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Note

# Facile synthesis and cytotoxicity of triterpenoid saponins bearing a unique disaccharide moiety: hederacolchiside $A_1$ and its analogues

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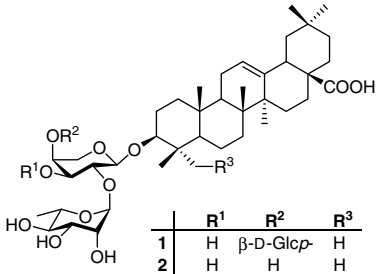
**Abstract**—An improved synthetic approach toward hederacolchiside $A_1$, an antitumor triterpenoid saponin bearing a unique disaccharide moiety, was established. This approach began from a partially protected intermediate and avoided tedious protection–deprotection manipulation. An abnormal ring conformation ($^1C_4$) of the center arabinose residue was found in the intermediate, which may account for the unusual regioselectivity between 3-OH and 4-OH of arabinose. Two analogues of hederacolchiside $A_1$ were then facilely prepared by this approach and exhibited significant cytotoxicity in preliminary in vitro assay.


*Keywords:* Hederacolchiside $A_1$; Triterpenoid saponin; Cytotoxicity; Regioselective glycosylation; $^1C_4$ conformation; Arabinose

Hederacolchiside $A_1$ (**1**) (Fig. 1), namely oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside, is an important triterpenoid saponin that has prominent inhibitory activity against many tumor cell lines.[1] Its structure contains a unique disaccharide substructure, α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, which is generally hypothesized to be a characteristic sugar sequence strongly inducing the antitumor activity of oleanane-type glycosides.[1–3] Many triterpenoid saponins bearing this disaccharide moiety commonly present significant antitumor activity, such as **2**–**4**.[1,3,4] Moreover, as a natural derivative of **1**, hederacolchiside A (**5**) exhibits excellent in vivo antitumor activity against various solid tumors in animal experiments.[5] Therefore, much attention has been paid to the further study of this series of saponins, including antitumor mechanism study, structural modification, and investigations into the structure–activity relationships.[6–8]

In 2006, we reported the synthesis of hederacolchiside $A_1$ (**1**) and β-hederin (**2**) from commercially available oleanolic acid.[9] However, the synthesis of **1** had necessary tedious protective group manipulation, and laborious column chromatography was required for purification of some intermediates. During the successive work on the structure modification of these two saponins, we found a more convenient method to prepare **1** and its analogues.

Compound **6**[9] (Scheme 1) is an important intermediate in the previous synthesis of **1**. In general, the equatorial 3-OH of arabinose is considered to be more reactive than the axial 4-OH. Thus, we attempted to glycosylate




| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| 1 | H | β-D-Glc*p*- | H |
| 2 | H | H | H |
| 3 | β-D-Glc*p*- | H | H |
| 4 | H | H | OH |
| 5 | H | β-D-Glc*p*- | OH |

**Figure 1.**

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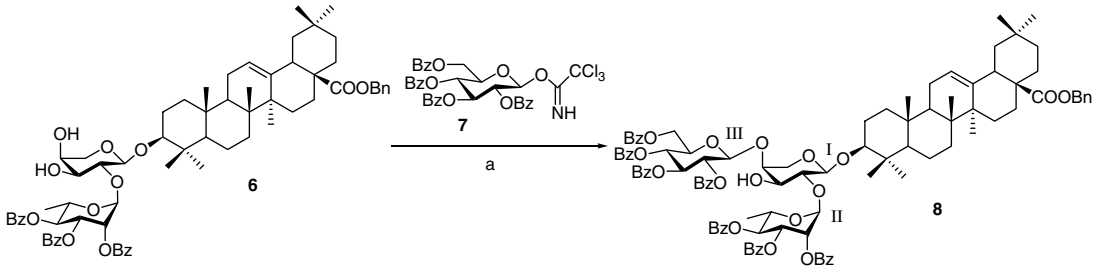



**Scheme 1.** Reagents and conditions: (a) TMSOTf, $CH_2Cl_2$, 4 Å MS, rt, 65%.

**6** with donor **7**[10] to prepare 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyl oleanolic acid (**3**), which is also a natural triterpenoid saponin with high cytotoxicity.[2] The glycosylation yield was 65%; however, NMR spectra showed that the glucose moiety had been introduced to $4^{I}$-OH, and no by-product with (1→3) linkage was found.

