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ORIGINAL ARTICLE

Synthesis and α -glucosidase inhibitory activity of oleanolic acid derivatives

Shan Qian^{a1}, Jiao Hai Li^{a2}, Yu Wei Zhang^{b3}, Xin Chen^{b4} and Yong Wu^{a*}

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Glucosidations of oleanolic acid (1) and its dihydroxy-olide derivatives (2) were carried out to provide eight glycosides. All synthesized compounds were evaluated by *in vitro* α -glucosidase inhibitory activity assay. 3-Acetyl dihydroxy-olide oleanolic derivative 15 showed the most potent inhibitory effect. Structure–activity relationships within these compounds are discussed.

Keywords: oleanolic acid; glycosides; α -glucosidase; inhibition ratio

1. Introduction

Pentacyclic triterpenoids are widely distributed in plants, and they have attracted much attention due to their broad biological activities. The most well-known member of this family of compounds is probably oleanolic acid (OA, 1; Figure 1), which has been in active clinical use as an anti-hepatitis drug in China for over 20 years, and possesses some attractive biological activities including protection of the liver against toxic injury [1], anti-inflammation [2], anti-HIV [3], anti-tumor [4], anti-hyperglycemia [5], etc. However, it is well known that pentacyclic triterpenes including OA have very poor H₂O solubility and thus have poor bioavailability. Given the significant biological importance and potentially new clinical utilities of OA as a promising modulator of glycogen metabolism, it is highly desirable to synthesize and biologically evaluate

new OA derivatives in order to find more potent therapeutic agents with better bioavailability [6,7].

α -Glucosidase is a membrane-bound enzyme at the epithelium of the small intestine, which hydrolyses the cleavage of glucose from disaccharides and oligosaccharides. The inhibitors of this enzyme delay carbohydrate digestion, prolong the overall carbohydrate digestion time, and thus cause a reduction in the rate of glucose absorption and lower the post-prandial rise in blood glucose. Therefore, inhibition of α -glucosidase is considered important in managing non-insulin-dependent diabetes [8].

Previously, Ali *et al.* [9] reported the synthesis and biological evaluation of some oleanolic derivatives as α -glucosidase inhibitors. Among the tested samples, dihydroxy-olide derivatives (2; Figure 1) were found to be the most active inhibitor

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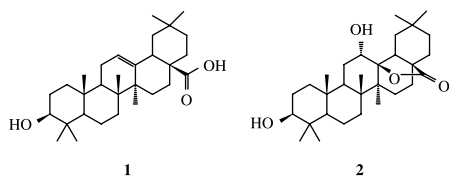


Figure 1. Structures of compounds **1** and **2**.

of α -glucosidase. On the other hand, the activity of saponins, which are glycosides of terpenoid, is well known, and strongly depends on the linkage between the glucose units and on their configurations [10]. Here, we report, for the first time, the synthesis of glycosides of **2** by conjugating **2** with sugar moieties for investigating whether the influence of the carbohydrate residue is comparable. As a result of our interest in chemical studies on the glycosidic constituents of oleanolic derivatives, we synthesized a series of OA glycosides by introducing different sugar moieties in C-3, and their inhibitor activities against α -glucosidase were evaluated *in vitro* to study their structure–activity relationships.

