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Research paper

Synthesis, biological evaluation and structure-activity relationship studies of hederacolchiside E and its derivatives as potential anti-Alzheimer agents

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ABSTRACT

Inspired by the previously reported neuroprotective activity of hederacolchiside E (**1**), we synthesized hederacolchiside E for the first time along with eleven of its derivatives. The neuroprotective effects of these compounds were further evaluated against H_2O_2- and A$\beta_{1\text{-}42}$-induced injury using cell-based assays. The derivatives showed obvious differences in activity due to structural variations, and two of them exhibited better neuroprotective effects than **1** in the A$\beta_{1\text{-}42}$-induced injury model. Compound **7** was the most active derivative and had a relatively simple chemical structure. Moreover, **1** and **7** can significantly reduce the release of lactate dehydrogenase (LDH), level of intracellular reactive oxygen species (ROS) and extent of malondialdehyde (MDA) increase resulting from A$\beta_{1\text{-}42}$ treatment, which demonstrated that these kinds of compounds show neuroprotective effects in Alzheimer's disease (AD) models via modulating oxidative stress. Compound **7** could be used as promising lead for the development of a new type of neuroprotective agent against AD.

1. Introduction

Alzheimer's disease (AD), characterized by progressive loss of memory and cognitive function, dementia and eventually death, is the most common neurodegenerative disorder of this era that substantially lowers the life quality of patients [1]. According to Alzheimer's Disease International (ADI) [2], there were 46.8 million people worldwide living with dementia in 2015; a number that is estimated to reach 131.5 million by 2050. However, existing drug treatments are insufficient in preventing or reversing its inexorable neurodegenerative progress [3] and show only marginal symptomatic improvement in moderately to severely affected patients [4]. Although the mechanism of this dementia has not been thoroughly elucidated, the abnormal accumulation of β-amyloid (Aβ) in the hippocampal and cortical regions is hypothesized to be a major pathological hallmark of AD [5–7]. In addition, some studies have reported evidence that Aβ-induced oxidative stress and mitochondrial dysfunctions also play roles in this kind of neuronal cytotoxicity [8,9]. Therefore, searching for antioxidants or free radical scavengers is one strategy for the treatment of AD.

For centuries, natural products and natural product-related structures have played a significant role in the drug discovery and development process. Triterpenoid saponins are a large family of natural products with diverse structures and bioactivities [10]. Many of them have been shown to cause improvements against neurotic atrophy and memory defect in dementia research [11]. For example, uncarinic acid C, extracted from *U. rhynchophylla* [12], could effectively inhibit Aβ aggregation, and a large number of oleanane triterpenes from the root of *Bupleurum chinese* [13] and the leaves of *Eleutherococcus senticosus* [14] also showed potent neuroprotective activities. However, the isolation of pure saponins from crude plant extracts, especially in suitable amounts, is relatively tedious and difficult because of the microheterogeneity of these complex structures [15]. Chemical synthesis would therefore provide us a feasible route to homogeneous saponins, as well as

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their derivatives, in sufficient amounts for elucidating the structure activity relationships (SARs), the biological mechanisms and for potential commercial applications.

Hederacolchiside E (**1**), namely 3-*O*-(α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl) oleanolic acid α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, is a bidesmosidic oleanane saponin first isolated in 1970 from the leaves of *Hedera colchica* (Fig. 1) [16]. Previously, Han's group reported that hederacolchiside E, the main bioactive component in the root extract of *Pulsatilla koreana*, had prominent cognition-enhancing abilities and neuroprotective effects in preliminary *vitro* and *vivo* activity-guided fractionation studies [17]. In the subsequent study, researchers found that the oleanolic-glycoside saponins enriched fraction SK-PC-B70M, which contained hederacolchiside E as the most active ingredient, could attenuate AD-like pathology in the brain of Tg2576 mice [18]. The antioxidative activity of hederacolchiside E demonstrated in previous reports may play an important role in the mechanism of its neuroprotective effect against Aβ [19].