Similar unusual regioselectivity in glycosylation of 3-OH and 4-OH of arabinose has been reported previously[11] and the reason was suggested to be the reduced activity of 3-OH by the bulky group at 2-OH. However, the small $^1$H NMR coupling constant of arabinose in **6** ($J_{1-2} = 1.3$) and **8** ($J_{1-2} = 3.5$, $J_{2-3} = 4.8$) indicated that the arabinose ring may adopt the unusual $^1C_4$ conformation rather than the $^4C_1$ form. Signal peaks of H-$2^{I}$, $3^{I}$, and $4^{I}$ in **6** were overlapped, such that the coupling constants cannot be calculated; nevertheless, the $3^{I}$-benzoate of **6** (that is, intermediate **22** in Ref. 9) showed small $J$ values ($J_{1-2} = 3.8$, $J_{2-3} = 2.8$, $J_{3-4} = 5.8$) in 600 MHz $^1$H NMR spectra, suggesting a $^1C_4$ conformation of arabinose. This unusual chair form had been observed on arabinose, xylose, and rhamnose during glycosylation,[12–14] especially when bulky protective groups are present in two adjacent positions; however, they usually return to normal chair forms ($^4C_1$ for arabinose and xylose, while $^1C_4$ for rhamnose) after deprotection. Therefore, structures of **6** and **8** should be **6′** and **8′**, respectively (Scheme 2). In this case, $4^{I}$-OH of **6′** was in the equatorial position and should have a higher reactivity than $3^{I}$-OH in the glycosylation.

Thereupon, we checked the $^1$H NMR spectra of intermediates in our previous work[9] and found that the arabinose kept the normal $^4C_1$ form in the first several steps and did not convert to $^1C_4$ until the isopropylidene was removed. Apparently, this $^1C_4$ form allows two bulky groups (aglycon and benzoylated rhamnose) at $1^{I}$-OH and $2^{I}$-OH an antiperiplanar conformation, which is more favorable than the synclinal conformation in the $^4C_1$ form. It is conceivable that the arabinose ring has been inclined to convert into the $^1C_4$ form since the installation of rhamnose, while the presence of isopropylidene prevented this conversion. That is, arabinose of intermediates **17**–**25** (except sugar donor **23**) in Ref. 9 should be in the $^1C_4$ form. Fortunately, it returned to the normal $^4C_1$ conformation after deprotection and yielded the final products, β-hederin and hederacolchiside $A_1$, correctly.

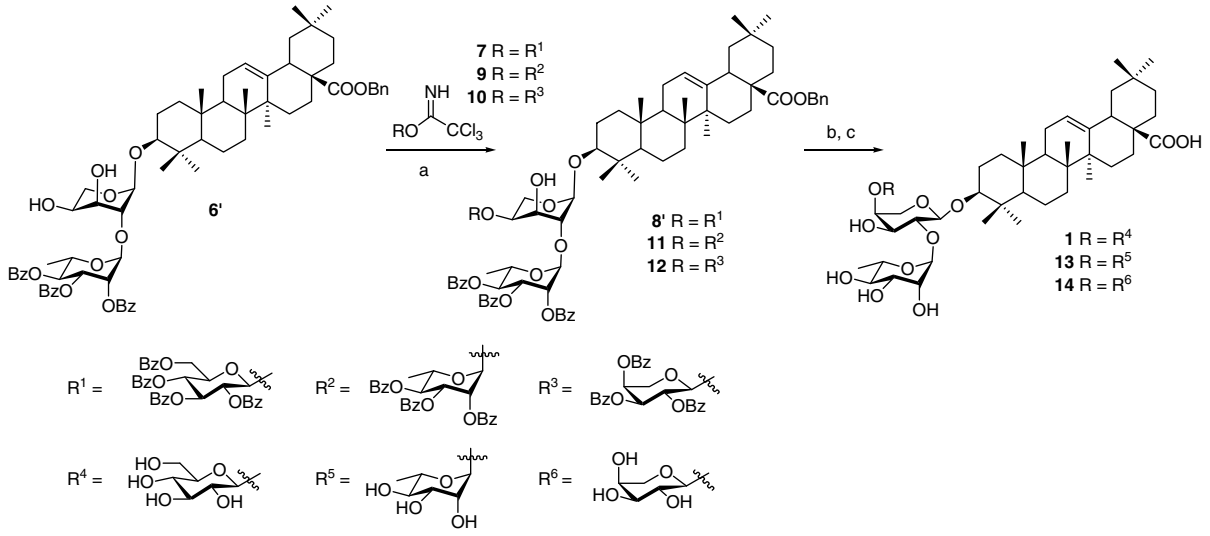
Afterward, benzyl group and benzoyl groups of **8′** were removed by catalytic hydrogenolysis and ester interchange in turn to give **1** at 89% yield. The $^4C_1$ conformation of arabinose was found in the product ($J_{1-2} = 5.9$). However, some differences were found between the $^1$H NMR spectra of **1** and those reported previously[9,15] (Table 1).

