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 Wang[a]

[a] *Bioengineering College, Xihua University; Chengdu 610039, P.R. China: and* [b] *West China School of Pharmacy, Sichuan University; Chengdu 610041, P.R. China.*

Received February 10, 2014; accepted May 16, 2014

First, Raddeanin A, a cytotoxic oleanane-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 ^{1}H-NMR, ^{13}C-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 oleanolic 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 oleanane-type triterpenoid saponins have been identified.[4–8] Recent studies have suggested that one of these triterpenoid saponins, Raddeanin A (**1**), 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 **1** and preliminarily evaluated its biological activities.

Results and Discussion

1 was elucidated as 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranoside oleanolic acid[13] (Fig. 1). This oleanane triglycoside bears three 1,2-*trans*-pyranosidic 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 glycoside analogues for structure–activity research (SAR) by altering monosaccharide donors. Therefore, stepwise glycosylation was adopted in our work.

As shown in Chart 1, oleanolic acid (**2**) was converted to its benzyl ester **3** by treating **2** with BnBr and K_2CO_3 in aqueous *N*,*N*-dimethylformamide (DMF) in high yield (98%).[14] The remaining oleanane 3-OH was glycosylated with *α*-L-arabinosyl trichloroacetimidate **4**[15] as the first monosaccharide unit in the presence of trimethylsilyl trifluoromethanesulfinate (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 oleanolate 3-*O*-*α*-L-arabinopyranoside **6**.[15] 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**.[15] Subsequently, during the preparation of oleanane diglycoside **9**, glucosyl trichloroacetimidate **8**[15] was detected to be completely consumed to produce 2,3,4,6-tetrabenzoyl-glucopyranoside 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 completed 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*-toluenesulfonic acid in dry DMF to give diol **11**.[16,17]

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 oleanolic triglycoside **1**. The previous study[17] has reported the 2″-OH and 3″-OH could be protected regioselectively with a *tert*-butyldimethylsilyl 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 **13**[18] with ethanol–triethylamine in the presence of tetrabutylammonium bromide. Subsquent 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 monoacetylated 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-

