Synthesis and Biological Evaluation of Raddeanin A, a Triterpene Saponin Isolated from Anemone raddeana

Shan Qian,*a Quan Long Chen,a Jin Long Guan,a Yong Wu,b and Zhou Yu Wanga

aBioengineering College, Xihua University; Chengdu 610039, P. R. China; and bWest China School of Pharmacy, Sichuan University; Chengdu 610041, P. R. China.

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First, Raddeanin A, a cytotoxic oleane-type triterpenoid saponin isolated from Anemone raddeana Regel, was synthesized. Stepwise glycosylation was adopted in the synthesis from oleanolic acid, employing arabinosyl, glucosyl and rhamnosyl trichloroacetimidate as donors. The chemical structure of Raddeanin A was confirmed by means of 1H-NMR, 13C-NMR, IR, MS and elemental analysis, which elucidated the structure to be 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside oleic acid. Biological activity tests showed that in the range of low concentrations, Raddeanin A displayed moderate inhibitory activity against histone deacetylases (HDACs), indicating that the HDACs’ inhibitory activity of Raddeanin A may contribute to its cytotoxicity.

Key words Raddeanin A; Anemone raddeana; oleanolic acid; cytotoxicity; histone deacetylase

In the history of traditional Chinese medicine, medicinal plants and their extracts were used to treat various diseases. The dry rhizome of Anemone raddeana Regel (Ranunculaceae), a very important Chinese folk medicine, was used to treat rheumatism and neuralgia.1–3 During the past 30 years, most of the studies focused on the isolation of alcohol extracts of this medicinal plant and more than thirty oleane-type triterpenoids have been identified.4–8 Recent studies have suggested that one of these triterpenoids, Raddeanin A (I), exhibited cytotoxicity in vitro. It had significant inhibitory effect on the growth of the tumor cells such as liver cancer, lung cancer and gastric cancer cells.9,12 Given its significant biological importance and the potentially clinical utilities as a promising antitumor drug, we have investigated the synthesis of I and preliminarily evaluated its biological activities.

Results and Discussion

I was elucidated as 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside oleic acid3 (Fig. 1). This oleane triglycoside bears three 1,2-transpyranosidic linkages, which could be constructed stereoselectively by stepwise glycosylation with a sugar donor equipped with a participating group at its 2-OH. Besides, stepwise glycosylation was adopted to construct the oligosaccharide moiety because it is a preferable method of preparing glycosides for structure–activity research (SAR) by altering monosaccharide donors. Therefore, stepwise glycosylation was adopted in our work.

As shown in Chart 1, oleanic acid (2) was converted to its benzyl ester 3 by treating 2 with BnBr and K₂CO₃ in aqueous N,N-dimethylformamide (DMF) in high yield (98%).14 The remaining oleane 3-OH was glycosylated with α-L-arabinosyl trichloroacetimidate 45) as the first monosaccharide unit in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.1 eq) to provide the 3-O-α-glycoside 5 in an excellent 92% yield. Debenzoylation of 5 in NaOMe–MeOH without influencing benzyl ester at C-28 produced benzyl oleonolate 3-O-α-L-arabinopyranoside 6.5 Selective shelter of hydroxyl groups at C-3’ and C-4’ of arabinose residue was successfully carried out using 2,2-dimethoxypropane give alcohol 7.5) Subsequently, during the preparation of oleane diglycoside 9, glucosyl trichloroacetimidate 85) was detected to be completely consumed to produce 2,3,4,6-tetrabenzoyl-gluco pyranoside under the similar conditions described above (TMSOTf, −40°C), instead of diglycoside 9. Therefore, the reaction temperature was raised to 0°C, so that the reaction could be completely successfully in 1 h with a 75% yield of the product 9. Then, the O-benzoyl groups of 9 were removed by NaOMe–MeOH to give 10, and benzylidene was introduced to protect 4°,6°-OH by the reaction of 10 with PhCH(OMe)₂ and p-toluensulfonic acid in dry DMF to give diol 11.10,17

The key step is the regioselective protection of the hydroxyl groups at C-2” and C-3” of 11. The disaccharide with free 2°-OH could only be glycosylated by rhamnosyl trichloroacetimidate as the third monosaccharide in the presence of TMSOTf and then gave the oleanic triglycoside 1. The previous study7) has reported the 2°-OH and 3°-OH could be protected regioselectively with a tert-butylimidemethylsilyl group (TBDMS), but this reaction was found to be very difficult in our experiment. No reaction product 12 was detected from silica gel TLC after the protection of 11 with a TBDMS ether and this reaction could not be improved by modification of the reaction conditions, such as change of reaction temperature or catalyst. 2°-OH and 3°-OH of 11 have been found to be extremely unreactive sugar acceptors during glycosylation due to these high steric hindrances.