Because of the unique biological activity in combination with isolation and purification difficulties, herein we report the chemical synthesis and biological evaluation of hederacolchiside E and its derivatives. Since the α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl moiety at 3-OH usually appears in oleanane-type triterpenoid saponins, we replaced the glucopyranosyl on the 4-OH of the arabinose by other monosaccharide moieties; thus, compounds **2-6** were designed. To elucidate the contribution of each sugar moiety in the biological activity, we removed one, two or three sugar moieties to give compounds **7-12** (Fig. 2). The SAR study of these natural and non-natural saponins against H_2O_2-induced oxidative damage, as well as Aβ-mediated neuronal cytotoxicity, is discussed. Further investigation of compounds **1** and **7** on related neuroprotective indicators demonstrated that the neuroprotective activities of this kind of compound are highly correlated with their antioxidative activities.

2. Chemistry

The synthesis of the naturally occurring saponin hederacolchiside E and its glycosylate-replaced and deglycosylated derivatives is described herein. Intermediates **18-23** were synthesized by routes analogous to the efficient synthetic route to **9** reported previously by our group (Scheme 1) [20]. Deprotection of the benzoyl groups of **13** generated intermediate **14**. The 3- and 4-OH groups of **14** were selectively protected as an isopropylidene acetal, and the 2-OH group was then glycosylated with a peracetylated rhamnopyranosyl donor to afford compound **16**. The isopropylidene group was hydrolyzed using *p*-TsOH, producing **18** with an abnormal $^1C_4$ conformation of the arabinopyranosyl residue (3-*O*-α-Ara H-1 J_{1-2} = 3.0) [21]. The conformation changing did not influence the subsequent selective glycosylation of 4-OH of the arabinopyranosyl residue by different trichloroacetimidate donors [22] under the promotion of trimethylsilyl triflate (TMSOTf) to provide intermediates **18-23**.

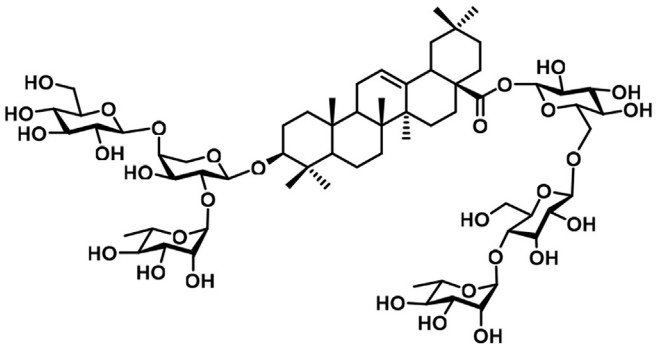


Fig. 1. The structure of hederacolchiside E (**1**).

As shown in Scheme 2, trichloroacetimidate donors linked with the carboxyl group of C-28 were synthesized in a straightforward manner from known 1-thio-β-D-glucopyranoside **24**. Regioselective silylation of the 6-OH of **24** by *tert*-butyldimethylsilyl chloride (TBSCl) in pyridine was followed by acetylation with acetic anhydride in one pot to afford **25** in excellent yield. The Lewis acid $BF_3 \cdot Et_2O$ was used to remove the TBS group from **25** to efficiently generate **26** with no observable acetyl migration [23]. Glycosylation of **26** with perbenzoylated glucopyranosyl trichloroacetimidate afforded **27**. Hydrolyzation of the thioglycoside using the efficient green reagent trichloroisocyanuric acid (TCCA) [24] followed by trichloroacetimidation with CCl_3CN catalyzed with 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) furnished the disaccharide building block **28**. To prepared trisaccharide donor **36**, the 4- and 6-OH groups of **24** were first protected using benzaldehyde dimethyl acetal to provide **29**. Acetylation of the two remaining hydroxyl groups followed by removal of the benzylidene group by *p*-TsOH afforded **31**. Regioselective benzoylation of **31** on the 6-OH with benzoyl chloride in pyridine at 0 °C generated **32** in 85% yield. Coupling **32** with a peracetylated rhamnopyranosyl donor provided **33**, which was followed by trichloroacetimidation to generate **34**. Glycosylation of **26** and **34** using catalytic amounts of TMSOTf successfully gave the trisaccharide **35**. The final trisaccharide donor **36** was prepared via hydrolysis and trichloroacetimidation of **35** in the same manner as above.