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: qians33@163.com

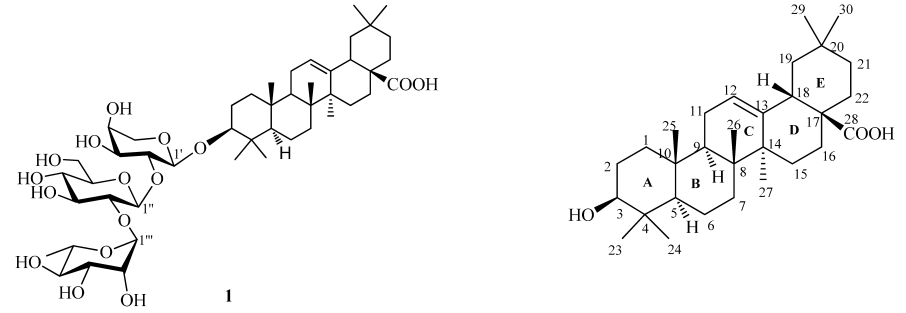
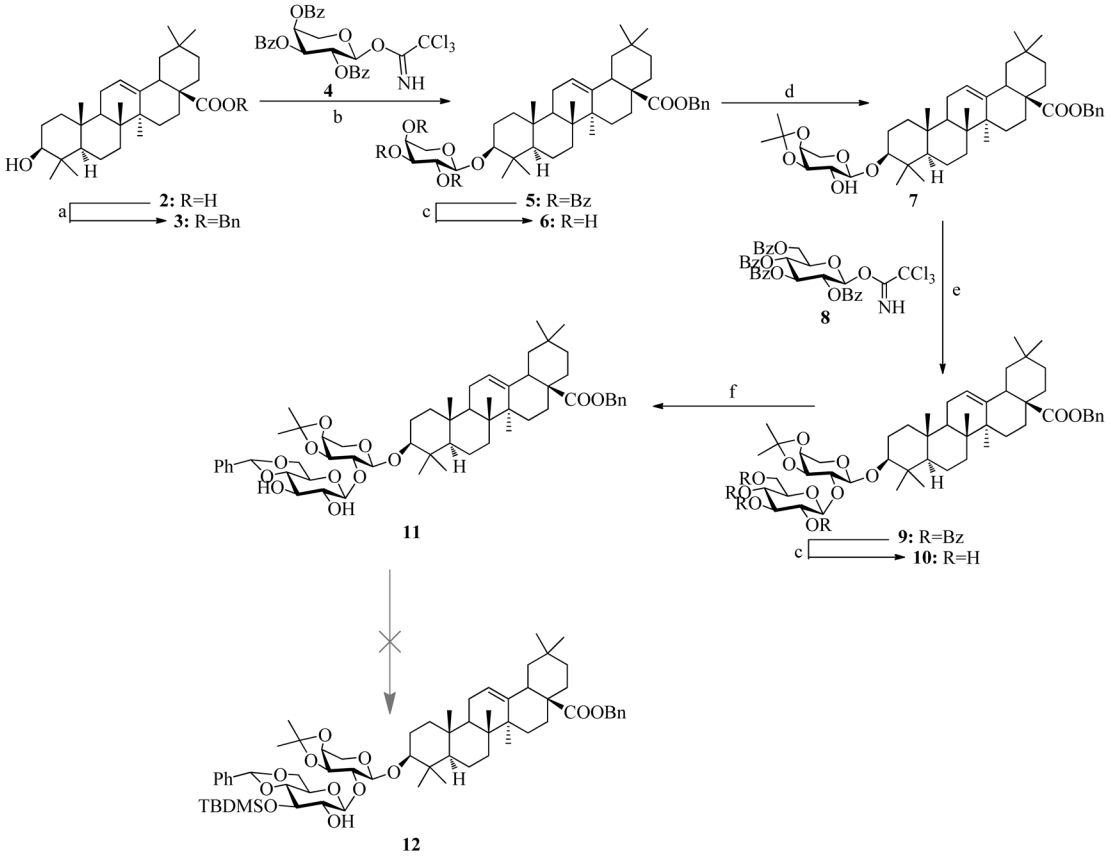
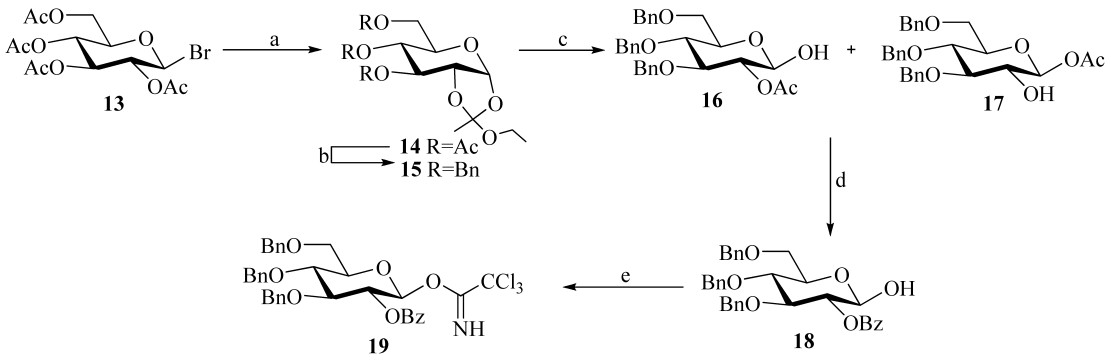


Fig. 1. Structures of Raddeanin A (**1**) and Oleanolic Acid (**2**)



