpene **10** was the best one. Structure-activity relationship (SAR) is also discussed.

Keywords: Diabetes, Glycogen phosphorylase inhibitors, Oleanolic acid, PPARγ agonists, Pentacyclic triterpene, Ursolic acid.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic multifactorial metabolic disease characterized by an elevated blood glucose concentration resulting from abnormal insulin sensitivity and excessive hepatic glucose production [1]. It can be controlled mainly by the use of hypoglycemic agents. However, most of the agents have limited efficacy with undersirable side-effects, including hypoglycemia, weight gain, and edema [2-3]. Therefore, much more effective agents with little side-effects are urgently needed.

Peroxisome proliferator-activated recepter γ (PPARγ) is a member of nuclear receptor superfamily of ligand dependent transcription factors, which play an important role in regulating insulin sensitivity and glucose homeostasis [4]. The activation of PPARγ by thiazolidine-2,4-dione or L-tyrosine derivatives resulted in insulin sensibilization and antidiabetic action. The thiazolidinedione and L-tyrosine moieties are considered to be the pharmcophores of the agonists [5-6]. On the other hand, glycogen phosphorylase (GP) catalyzes the process of glycogenolysis, which is the key enzyme relevant to the control of hepatic glucose production. It is thought that the inhibition of GP may provide a potential new treatment for T2DM [7]. We previously reported that oleanolic acid (**OA**, **1**), ursolic acid (**UA**, **2**) and related derivatives, represented a novel class of inhibitors of GP [8], that discovery afforded novel pharmacophores for GP inhibitors.

Combination of two active pharmacophores into one molecule is one of the rational drug designing techniques used in drug discovery program. This approach led to the design of **OA** and **UA** derivatives containing a thiazolidinedione or a L-tyrosine moiety. This manuscript describes the synthesis and biological evaluation of the above-mentioned derivatives.

RESULTS AND DISCUSSION

Synthesis

The synthesis of derivatives containing a thiazolidinedione moiety is summarized in Scheme **1**. 4-Nitrophenol **3** was treated with 1, 2-dibromoethane in ethanol to give the intermediate **4**. According to the procedures reported previously [8], treatment of oleanolic acid **1** with intermediate **4** afforded ester **5**, which was then reduced to **7** over Pd-C in THF. Meerwein arylation was carried out on compound **7** to give bromo ester **9**. Compound **9** gave thiazolidinedione derivative **11** by reaction with thiourea and subsequent hydrolysis in ethanolic hydrochloric acid. In the same fasion, ursolic acid derivative **12** was synthesized starting from ursolic acid.

As shown in Scheme **2**, treatment of t-Boc-L-tyrosine methyl ester **13** with 1, 2-dibromoethane in the presence of 18-Crown-6 at 80°C afforded the intermediate **14** in 70% yield. Reaction of oleanolic acid **1** with intermediates **14** in the presence of K_2CO_3 at room temperature afforded ester