Thereupon, $^1$H NMR data of the original synthetic sample[9] were measured again at 600 MHz. Surprisingly, the new spectra of the original sample agreed with that of the new product, but differed from data determined previously.[9] Nevertheless, other physical properties and analytical data (such as $^{13}$C NMR spectra) were in good agreement.[9,15,16] Chemical shift ($\delta$) of carbohydrate protons of saponins is sensitive to various factors (such as temperature and residual water in NMR solvent) and sometimes show considerable instability, while $^{13}$C chemical shifts are much less variable. Although isolation and structure characterization of **1** have been reported repeatedly,[4,15–18] $^1$H NMR data were rarely presented in the literatures except Ref. 15. Interestingly, Lu et al. isolated **1** from rhizome of *Anemone raddeana* and we found that our new $^1$H NMR spectra were in agreement with these data.[19,20]

In succession, analogues of hederacolchiside $A_1$ were facilely prepared through this route (Scheme 2). Trichloroacetimidates **9** and **10** were used in the regioselective glycosylation of **6′** according to the same procedure. After deprotection with catalytic hydrogenolysis and ester interchange, two analogues **13** and **14** were furnished in moderate yields (24% and 47% from **6′**, respectively).

A preliminary in vitro pharmacology assay was then performed to evaluate the cytotoxicity of these saponins against HeLa cell using a standard MTT assay. Synthetic β-hederin (**2**) was used as a reference compound.

As shown in Table 2, **14** exhibited a considerable cytotoxicity against HeLa cells, while the activity of **13** was a bit lower ($IC_{50} > 10$ μM). However, compared to the leading compounds **1** and **2**, the cytotoxicity was remarkably reduced after structural modification. This




**Scheme 2.** Reagents and conditions: (a) TMSOTf, $CH_2Cl_2$, 4 Å MS, rt; (b) 10% Pd–C, $H_2$, EtOAc, reflux; (c) NaOMe, $CH_2Cl_2$–MeOH, rt.