Therefore, an alternate approach was investigated. The ortho-ester method is effective for weakly reactive hydroxyl groups during glycosylation. As depicted in the Chart 2, acceptor 19 was efficiently obtained starting from acetobromoglucose 13. Initially, ortho-ester 14 was smoothly generated from acetobromoglucose 138) with ethanol–triethylamine in the presence of tetrahydrothiophenium bromide. Subsequent exchange of acetyl against benzyl blocking groups, i.e., 14→15, was performed with BnBr–KOH in tetrahydrofuran (THF).19 Compound 15 was submitted to sequential acid-mediated cyclic ortho-ester opening to yield the monoacycated intermediates 16 and 17 in the ratio of 1:3, which could be separated by careful chromatography on a silica gel column.20 The ben-
Fig. 1. Structures of Raddeanin A (1) and Oleanolic Acid (2)

Reagents and conditions: (a) \(\text{BnBr, } K_2CO_3, \text{ DMF, 50°C, 3 h, 98%} \); (b) \(4, \text{TMSOTf, 4 Å MS, CH}_2\text{Cl}_2, -40°C, 1 h, 92% \); (c) \(\text{NaOMe, CH}_2\text{Cl}_2–\text{MeOH, rt., 2 h, 80%} \); (d) \(\text{Me}_2\text{C(OMe)}_2, \text{TsOH, acetone, 0°C to rt., 1 h, 85%} \); (e) \(8, \text{TMSOTf, CH}_2\text{Cl}_2, 4 Å MS, 0°C, 1 h, 75% \); (f) \(\text{PhCH(OMe)}_2, \text{TsOH, DMF, 50°C, 30 min, 92%} \).

Chart 1

Reagents and conditions: (a) \(\text{tetrabutylammonium bromide, ethanol–triethylamine, CH}_2\text{Cl}_2, \text{rt., 24 h, 53%} \); (b) \(\text{BnBr–KOH, THF, 60°C, 3 h, 84%} \); (c) \(80% \text{ HOAc, rt., 1 h, 87%} \); (d) i. \(\text{Et}_3\text{N, CH}_2\text{OH, H}_2\text{O, rt., 3 h; ii. BzCl, py., rt., overnight} \); iii. \(\text{CH}_3\text{NH}_2, \text{rt., 72% for three steps} \); (e) \(\text{CCl}_3\text{CN, DBU, CH}_2\text{Cl}_2, \text{rt., 91%} \).

Chart 2
zoyl group has proved more favorable than the acetyl group at 2-OH, since more stable is observed. The conversion of 16 and 17 into 2-benzoyl-3,4,6-tribenzyl-glucopyranose 18 could be readily accomplished in a one-pot procedure by a rapid sequence of three reactions, without any chromatographical purification of intermediates. Finally, this key building block 18 was directly converted to the corresponding glucosyl trichloroacetimidate 19 under the agency of CCl₃CN and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

The diglycoside 20 was prepared from benzyl ester 7 and glucosyl trichloroacetimidate 19 according to the same procedure described for diglycoside 9. With the monoglycoside O-benzoyl group of 20 was removed by NaOMe–MeOH to give 21. The treatment of free 2'-OH of 21 with the rhamnosyl trichloroacetimidate 22 under TMSOTf gave the expected glycosylation product 23 in an 82% yield. Finally, deisopropylidenation of 23 under 80% HOAc, followed by removal of benzoyl group in NaOMe–MeOH and benzyl group through catalytic hydrogenation afforded the target compound 1 (Chart 3). The structure of 1 was confirmed by ¹H-NMR, ¹³C-NMR, IR, electrospray ionization (ESI)-MS and elemental analyses, and physical data are in agreement with the literature reported. The oleanolic triglycoside 1 exhibited cytotoxicity in vitro and was potential antitumor drug. However, the related mechanism has not yet been reported. Histone deacetylases (HDACs) are significant enzymes involved in tumor genesis and development, and inhibition of HDACs has become a novel and validated therapeutic strategy against cancers. In this article, HDACs inhibitory activities of 1 and its synthetic derivatives had been evaluated (Table 1), in order to reveal the relationship between HDACs inhibitory activities and cytotoxicity of these compounds. Oleanolic acid 2 displayed poor HDACs inhibitory activities. Esterification of the C-28