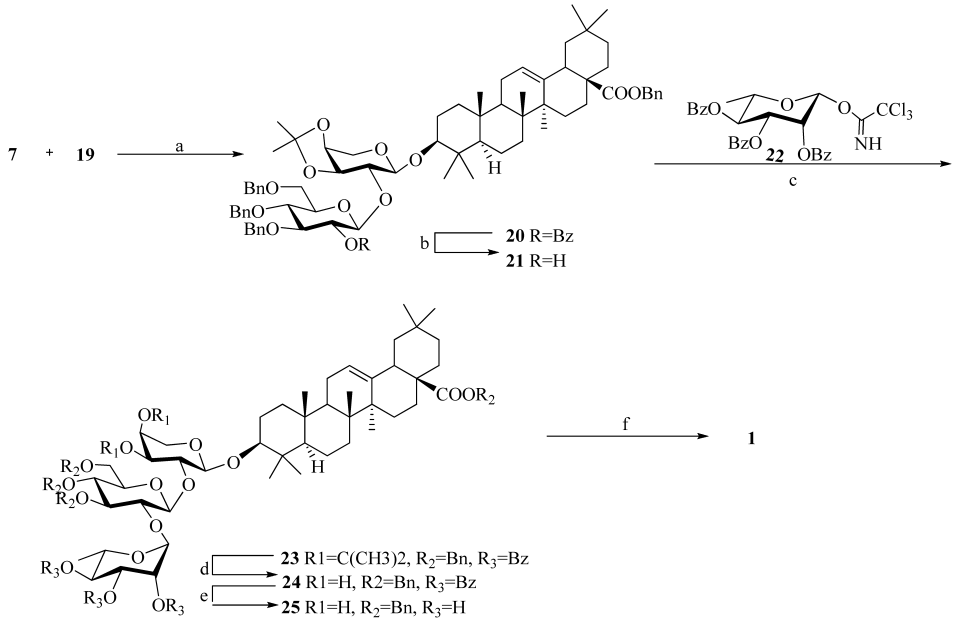
Reagents and conditions: (a) BnBr, K_2CO_3, DMF, 50°C, 3 h, 98%; (b) **4**, TMSOTf, 4 Å MS, CH_2Cl_2, −40°C, 1 h, 92%; (c) NaOMe, CH_2Cl_2–MeOH, rt., 2 h, 80%; (d) $Me_2C(OMe)_2$, TsOH, acetone, 0°C to rt., 1 h, 85%; (e) **8**, TMSOTf, CH_2Cl_2, 4 Å MS, 0°C, 1 h, 75%; (f) $PhCH(OMe)_2$, TsOH, DMF, 50°C, 30 min, 92%.

Chart 1



Reagents and conditions: (a) tetrabutylammonium bromide, ethanol–triethylamine, CH_2Cl_2, rt., 24 h, 53%; (b) BnBr–KOH, THF, 60°C, 3 h, 84%; (c) 80% HOAc, rt., 1 h, 87%; (d) i. Et_3N, CH_3OH, H_2O, rt., 3 h; ii. BzCl, py., rt., overnight; iii. CH_3NH_2, rt., 72% for three steps; (e) CCl_3CN, DBU, CH_2Cl_2, rt., 91%.

Chart 2



Reagents and conditions: (a) TMSOTf, 4 Å MS, CH_2Cl_2, 0°C, 1 h, 81%; (b) NaOMe, CH_2Cl_2–MeOH, rt., 2 h, 100%; (c) **22**, TMSOTf, 4 Å MS, CH_2Cl_2, 0°C, 1 h, 82%; (d) 80% AcOH, 70°C, 86%; (e) NaOMe, CH_2Cl_2–MeOH, rt., 2 h, 76%; (f) H_2, Pd-C (10%), MeOH, rt., 24 h, 83%.

Chart 3

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

Compound	HDACs[b]	α-Glu[c]	PTP-1B[d]	DPP-IV[e]	GLP-1R[f]	PPRE[g]
	% Inhibition[a]				% Activation[a]	
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[h]	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 0.26 μg/mL. *c*) The % inhibition of Voglibose was 91.83%, of which the concentration was 1.00 μg/mL. *d*) The % inhibition of sodium *ortho*-vanadate was 96.36%, of which the concentration was 10 μg/mL. *e*) The % inhibition of KR-62436 was 79.95%, of which the concentration was 0.30 μg/mL. *f*) The % inhibition of Exenatide was 98.95%, of which the concentration was 0.50 μg/mL. *g*) The % inhibition of Piglitazone was 95.35%, of which the concentration was 0.50 μg/mL. *h*) NA means that the datum is not available.

zoyl group has proved more favorable than the acetyl group at 2-OH, since more stable is observed.[21] 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.[15,22] Finally, this key building block **18** was directly converted to the corresponding glucosyl trichloroacetimidate **19** under the agency of CCl_3CN 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**[15] 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 ^{1}H-NMR, ^{13}C-NMR, IR, electrospray ionization (ESI)-MS and elemental analyses, and physical data are in agreement with the literature reported.[13]