**Table 1.** $^1$H NMR and $^{13}$C NMR data of **1** (carbohydrate moiety)

| Position | $^{13}$C[a] | $^1$H NMR[a] | | $^1$H NMR[b] | |
|---|---|---|---|---|---|
| | $\delta$ (ppm) | $\delta$ (ppm) | $J$ (Hz) | $\delta$ (ppm) | $J$ (Hz) |
| $1^{I}$ | 105.0 | 4.77, d | 5.9 | 4.76, d | 6.2 |
| $2^{I}$ | 76.4 | 4.50–4.47, m | | 4.67–4.60, m | |
| $3^{I}$ | 74.2[c] | 4.29–4.25, m | | 4.30–4.16, m | |
| $4^{I}$ | 79.8 | 4.29–4.25, m | | 4.30–4.16, m | |
| $5^{I}$-a | 64.7 | 4.40, dd | 12.2, 4.0 | 4.41–4.34, m | |
| $5^{I}$-b | | 3.81, br d | 11.0 | 3.80, d | 11.3 |
| $1^{II}$ | 101.8 | 6.13, br s | | 6.20, s | |
| $2^{II}$ | 72.4 | 4.71, t | 1.6 | 4.75–4.73, m | |
| $3^{II}$ | 72.5 | 4.60–4.57, m | | 4.67–4.60, m | |
| $4^{II}$ | 74.1[c] | 4.29–4.25, m | | 4.41–4.34, m | |
| $5^{II}$ | 69.9 | 4.62–4.59, m | | 4.67–4.60, m | |
| $6^{II}$ | 18.5 | 1.63, d | 6.1 | 1.64, d | 6.2 |
| $1^{III}$ | 106.5 | 5.12, d | 7.9 | 5.14, d | 7.8 |
| $2^{III}$ | 75.5 | 4.01, dd | 8.8, 8.1 | 4.03, t | 7.9 |
| $3^{III}$ | 78.6 | 4.17, dd | 9.0, 8.9 | 4.30–4.16, m | |
| $4^{III}$ | 71.3 | 4.22, t | 9.1 | 4.30–4.16, m | |
| $5^{III}$ | 78.9 | 3.90–3.87, m | | 3.91, m | |
| $6^{III}$-a | 62.6 | 4.50–4.47, m | | 4.53–4.48, m | |
| $6^{III}$-b | | 4.36, dd | 11.7, 4.8 | 4.53–4.48, m | |
| 3 | 88.7 | 3.22, dd | 11.7, 4.1 | 3.22, dd | 11.5, 3.8 |
| 12 | 122.6 | 5.47, br s | | 5.46, br s | |
| 18 | 42.0 | 3.29, dd | 13.7, 3.8 | 3.27, br d | 10.2 |

[a] Synthetic product of the present work. Data were determined at 600 MHz in pyridine-$d_5$.
[b] Spectra of Ref. 9.
[c] Assignments may be interchangeable.

result provided further support for the hypothesis that this unique disaccharide contributes essentially to the cytotoxicity of this class of triterpenoid saponins, and on the other hand, substituents on the arabinose residue may influence the bioactivity significantly. Consequently, structural modification of this class of triterpenoid saponin using β-hederin as a core structure is expected to provide novel compounds with higher antitumor activity.

In conclusion, an improved synthesis of hederacolchiside $A_1$ has been achieved from intermediate **6′** through three steps at a yield of 58%, while seven steps were

**Table 2.** $IC_{50}$ values of synthetic triterpenoid saponins against HeLa cell

| Compound | **1** | **2** | **13** | **14** |
|---|---|---|---|---|
| $IC_{50}$ (μM) | 0.69 | 0.80 | 12.80 | 4.86 |

needed to convert **6′** to the target molecule in our former approach. This newly developed approach avoids tedious protection–deprotection operation. From this approach, two analogues of hederacolchiside $A_1$ were facilely prepared in moderate yields, and exhibited significant cytotoxicity in preliminary in vitro study. Moreover, $^1C_4$ conformation of arabinose was found when its 1-OH and 2-OH were occupied by some bulky groups, which may be meaningful in the synthesis of complex arabinosides.

## 1. Experimental

### 1.1. Chemical synthesis

**1.1.1. General methods.** Commercial reagents were used without further purification. Boiling range of petroleum ether was 60–90 °C. Analytical TLC was performed using TLC plates pre-coated with silica HF254 and visualized by UV radiation (254 nm) or $I_2$ fuming. Preparative column chromatography was performed with silica gel (200–300 mesh). Melting points were detected with BÜCHI Melting Point B-540. Optical rotations were measured at the sodium D-line at room temperature with a Perkin–Elmer 241 MC polarimeter. NMR spectra were recorded on Avance AV 600 MHz spectrometer. *J* values were given in hertz. ESI mass spectra were obtained on a Waters Quattro Micro mass spectrometer. HRMS was detected on High resolution ESI-FTICR mass spectrometry (Ion spec 7.0T).