*Address correspondence to these authors at the Jiangsu Center for Drug Screening, China Pharmaceutical University, Nanjing 21009, People's Republic of China; and Center of Drug Discovery, College of Pharmacy, China Pharmaceutical University, Nanjing 21009, People's Republic of China; Tel: +86-(0)25-83271198; Fax: +86-(0)25-83271198;
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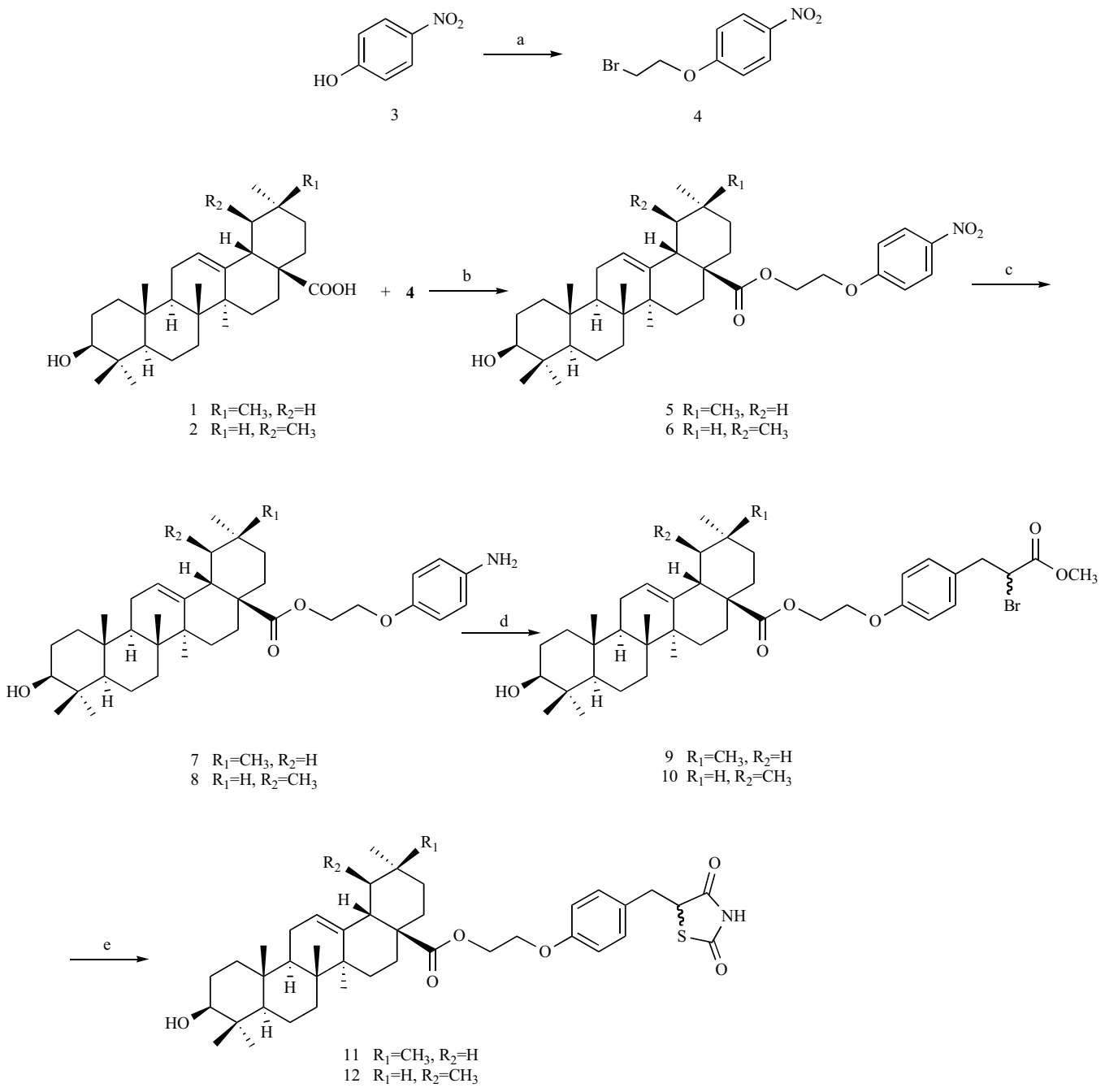
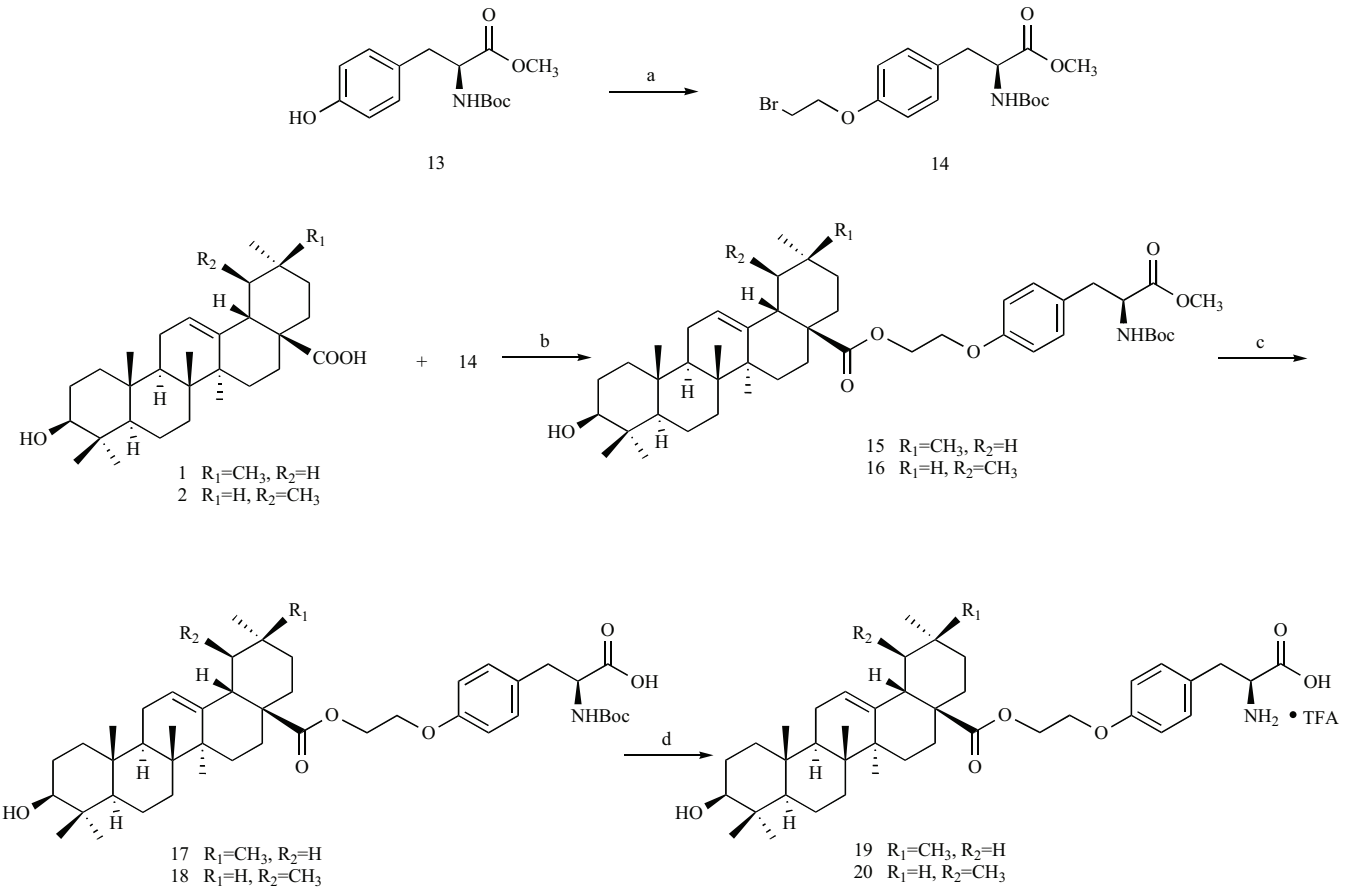


Scheme 1. The synthesis of compounds 5-12. Reagents and conditions: (a) $BrCH_2CH_2Br$, NaOH, ethanol, reflux; (b) K_2CO_3, DMF, r.t.; (c) H_2, Pd-C, THF, r.t.; (d) 47% Hydrobromic acid, $NaNO_2$, CH_2=CHCOOCH$_3$, Cu_2O, 40 °C; (e) (i) Thiourea, ethanol, reflux; (ii) 2M HCl, reflux.

15. Hydrolyzation of **15** with aqueous NaOH gave the corresponding acid **17**. Treatment of **17** with trifluoroacetic acid in CH_2Cl_2 at rome temperature gave the trifluoroacetate **19**. In the same fasion, ursolic acid derivative **20** was synthesized starting from ursolic acid.

Biological Activity

The above-synthesized triterpene derivatives **11**, **12**, **17**, **19** and rosiglitazone (**ROS**, a PPARγ full agonist as a positive control) were evaluated in an cell-based transactivation assay, according to the literature methods [9-10]. The cells were transfected with an expression plasmid for PPARγ and the activation of luciferase gene was measured. As shown in Fig. **1A** and **1B**, the compounds **11**, **12**, **17** and **19** were obviously able to enhance the expression of reporter gene as compared to **ROS**, indicating the derivatives might be potential PPARγ agoinsts. Moreover, the compounds **11** and **17** could activate the transcription activity of PPARγ in a dose-dependent manner. Among these compounds, the L-tyrosine substituted derivative **17** exhibited the most potent activity.