With key trisaccharide trichloroacetimidate donor **36** in hand, we started to formally assemble target saponins **1-8**. As shown in Scheme 3, ready removal of the benzyl group through catalytic hydrogenation provided intermediates **37-42**. Esterification of **37-42** with trichloroacetimidate donor **36** promoted by TMSOTf in dry CH_2Cl_2 under an atmosphere of N_2, followed by global deprotection of the benzoyl and acetyl groups by 1 M NaOMe in the presence of the C-28 ester glycosidic linkage, afforded target compound **1** and its derivatives **2-6**. The conformation of the arabinopyranosyl moiety returned to the normal $^4C_1$ form (3-*O*-α-Ara H-1 $J_{1-2} = 6.0$) [20,21,25] after deprotection due to the attenuated steric hindrance of the rhamnopyranosyl moiety and led to the correct final products.

The preparation of target compounds **7** and **8** were similar to the synthetic route described above (Scheme 3). Catalytic hydrogenation of **13** afforded intermediate **49**, which was converted to compound **7** by esterification and global deprotection via the general procedure. Deprotecting the carboxyl group of compound **15** followed by esterification with **36** furnished compound **52**. Deprotection of the isopropylidene by *p*-TsOH and benzoyl and acetyl groups by NaOMe afforded compound **8**. Changing substituent groups on the carboxyl group of **37** led to compounds **9-12**. Direct removal of the benzoyl and acetyl groups of **37** furnished compound **9**. Coupling **37** with perbenzoylated glucopyranosyl trichloroacetimidate (or **28**, **34**) followed by global deprotection of the benzoyl and acetyl groups to provide compounds **10-12** (Scheme 4).

3. Results and discussion

3.1. Neuroprotective effects on H_2O_2-induced apoptosis in PC12 cells

It has been demonstrated that neurodegenerative disorders are a class of disease that induce cellular injury by oxidative stress. H_2O_2 can generate exogenous free radicals and simulate damage caused by oxidative stress in nerve cells [26]. High concentrations of H_2O_2 can help us investigate neuroprotective activities of