Table 1. Inhibition or Activation Percentages Observed with These Compounds at a Single-Dose Concentration of 10µg/mL over the Tested Proteins

| Compound | HDACs | α-Glu | PTP-1B | DPP-IV | GLP-1R | PPRE |
|----------|-------|-------|--------|--------|--------|------|
|          | % Inhibition | % Activation | % Inhibition | % Activation | % Inhibition | % Activation |
| 1        | 30.19 | 19.67 | 2.15   | 5.22   | 6.35   | 18.08 |
| 2        | 9.66  | 2.92  | 11.69  | NA     | 1.59   | 15.21 |
| 3        | 0.39  | 10.82 | 7.38   | NA     | 1.56   | 17.63 |
| 6        | 5.01  | 11.48 | 6.94   | 0.73   | 1.15   | 24.33 |
| 7        | 30.72 | 22.42 | 36.58  | 5.11   | 2.26   | 8.37  |
| 10       | 33.7  | 23.54 | NA     | 6.74   | NA     | 9.69  |
| 11       | 24.12 | 17.44 | 48.59  | 2.09   | 0.89   | NA    |
| 23       | 27.52 | 19.74 | 10.12  | 3.39   | 4.3    | 4.21  |
| 24       | 19.12 | 14.54 | 18.33  | 0.79   | NA     | 17.52 |
| 25       | 26.31 | 17.76 | 38.82  | 3.36   | NA     | 13.16 |

a) Values are means of at least two measurements. b) The % inhibition of Vorinostat was 87.07%, of which the concentration was 0.26µg/mL. c) The % inhibition of Voglibose was 91.83%, of which the concentration was 1.00µg/mL. d) The % inhibition of sodium ortho-vanadate was 96.36%, of which the concentration was 10µg/mL. e) The % inhibition of KR-62436 was 79.95%, of which the concentration was 0.30µg/mL. f) The % inhibition of Exenatide was 98.95%, of which the concentration was 0.50µg/mL. g) The % inhibition of Pigtizazole was 95.35%, of which the concentration was 0.50µg/mL. h) NA means that the datum is not available.
carboxylic acid group (3) eliminated the inhibitory activities compared to 1. The monoglycoside 6 displayed lower HDACs inhibitory activities than its isopropylidene derivative 7. The diglycoside 10, 11 and triglycoside 23-25 exhibited more potent compared to monoglycoside 6, indicating that extension of the saccharide moiety resulted in an increase in activity. The target product 1 exhibited moderate HDACs inhibitory activity, with an inhibition ratio of 30.19%. The result revealed that the HDACs inhibitory activities of 1 might contributed to its cytotoxicity.