The oleanolic triglycoside **1** exhibited cytotoxicity *in vitro* and was potential antitumor drug.[9–12] 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.[23–26] 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

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 with diabetes mellitus. In α-glucosidase (α-glu) inhibition assays,[27] oleanolic acid **2** exhibited low a-glucosidase inhibitory activity with an inhibition ratio of 2.92%. Benzyl oleanolate **3** was fourfold more active than the parent acid **2**. Introduction of the sugar moiety at the C-3 position of **2** and benzyl oleanolate **3** resulted in an increase in inhibitory activity. In protein tyrosine phosphatase 1B (PTP-1B) inhibition assays,[28] esterification of the C-28 carboxylic acid of **2** diminished the PTP-1B inhibitory activities, indicating that C-28 carboxylic acid groups contribute to PTP-1B inhibition. Although monoglycoside **7**, diglycoside **11** and triglycoside **25** displayed moderate activities, with an inhibition ratio of 36.58%, 48.59% and 38.82%, respectively, the title product **1** displayed disappointing PTP-1B inhibitory activity. In dipeptidyl peptidase-IV (DPP-IV) inhibition assays,[29] glucagon-like peptide-1 receptor (GLP-1R)[30] activation assays and peroxisome proliferator-activated receptor response element (PPRE)[31] activation assays, all compounds didn't exhibited satisfactory enzyme activities. Besides, the potent oleanane-type candidates of DPP-IV, GLP-1R or PPRE are rare reported during the last decade, indicating that oleanane-type saponins probably are of no worth for further study against these three proteins.

Conclusion

In summary, the oleanane-type triglycoside **1**, isolated from *Anemone raddeana* Regel, was firstly synthesized effectively by the means of stepwise glycosylation employing glycosyl trifluoroacetimidates **4**, **19** and **22** as sugar donors. **1** exhibited potent HDACs inhibitory activity *in vitro*, which might contributed to its cytotoxicity. On the basis of these results, further biological evaluation of **1** and its derivatives as promising drugs are ongoing in our laboratories, and results will be reported in due course.

Experimental

General ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian INOVA 400 or Bruker DRX-300. Chemical shifts were expressed in δ (ppm) with tetramethylsilane (TMS) as the internal reference and coupling constants (J) were expressed in *Hz*. Mass spectra were recorded on an Agilent 1946B ESI-MS instrument. IR spectra were obtained on a Perkin-Elmer 983. Elemental analyses was performed by Atlantic Microlab, Atlanta, GA. Melting point was measured on an YRT-3 melting point apparatus. Optical rotations were determined at room temperature with a Perkin-Elmer 141 polarimeter. TLC was performed using precoated silica gel GF254 (0.2 mm), and flash column chromatography was performed using silica gel (200–300 mesh). All solvents were reagent grade and, when necessary, were purified and dried by standard methods.

Benzyl Oleanolate 3-*O*-α-L-Arabinopyranoside (6) According to the reported method,[14,15] benzyl oleanolate 3-*O*-α-L-arabinopyranoside **6** was obtained as a white amorphous solid from starting material **2**, yield 72% for three steps. ^{1}H-NMR (DMSO-d_6) 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, dd, 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 $[M+H]^+$.

Benzyl Oleanolate 3-*O*-3′,4′-*O*-Isopropylidene-α-L-arabinopyranoside (7) According to the reported method,[15] benzyl oleanolate 3-*O*-3′,4′-*O*-isopropylidene-α-L-arabinopyranoside (**7**) was obtained as white amorphous solid on chromatography (PE : EA=20 : 1), from **6** yield 85% . ^{1}H-NMR ($CDCl_3$) δ: 7.34 (5H, s), 5.29 (1H, t, J=3.6 Hz), 5.09 (1H, d, J=12.8 Hz), 5.04 (1H, d, J=12.4 Hz), 4.20 (2H, m), 4.05 (1H, dd, J=7.8, 6.0 Hz), 3.75 (1H, dd, J=14.0, 3.4 Hz), 3.63 (1H, t, J=7.8 Hz), 3.12 (1H, dd, J=11.6, 4.6 Hz), 2.90 (1H, dd, J=13.8, 3.3 Hz), 1.54 (3H, s), 1.38 (3H, s), 1.12, 0.98, 0.86, 0.85, 0.84, 0.81, 0.60 (3H each, 21H, s each). MS m/z: 719 $[M+H]^+$. $[\alpha]_D^{20}$ +13.8 (c=0.5, MeOH).