Scheme 2. The synthesis of compounds 15-20. Reagents and conditions: (a) $BrCH_2CH_2Br$, 18-Crown-6, 80 °C; (b) K_2CO_3, DMF, r.t.; (c) 4 M NaOH, MeOH/THF, r.t.; (d) Trifluoroacetic acid, CH_2Cl_2 , r.t.

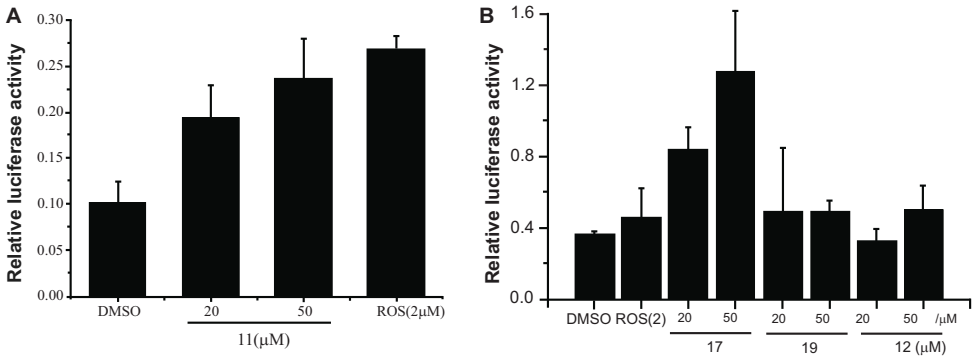


Fig. (1). Effects of componuds **11**, **12**, **17**, **19** on the transactivation activity of PPARγ.

In addition, the thiazolidinedione substituted compound **12** showed weaker activity compared to **17** and **19**, suggesting the introduction of L-tyrosine moiety at C-28 position was preferable than thiazolidinedione moiety for the transactivation activity.

All the synthesized triterpene derivatives were biologically evaluated for their inhibitory activities against rabbit muscle glycogen phosphorylase a (RMGPa). As described previously, the activity of RMGPa was measured through detecting the release of phosphate from glucose-1-phosphate in the direction of glycogen synthesis [11]. The assay results showed that most of the synthesized triterpenes exhibited inhibitory activities against rabbit muscle GPa with IC_{50} values in the range of 40.6-425.6 μM (Table **1**). Compared to the parent compounds **1** and **2**, modifications at C-28 position with thiazolidinedione or L-tyrosine moiety resulted in loss of activity (**11**) or no activity (**12**, **19-20**). SAR analysis shows that the potency trend of the triterpene skeletons is not clear. Compounds with the ursane skeleton showed better inhibitory than the oleanane skeleton in some cases (*e.g.* **5** vs **6**; **7** vs **8**; **9** vs **10**; **17** vs **18**). On the other hand, there is a reverse trend among the compounds **11** and **12**. The introduction of hydrophilic substituents at the benzene moiety reduced GP inhibitory activity compared with hydrophobic groups (*e.g.* **15** vs **17** & **19**; **16** vs **18** & **20**). Within this se-

Table 1. Inhibition of Rabbit Muscle GPa by Synthesized Triterpene Derivatives

Compound	RMGPa IC_{50}[a] (μM)	Compound	RMGPa IC_{50} (μM)
1 (OA)	14	**12**	NI[b]
2 (UA)	9	**15**	86.3
5	425.6	**16**	165
6	97.8	**17**	102.6
7	322	**18**	73.9
8	97.8	**19**	NI
9	52.9	**20**	NI
10	40.6	**Caffeine**[c]	144
11	125.1		

[a] Values are means of three experiments.
[b] NI means no inhibition.
[c] Caffeine was used as positive control.

ries of compounds, **10** (IC_{50} = 40.6 μM) displayed the most efficient inhibition, with a lower IC_{50} value than its parent compound ursolic acid **2** (IC_{50}=9 μM). These results suggested that the carboxy group at C-17 position played an important role in inhibitory activity against RMGPa, and that the introduction of substituents on the carboxy group did not seem to be well tolerated, probaly due to steric factors.

CONCLUSION

In summary, a series of triterpene derivatives containing a thiazolidinedione or a L-tyrosine moiety were synthesized, and the derivatives were tested for their PPARγ agonistic activities and glycogen phosphorylase inhibitory activities. Of the compounds evaluated, triterpenes **11** and **17** activated the transcription activity of PPARγ in a dose-dependent manner, while compound **10** was the most potent RMGPa inhibitor.

EXPERIMENTAL SECTION

Reactions and the resulted products were monitored by thin-layer chromatography (TLC) on precoated silica gel GF254 plates. Melting points (uncorrected) were determined on RY-1 MP apparatus (Tianjin Analytical Apparatus Corp, Tianjin, China). Mass spectral data were obtained on Agilent 1100 LC/DAD/MSD or Q-Tof Micro MS/MS. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ or d_6-DMSO on a Bruker AV-300 MHz spectrometer with TMS as internal standard. IR spectra were recorded on Shimadzu FTIR-8400S spectrometer.

Chemistry

Oleanolic acid (**1**) and ursolic acid (**2**) were purchased from Zelang Medical Technology Company (Nanjing, China). All other commercially available solvents and reagents were of AR grade and used without further purification. Solvents for reactions under anhydrous conditions were dried according to standard procedures.

Preparation of 2-(4-Nitrophenoxy)-1-bromoethane (4)

A solution of 4-nitrophenol (1.0 g, 7.3 mmol) in anhydrous alcohol (15 mL) was added NaOH (0.29 g, 7.2 mmol) in three portions. The mixture was refluxed for 1 h. After cooling to room temperature, 1, 2-dibromoethane (1.90 mL, 21.9 mmol) was added dropwise and heated to reflux for 20 h. Then, the mixture was filtered and concentrated. The residue was redissolved in CH_2Cl_2 and washed with water, dried over Na_2SO_4 and concentrated. The residue was purified over a column of silica gel (200-300 mesh) with petroleum ether/ethyl acetate 50:1 as eluent to get compound **4** as white solid 0.76 g, yield 44%, m.p. 62-64 °C.