In order to investigate more potential biological activities of these saponins, we subsequently evaluate in vitro activities against several target proteins related to diabetes mellitus. In α-glucosidase (α-glu) inhibition assays,25 oleanolic acid 2 exhibited low α-glucosidase inhibitory activity with an inhibition ratio of 2.92%. Benzyl oleanolate 3 was fourfold more active than the parent acid with an inhibition ratio of 2.92%. Benzyl oleanolate 3-4-O-benzoyl-β-D-glucopyranosyl-(1→2)-3′,4′-O-isopropylidene-α-L-arabinopyranoside (9) Benzyl ester 7 (512.3 mg, 0.7 mmol), trichloroacetimidate 8 (1.6 g, 21 mmol) and powdered 4 Å molecular sieves (2.0 g) were stirred for 15 min at rt. in dry CH2Cl2 (10 mL), and then cooled to 0°C. TMSOTf (38.9 µL, 0.2 mmol) was added and the mixture was stirred at 0°C for 1 h before the reaction was quenched by Et3N (0.2 mL). The suspension was then filtered and the filtrate was concentrated and subjected to a silica gel chromatography (PE:CH2Cl2:CH3OH=15:1) to furnish the product 9 (0.7 g, 75%) as white foam. 1H-NMR (CDCl3) δ: 8.04-8.02 (4H, m), 7.89-7.82 (4H, m), 7.56-7.28 (17H, m), 5.80 (1H, t, J=9.6 Hz), 5.72 (1H, t, J=9.6 Hz), 5.50 (2H, dd, J=9.6, 8.2 Hz), 5.30 (1H, d, J=8.0 Hz), 5.26 (1H, t, J=3.4 Hz), 5.10 (1H, d, J=12.8 Hz), 5.04 (1H, d, J=12.4 Hz), 4.58 (1H, dd, J=12.4, 3.0 Hz), 4.48 (1H, d, J=12.4, 4.2 Hz), 4.36 (1H, d, J=6.81 Hz), 4.12 (2H, m), 3.80 (2H, m), 3.68 (1H, dd, J=12.8, 4.81 Hz), 3.00 (1H, dd, J=11.2, 4.8 Hz), 2.90 (1H, d, J=13.6, 4.0 Hz), 1.50 (3H, s), 1.24 (3H, s), 1.10, 0.95, 0.92, 0.90, 0.79, 0.72, 0.58 (3H each, 21H, s each). MS m/z: 1297 [M+H]+. [α]D20 +13.8 (c=0.5, MeOH).

Benzyl Oleanolate 3-3′,4′,6′-Tetra-O-benzyl-β-D-glucopyranosyl-(1→2)-3′,4′-O-isopropylidene-α-L-arabinopyranoside (10) According to the reported method,25 diglycoside 10 was obtained as white crystals on chromatography (CH2Cl2:CH3OH=50:1) from 9, yield 85%. 1H-NMR (DMSO-d6) δ: 7.40 (5H, m), 5.34 (1H, d, J=4.0 Hz), 5.15 (1H, s), 5.08 (1H, d, J=12.8 Hz), 5.03 (1H, d, J=12.8 Hz), 4.98 (1H, d, J=2.0 Hz), 4.92 (2H, d, J=4.0 Hz), 4.63 (1H, d, J=4.0 Hz), 4.40 (1H, d, J=4.8 Hz), 4.35 (1H, d, J=7.6 Hz), 4.21 (1H, t, J=5.0 Hz), 3.56-3.68 (7H, m), 3.51-2.94 (6H, m), 2.75 (1H, d, J=9.2, 4.4 Hz), 1.10, 0.98, 0.88, 0.87, 0.75, 0.71 (3H each, 21H, s each). IR (KBr) cm−1: 3421, 2924, 1693, 1643, 1460, 1386, 1079. MS m/z: 881 [M+H]+

Benzyl Oleanolate 3-3′,4′,6′-O-Benzylidene-β-D-glucopyranosyl-(1→2)-3′,4′-O-isopropylidene-α-L-arabinopyranoside (11) According to the reported method,25 benzyl oleanolate 3-3′,4′,6′-O-Benzylidene-β-D-glucopyranosyl-(1→2)-3′,4′-O-isopropylidene-α-L-arabinopyranoside (11) was obtained as a white amorphous solid on chromatography (PE:EtOAc=20:1) from 10, yield 88%. 1H-NMR (CDCl3) δ: 7.34 (5H, s), 5.19 (1H, t, J=3.6 Hz), 5.09 (1H, d, J=12.4 Hz), 5.04 (1H, d, J=12.4 Hz), 4.80 (1H, d, J=4.4 Hz), 4.48 (1H, d, J=15.6, 5.4 Hz), 4.14 (1H, d, J=5.4 Hz), 3.64 (1H, dd, J=12.0, 3.2 Hz), 3.59 (1H, s), 2.91-3.08 (4H, m), 3.00 (1H, dd, J=11.6, 4.4 Hz), 2.60 (1H, dd, J=13.6, 4.0 Hz), 1.10, 0.98, 0.88, 0.87, 0.86, 0.75, 0.71 (3H each, 21H, s each). ESI-MS m/z: 679 [M+H]+.
**pyranoside (11)** According to the reported method, diglycoside 11 was obtained as white crystals on chromatography (CH$_2$Cl$_2$:CH$_3$OH=200:1) from 10, yield 92%. $^1$H-NMR (CDCl$_3$) $\delta$: 7.50 (3H, m), 7.37 (7H, m), 5.59 (1H, s), 5.29 (1H, s), 5.08 (1H, d, $J=12.8$ Hz), 5.03 (1H, d, $J=12.8$ Hz), 4.64 (1H, d, $J=7.6$ Hz), 4.43 (1H, d, $J=7.6$ Hz), 4.31 (2H, m), 4.19 (1H, t, $J=7.6$ Hz), 4.02 (1H, d, $J=13.2$, 4.0 Hz), 3.81 (1H, t, $J=9.2$ Hz), 3.78 (7H, m), 3.57 (2H, m), 3.45 (1H, m), 3.11 (1H, d, $J=11.6$, 4.4 Hz), 2.89 (1H, d, $J=10.4$ Hz), 1.55 (3H, s), 1.36 (3H, s), 1.10, 1.00, 0.91, 0.87, 0.88, 0.81, 0.60 (3H each, 21H, s each). MS $m/z$: 969 [M+H]$^+$. mp 92–94°C. $^{13}$C NMR. Anal. Calcd for C$_{36}$H$_{52}$O$_{13}$: C, 75.57; H, 8.78. Found: C, 75.68; H, 7.90.