**1.1.2. Benzyl oleanolate 3-*O*-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside (8′).** A suspension of **6′** (133 mg, 0.117 mmol), **7** (92 mg, 0.124 mmol), and powdered 4 Å molecular sieves (400 mg) in dry $CH_2Cl_2$ (4 mL) was stirred for 30 min at rt. A solution of TMSOTf in dry $CH_2Cl_2$ (5% v/v, 95 μL, 0.024 mmol) was added and the mixture was stirred for 40 min before $Et_3N$ (0.10 mL) was added to quench the reaction. The mixture was then filtered and the filtrate was concentrated and purified by a flash column chromatography (3:1, petroleum ether–EtOAc) to afford **8′** (131 mg, 65%) as a white foam. $[\alpha]_D$ +56.7 (*c* 0.61, $CH_2Cl_2$); $R_f = 0.38$ (2:1, petroleum ether–EtOAc); $^1$H NMR ($CDCl_3$): $\delta$ 8.09–7.81 (m, 14H), 7.62 (m, 1H), 7.53–7.48 (m, 5H), 7.43–7.37 (m, 6H), 7.36–7.31 (m, 6H), 7.30–7.24 (m, 8H) (aromatic H), 5.94 (t, 1H, *J* 9.6, H-3$^{III}$), 5.79 (dd, 1H, *J* 10.1, 2.5, H-3$^{II}$), 5.71 (dd, 1H, *J* 9.7, 9.6, H-4$^{III}$), 5.65–5.61 (m, 2H, H-2$^{II}$, H-4$^{II}$), 5.59 (m, 1H, H-2$^{III}$), 5.29 (br s, 1H, H-12), 5.20 (br s, 1H, H-1$^{II}$), 5.13 (d, 1H, *J* 7.8, H-1$^{III}$), 5.07 (dd, 2H, *J* 33.4, 12.5, PhC$H_2$), 4.71 (dd, 1H, *J* 12.1, 2.4, H-6$^{III}$-a), 4.65 (d, 1H, *J* 3.5, H-1$^{I}$), 4.52 (dd, 1H, *J* 12.0, 4.7, H-6$^{III}$-b), 4.32 (m, 1H, H-5$^{II}$), 4.25 (m, 1H, H-5$^{III}$), 4.12 (m, 1H, H-5$^{I}$-a), 4.08 (m, 1H, H-4$^{I}$), 3.89 (m, 1H, H-3$^{I}$), 3.84 (dd, 1H, *J* 3.6, 4.8, H-2$^{I}$), 3.69 (br d, 1H, *J* 9.7, H-5$^{I}$-b), 3.10 (dd, 1H, *J* 11.6, 3.8, H-3), 2.91 (dd, 1H, *J* 13.8, 3.2, H-18), 1.29 (d, 3H, *J* 6.1, H-6$^{II}$), 1.11, 1.03, 0.92, 0.90, 0.86, 0.81, 0.60 (s, 7 × 3H, $CH_3$); $^{13}$C NMR ($CDCl_3$): $\delta$ 177.4 (C-28), 166.1, 165.8, 165.7, 165.5, 165.4, 165.3, 165.1 (7 × Ph*C*O), 143.6 (C-13), 136.4, 133.5–133.1, 129.9–127.9 (aromatic C), 122.5 (C-12), 102.5 (C-1$^{I}$), 101.9 (C-1$^{III}$), 97.8 (C-1$^{II}$), 90.1 (C-3), 76.2 (C-4$^{I}$), 76.0 (C-2$^{I}$), 72.8 (C-3$^{III}$), 72.3 (C-5$^{III}$), 72.0 (C-2$^{II}$), 71.7 (C-4$^{II}$), 70.7 (C-3$^{I}$, C-2$^{III}$), 69.8 (C-3$^{II}$), 69.6 (C-4$^{III}$), 67.0 (C-5$^{II}$), 65.9 (Ph*C*$H_2$), 63.0 (C-6$^{III}$), 60.8 (C-5$^{I}$), 55.6, 47.6, 46.7, 45.9, 41.6, 41.4, 39.3, 39.1, 38.6, 36.7, 33.8, 33.1, 32.7, 32.4, 30.7, 28.2, 27.6, 25.9, 25.8, 23.6, 23.4, 23.0, 18.2 (C-6$^{II}$), 17.5, 16.9, 16.5, 15.3; ESI-MS *m*/*z*: 1738.7 ([M+Na]$^+$); 1714.8 ([M−H]$^-$).