Preparation of 2-(4-Nitrophenoxy)ethyl 3β-hydroxyolean-12-en-28-oate (5)

To a mixture of oleanoic acid (**1**, 1.40 g, 3.07 mmol) and K_2CO_3 (0.85 g, 6.14 mmol) in anhydrous DMF (15 mL) was added **4** (0.76 g, 3.07 mmol). The mixture was stirred at room temperature over night and then filtered. The filtrate was diluted with water (30 mL), extracted with ethyl acetate (15 mL). The organic layer was washed with 1M HCl (2 × 30mL), water (2 × 30mL), satd $NaHCO_3$ (2 × 30mL) and brine (2 × 30mL), dried over Na_2SO_4 and concentrated. The residue was purified over a column of silica gel (200-300 mesh) with petroleum ether/ethyl acetate 6:1 as eluent to get compound **5** as light yellow solid 0.98 g, yield 52%, m.p. 190-192 °C. **IR (**KBr, ν**)**: 3544, 2948, 2860, 1723, 1516; **^{1}H NMR ($CDCl_3$, 300 MHz,** δppm**)**: 0.66 (s, 3H), 0.77 (s, 3H), 0.83 (s, 3H), 0.90 (s, 3H), 0.91 (s, 3H), 0.98 (s, 3H), 1.12 (s, 3H), 2.86 (dd, 1H, *J* = 2.61, 8.34 Hz)**,** 3.18-3.21 (m, 1H), 4.25 (t, 2H, *J* = 2.88 Hz), 4.40-4.42 (m, 2H), 5.24 (t, 1H, *J* = 2.07 Hz), 6.94-7.00 (m, 2H), 8.20-8.23 (m, 2H); **^{13}C NMR ($CDCl_3$, 75 MHz,** δppm**)**: 177.7, 143.6, 126.0, 122.6, 114.6, 79.0, 66.7, 62.0, 55.2, 47.6, 46.9, 45.8, 41.7, 41.3, 39.3, 38.7, 38.4, 37.0, 33.8, 33.0, 32.7, 32.4, 30.7, 28.1, 27.6, 27.2, 25.8, 23.6, 23.4, 23.0, 18.2, 17.0, 15.5, 15.2; **MS (ESI, m/z)**: 620.1 $[M-H]^-$ (2).

Preparation of 2-(4-Nitrophenoxy)ethyl 3β-hydroxyurs-12-en-28-oate (6)

Compound **6** could be prepared following the procedure described for **5**. White solid, yield 93%. m.p. 184-185 °C. **IR (**KBr, ν**)**: 3437, 2945, 1724, 1515; **^{1}H NMR ($CDCl_3$, 300 MHz,** δppm**)**: 0.69 (s, 3H), 0.77 (s, 3H), 0.84 (d, 6H, *J* = 6.70 Hz), 0.97 (d, 6H, *J* = 10.4 Hz), 1.07 (s, 3H), 2.24 (d, 1H, *J* = 11.2Hz**),** 3.18-3.23 (m, 1H), 4.25 (t, 2H, *J* = 4.7 Hz), 4.33-4.45 (m, 2H), 5.21 (t, 1H, *J* = 3.4 Hz), 6.94-6.99 (m, 2H), 8.20-8.25 (m, 2H); **^{13}C NMR ($CDCl_3$, 75 MHz,** δppm**)**: 138.0, 125.9, 125.7, 114.5, 79.0, 66.7, 62.0, 55.2, 52.8, 48.3, 47.5, 42.0, 39.6, 39.0, 38.9, 38.7, 38.6, 36.9, 36.7, 33.0, 30.6, 28.1, 27.9, 27.2, 24.2, 23.5, 23.3, 21.1, 18.3, 17.1, 17.0, 16.0, 15.3; **MS (ESI, m/z)**: 622.4 $[M+H]^+$(26).

Preparation of 2-(4-Aminophenoxy)ethyl 3β-hydroxyolean-12-en-28-oate (7)

A mixture of **5** (1.64 g, 2.64 mmol) and 10% Pd/C (160 mg) in THF (50 ml) was stirred at room temperature under hydrogen atmosphere for 12 h. The mixture was filtered through Celite, and the insoluble substance was washed with THF. The filtrate was concentrated and the residue was purified over a column of silica gel (200-300 mesh) with petroleum ether/ethyl acetate 3:1 as eluent to get compound **7** as white solid 1.0 g, yield 64%, m.p. 93-95 °C. **IR (**KBr, ν**)**: 3366, 2950, 1722, 1511; **^{1}H NMR ($CDCl_3$, 300 MHz,** δppm**)**: 0.70 (s, 3H), 0.78 (s, 3H), 0.87 (s, 3H), 0.89 (s, 3H), 0.92 (s, 3H), 0.98 (s, 3H), 1.15 (s, 3H), 2.86 (dd, 1H, *J* = 2.75, 8.33 Hz**),** 3.19-3.22 (m, 1H), 4.07-4.09 (m, 2H), 4.29-4.35 (m, 2H), 5.27 (t, 1H, *J* = 2.1 Hz), 6.68-6.70 (m, 2H), 6.73-6.77 (m, 2H); **^{13}C NMR ($CDCl_3$, 75 MHz,** δppm**)**: 177.7, 152.3, 143.7, 139.3, 122.6, 116.9, 116.1, 79.1, 66.9, 62.7, 55.3, 47.7, 46.8, 45.9, 41.7, 41.3, 39.4, 38.8, 38.5, 37.0, 33.9, 33.1, 32.7, 32.4, 30.7, 28.1, 27.7, 27.2, 25.8, 23.6, 23.4, 23.0, 18.3, 17.0, 15.5, 15.2; **MS (ESI, m/z):** 1183.9 ($2M+H^+$, base peak).