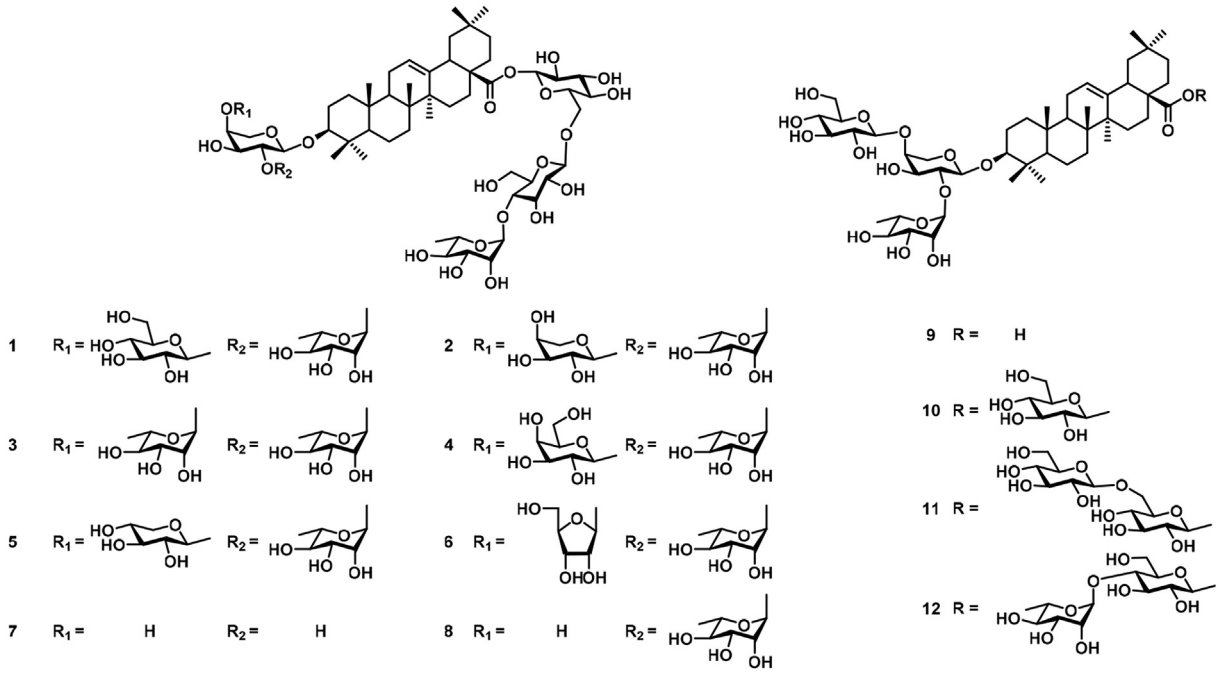
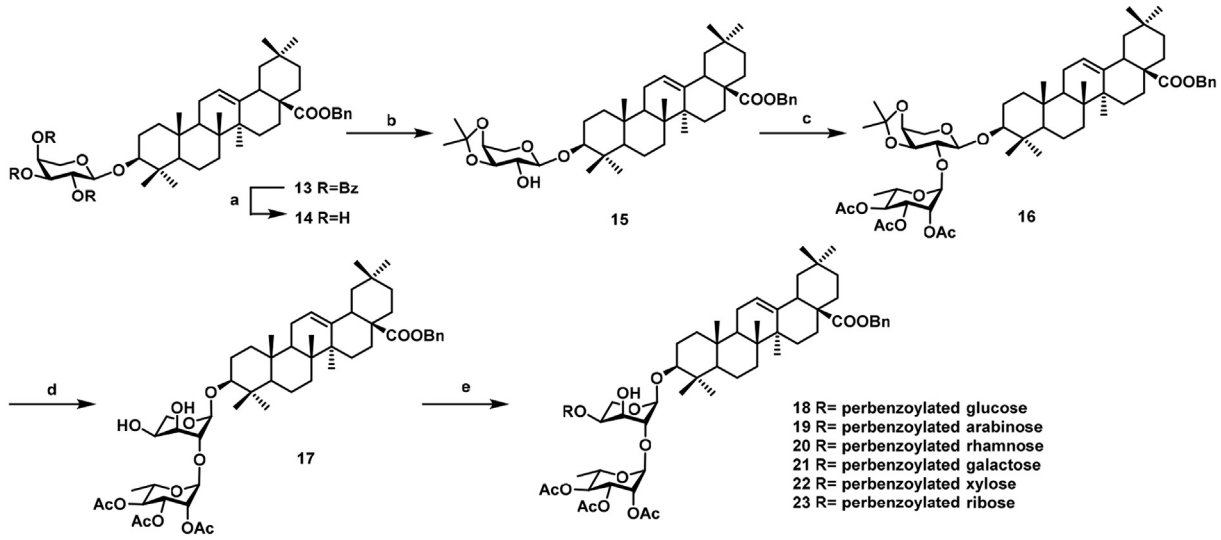