**Benzyl Oleanolate 3-O-2",3",4",6"-Tri-O-benzyl-α-1,3-rhamnopyranosyl (1→2)-3",4",6"-O-benzyl-β-D-glucopyranosyl (1→2)-3",4",6"-O-isopropylidene-α-1,3-arabinopyranoside (23)** Diglycoside 20 was dissolved in dry CH$_2$Cl$_2$:MeOH (1:2, 18 mL), to which a newly prepared NaOMe in MeOH solution (1.0 mol/L, 0.8 mL) was added. The mixture was stirred at rt. for 2h and then neutralized with Et$_3$N resin to pH 7. The mixture was then filtered and the filtrate was concentrated to dryness to afford crude 21 as colorless oil. Triglycoside 23 was prepared from crude 21 and rhamnosyl trichloroacetimidate 22 according to the same procedure described for diglycoside 9 as white crystals on chromatography (PE:EtOAc=10:1), yield 82%. $^1$H-NMR (CDCl$_3$) $\delta$: 8.01 (2H, d, $J=7.2$ Hz), 7.92 (2H, d, $J=7.6$ Hz), 7.79 (2H, d, $J=7.2$ Hz), 7.61 (1H, m), 7.20–7.41 (22H, m), 7.09 (3H, m), 5.86 (1H, d, $J=10.0$ Hz), 5.74 (1H, d, $J=3.2$, 1.6 Hz), 5.60 (1H, t, $J=10.0$ Hz), 5.47 (2H, m), 5.26 (1H, s), 5.08 (1H, d, $J=12.4$ Hz), 5.03 (1H, d, $J=12.4$ Hz), 4.95 (1H, d, $J=10.8$ Hz), 4.90 (1H, d, $J=7.6$ Hz), 4.85 (1H, d, $J=10.8$ Hz), 4.79 (1H, d, $J=11.2$ Hz), 4.65 (3H, m), 4.55 (2H, m), 4.39 (2H, m), 4.09 (1H, t, $J=5.2$ Hz), 3.95 (1H, dd, $J=12.8$, 5.2 Hz), 3.95 (1H, dd, $J=12.8$, 5.2 Hz), 3.80 (5H, m), 3.78 (5H, m), 3.73 (1H, d, $J=12.1$ Hz). MS $m/z$: 1609 [M+H]$^+$. mp 87–90°C. Anal. Calcd for C$_{108}$H$_{152}$O$_{33}$: C, 73.86; H, 7.26. Found: C, 73.22; H, 7.08.