Preparation of 2-(4-Aminophenoxy)ethyl 3β-hydroxyurs-12-en-28-oate (8)

Compound **8** could be prepared following the procedure described for **7**. White solid, yield 60%, m.p. 94-95 °C. **IR (**KBr, ν**)**: 3537, 2862, 1731, 1513; **^{1}H NMR ($CDCl_3$, 300 MHz,** δppm**)**: 0.73 (s, 3H), 0.78 (s, 3H), 0.86 (t, 6H, *J* = 6.1 Hz), 0.93 (d, 3H, *J* = 5.5 Hz), 0.98 (s, 3H), 1.07 (s, 3H), 2.24 (d, 1H, *J* = 11.2 Hz**),** 3.20-3.23 (m, 1H), 4.06 (t, 2H, *J* = 4.9 Hz), 4.28-4.32 (m, 2H), 5.23 (t, 1H, *J* = 3.4 Hz), 6.63-6.67 (m, 2H), 6.72-6.77 (m, 2H); **^{13}C NMR ($CDCl_3$, 75 MHz,** δppm**)**: 177.4, 151.9, 140.2, 138.0, 125.7, 116.4, 116.0, 79.0, 66.8, 62.7, 55.3, 52.8, 48.2, 47.6, 42.1, 39.6, 39.1, 38.8, 38.7, 38.7, 37.0, 36.6, 33.0, 30.7, 28.1, 28.0, 27.3, 24.2, 23.5, 23.3, 21.1, 18.3, 17.1, 16.9, 15.6, 15.4; **MS (ESI, m/z)**: 592.3 ($M+H^+$, base peak).

Preparation of 2-[4-(2-Bromo-3-methoxy-3-oxopropyl)phenoxy]ethyl 3β-hydroxyolean-12-en-28-oate (9)

To a stirred solution of **7** (1 g, 1.69 mmol) in MeOH (6.2 mL) and acetone (1.54 mL) was added aqueous HBr (47%, 1.1 mL) and stirred for 10 min at 0°C. Then a aqueous solution of $NaNO_2$ (0.48 g/mL, 0.384 mL) was added slowly dropwise at 0°C and stirred for 20 min. The reaction mixture was added methyl acrylate (1.28 mL, 14.12 mmol) and allowed to warm to 30°C. Catalytic amount of cuprous oxide (54 mg, 0.38 mmol) was added and the reaction mixture was stirred for 1 h at 40 °C. The solvent was removed under reduced pressure and the resultant residue was redissolved in ethyl acetate (10 mL). The organic layer was washed with dilute ammonia solution (2 × 20mL), water (2 × 20mL) and brine (2 × 20mL), dried over Na_2SO_4 and concentrated. The residue was purified over a column of silica gel (200-300 mesh) with petroleum ether/ethyl acetate 12:1 as eluent to get compound **9** as white solid 0.48 g, yield 38%, m.p. 64-66 °C. **IR (**KBr, ν**)**: 3451, 2946, 1742, 1512; **^{1}H NMR ($CDCl_3$, 300 MHz,** δppm**)**: 0.67 (s, 3H), 0.77 (s, 3H), 0.89 (s, 3H), 0.91 (s, 3H), 0.95 (s, 3H), 0.98 (s, 3H), 1.11 (s, 3H), 2.86 (dd, 1H, *J* = 4.47, 14.3 Hz**),** 3.13-3.22 (m, 2H), 3.35-3.43 (m, 1H), 3.73 (s, 3H), 4.12 (t, 2H, *J* = 4.6 Hz), 4.32-4.38 (m, 2H), 5.25 (br *s*, 1H), 6.81 (dd, 2H, *J* = 8.6, 88.5 Hz), 7.11 (dd, 2H, *J* = 8.5, 88.5 Hz); **^{13}C NMR ($CDCl_3$, 75 MHz,** δppm**)**: 177.7, 169.9, 157.9, 143.6, 130.3, 129.2, 122.5, 114.8, 79.0, 65.9, 62.5, 55.2, 52.9, 47.6, 46.8, 45.9, 45.4, 41.7, 41.3, 40.3, 39.4, 38.7, 38.5, 37.0, 33.9, 33.1, 32.7, 32.4, 30.7, 28.1, 27.6, 27.2, 25.9, 23.6, 23.4, 23.0, 18.3, 17.0, 15.6, 15.3; **MS (ESI, m/z)**: 759.7 ($M+NH_4^+$, base peak).

Preparation of 2-[4-(2-Bromo-3-methoxy-3-oxopropyl)phenoxy]ethyl 3β-hydroxyurs-12-en-28-oate (10)

Compound **10** could be prepared following the procedure described for **9**. White solid, yield 37%, m.p. 71-72 °C. **IR (**KBr, ν**)**: 3558, 3423, 2917, 1743, 1512; **^{1}H NMR ($CDCl_3$, 300 MHz,** δppm**)**: 0.71 (s, 3H), 0.78 (s, 3H), 0.84 (d, 6H, *J* = 6.7 Hz), 0.94 (s, 3H), 0.99 (s, 3H), 1.07 (s, 3H), 2.24 (d, 1H, *J* = 11.0 Hz), 3.14-3.22 (m, 2H), 3.36-3.44 (m, 1H), 3.73 (s, 3H), 4.12 (t, 2H, *J* = 4.8 Hz), 4.32-4.35 (m, 2H), 5.20 (t, 1H, *J* = 3.5 Hz), 6.81-6.85 (m, 2H), 7.11-7.14 (m, 2H); **MS (ESI, m/z)**: 759.7 ($M+NH_4^+$, base peak).

Preparation of 2-{4-[(2,4-Dioxothiazolidin-5-yl)methyl]phenoxy}ethyl 3β-hydroxyolean-12-en-28-oate (11)

To a stirred solution of **9** (44 mg, 0.06 mmol) in anhydrous ethanol (2 mL) was added thiourea (37 mg, 0.47 mmol) and refluxed over night. After cooling to room temperature, 2M HCl (1.5mL) was added and then heated to reflux for 5 h. The reaction mixture was concentrated and redissolved in ethyl acetate (5 mL). The organic layer was washed with water (2 × 10mL), dried over Na_2SO_4 and concentrated. The residue was purified over a column of silica gel (200-300 mesh) with petroleum ether/ethyl acetate 6:1 as eluent to get compound **11** as white solid 15 mg, yield 36%, m.p. 121-123 °C. **IR (**KBr, ν**)**: 3537, 2946, 1702, 1512; **^{1}H NMR ($CDCl_3$, 300 MHz,** δppm**)**: 0.67 (s, 3H), 0.77 (s, 3H), 0.84 (s, 3H), 0.89 (s, 3H), 0.91 (s, 3H), 0.98 (s, 3H), 1.11 (s, 3H), 2.86 (dd, 1H, *J* = 3.82, 9.59 Hz), 3.04-3.12 (m, 1H), 3.19-3.24 (m, 1H), 3.44-3.50 (m, 1H), 4.10-4.15 (m, 2H), 4.35-4.39 (m, 2H), 4.48 (dd, 1H, *J* = 3.88, 9.72 Hz), 5.25 (br *s*, 1H), 6.82-6.86 (m, 2H), 7.09-7.16 (m, 2H), 8.69 (br *s*, 1H); **^{13}C NMR ($CDCl_3$, 75 MHz,** δppm**)**: 177.7, 174.0,