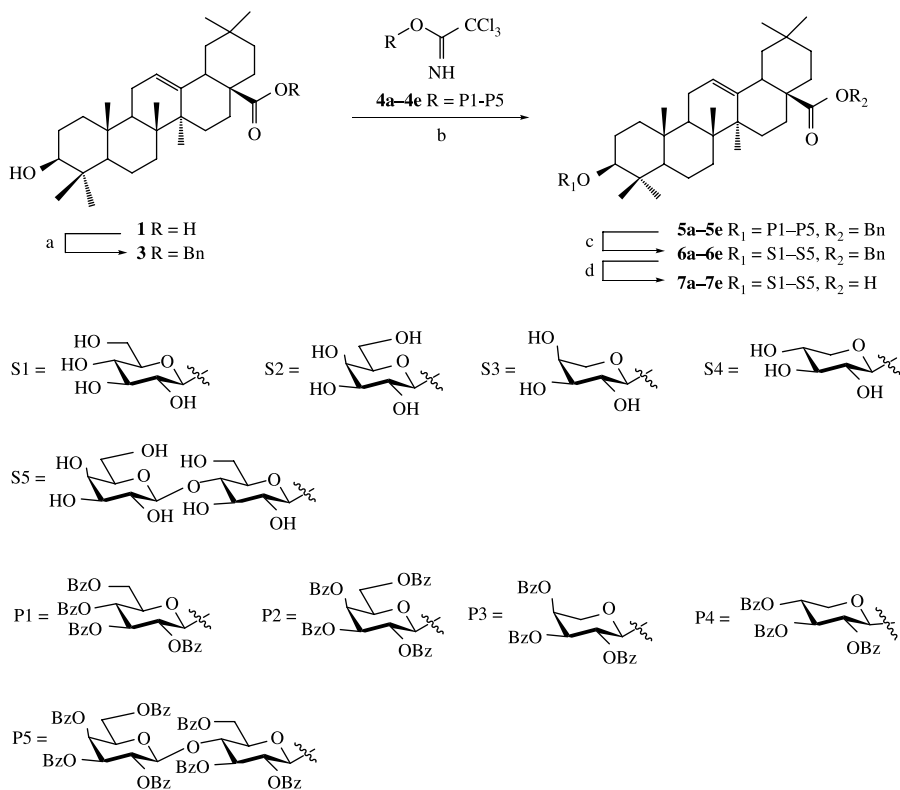
2. Results and discussion

Five 3-glycosides of **1** bearing different natural monosaccharides and disaccharides were easily prepared in four steps from OA (**1**), as described in the literature [11]. First, **1** was reacted with BnBr and K_2CO_3 in dry DMF, which gave benzyl oleanate (**3**) in a high yield. Esterification of **1** was followed by glycosylation with trichloroacetimidates (**4**) under promotion with trimethylsilyl trifluoromethanesulfonate (TMSOTf). Consequently, removal of benzoyl on **5** through ester exchange in NaOMe–MeOH afforded glycosides **6**. The benzyl moiety was then removed under catalytic hydrogenolysis to give free carboxylic acids **7** (Scheme 1).

In order to investigate whether the influence of the carbohydrate residue of oleanane saponins is comparable, we used the disaccharides, β -D-glucopyranosyl-

(1 \rightarrow 2)- α -L-arabinopyranosyl moiety, as glycosyl donors. So far, no α -glucosidase inhibitory activity evaluation toward OA glycoside bearing this disaccharide has been reported. As described by Cheng *et al.* [12], selective shelter of hydroxyl groups at C-3 and C-4 of the arabinose residue on **8** was successfully carried out using 2,2-dimethoxypropane. Coupling of **8** and **4a** promoted by TMSOTf gave glycoside **9**. Et_3N was added after glycosylation to neutralize the Lewis acid, and an additional step of deprotection was performed to provide intermediate **10**, followed by removal of the benzoyl group in NaOMe–MeOH to afford **11**. Finally, removal of the benzyl group through catalytic hydrogenation afforded **12** (Scheme 2).

Treatment of **1** with ozone produced dihydroxy-olide derivatives **2** [9] (Scheme 3). As shown in the 1H NMR spectrum, the formation of **2** could be explained by the resonances of an olide-type bridge between C-13 and C-28. Subsequently, we planned to introduce sugar moieties in C-3 and C-12 of **2** by glycosylation with trichloroacetimidates (**4a**) under promotion with TMSOTf. Initially, we failed to obtain the target product **13**. Instead of **13**, trichloroacetimidates **4a** were unexpectedly transferred to 2,3,4,6-benzoyl-glucopyranose under effectiveness of Lewis acid. We were faced with the problem of glycosylation of hindered C-3 and C-12 positions on **2**. We have probed different glycosylation methods in the experiment. AgOTf, Ag_2CO_3 , etc. have been employed to promote the glycosylation. Finally, hydroxyls of **2** were glycosylated successfully under promotion with TMSOTf. In a typical experiment, **2** and excess of trichloroacetimidates (**4a**) were dissolved in absolute CH_2Cl_2 in the presence of 4 Å molecular sieves. The vigorously stirred mixture was treated at $-40^\circ C$ with TMSOTf added dropwise. After standing overnight, the reaction was complete and the product was obtained in a satisfactory yield.