**Benzyl Oleanolate 3-O-2",3",4",6"-Tri-O-benzyl-α-1,3-rhamnopyranosyl (1→2)-3",4",6"-O-benzyl-β-D-glucopyranosyl (1→2)-α-1,3-arabinopyranoside (24)** According to the reported method, compound 24 was obtained as white amorphous solid from starting material 23, yield 86%. $^1$H-NMR (CDCl$_3$) $\delta$: 8.01 (4H, dd, $J=8.8$, 7.2 Hz), 7.79 (2H, d, $J=6.8$ Hz), 7.61 (1H, t, $J=7.6$ Hz), 7.20–7.51 (27H, m), 7.09 (3H, m), 5.82 (1H, dd, $J=10.4$, 3.2 Hz), 5.70 (1H, dd, $J=3.2$, 1.6 Hz), 5.62 (1H, t, $J=10.0$ Hz), 5.54 (2H, m), 5.28 (1H, s), 5.09 (1H, d, $J=12.4$ Hz), 5.04 (1H, d, $J=12.4$ Hz), 4.95 (2H, d, $J=8.0$ Hz), 4.80 (2H, t, $J=11.2$ Hz), 4.60 (5H, m), 4.15 (3H, m), 3.50–3.88 (7H, m), 3.07 (1H, d, $J=11.6$, 4.4 Hz), 2.91 (1H, d, $J=9.6$ Hz), 1.33 (3H, d, $J=6.4$ Hz), 1.11, 0.92, 0.90, 0.89, 0.83, 0.78, 0.61 (3H each, 21H, s each). IR (KBr) cm$^{-1}$: 3742, 3449, 2943, 1725, 1662, 1459, 1382, 1092. MS $m/z$: 1695 [M+H]$^+$. Anal. Calcd for C$_{107}$H$_{151}$O$_{34}$: C, 73.45; H, 7.19. Found: C, 73.06; H, 7.04.

**Benzyl Oleanolate 3-O-2",3",4",6"-Tri-O-benzyl-α-1,3-rhamnopyranosyl (1→2)-3",4",6"-O-benzyl-β-D-glucopyranosyl (1→2)-α-1,3-arabinopyranoside (25)** Compound 25 was prepared as white crystals from triglycoside 24 according to the same procedure described for diglycoside 21 form on chromatography (CH$_2$Cl$_2$:CH$_3$OH=20:1), yield 76%. $^1$H-NMR (CDCl$_3$) $\delta$: 7.26–7.36 (14H, m), 7.18 (2H, m), 5.28 (1H, m), 5.22 (1H, s), 5.19 (1H, d, $J=12.4$ Hz), 5.04 (1H, d, $J=12.8$ Hz), 4.91 (1H, m), 4.87 (1H, d, $J=7.2$ Hz), 4.76 (1H, d, $J=10.8$ Hz), 4.68 (1H, d, $J=11.2$ Hz), 4.56 (3H, m), 4.43 (1H, d, $J=7.2$ Hz), 3.98 (4H, m), 3.60–3.75 (9H, m), 3.37 (2H, m), 3.04 (1H, d, $J=11.2$, 4.0 Hz), 2.91 (1H, d, $J=10.0$ Hz), 1.27 (3H, d, $J=6.0$ Hz), 1.30, 0.91, 0.89, 0.84, 0.83, 0.74, 0.61 (3H each, 21H, s each). MS $m/z$:
1257 [M+H]⁺. mp 110–112°C. [α]D²⁰ −7.3 (c=0.5, CH₂Cl₂). Anal. Calcd for C₇₅H₁₀₀O₁₆: C, 71.63; H, 8.01. Found: C, 71.58; H, 8.06.

Oleanolate 3-O-α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside (I) The triglyco-side 25 (1.3 g, 1.0 mmol) and 10% Pd–C (0.5 g) was dispersed in acetic acid (30 mL), and stirred under H₂ at 0.8 MPa for 24h. The mixture was filtered, the filtrate concentrated to dryness, and the residue purified by column chromatography (CH₂Cl₂ : CH₂OH·H₂O : CH₃CO₂H = 40 : 10 : 1) to get the pure title compound 1 (0.8 g, 83%) as white powders. ¹H-NMR (pyridine-d₅): δ: 6.43 (1H, s), 5.46 (1H, s), 5.31 (1H, s), 5.29 (1H, s), 3.15 (1H, dd, J=11.7, 4.6Hz), 1.80 (3H, d, J=6.4Hz), 1.27, 1.19, 1.00, 0.97, 0.95, 0.94, 0.79 (3H each, 2H, s each). ¹³C-NMR (pyridine-d₅): δ: 180.5 (s), 144.6 (s), 125.0 (d),109.8 (d), 112.3 (d), 123.0 (d). IR (KBr) cm⁻¹: 3420, 2941, 1695, 1644, 1460, 1387, 1076. MS m/z: 897 [M+H]⁺, mp >250°C. [α]D²⁰ +4.6 (c=0.5, CH₂Cl₂). Anal. Calcd for C₇₅H₁₀₀O₁₆: C, 62.88; H, 8.50.