170.1, 158.1, 143.6, 130.3, 128.2, 122.5, 115.0, 79.1, 66.0, 62.5, 55.2, 53.7, 47.6, 46.8, 45.9, 41.7, 41.3, 39.4, 38.7, 38.5, 37.9, 37.0, 33.9, 33.1, 32.7, 32.4, 30.7, 28.1, 27.6, 27.2, 25.9, 23.6, 23.4, 23.0, 18.3, 17.0, 15.6, 15.3; **MS (ESI, m/z)**: 704.5 (M-H$^-$, base peak); **HRMS for $C_{42}H_{59}N_1O_6S_1$+Na** calcd 728.39608, found 728.39553.

Preparation of 2-{4-[(2,4-Dioxothiazolidin-5-yl)methyl]phenoxy}ethyl 3β-hydroxyurs-12-en-28-oate (12)

Compound **12** could be prepared following the procedure described for **11**. White solid, yield 67%, m.p. 127-129 °C. **IR (**KBr, ν**)**: 3516, 2939, 1703, 1512; **^{1}H NMR ($CDCl_3$, 300 MHz,** δppm**)**: 0.70 (s, 3H), 0.76 (s, 3H), 0.83 (d, 6H, *J* = 6.2 Hz), 0.94 (s, 3H), 0.97 (s, 3H), 1.06 (s, 3H), 2.24 (d, 1H, *J* = 10.9 Hz), 3.04-3.12 (m, 1H), 3.18-3.23 (m, 1H), 3.43-3.49 (m, 1H), 4.11 (t, 2H, *J* = 4.8 Hz), 4.27-4.39 (m, 2H), 4.48 (dd, 1H, *J* = 3.9, 9.7 Hz), 5.21 (br *s*, 1H), 6.82-6.85 (m, 2H), 7.12-7.15 (m, 2H); **^{13}C NMR ($CDCl_3$, 75 MHz,** δppm**)**: 177.5, 158.2, 138.0, 130.3, 128.1, 125.7, 115.0, 79.1, 66.0, 62.5, 55.2, 53.7, 52.9, 48.2, 47.6, 42.1, 39.6, 39.1, 38.8, 38.7, 38.7, 37.8, 37.0, 36.6, 33.0, 30.7, 28.1, 28.0, 27.2, 24.2, 23.5, 23.3, 21.1, 18.3, 17.1, 17.0, 15.6, 15.4; **MS (ESI, m/z)**: 723.9 $[M+NH_4]^+$(50); **HRMS for $C_{42}H_{59}N_1O_6S_1$+Na** calcd 728.39608, found 728.39553.

Preparation of (*S*)-Methyl 3-[4-(2-bromoethoxy)phenyl]-2-(*tert*-butoxycarbonylamino)propanoate (14)

A mixture of t-Boc-L-tyrosine methyl ester (0.1 g, 0.34 mmol), 1, 2-dibromoethane (0.2 mL, 2.3 mmol), potassium carbonate (0.32 g, 2.3 mmol) and 18-crown-6 (0.01 g, 0.04 mmol) was heated at 80 °C for 12 h. After cooling to room temperature, the mixture was resuspended in ethyl acetate, filtered and the insoluble substance was washed with ethyl acetate. The filtrate was concentrated and the residue was purified over a column of silica gel (200-300 mesh) with petroleum ether/ethyl acetate 4:1 as eluent to get compound **14** as white solid 0.1 g, yield 70%.

Preparation of (S)2-{4-[2-(*tert*-Butoxycarbonylamino)-3-methoxy-3-oxopropyl]phenoxy}ethyl 3β-hydroxyolean-12-en-28-oate (15)

Compound **15** could be prepared following the procedure described for **5**. White solid, yield 88%, m.p. 113-115 °C. **IR (**KBr, ν**)**: 2946, 2854, 1720, 1510; **^{1}H NMR ($CDCl_3$, 300 MHz,** δppm**)**: 0.69 (s, 3H), 0.77 (s, 3H), 0.86 (s, 3H), 0.89 (s, 3H), 0.91 (s, 3H), 0.98 (s, 3H), 1.12 (s, 3H), 1.42 (s, 9H), 2.84 (dd, 1H, *J* = 4.2, 13.8 Hz)**,** 2.97-3.07 (m, 2H), 3.18-3.23 (m, 1H), 3.71 (s, 3H), 4.12 (t, 2H, *J* = 4.8 Hz), 4.29-4.42 (m, 2H), 4.51-4.55 (m, 1H), 4.96 (d, 1H, *J* = 8.5 Hz), 5.26 (t, 1H, *J* = 3.3 Hz), 6.80 (dd, 2H, *J* = 8.6, 64.8 Hz), 7.03 (dd, 2H, *J* = 8.5, 64.7 Hz); **^{13}C NMR ($CDCl_3$, 75 MHz,** δppm**)**: 177.7, 172.3, 157.7, 143.6, 130.3, 128.4, 122.5, 114.7, 79.0, 65.9, 62.5, 55.2, 52.1, 47.6, 46.8, 45.9, 41.7, 41.3, 39.4, 38.8, 38.5, 37.0, 33.9, 33.1, 32.7, 32.4, 30.7, 28.3, 28.1, 27.7, 27.2, 25.9, 23.6, 23.4, 23.0, 18.3, 17.0, 15.6, 15.3; **MS (ESI, m/z)**: 795.5 ($M+NH_4^+$, base peak).