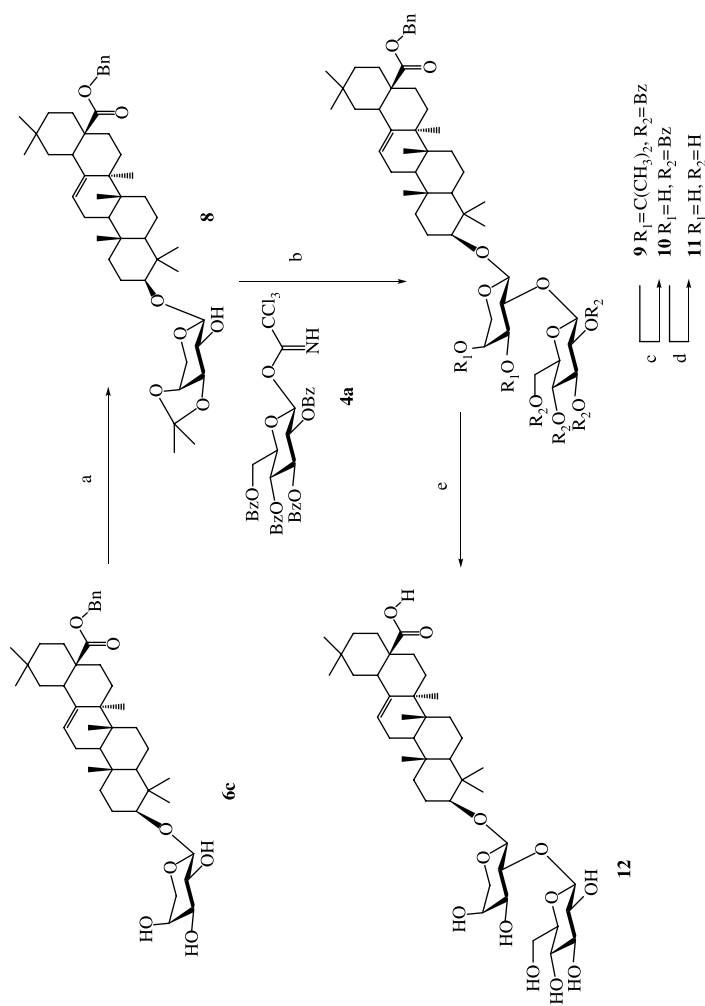


Scheme 1. The synthetic route of **7a–7e**. Reagents and conditions: (a) BnBr, K_2CO_3 , DMF, $50^\circ C$, 97%; (b) TMSOTf, 4 Å MS, CH_2Cl_2 , $-40^\circ C$; (c) NaOMe, CH_2Cl_2 –MeOH, r.t., 75–80% for two steps; (d) H_2 , Pd/C (10%), MeOH, r.t., 90%.

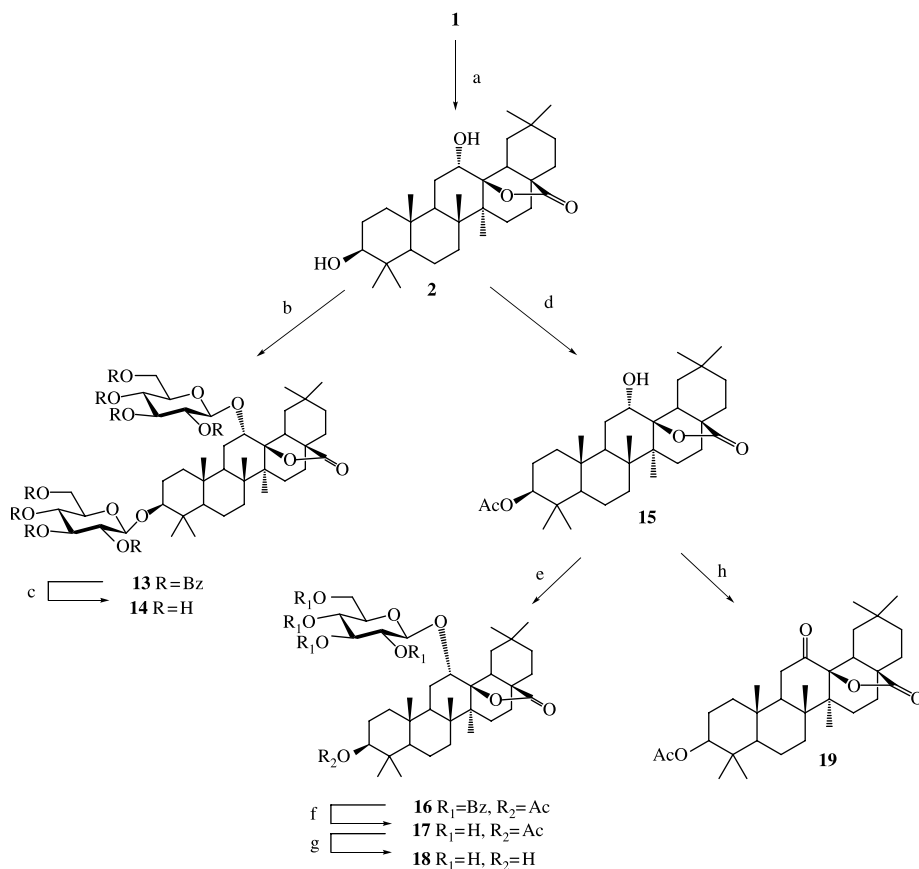
Removal of benzoyl on **13** through ester exchange in NaOMe–MeOH afforded target glycoside **14**. Compound **2** containing two hydroxyl functions introduced acetyl in C-3 selectively with acetic anhydride in pyridine to obtain **15** [9]. Compound **15** was glycosylated with trichloroacetimidates **4a** under promotion with TMSOTf to get **16**. All the benzoyl groups on the sugar parts were removed by NaOMe–MeOH, while the acetyl linkage was not affected under the condition. Compound **17** was hydrolyzed under the effectiveness of NaOH to afford **18**. The configuration of the glucosidic bond was determined from the 1H NMR spectra of the products. The stereochemistry of the glycosides was assigned on the basis of coupling constants. The coupling constants of H-1' proton ($J_{1',2'}$) in products **14** and **18**

(6.0–8.0 Hz) indicated the β -glucosidic bond junction between pyranose **4a** and A ring of **2**. The hydroxyl on C-12 of **2** was converted into ketonic functions **19** by treating with Jones reagent [9]. The derivatization of **2** into derivatives **13–19** is summarized in Scheme 3, and the glycosides **13**, **14**, **16–18** were the new compounds.