HDCAs Inhibition Assays HDCAs were extracted from HeLa nuclear. Substrate for HDCAs assays is a fluorogenic peptide (ZBoc-lys(AC)-AMC). HDACs and the tested compounds were dissolved in a 200 μL solution with assay buffer (50 mM Tris–HCl). Vorinostat (supplied from Cayman) was used as positive control. The reaction system was stirred for 10 min at 37°C, and the substrate of PTP (AG-D-gluc (Sigma G-0660), was measured spectrophotometrically at pH 6.8 and at 37°C using 0.2 units/mL of PTP. The reaction system was stirred for 10 min at room temperature, and then stopped by addition of trypsin. Fluorescence was monitored after 30 min at excitation and emission wavelengths of 355 and 460 nm respectively.

α-Glu Inhibition Assays The inhibitory activity of all samples against α-glu (Sigma G-0660), was measured spectrophotometrically at pH 6.8 and at 37°C using 0.2 units/mL enzyme in 0.67 mM sodium phosphate buffer. Voglibose was selected as the reference inhibitor. The reaction system was stirred at 37°C for 10 min, and 0.1 mol/L maltose was added. After 10 min, the reagent (200 μL) for detecting glucose was added and the optical density (OD) value in absorption at 490 nm was monitored continuously with the spectrophotometer.

PTP-1B Inhibition Assays With a spectrophotometer, the inhibitory activities of all samples against PTP-1B (recombinant protein obtained from Escherichia coli BL21 expression system) were measured at 37°C with 0.2 units/mL enzyme in a buffer (25 mM N-(2-hydroxyethyl)piperazine-N’-2-ethanesulfonic acid (HEPES), 50 mM NaCl, 2.5 mM ethylene-diaminetetraacetic acid (EDTA), 0.1% bovine serum albumin (BSA), pH 7.2). Sodium orthovanadate was used as positive control. The reaction system was stirred for 10 min at 37°C, and the substrate of PTP (p-nitrophenyl phosphate disodium hexahydrate) was added. After 30 min, 2 mol/mL Na₂CO₃ was added to terminate the reaction. The OD value was monitored continuously with a spectrophotometer at 405 nm.

DPP-IV Inhibition Assays The assay of inhibition of DPP-IV activity was determined by measuring the rate of hydrolysis of a surrogate substrate, GLY-PRO-GLY-GLY. Recombinant human DPP-IV and the tested compounds were dissolved in a 200 μL solution with 25 mM HEPES buffer (140 mM NaCl, 1% BSA, 80 mM MgCl₂). KR-62436 (supplied from Sigma) was selected as the reference inhibitor. The DPP-IV substrate was added, and reaction was allowed to proceed for 30 min at room temperature. Fluorescence was monitored at excitation and emission wavelengths of 355 and 460 nm respectively.

GLP-1R Activation Assays In vitro GLP-1R activation was measured by the fluorimetric assay as previously described. Briefly, the HEK293 cell line was transfected with GLP-1R expression plasmid and luciferase expression plasmid contained CRE response element. The cell line was cultured in 96-well plates overnight and added Dulbecco’s modified Eagle’s medium (DMEM) medium of the tested compounds. Exemata was used as a positive control. Fluorescence was monitored after 24h at excitation and emission wavelengths of 355 and 460 nm respectively.

PPRE Activation Assays In vitro PPRE activation was measured by the assay as previously described. Briefly, Luciferase was selected as the report gene. PPRE was selected as the target sequence. The HepG2 cell line was stably expressed by PPRE-Luc, and then cultured in 96-well plates overnight and added DMEM medium of the tested compounds. Pigliatone was used as a positive control. Fluorescence was monitored after 24h at excitation and emission wavelengths of 355 and 460 nm respectively.

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