Preparation of (S)2-{4-[2-(*tert*-Butoxycarbonylamino)-3-methoxy-3-oxopropyl]phenoxy}ethyl 3β-hydroxyurs-12-en-28-oate (16)

Compound **16** could be prepared following the procedure described for **5**. white solid, yield 79%, m.p. 88-90 °C. **IR (**KBr, ν**)**: 3541, 2924, 1720, 1512; **^{1}H NMR ($CDCl_3$, 300 MHz,** δppm**)**: 0.72 (s, 3H), 0.77 (s, 3H), 0.84 (d, 3H, *J* = 6.5 Hz), 0.87 (s, 3H), 0.94 (s, 3H), 0.98 (s, 3H), 1.06 (s, 3H), 1.42 (s, 9H), 2.24 (d, 1H, *J* = 11.32 Hz)**,** 2.97-3.07 (m, 2H), 3.18-3.23 (m, 1H), 3.71 (s, 3H), 4.10 (t, 2H, *J* = 4.7 Hz), 4.33 (t, 2H, *J* = 4.2 Hz), 4.56-4.57 (m, 1H), 4.95-4.98 (m, 1H), 5.22 (t, 1H, *J* = 3.3 Hz), 6.80 (dd, 2H, *J* = 8.62, 65.2 Hz), 7.03 (dd, 2H, *J* = 8.54, 65.1 Hz); **^{13}C NMR ($CDCl_3$, 75 MHz,** δppm**)**: 177.4, 172.3, 157.8, 138.0, 130.3, 128.4, 125.7, 114.7, 79.0, 65.9, 62.5, 55.3, 52.9, 52.1, 48.2, 47.6, 42.1, 39.6, 39.1, 38.8, 38.7, 38.7, 37.0, 36.6, 33.0, 30.7, 28.3, 28.1, 28.0, 27.3, 24.2, 23.5, 23.3, 21.1, 18.3, 17.1, 17.0, 15.6, 15.4; **MS (ESI, m/z)**: 795.5 ($M+NH_4^+$, base peak).

Preparation of (S)2-{4-[2-(*tert*-Butoxycarbonylamino)-2-carboxyethyl] phenoxy}ethyl 3β-hydroxyolean-12-en-28-oate (17)

To a solution of **15** (0.65 g, 0.84 mmol) in MeOH (11.7 mL) and THF (18 mL), 4 M NaOH (5 mL) was added dropwise, and the resulting mixture was stirred at room temperature over night. After neutralized with 1M aqueous solution of $KHSO_4$, the product was extracted with ethyl acetate (30 mL). The organic phase was washed with water (2 × 60mL), dried over Na_2SO_4 and concentrated. The residue was purified over a column of silica gel (200-300 mesh) with petroleum ether/ethyl acetate 1:1 as eluent to get compound **17** as white solid 0.27 g, yield 42%, m.p. 133-135 °C. **IR (**KBr, ν**)**: 3534, 2945, 1725, 1512; **^{1}H NMR ($CDCl_3$, 300 MHz,** δppm**)**: 0.70 (s, 3H), 0.78 (s, 3H), 0.86 (s, 3H), 0.89 (s, 3H), 0.92 (s, 3H), 0.98 (s, 3H), 1.12 (s, 3H), 1.42 (s, 9H), 2.89 (dd, 1H, *J* = 3.7, 13.8 Hz), 3.03-3.16 (m, 2H), 3.20-3.25 (m, 1H), 4.12 (t, 2H, *J* = 4.5 Hz), 4.31-4.40 (m, 2H), 4.55 (br *s*, 1H), 4.93 (br *s*, 1H), 5.26 (br *s*, 1H), 6.82 (dd, 2H, *J* = 8.43, 80.9 Hz), 7.09 (dd, 2H, *J* = 8.26, 81.1 Hz); **^{13}C NMR ($CDCl_3$, 75 MHz,** δppm**)**: 177.7, 174.9, 157.8, 155.5, 143.6, 130.0, 128.3, 122.5, 114.8, 79.1, 65.9, 62.6, 55.3, 47.6, 46.8, 45.9, 41.7, 41.3, 39.4, 38.8, 38.5, 37.0, 33.9, 33.1, 32.7, 32.4, 30.7, 28.3, 28.1, 27.7, 27.2, 25.9, 23.6, 23.4, 23.0, 18.3, 17.0, 15.6, 15.3; **MS (ESI, m/z)**: 762.6 (M-H$^-$, base peak).

Preparation of (S)2-{4-[2-(*tert*-Butoxycarbonylamino)-2-carboxyethyl] phenoxy}ethyl 3β-hydroxyurs-12-en-28-oate (18)

Compound **18** could be prepared following the procedure described for **17**. White solid, yield 38%, m.p. 151-153 °C. **IR (**KBr, ν**)**: 3439, 2917, 1723, 1512; **^{1}H NMR ($CDCl_3$, 300 MHz,** δppm**)**: 0.72 (s, 3H), 0.77 (s, 3H), 0.85 (d, 6H, *J* = 8.1 Hz), 0.93 (d, 3H, *J* = 5.4 Hz), 0.98 (s, 3H), 1.07 (s, 3H), 1.42 (s, 9H), 2.22-2.28 (m, 2H), 3.06-3.17 (m, 2H), 3.19-3.25 (m, 1H), 4.11 (t, 2H, *J* = 4.6 Hz), 4.31-4.34 (m, 2H), 4.54-4.56 (m, 1H), 4.91-4.94 (m, 1H), 5.23 (br *s*, 1H), 6.83 (dd, 2H, *J* = 8.5, 80.8 Hz), 7.09 (dd, 2H, *J* = 8.5, 80.7); **^{13}C NMR**

($CDCl_3$, 75 MHz, δppm**)**: 177.5, 157.8, 138.0, 130.4, 128.2, 125.8, 114.8, 79.1, 65.9, 62.5, 55.2, 52.9, 48.2, 47.6, 42.1, 39.6, 39.1, 38.9, 38.7, 38.7, 37.0, 36.7, 33.0, 30.7, 28.3, 28.2, 28.0, 27.2, 24.2, 23.6, 23.3, 21.1, 18.3, 17.1, 17.0, 15.6, 15.4; **MS (ESI, m/z)**: 762.5 ($M-H^-$, base peak).