All the derivatives of OA were screened for their *in vitro* α -glucosidase inhibitory activity for the determination of inhibition ratio (%). The inhibition ratio of the compounds is reported in Table 1. The known active compound against α -glucosidase, voglibose, was included in the assays as controls and reference points. Its inhibition ratio is 92.63% (1 $\mu g/ml$).



Scheme 2. The synthetic route of **12**. Reagents and conditions: (a) $Me_2C(OMe)_2, TsOH$, acetone, $0^\circ C$ to r.t., 85%; (b) **4a**, TMSOTf, $CH_2Cl_2, 4 \text{ \AA MS}$, $-40^\circ C$, 75%; (c) $TsOH, CH_2Cl_2-MeOH$, r.t., 90%; (d) $NaOMe, CH_2Cl_2-MeOH$, r.t.; (e) $Pd/C, H_2, EtOAc$, r.t., 82% for two steps.



Scheme 3. The synthetic route of **13–19**. Reagents and conditions: (a) O₃, CH₂Cl₂, –70°C, 62%; (b) **4a**, TMSOTf, CH₂Cl₂, 4 Å MS, –40°C, 57%; (c) NaOMe, CH₂Cl₂–MeOH, r.t., 85%; (d) Ac₂O, pyridine, 0°C, 64%; (e) **4a**, TMSOTf, CH₂Cl₂, 4 Å MS, –40°C; (f) NaOMe, CH₂Cl₂–MeOH, r.t., 41% for two steps; (g) NaOH, THF–MeOH, 67%; (h) 3 mol/l Jones reagent, 0°C, 92%.

OA (**1**) exhibited low α -glucosidase inhibitory activity with an inhibition ratio of 2.92%. Benzyl oleanolate (**3**) was fourfold more active than the parent acid **1**. Introduction of the sugar moiety at the C-3 position of OA (**1**) and benzyl oleanolate (**3**) resulted in an increase in inhibitory activity of the parent triterpenes. On the other hand, the corresponding glycosides of **1** and glycosides of **3** exhibited similar inhibitory activity. 3-(β -D-Glucopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosyl) benzyl oleanolate (**11**) showed the highest activity of OA glycosides, with an inhibition ratio of 15.77%.

In the derivatives of dihydroxy-oleanolide (**2**), almost all compounds exhibited α -glucosidase inhibitory activity. Compound **2** showed higher activity than the parent acid **1**, with an inhibition ratio of 11.92%. The corresponding acetate **15** showed the highest activity, with an inhibition ratio of 17.50%. The results indicated that acetylation of the hydroxyl group of **2** resulted in an increase in activity. Incorporation of the sugar moiety at the C-3 and C-12 positions of **2** and at the C-12 position of acetate **15** resulted in a slight increase in α -glucosidase inhibitory potency. The ketonic derivative **19** showed less

Table 1. α -Glucosidase inhibitory activity (*in vitro*) of the derivatives of OA.

Compound	Inhibition (%)	Compound	Inhibition (%)
1	2.92	7d	14.71
2	11.92	7e	14.38
3	10.82	11	15.77
6a	13.33	12	12.32
6b	8.97	13	13.92
6c	10.48	14	16.8
6d	15.11	15	17.5
6e	15.18	16	15.83
7a	11.35	17	17.17
7b	15.49	18	15.79
7c	9.04	19	14.07

Notes: The concentrations of all the determined compounds were 5 μ g/ml. The standard drug, voglibose, showed an inhibition ratio of 92.63% (1 μ g/ml).

activity than the parent compound **15**, indicating a preference for hydrophilic groups over hydrophobic groups at C-12. Further biological evaluation of these oleanolic derivatives and extensive lead optimizations are ongoing in our laboratory, and more results will be reported in due time.

3. Experimental

3.1 General experimental procedures

TLC was performed using precoated silica gel GF254 (0.2 mm; Chemical Industry Institute, Yantai, China), and column chromatography was performed using silica gel (100–200 mesh; Haiyang Chemical Co., Ltd, Qingdao, China). Melting points were observed in an open capillary tube and are uncorrected. Optical rotations were determined on a Perkin-Elmer model 241 polarimeter. IR spectra were obtained on a Perkin-Elmer 983. Elemental analyses were performed by Atlantic Microlab (Atlanta, GA, USA). NMR spectra were taken on a Varian INOVA 400 and on a Bruker AC-E200. Chemical shifts (δ) are expressed in ppm, tetramethylsilane (TMS) used as the internal reference, and coupling constants

(*J*) are expressed in Hz. Mass spectra were recorded on an Agilent 1946B ESI-MS instrument.