**1.1.3. Oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside (1).** A suspension of **8′** (90 mg, 0.0524 mmol) and 10% Pd–C (40 mg) in EtOAc (8 mL) was refluxed and bubbled up with $H_2$ (20 mL/min). When TLC (2:1, petroleum–EtOAc) showed that the reaction had completed, Pd–C was removed through filtration and the filtrate was concentrated to dryness. The resulted amorphous solid was dissolved in dry $CH_2Cl_2$–MeOH (1:2, 24 mL), to which a newly prepared NaOMe/MeOH (1.0 mol/L, 1.20 mL) was added. The solution was stirred at rt for 2 h and then neutralized with Dowex $H^+$ resin to pH 7 and filtered. The filtrate was concentrated and subjected to a flash column chromatography ($CHCl_3$–MeOH–$H_2O$ 7:3:1, organic layer) to give **1** (42 mg, 89%) as a white powder. Mp 249–251 °C, lit.[9] 251–253 °C, lit.[16] 250–260 °C; $[\alpha]_D$ +0.8 (*c* 0.33, MeOH), lit.[16] 0 (*c* 2.97, MeOH); $R_f = 0.24$ ($CHCl_3$–MeOH–$H_2O$ 7:3:1, organic layer); $^1$H NMR: see Table 1; $^{13}$C NMR (pyridine-$d_5$): aglycon moiety $\delta$ 180.2 (C-28), 144.9 (C-13), 122.6 (C-12), 88.7 (C-3), 56.0 (C-5), 48.0 (C-9), 46.7 (C-17), 46.5 (C-19), 42.2 (C-14), 42.0 (C-18), 39.7 (C-4), 39.5 (C-8), 38.9 (C-1), 37.1 (C-10), 34.2 (C-21), 33.3 (C-29), 33.2, 33.1 (C-7, C-22), 31.0 (C-20), 28.3 (C-15), 28.1 (C-23), 26.7 (C-2), 26.2 (C-27), 23.8 (C-30), 23.7 (C-11, C-16), 18.7 (C-6), 17.4 (C-26), 17.1 (C-24), 15.6 (C-25), carbohydrate moiety: see Table 1; HRMS *m*/*z*: calcd for $[C_{47}H_{76}NaO_{16}]^+$ ([M+Na]$^+$) 919.5026, found: 919.5030.

**1.1.4. Oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-α-L-arabinopyranoside (13).** Compound **13** was prepared from **6′** by the same procedure as for **1**. Yield: 24%; white powder, $[\alpha]_D$ −15.4 (*c* 0.44, MeOH); $R_f = 0.33$ ($CHCl_3$–MeOH–$H_2O$ 7:3:1, organic layer); $^1$H NMR (pyridine-$d_5$): $\delta$ 5.97 (s, 1H, H-1$^{II}$), 5.72 (s, 1H, H-1$^{III}$), 5.43 (br s, 1H, H-12), 4.87 (d, 1H, *J* 4.3, H-1$^{I}$), 4.70 (br s, 1H), 4.59 (br d, 1H, *J*

9.4), 4.55–4.50 (m, 2H), 4.49–4.45 (m, 2H), 4.42 (dd, 1H, *J* 11.6, 4.6), 4.37–4.31 (m, 3H), 4.28 (t, 1H, *J* 9.2), 4.23 (t, 1H, *J* 9.2), 3.84 (br d, 1H, *J* 11.2), 3.26 (br d, 1H, *J* 12.8, H-18), 3.18 (dd, 1H, *J* 11.9, 3.4, H-3), 1.64 (d, 3H, *J* 5.8, H-6$^{II}$), 1.58 (d, 3H, *J* 5.9, H-6$^{III}$), 1.26, 1.13, 1.02, 0.97, 0.95, 0.92, 0.78 (s, 7 × 3H, $CH_3$); $^{13}$C NMR (pyridine-$d_5$): $\delta$ 180.2 (C-28), 144.8 (C-13), 122.5 (C-12), 104.9 (C-1$^{I}$), 102.6 (C-1$^{III}$), 102.0 (C-1$^{II}$), 89.0 (C-3), 76.4 (C-2$^{I}$), 75.0 (C-4$^{I}$), 74.1 (C-3$^{I}$, C-4$^{II}$), 72.6 (C-2$^{II}$, C-3$^{II}$), 72.4, 72.2, 72.1 (C-2$^{III}$, C-3$^{III}$, C-4$^{III}$), 70.3, 70.2 (C-5$^{II}$, C-5$^{III}$), 63.0 (C-5$^{I}$), 55.9 (C-5), 39.8 (C-4), 39.5 (C-8), 38.8 (C-1), 37.0 (C-10), 34.3 (C-21), 33.3 (C-29), 33.2, 33.2 (C-7, C-22), 31.0 (C-20), 28.3 (C-15), 28.1 (C-23), 26.5 (C-2), 26.2 (C-27), 23.8, 23.8, 23.7 (C-30, C-11, C-16), 18.7 (C-6$^{II}$), 18.6 (C-6$^{III}$), 18.6 (C-6), 17.4 (C-26), 16.9 (C-24), 15.5 (C-25); HRMS *m*/*z*: calcd for $[C_{47}H_{76}ClO_{15}]^-$ ([M+Cl]$^-$) 915.4878, found: 915.4881.