Preparation of (S)2-[4-(2-Amino-2-carboxyethyl)phenoxy] ethyl 3β-hydroxyolean-12-en-28-oate (19)

To a solution of **17** (0.17 g, 0.22 mmol) in CH_2Cl_2 (10 mL) was added trifluoroacetic acid (2 mL, 40 mmol), the reaction mixture was stirred at 0 °C for 2 h. Then the reaction mixture was concentrated and the residue was purified over a column of silica gel (200-300 mesh) with CH_2Cl_2/MeOH 50:1 as eluent to get compound **19** as white solid 0.11 g, yield 66%, m.p.159-161 °C. **IR (**KBr, ν**)**: 3534, 2944, 1780, 1725, 1512; **^{1}H NMR (d_6-DMSO, 300 MHz,** δppm**)**: 0.70 (s, 3H), 0.78 (s, 3H), 0.83 (s, 3H), 0.86 (s, 6H), 0.91 (s, 6H), 1.12 (s, 3H), 2.38 (d, 1H, *J* = 13.8 Hz), 2.67-2.82 (m, 2H), 3.03-3.09 (m, 1H), 4.11 (br *s*, 2H), 4.35 (br *s*, 2H), 4.70-4.73 (m, 1H), 6.81 (dd, 2H, *J* = 8.5, 104.1 Hz), 7.16 (dd, 2H, *J* = 8.5, 104.0 Hz); **^{13}C NMR (d_6-DMSO, 75 MHz,** δppm**)**: 175.5, 156.9, 137.6, 130.3, 129.8, 127.3, 114.3, 86.2, 65.2, 62.4, 55.5, 54.0, 49.6, 47.8, 43.9, 40.7, 40.6, 37.6, 36.6, 36.2, 35.3, 34.1, 32.4, 32.2, 31.8, 27.4, 26.5, 24.6, 23.8, 22.6, 21.2, 20.7, 17.6, 17.1, 16.1, 15.9; **MS (ESI, m/z)**: 662.4 ($M-H^-$, base peak); **HRMS for $C_{43}H_{61}F_3NO_7$** calcd 760.44001, found 760.43946.

Preparation of (S)2-[4-(2-Amino-2-carboxyethyl)phenoxy] ethyl 3β-hydroxyurs-12-en-28-oate (20)

Compound **20** could be prepared following the procedure described for **19**. White solid, yield 50%, m.p. 111-113 °C. **IR (**KBr, ν**)**: 3480, 2924, 1730, 1516; **^{1}H NMR (d_6-DMSO, 300 MHz,** δppm**)**: 0.65 (s, 3H), 0.81 (d, 3H, *J* = 6.2 Hz), 0.85 (s, 9H), 0.92 (s, 3H), 1.05 (s, 3H), 2.14 (d, 1H, *J* = 11.4 Hz), 2.75-2.83 (m, 1H), 3.04-3.09 (m, 1H), 3.34-3.36 (m, 1H), 4.12 (br *s*, 2H), 4.24 (br *s*, 2H), 4.71(m, 1H), 5.12 (br *s*, 1H), 6.83 (dd, 2H, *J* = 8.4, 102.3 Hz), 7.16 (dd, 2H, *J* = 8.4, 102.3 Hz); **^{13}C NMR (d_6-DMSO, 75 MHz,** δppm**)**: 176.3, 169.4, 156.9, 137.7, 130.3, 129.7, 124.8, 114.3, 86.1, 65.6, 62.4, 55.5, 53.8, 52.3, 47.4, 46.5, 41.5, 40.3, 37.5, 37.3, 36.2, 36.1, 32.2, 29.9, 27.4, 23.7, 23.2, 22.8, 22.5, 20.9, 17.5, 16.9, 16.6, 16.3, 14.9; **MS (ESI, m/z)**: 662.5 ($M-H^-$, base peak); **HRMS for $C_{43}H_{61}F_3NO_7$+H** calcd 761.44784, found 761.44729.

IN VITRO ACTIVITIES

Reagents and Chemicals

Rosiglitazone was obtained from Cayman Chem Co (Ann Arbor, MI, USA). All cell culture reagents were obtained from GIBCO. Lipofectamine-2000 was obtained from Invitrogen. All other reagents and solvents were purchased commercially and used without further purifications.

Cell Culture

HEK 293T (Human embryonic kidney) cells were kindly provided by Dr. Shen' lab in Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China. The cells were prepared and saved by conventional method [9].

Transactivation Assay

The transactivation assay was performed by the published method with some modifications [9-10]. The cells were seeded into a 24-well plate at 100,000 cells per well, and when the cell confluence had reached about 70%, pSV-PPRE-Luc luciferase reporter gene, pCDNA3.1-PPARγ, and pCDNA3.1-RXRα, together with the control pSV-β-galactosidase (0.3 μg), were transiently transfected into the cells with Lipofectamine 2000 (2 μL per well). After transfection (24 h), the medium was replaced with fresh medium containing either rosiglitazone (**Ros**, 2 μM) or different concentrations of test compound (20 μM, 50 μM). After a further 24 h incubation, expression of the reporter gene was measured through the activity of firefly luciferase with the luciferase assay system (Promega), and the firefly luciferase activity was corrected for transfection efficiency based on the activity of internal control β-galactosidase.

Glycogen Phosphorylase Activity

The inhibitory activity of the synthesized compounds against rabbit muscle glycogen phosphorylase a (GPa) was monitored using microplate reader (BIO-RAD) based on the published method [11]. In brief, GPa activity was measured in the direction of glycogen synthesis by the release of phosphate from glucose-1-phosphate. Each test compound was dissolved in DMSO and diluted at different concentrations for IC_{50} determination. The enzyme was added into 100 μL of buffer containing 50 mM Hepes (pH = 7.2), 100 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM glucose-1-phosphate, 1 mg/mL glycogen and the test compound in 96-well microplates (Costar). After the addition of 150 μL of 1 M HCl containing 10 mg/mL ammonium molybdate and 0.38 mg/mL malachite green, reactions were run at 22°C for 25 min, and then the phosphate absorbance was measured at 655 nm. The IC_{50} values were estimated by fitting the inhibition data to a dose-dependent curve using a logistic derivative equation.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

The work was supported by program for Changjiang Scholars and Innovative Research Team in University (PCSIRT-IRT1193), the National Natural Science Fundation of China (No. 81001401) and the Natural Science Foundation of Hebei Province (No. H2012406011).

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Received: May 17, 2011 Revised: May 14, 2012 Accepted: June 14, 2012