3.2 General procedures for the synthetic compounds

3.2.1 Oleanolic acid

3-O- β -D-glucopyranoside (**7a**)

According to the reported method [11], 3-O- β -D-glucopyranoside benzyl oleanolate (**6a**) was obtained as a white amorphous solid from starting material **1**, yield 72% for three steps. Mp 152°C; $[\alpha]_D^{20} = +17.1$ (*c* = 0.5, methanol); IR (KBr) ν_{\max} : 3418, 2945, 1727, 1668, 1459, 1366, 1161, 1078 cm^{-1} ; ^1H NMR (CDCl_3): δ 7.38 (s, 5H), 5.30 (s, 1H), 5.10 (d, *J* = 12.4 Hz, 1H), 5.04 (d, *J* = 12.4 Hz, 1H), 4.33 (d, *J* = 6.8 Hz, 1H), 3.80 (s, 2H), 3.48–3.64 (m, 5H), 3.10–3.40 (m, 3H), 2.90 (d, *J* = 12.8 Hz, 1H), 1.13 (s, 3H), 0.99 (s, 3H), 0.89–0.91 (s each, 3H each, 9H), 0.80 (s, 3H), 0.64 (s, 3H); ESI-MS *m/z*: 709 $[\text{M}+\text{H}]^+$. Compound **7a** was obtained as a white amorphous solid from **6a** according to the reported method [11], yield 90%. Mp 240–241°C; $[\alpha]_D^{20} = +21.3$ (*c* = 0.5, methanol). IR (KBr) ν_{\max} : 3422, 2945, 2653, 1694, 1647, 1462, 1387, 1364, 1077, 1022 cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$): δ 12.00 (br, 1H), 5.19 (s, 1H), 4.90 (m, 3H), 4.38 (t, *J* = 5.4 Hz, 1H), 4.13 (d, *J* = 7.6 Hz, 2H), 3.64 (dd, *J* = 11.2 Hz, 4.6, 1H), 3.06–3.36 (m, 5H), 2.72 (d, *J* = 10.0 Hz, 1H), 1.09 (s, 3H), 0.98 (s, 3H), 0.89–0.91 (s each, 3H each, 9H), 0.75 (s, 3H), 0.70 (s, 3H); ESI-MS *m/z*: 620 $[\text{M}+\text{H}]^+$.

Compounds **7b–7e** were prepared according to the same procedure described for **7a**.

3.2.1.1 Oleanolic acid 3-O- β -D-galactopyranoside (**7b**). Mp 235–236°C; $[\alpha]_D^{20} = +19.0$ (*c* = 0.5, methanol); ^1H NMR ($\text{DMSO}-d_6$): δ 12.00 (s, 1H), 5.15 (s, 1H), 4.75–4.29 (br, 2H), 4.10 (d,

$J = 6.4$ Hz, 1H), 3.60–3.20 (m, 8H), 3.03 (d, $J = 7.6$ Hz, 1H), 2.73 (d, $J = 12.8$ Hz, 1H), 1.09 (s, 3H), 0.98 (s, 3H), 0.89–0.91 (s each, 3H each, 9H), 0.75 (s, 3H), 0.70 (s, 3H); ESI-MS m/z : 619 [M+H]⁺.

3.2.1.2 Oleanolic acid 3-O-β-D-arabinopyranoside (7c). Mp 236–238°C; $[\alpha]_D^{20} = +18.7$ ($c = 0.5$, methanol); ¹H NMR (DMSO-*d*₆): δ 12.0 (br, 1H), 5.19 (s, 1H), 4.90 (br, 1H), 4.18 (d, $J = 7.6$ Hz, 1H), 3.63 (dd, $J = 11.2, 5.4$ Hz, 1H), 2.91–3.08 (m, 5H), 2.60 (dd, $J = 13.6, 4.0$ Hz, 1H), 1.09 (s, 3H), 0.98 (s, 3H), 0.89–0.91 (s each, 3H each, 9H), 0.75 (s, 3H), 0.70 (s, 3H); ESI-MS m/z : 590 [M+H]⁺.

3.2.1.3 Oleanolic acid 3-O-β-D-glucopyranoside (7d). Mp 238–239°C; $[\alpha]_D^{20} = +18.6$ ($c = 0.5$, methanol); ¹H NMR (DMSO-*d*₆): δ 5.19 (s, 1H), 4.62 (d, $J = 6.4$ Hz, 1H), 4.20 (dd, $J = 10.8, 4.6$ Hz, 1H), 4.15 (m, 2H), 2.89 (m, 1H), 3.54 (m, 1H), 3.21 (d, $J = 10.0$ Hz, 1H), 2.98 (dd, $J = 11.2, 3.8$ Hz, 1H), 1.09 (s, 3H), 0.98 (s, 3H), 0.89–0.91 (s each, 3H each, 9H), 0.75 (s, 3H), 0.70 (s, 3H); ESI-MS m/z : 589 [M+H]⁺.