Benzyl Oleanolate 3-*O*-2″,3″,4″,6″-Tetra-*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, 2.1 mmol) and powdered 4 Å molecular sieves (2.0 g) were stirred for 15 min at rt. in dry CH_2Cl_2 (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 1h before the reaction was quenched by Et_3N (0.2 mL). The suspension was then filtered and the filtrate was concentrated and subjected to a silica gel chromatography (PE : EtOAc=15 : 1) to furnish the product **9** (0.7 g, 75%) as white foam. ^{1}H-NMR ($CDCl_3$) 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, dd, J=12.4, 4.2 Hz), 4.36 (1H, d, J=6.8 Hz), 4.12 (2H, m), 3.80 (2H, m), 3.68 (1H, dd, J=12.8, 4.8 Hz), 3.00 (1H, dd, J=11.2, 4.8 Hz), 2.90 (1H, dd, 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 $[M+H]^+$. $[\alpha]_D^{20}$ +18.2 (c=0.5, MeOH). *Anal.* Calcd for $C_{79}H_{92}O_{16}$: C, 73.13; H, 7.15. Found: C, 73.02; H, 7.52.

Benzyl Oleanolate 3-*O*-β-D-Glucopyranosyl-(1→2)-3′,4′-*O*-isopropylidene-α-L-arabinopyranoside (10) According to the reported method,[14] diglycoside **10** was obtained as white crystals on chromatography (CH_2Cl_2 : CH_3OH=50 : 1) from **9**, yield 85%. ^{1}H-NMR (DMSO-d_6) 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, dd, J=9.2, 4.4 Hz), 1.10, 0.98, 0.88, 0.87, 0.86, 0.75, 0.71 (3H each, 21H, s each). IR (KBr) cm^{-1}: 3421, 2942, 1693, 1643, 1460, 1386, 1079. MS m/z: 881 $[M+H]^+$.

**Benzyl Oleanolate 3-*O*-4″,6″-*O*-Benzylidene-β-D-glucopyranosyl-(1→2)-3′,4′-*O*-isopropylidene-α-L-arabino-

pyranoside (11) According to the reported method,[16,17] diglycoside **11** was obtained as white crystals on chromatography (CH_2Cl_2 : CH_3OH=200 : 1) from **10**, yield 92%. ^{1}H-NMR ($CDCl_3$) δ: 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 (3H, m), 3.57 (2H, m), 3.45 (1H, m), 3.11 (1H, dd, 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. $[\alpha]_D^{20}$ +37.5 (c=0.5, CH_2Cl_2). *Anal*. Calcd for $C_{58}H_{80}O_{12}$: C, 71.87; H, 8.32. Found: C, 72.02; H, 8.45.

3,4,6-Tri-*O*-acetyl-D-glucosyl-1,2-*ortho*-ester (14) 2,3,4,6-Tetra-*O*-acetyl-l-bromoglucose (**13**) (2.0 g, 4.9 mmol), tetrabutylammonium bromide, (0.6 g, 0.2 mmol), triethylamine (3 mL, 19.6 mmol) and dry ethanol (2 mL, 30.0 mmol) were stirred at rt. for 24 h in dry CH_2Cl_2 (20 mL). The solution was then concentrated and subjected to a silica gel chromatography (PE : EtOAc=5 : 1) to furnish the product **14** (1.0 g, 53%) as white foam. ^{1}H-NMR ($CDCl_3$) δ: 5.71 (1H, d, J=5.2 Hz), 5.21 (1H, t, J=2.8 Hz), 4.90 (1H, m), 4.32 (1H, dd, J=5.2, 2.8 Hz), 4.20 (2H, m), 3.96 (1H, m), 3.55 (2H, q, J=7.2 Hz), 2.11, 2.10, 2.09 (3H each, 9H, s each), 1.72 (3H, s), 1.18 (3H, t, J=7.2 Hz). MS m/z: 377 $[M+H]^+$.