Fig. 2. Chemical structures of target compounds **1-12.**

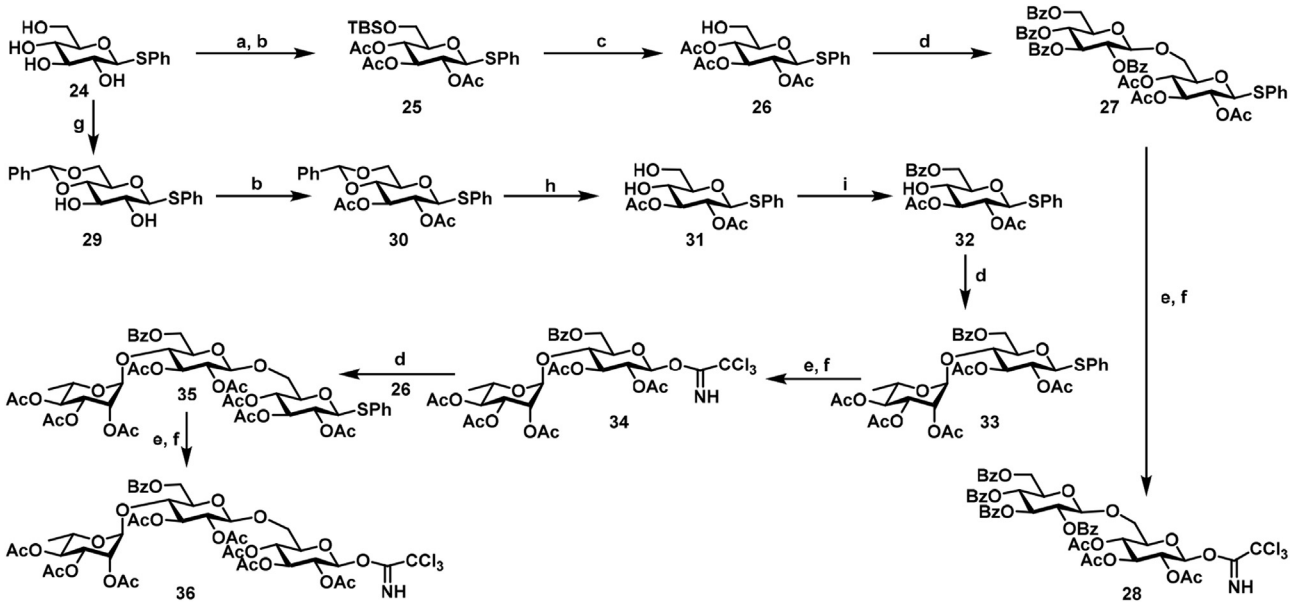


Scheme 1. Synthesis of intermediates **18-23**. Reagents and conditions: (a) NaOMe, MeOH-CH_2Cl_2 2:1, v/v, rt, 10 min; (b) $Me_2C(OMe)_2$, *p*-TsOH, CH_2Cl_2, rt, 5 h; (c) peracetylated rhamnopyranosyl trichloroacetimidate, TMSOTf, CH_2Cl_2, 4 Å MS, 0 °C, 30 min; (d) *p*-TsOH, MeOH-CH_2Cl_2 2:1, v/v, rt, 2 h; (e) perbenzoylated sugar trichloroacetimidates, TMSOTf, CH_2Cl_2, 4 Å MS, 0 °C, 30 min.

compounds in an economical way. The neuroprotective effects of compounds **1-12** on the H_2O_2-induced decrease in cell viability in rat pheochromocytoma (PC12) cells were assessed in preliminary screenings using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and the results are expressed as a percentage of untreated control. As shown in Fig. 3, the cell viabilities decreased to approximately 58% when treated with H_2O_2 alone. Natural saponin **1** exhibited a significant and dose-dependent effect on protecting PC12 cells from H_2O_2 damage, and derivatives **2-6**, which kept the six-sugar skeleton, also showed dose-dependent activities. The antioxidant activities of these compounds were equivalent to Edaravone, the positive control. Compound **7** displayed a moderate activity and compound **8** showed potent neuroprotective effects at 1 and 10 μM, however as the concentration of the compounds increased to 50 μM, some toxicity appeared. Compound **9**, with no substituent on carboxyl group of C-28, is another natural product, hederacolchiside A_1, and it showed potent cytotoxicity against PC12 cells which is consistent with previous reports [27]. Derivatives **10** and **11** showed cytotoxicity at high concentration; however, compound **12** showed moderate neuroprotective effects in this model test.