3.2.1.4 Oleanolic acid 3-O-β-D-glucopyranoside (7e). Mp 220–221°C; $[\alpha]_D^{20} = +14.9$ ($c = 0.6$, methanol); ¹H NMR (DMSO-*d*₆): δ 5.18 (s, 1H), 5.10 (d, $J = 4.0$ Hz, 1H), 5.05 (d, $J = 4.4$ Hz, 1H), 4.80 (d, $J = 4.8$ Hz, 1H), 4.64–4.68 (m, 2H), 4.53 (d, $J = 4.4$ Hz, 1H), 4.46 (t, $J = 6.0$ Hz, 1H), 4.18–4.22 (m, 2H), 3.72 (dd, $J = 11.2, 5.0$ Hz, 1H), 3.40–3.60 (m, 4H), 3.14 (m, 1H), 2.78 (m, 1H), 1.09 (s, 3H), 0.98 (s, 3H), 0.89–0.91 (s each, 3H each, 9H), 0.75 (s, 3H), 0.70 (s, 3H); ESI-MS m/z : 781 [M+H]⁺.

The spectral data of **7a–7e** are similar to those reported in the literature [11].

3.2.2 Benzyl oleanolate 3-O-3,4-O-isopropylidene-α-L-arabinopyranoside (8)

According to the reported method [12], **8** was obtained as a white foam from **6c**, yield 85%. $[\alpha]_D^{20} = +13.8$ ($c = 0.5$, methanol); ¹H NMR (400 MHz, CDCl₃): δ 7.34 (s, 5H), 5.29 (t, $J = 3.6$ Hz, 1H), 5.09 (d, $J = 12.8$ Hz, 1H), 5.04 (d, $J = 12.4$ Hz, 1H), 4.20 (m, 3H), 4.05 (dd, $J = 7.8, 6.0$ Hz, 1H), 3.75 (dd, $J = 14.0, 3.4$ Hz, 1H), 3.63 (t, $J = 7.8$ Hz, 1H), 3.12 (dd, $J = 11.6, 4.6$ Hz, 1H), 2.90 (dd, $J = 13.8, 3.3$ Hz, 1H), 1.54, 1.38, 1.12, 0.98, 0.86, 0.85, 0.84, 0.81, 0.60 (s each, 3H each); ESI-MS m/z : 719 [M+H]⁺. The spectral data of **8** are similar to those reported in the literature [12].

3.2.3 Benzyl oleanolate 3-O-2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl-(1 → 2)-3,4-O-isopropylidene-α-L-arabinopyranoside (9)

Compound **9** was prepared from **8** according to the same procedure described for **7a**, yield 75%. $[\alpha]_D^{20} = +18.2$ ($c = 0.5$, methanol); ¹H NMR (400 MHz, CDCl₃): δ 8.03–7.28 (m, 25H), 5.80 (t, $J = 9.6$ Hz, 1H), 5.72 (t, $J = 9.6$ Hz, 1H), 5.50 (dd, $J = 9.6, 8.2$ Hz, 2H), 5.42 (d, $J = 8.0$ Hz, 1H), 5.38 (t, $J = 3.4$ Hz, 1H), 5.10 (d, $J = 12.8$ Hz, 1H), 5.04 (d, $J = 12.4$ Hz, 1H), 4.58 (dd, $J = 12.4, 3.0$ Hz, 1H), 4.48 (dd, $J = 12.4, 4.2$ Hz, 1H), 4.36 (d, $J = 6.8$ Hz, 1H), 4.12 (m, 2H), 3.80 (m, 3H), 3.68 (dd, $J = 12.8, 4.8$ Hz, 1H), 3.00 (dd, $J = 11.2, 4.8$ Hz, 1H), 2.90 (dd, $J = 13.6, 4.0$ Hz, 1H), 1.50, 1.24, 1.10, 0.95, 0.92, 0.90, 0.79, 0.72, 0.58 (s each, 3H each); ESI-MS m/z : 1298 [M+H]⁺. The spectral data of **9** are similar to those reported in the literature [12].

3.2.4 Oleanolic acid 3-O-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside (12)

Compound **12** was prepared from **9** according to the reported method [12],

yield 74% for three steps. Mp 220–221°C; $[\alpha]_D^{20} = +16.0$ ($c = 0.5$, methanol); IR (KBr) ν_{\max} : 3421, 2942, 1693, 1643, 1460, 1386, 1079 cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6): δ 5.34 (d, $J = 4.0$ Hz, 1H), 5.15 (s, 1H), 4.98 (d, $J = 2.0$ Hz, 1H), 4.92 (d, $J = 4.0$ Hz, 2H), 4.63 (d, $J = 4.0$ Hz, 1H), 4.40 (d, $J = 4.8$ Hz, 1H), 4.35 (d, $J = 7.6$ Hz, 1H), 4.21 (t, $J = 5.0$ Hz, 1H), 3.56–3.68 (m, 5H), 3.44–3.51 (m, 1H), 2.94–3.17 (m, 5H), 2.75 (dd, $J = 9.2, 4.4$ Hz, 1H), 1.09 (s, 3H), 0.98 (s, 3H), 0.82–0.88 (s each, 3H each, 9H), 0.74 (s, 3H), 0.72 (s, 3H); ESI-MS m/z : 751 $[\text{M} + \text{H}]^+$. The spectral data of **12** are similar to those reported in the literature [12].