2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-D-glucopyranose (18) *ortho*-Ester **14** (6.2 g, 16.5 mmol) was dissolved in dry THF (40 mL), to which powdered KOH (10.2 g, 0.2 mol) and benzyl bromine (6 mL, 53.0 mmol) was added. The reaction mixture was heated under reflux for 3 h, and then cooled to room temperature. The mixture was washed with water and sat. $NaHCO_3$ and dried to get the 3,4,6-tribenzyl-1,2-glucosyl orthoester **15** (7.2 g, 84%). *ortho*-Ester **15** was dissolved in 80% HOAc (30 mL). After stirred at rt. for 1 h, this solution was diluted with CH_2Cl_2, and washed with water and sat. $NaHCO_3$ and dried to get the mixture of monoacetylated intermediates **16** and **17** in the ratio of 1 : 3. According to the reported method,[15,22] the product **18** was obtained as white crystals on chromatography (CH_2Cl_2 : CH_3OH=5 : 1) from the mixture of **16** and **17** in a one-pot procedure by a rapid sequence of three reactions, without any purification of intermediates, yield 72% for three steps. ^{1}H-NMR ($CDCl_3$) δ: 7.96–7.20 (20H, m), 5.38 (1H, d, J=10.0 Hz), 4.82 (3H, m), 4.65 (1H, d, J=12.0 Hz), 4.58 (1H, m), 4.52 (1H, d, J=12.0 Hz), 4.26 (1H, d, J=9.6 Hz), 4.07 (2H, m), 3.96 (1H, d, J=9.6 Hz), 3.84 (1H, d, J=11.2 Hz), 3.73 (1H, d, J=11.2 Hz). MS m/z: 555 $[M+H]^+$.

Benzyl Oleanolate 3-*O*-2″-Benzoyl-3″,4″,6″-tri-*O*-benzyl-*β*-D-glucopyranosyl-(1→2)-3′,4′-*O*-isopropylidene-*α*-L-arabinopyranoside (20) According to the reported method,[15] 2-benzoyl-3,4,6-tri-*O*-benzyl-glucosyl trichloroacetimidate **19** was obtained as white amorphous solid from **18**, yield 91%. Compound **20** was prepared from benzyl ester **7** and glucosyl trichloroacetimidate **19** according to the same procedure described for diglycoside **9**, yield 81%. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (2H, m), 7.55 (1H, dt, J=7.2, 1.6 Hz), 7.43 (2H, t, J=7.6 Hz), 7.18–7.39 (20H, m), 5.27 (1H, t, J=3.6 Hz), 5.23 (1H, d, J=8.4 Hz), 5.09 (1H, d, J=12.4 Hz), 5.04 (1H, d, J=12.4 Hz), 4.94 (1H, d, J=4.0 Hz), 4.82 (1H, d, J=10.8 Hz), 4.73 (1H, d, J=11.6 Hz), 4.63 (3H, d, J=11.6 Hz), 4.53 (1H, d, J=12.0 Hz), 4.35 (1H, d, J=6.0 Hz), 4.06 (1H, dd, J=11.2, 4.4 Hz), 3.78–3.92 (6H, m), 3.62 (1H, dd, J=12.8, 4.4 Hz), 3.51 (1H, d, J=7.2 Hz), 3.00 (1H, dd, J=11.2, 4.4 Hz), 2.90 (1H, dd, J=14.4, 3.6 Hz), 1.40 (3H, s), 1.25 (3H, s), 1.11, 1.02, 0.91, 0.89, 0.84, 0.79, 0.64 (3H each, 21H, s each). MS m/z: 1255 $[M+H]^+$. *Anal*. Calcd for $C_{79}H_{98}O_{13}$: C, 75.57; H, 7.87. Found: C, 75.68; H, 7.90.