3.2. Neuroprotective effects on $A\beta_{1\text{-}42}$-induced apoptosis in PC12 cells

To further examine the neuroprotective effects of these



Scheme 2. Synthesis of intermediates **28**, **34** and **36**. Reagents and conditions: (a) TBSCl, DMAP, pyridine, rt, 24 h; (b) Ac_2O, pyridine, 0 °C to rt, 5 h; (c) $BF_3 \cdot Et_2O$, CH_2Cl_2, rt, 1 h; (d) sugar trichloroacetimidates, TMSOTf, CH_2Cl_2, 4 Å MS, 0 °C, 30 min; (e) TCCA, acetone-H_2O 9:1, v/v, 0 °C to rt, 1 h; (f) CCl_3CN, DBU, CH_2Cl_2, rt, 1 h; (g) $PhCH(OMe)_2$, *p*-TsOH, CH_3CN, 50 °C, 1 h; (h) *p*-TsOH, MeOH-CH_2Cl_2 2:1, v/v, rt, 2 h; (i) BzCl, pyridine, 0 °C to rt, 6 h.

compounds, a model of A$\beta_{1\text{-}42}$-induced damage was chosen. Aβ is widely used to generate AD-like models to analyze the protective effects and mechanisms of action of new pharmacotherapies for AD. H_2O_2-mediated toxicity to neurons in this system occurs through covalent cross-linking of Aβ [28]. The results showed that the neuroprotective effects of these compounds against A$\beta_{1\text{-}42}$-induced damage had obvious structure-based activity differences that were not exactly in line with H_2O_2-induced damage (Table 1). Based on the data, the preliminary SARs were analyzed and were found to be the following:

a) The glycosyl residue on 4-OH of arabinose is crucial for the activity since the six-sugar skeleton compounds **1-6** showed different effects against A$\beta_{1\text{-}42}$-induced damage. Natural product **1** showed remarkable activity at 50 and 100 μM. However, when the glucopyranosyl moiety was substituted with a rhamnopyranosyl moiety, compound **3** showed more potent anti-A$\beta_{1\text{-}42}$ activity than **1**. Compounds **2**, **4**, **5** and **6** that had alternative sugar substituents showed diminished neuroprotective activity in this model test.

b) For the substituent group on C-3 position, the situation was mixed. Compound **7**, bearing only an arabinopyranosyl moiety on C-3, displayed more potent activity against A$\beta_{1\text{-}42}$-induced damage than compound **1** did at 50 and 100 μM. Compound **8**, with the α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl moiety, was less potent than **1** and showed only feeble activity at 50 μM.

c) The activities of compounds **10-12**, which were designed by changing the substituent group on C-28, were consistent in both H_2O_2- and A$\beta_{1\text{-}42}$-induced models. Only substitution by one or two glucopyranosyl moieties (**10**, and **11**) lead to cytotoxicity, and substitution with glucopyranosyl and rhamnopyranosyl (**12**) afforded comparable neuroprotective activity to that of **1**. The results indicated that the presence of the rhamnopyranosyl residue at the C-28 position is of vital importance for the neuroprotective activity.