3.2.5 12-Dihydroxy-olide-olean (2)

According to the reported method [9], **2** was obtained in a pure form on chromatography, yield 62%. Mp $>250^\circ\text{C}$; $[\alpha]_D^{20} = +19.5$ ($c = 0.5$, methanol); IR (KBr) ν_{\max} : 3516, 2948, 2871, 1740, 1705, 1463, 1393, 1256 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 3.90 (s, 1H), 3.22 (dd, $J = 11.2, 4.8$ Hz, 1H), 1.29, 1.13, 0.98, 0.89, 0.97, 0.86, 0.76 (s each, 3H each); elemental analyses found: C, 76.25%; H, 10.21%; O, 13.58%; calcd for $\text{C}_{30}\text{H}_{48}\text{O}_4$: C, 76.23%; H, 10.24%; O, 13.54%; ESI-MS m/z : 473 $[\text{M} + \text{H}]^+$. The spectral data of **2** are similar to those reported in the literature [9].

3.2.6 3,12- β -D-Glucopyranosyl-12-dihydroxy-olide-olean (14)

Compound **2** (0.2 g, 0.4 mmol), trichloroacetimidates **4a** (1.7 g, 2.0 mmol), and powdered 4 Å molecular sieves (4.0 g) were stirred for 30 min at -40°C in dry CH_2Cl_2 (20 ml). A solution of TMSOTf (23 μl) in dry CH_2Cl_2 was added dropwise at -40°C . The mixture was stirred for 24 h followed by addition of Et_3N (0.1 ml) and filtration. The mixture was concentrated and purified through silica gel column chromatography (CH_2Cl_2 –MeOH) to give **13** (0.4 g, 57%) as a white amorphous

solid. Mp 141–143°C; $[\alpha]_D^{20} = +13.4$ ($c = 0.6$, CH_2Cl_2).

To a solution of **13** (0.1 g, 0.06 mmol) in dry CH_2Cl_2 –MeOH (1:1, 15 ml), a newly prepared NaOMe in MeOH solution (30%, 65 μl) was added. The mixture was stirred at room temperature for 4 h and neutralized with acetic acid to pH 7. The solution was concentrated and the resulting residue was subjected to silica gel column chromatography (CH_2Cl_2 –MeOH– $\text{H}_2\text{O} = 5:1:0.05$) to give **14** (40.6 mg, 85%) as a white amorphous solid. Mp 182–184°C; $[\alpha]_D^{20} = +27.9$ ($c = 0.5$, methanol); IR (KBr) ν_{\max} : 3418, 2936, 2873, 1727, 1645, 1459, 1384, 1076, 1026 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 5.38 (d, $J = 8.0$ Hz, 1H), 5.21 (d, $J = 3.6$ Hz, 1H), 5.11 (d, $J = 6.4$ Hz, 1H), 4.96 (dd, $J = 10.4, 5.0$ Hz, 2H), 4.83–4.91 (m, 4H), 4.80 (s, 2H), 4.69 (dd, $J = 7.6, 4.8$ Hz, 2H), 4.40 (dd, $J = 10.8, 5.6$ Hz, 2H), 4.32 (t, $J = 5.6$ Hz, 1H), 4.15 (d, $J = 7.6$ Hz, 1H), 0.96, 0.93, 0.90, 0.84, 0.81, 0.74, 0.66 (s each, 3H each); elemental analyses found: C, 63.22%; H, 8.53%; O, 28.26%; calcd for $\text{C}_{42}\text{H}_{68}\text{O}_{14}$: C, 63.30%; H, 8.60%; O, 28.11%; ESI-MS m/z : 798 $[\text{M} + \text{H}]^+$.

3.2.7 3-Acetyl dihydroxy-olide oleanolic derivative (15)

According to the reported method [9], **15** was obtained (131.6 mg, 64%) as a white amorphous solid on chromatography. Mp $>250^\circ\text{C}$; $[\alpha]_D^{20} = +18.1$ ($c = 0.5$, methanol); ^1H NMR (CDCl_3): δ 4.50 (dd, $J = 10.4, 6.4$ Hz, 1H), 3.90 (s, 1H), 2.05 (s, 3H), 1.30 (s, 3H), 1.15 (s, 3H), 0.99 (s, 3H), 0.90–0.92 (s each, 3H each, 6H), 0.88 (s, 3H), 0.87 (s, 3H); ESI-MS m/z : 516 $[\text{M} + \text{H}]^+$. The spectral data of **15** are similar to those reported in the literature [9].