Benzyl Oleanolate 3-*O*-2‴,3‴,4‴-Tri-*O*-benzoyl-*α*-L-rhamnopyranosyl-(1→2)-3″,4″,6″-*O*-benzyl-*β*-D-glucopyranosyl-(1→2)-3′,4′-*O*-isopropylidene-*α*-L-arabinopyranoside (23) Diglycoside **20** was dissolved in dry CH_2Cl_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 2 h and then neutralized with Et_3N 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 ($CDCl_3$) δ: 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, dd, 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), 3.60–3.85 (5H, m), 3.47 (1H, d, J=9.2 Hz), 2.98 (1H, dd, J=11.2, 4.4 Hz), 2.89 (1H, d, J=10.0 Hz), 1.50 (3H, s), 1.42 (3H, s), 1.31 (3H, d, J=6.0 Hz), 1.11, 0.96, 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: 1609 $[M+H]^+$. mp 87–90°C. *Anal*. Calcd for $C_{99}H_{116}O_{19}$: C, 73.86; H, 7.26. Found: C, 73.22; H, 7.08.

Benzyl Oleanolate 3-*O*-2‴,3‴,4‴-Tri-*O*-benzoyl-*α*-L-rhamnopyranosyl-(1→2)-3″,4″,6″-tri-*O*-benzyl-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranoside (24) According to the reported method,[15] compound **24** was obtained as white amorphous solid from starting material **23**, yield 86%. ^{1}H-NMR ($CDCl_3$) δ: 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, dd, 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, 0.87, 0.79, 0.59 (3H each, 21H, s each). MS m/z: 1569 $[M+H]^+$. *Anal*. Calcd for $C_{96}H_{112}O_{19}$: C, 73.45; H, 7.19. Found: C, 73.06; H, 7.04.

Benzyl Oleanolate 3-*O*-*α*-L-Rhamnopyranosyl-(1→2)-3″,4″,6″-tri-*O*-benzyl-*β*-D-glucopyranosyl-(1→2)-*α*-L-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_2Cl_2 : CH_3OH=20 : 1), yield 76%. ^{1}H-NMR ($CDCl_3$) δ: 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. $[\alpha]_D^{20}$ −7.3 (c=0.5, CH_2Cl_2). *Anal*. Calcd for $C_{75}H_{100}O_{16}$: 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 (1) The triglycoside **25** (1.3 g, 1.0 mmol) and 10% Pd–C (0.5 g) was dispersed in acetic acid (30 mL), and stirred under H_2 at 0.8 MPa for 24h. The mixture was filtered, the filtrate concentrated to dryness, and the residue purified by column chromatography (CH_2Cl_2 : CH_3OH : H_2O=40 : 10 : 1) to get the pure title compound **1** (0.8 g, 83%) as white powders. ^{1}H-NMR (pyridine-d_5) δ: 6.43 (1H, s), 5.46 (1H, s), 5.31 (1H, s), 5.29 (1H, s), 3.15 (1H, dd, J=11.7, 4.6 Hz), 1.80 (3H, d, J=6.4 Hz), 1.27, 1.19, 1.00, 0.97, 0.95, 0.94, 0.79 (3H each, 21H, s each). ^{13}C-NMR (pyridine-d_5) 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. $[\alpha]_D^{20}$ +4.6 (c=0.5, CH_2Cl_2). *Anal*. Calcd for $C_{47}H_{76}O_{16}$: C, 62.93; H, 8.54. Found: C, 62.88; H, 8.50.

HDACs Inhibition Assays HDACs were extracted from HeLa nuclear. Substrate for HDACs 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 a positive control. Fluorogenic substrate (25 μL) was added, and reaction was allowed to proceed for 30 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 mL) 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 ethylenediaminetetraacetic 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 Na_2CO_3 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 $MgCl_2$). 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.[30] 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. Exenatide 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.[31] 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. Pigliazone was used as a positive control. Fluorescence was monitored after 24 h at excitation and emission wavelengths of 355 and 460 nm, respectively.

Acknowledgments This work was supported by the National Natural Science Foundation for the Youth of China (81302647), the Chunhui Project of Ministry of Education (z2011097) and the Scientific Research Fund of Sichuan Provincial Science and Technology Department. We also thank the drug screening center of Di'Ao Pharmaceutical Group for the enzymatic activity assays.