**1.1.5. Oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→4)]-α-L-arabinopyranoside (14).** Compound **14** was prepared from **6′** by the same procedure as for **1**. Yield: 47%; white powder, $[\alpha]_D$ 0.0 (*c* 0.31, MeOH); $R_f$ = 0.30 ($CHCl_3$–MeOH–$H_2O$ 7:3:1, organic layer); $^1$H NMR (pyridine-$d_5$): $\delta$ 6.12 (s, 1H, H-1$^{II}$), 5.43 (br s, 1H, H-12), 4.88 (d, 1H, *J* 7.5, H-1$^{I}$), 4.76 (d, 1H, *J* 6.1, H-1$^{III}$), 4.69 (m, 1H), 4.63–4.58 (m, 2H), 4.46–4.42 (m, 3H), 4.27 (t, 1H, *J* 9.4), 4.25–4.22 (m, 4H), 4.06 (dd, 1H, *J* 9.1, 3.2), 3.82 (br d, 1H, *J* 10.8), 3.66 (br d, 1H, *J* 10.9), 3.26 (dd, 1H, *J* 13.3, 3.8, H-18), 3.20 (dd, 1H, *J* 11.8, 4.1, H-3), 1.60 (d, 3H, *J* 6.1, H-6$^{II}$), 1.25, 1.15, 1.07, 0.97, 0.94, 0.92, 0.79 (s, 7 × 3H, $CH_3$); $^{13}$C NMR (pyridine-$d_5$): $\delta$ 180.2 (C-28), 144.8 (C-13), 122.6 (C-12), 107.0 (C-1$^{III}$), 105.0 (C-1$^{I}$), 101.8 (C-1$^{II}$), 88.8 (C-3), 79.3 (C-4$^{I}$), 76.4 (C-2$^{I}$), 74.9 (C-3$^{I}$, C-4$^{II}$), 74.1, 74.1 (C-2$^{III}$, C-3$^{III}$), 72.9 (C-4$^{III}$), 72.6, 72.4 (C-2$^{II}$, C-3$^{II}$), 69.9 (C-5$^{II}$), 67.5 (C-5$^{III}$), 64.6 (C-5$^{I}$), 56.0 (C-5), 48.1 (C-9), 46.7 (C-17), 46.5 (C-19), 42.2 (C-14), 42.0 (C-18), 39.8 (C-4), 39.6 (C-8), 38.9 (C-1), 37.1 (C-10), 34.3 (C-21), 33.3 (C-29), 33.2, 33.2 (C-7, C-22), 31.0 (C-20), 28.4 (C-15), 28.1 (C-23), 26.7 (C-2), 26.2 (C-27), 23.8, 23.8, 23.7 (C-11, C-16, C-30), 18.7 (C-6$^{II}$), 18.6 (C-6), 17.4 (C-26), 17.1 (C-24), 15.6 (C-25); HRMS *m*/*z*: calcd for $[C_{46}H_{74}NaO_{15}]^+$ ([M+Na]$^+$) 889.4920, found: 889.4924.

### 1.2. Pharmacology

Cytotoxicity of test compounds against HeLa cell line was evaluated by the MTT method in vitro. Cells were plated at a density of 4000 cells per well into 96-well plate and incubated at 37 °C with 5% $CO_2$ for 24 h. The cells were then treated with increasing concentrations of tested compounds and incubated at 37 °C with 5% $CO_2$ for 4 d. 100 μL MTT (1.0 mg/mL in nutrient medium) was added and cells continued to incubate for a further 4 h. Then, 150 μL DMSO was added to each well and the absorbance of samples was measured at 490 nm. The $IC_{50}$ values were calculated according to Logit method after getting the inhibitory rate.

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