3.2.8 Dihydroxy-olide oleanolic-12-glycosides (16)

Compound **16** was prepared from **15** according to the same procedure described

for **13**. $[\alpha]_{\text{D}}^{20} = +11.9$ ($c = 0.5$, methanol); $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 7.83–8.06 (m, 8H), 7.28–7.58 (m, 12H), 6.01 (m, 2H), 5.75 (m, 2H), 4.58 (dd, $J = 12.0$, 2.8 Hz, 1H), 4.49 (m, 2H), 4.30 (m, 1H), 2.75 (d, $J = 13.6$ Hz, 1H), 2.58 (d, $J = 4.4$ Hz, 1H), 2.03 (s, 3H), 0.89 (s, 3H), 0.86 (s, 3H), 0.79–0.81 (s each, 3H each, 9H), 0.65 (s, 3H), 0.62 (s, 3H); elemental analyses found: C, 72.54%; H, 7.03%; O, 20.44%; calcd for $\text{C}_{66}\text{H}_{76}\text{O}_{14}$: C, 72.51%; H, 7.01%; O, 20.49%; ESI-MS m/z : 1094 $[\text{M}+\text{H}]^+$.

Compound **17** was prepared from **16** according to the same procedure described for **14**, yield 41% for two steps. $[\alpha]_{\text{D}}^{20} = +12.3$ ($c = 0.5$, methanol); IR (KBr) ν_{max} : 3429, 2945, 2874, 1736, 1468, 1369, 1248, 1369, 1247, 1073, 1033 cm^{-1} ; $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 5.68 (d, $J = 7.6$ Hz, 1H), 4.50 (dd, $J = 11.2$, 4.8 Hz, 1H), 3.80 (m, 2H), 3.40–3.60 (m, 5H), 2.05 (s, 3H), 0.80–1.00 (s each, 21H); elemental analyses found: C, 67.40%; H, 8.98%; O, 23.62%; calcd for $\text{C}_{38}\text{H}_{60}\text{O}_{10}$: C, 67.43%; H, 8.93%; O, 23.64%; ESI-MS m/z : 678 $[\text{M}+\text{H}]^+$.

To a solution of **17** (90.0 mg, 0.14 mmol) in THF–MeOH (1.5:1, 15 ml), NaOH (4 mol/l, 0.72 ml) was added. The mixture was stirred at room temperature for 5 h and neutralized with dilute hydrochloric acid to pH 7. The solution was extracted with CH_2Cl_2 . The CH_2Cl_2 layer was separated and washed with water, dried over sodium sulfate, filtered and concentrated to a yellow solid, and the resulting residue was subjected to silica gel column chromatography (CH_2Cl_2 –MeOH) to give **18** (60.0 mg, 67%) as a white amorphous solid. $[\alpha]_{\text{D}}^{20} = +21.6$ ($c = 0.5$, methanol); IR (KBr) ν_{max} : 3422, 2931, 2868, 1727, 1692, 1467, 1367, 1074, 1034 cm^{-1} ; $^1\text{H NMR}$ ($\text{DMSO}-d_6$): δ 5.37 (d, $J = 8.4$ Hz, 1H), 5.29 (d, $J = 6.0$ Hz, 1H), 5.05 (d, $J = 4.8$ Hz, 1H), 4.98 (d, $J = 4.8$ Hz, 1H), 4.43 (t, $J = 5.6$ Hz, 1H), 4.32 (d, $J = 5.2$ Hz, 1H), 1.24 (s, 3H), 0.86–0.94 (s each, 3H each, 12H), 0.79 (s, 3H), 0.68 (s, 3H);

elemental analyses found: C, 68.14%; H, 9.23%; O, 22.63%; calcd for $\text{C}_{36}\text{H}_{58}\text{O}_9$: C, 68.11%; H, 9.21%; O, 22.68%; ESI-MS m/z : 635 $[\text{M}+\text{H}]^+$.

3.2.9 3-Acetyl dihydroxy-olide oleanolic-12-oxo-derivative (**19**)

According to the reported method [9], **19** was obtained as a white solid after concentration and repeated column chromatography, yield 92%. Mp $> 250^\circ\text{C}$; $[\alpha]_{\text{D}}^{20} = +16.1$ ($c = 0.5$, methanol); $^1\text{H NMR}$ (CDCl_3): δ 4.50 (dd, $J = 11.2$, 4.8 Hz, 1H), 2.71 (t, $J = 14.2$ Hz, 1H), 2.54 (t, $J = 8.2$ Hz, 1H), 2.39 (d, $J = 2.8$ Hz, 1H), 2.37 (d, $J = 3.2$ Hz, 1H), 1.32 (s, 1H), 0.96–0.98 (s each, 3H each, 9H), 0.94 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H); ESI-MS m/z : 514 $[\text{M}+\text{H}]^+$. The spectral data of **19** are similar to those reported in the literature [9].

3.3 Enzymatic activity assays

The inhibitory activity of all samples against α -glucosidase (Sigma G-0660), supplied by Sigma-Aldrich Co. Ltd (St Louis, MO, USA), 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 (1 $\mu\text{g}/\text{ml}$) was used as a positive control. The reaction system was stirred at 37°C for 10 min, and 0.1 mol/l maltose was added. After 10 min, a reagent (200 μl) for detecting glucose, supplied by Jiancheng Co. Ltd (Nanjing, China), was added and the OD value in absorption at 490 nm was monitored continuously with the spectrophotometer. The results are shown in Table 1.

Notes

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