References

1) Sun Y. X., Liu J. C., Liu D. Y., *Pharmazie*, **66**, 813–821 (2011).
2) Wu F. E., Koike K., Ohmoto T., Chen W. X., *Chem. Pharm. Bull.*, **37**, 2445–2447 (1989).
3) Lu J. C., Xu B. B., Zhang X. Y., Xin Y., Sun Q. S., *Yao Xue Xue Bao*, **37**, 709–712 (2002).
4) Wang X., Liu D. Y., Xia Z. T., *Chinese J. Anal. Chem.*, **32**, 587–592 (2004).
5) Xia Z. T., Liu D. Y., Wang X. Y., Liu K. F., Zhang P. C., *Acta Chimica Sinica*, **62**, 1935–1940 (2004).
6) Xia Z. T., Liu D. Y., Wang X. Y., *Chemical Journal of Chinese Universities*, **25**, 2057–2059 (2004).
7) Fan L., Lu J. C., Xu B. B., Gao S., Zhang H. F., Liu R. X., *Helv. Chim. Acta*, **93**, 58–64 (2010).
8) Li F., Sun C. R., Chen B., Ding L. S., Wang M. K., *Phytochem. Lett.*, **5**, 258–261 (2012).
9) Wang M. K., Ding L. S., Wu F. E., *Chinese Journal of Cancer*, **27**, 910–913 (2008).
10) Lin X. Q., Li D. L., Yang C. S., He X. H., Fang J., Li D. L., *Drugs & Clinic*, **24**, 49–51 (2009).
11) Gao Y. R., An J. X., Zhu Q. Y., Li P., Ma C. Y., Wnag M. K., Tang Y. X., *Chinese Journal of Applied and Environmental Biology*, **16**, 637–641 (2010).
12) Xue G., Zou X., Zhou J. Y., Sun W., Wu J., Xu J. L., Wang R. P., *Biochem. Biophys. Res. Commun.*, **439**, 196–202 (2013).
13) Wu F. E., Zhu Z. Q., *Acta Chimica Sinica*, **42**, 253–258 (1984).
14) Qian S., Li J. H., Chen Y., Zhang W. Y., Yang S. Y., Wu Y., *J. Nat. Prod.*, **73**, 1743–1750 (2010).
15) Qian S., Li J. H., Zhang Y. W., Chen X., Wu Y., *J. Asian Nat. Prod. Res.*, **12**, 20–29 (2010).
16) Pei S. C., Wu J. B., Lei F., Qian S., Hai L., Wu Y., *Chin. Chem. Lett.*, **23**, 403–406 (2012).
17) Li C., Yu B., Liu M., Hui Y., *Carbohydr. Res.*, **306**, 189–195 (1998).

18) Lichtenthaler F. W., Schneider-Adams T., *J. Org. Chem.*, **59**, 6728–6734 (1994).
19) Adinolfi M., Iadonisi A., Ravida A., Schiattarella M., *Tetrahedron Lett.*, **44**, 7863–7866 (2003).
20) Zhu C., Peng W., Li Y., Han X., Yu B., *Carbohydr. Res.*, **341**, 1047–1051 (2006).
21) Paulsen H., *Angew. Chem. Int. Ed. Engl.*, **21**, 155–173 (1982).
22) Zhu S., Li Y., Yu B., *J. Org. Chem.*, **73**, 4978–4985 (2008).
23) Zhang L., Lei J., Shan Y., Yang H., Song M., Ma Y., *Mini Rev. Med. Chem.*, **13**, 1999–2013 (2013).
24) Bolden J. E., Peart M. J., Johnstone R. W., *Nat. Rev. Drug Discov.*, **5**, 769–784 (2006).
25) Minucci S., Pelicci P. G., *Nat. Rev. Cancer*, **6**, 38–51 (2006).
26) Xu W. S., Parmigiani R. B., Marks P. A., *Oncogene*, **26**, 5541–5552 (2007).
27) Krentz A. J., Bailey C. J., *Drugs*, **65**, 385–411 (2005).
28) Pei Z., Liu G., Lubben T. H., Szczepankiewicz B. G., *Curr. Pharm. Des.*, **10**, 3481–3504 (2004).
29) Drucker D. J., Nauck M. A., *Lancet*, **368**, 1696–1705 (2006).
30) Madsbad S., *Diabetes Obes. Metab.*, **16**, 9–21 (2014).
31) Guilherme A., Virbasius J. V., Puri V., Czech M. P., *Nat. Rev. Mol. Cell Biol.*, **9**, 367–377 (2008).