3.3. Effects on A$\beta_{1\text{-}42}$-induced lactate dehydrogenase (LDH) leakage in PC12 cells

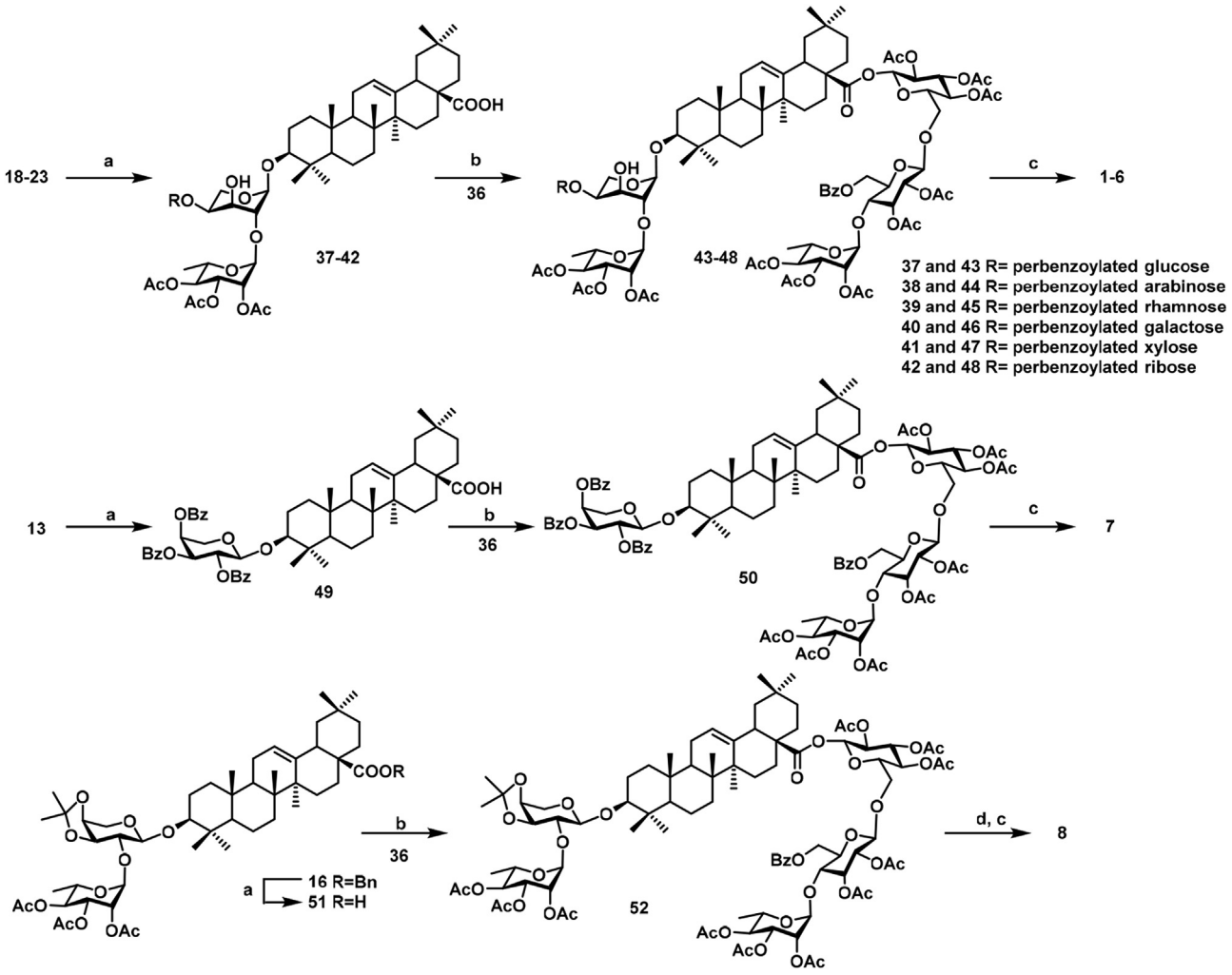
LDH is a soluble enzyme present in most eukaryotic cells, it released from damaged cells into the culture medium because of damage to the plasma membrane [29]. Measuring the activity of LDH can quantitatively assess cytotoxicity. As shown in Fig. 4, when PC12 cells were exposed to 40 μM A$\beta_{1\text{-}42}$ for 24 h, the LDH leakage was substantially increase to 153% compared with the control group (100%). Pretreatment of the cells with various concentrations (10, 50, and 100 μM) of compounds **1** and **7** led to significantly decreases in LDH. The LDH leakage percentages of samples treated with compound **1** were 147.4%, 138.5%, and 126.7%, respectively, and similar levels were found with compound **7** (143.7%, 134.4% and 126.3%, respectively). The results indicated that the two compounds can prevent A$\beta_{1\text{-}42}$-induced damage by reducing the permeability of the plasma membrane.

3.4. Effects on A$\beta_{1\text{-}42}$-induced intracellular reactive oxygen species (ROS) in PC12 cells

Overproduction of ROS can lead to severe impairment of cellular functions. Once ROS cannot be cleared by the antioxidant system, oxidative stress may occur. For instance, it can peroxidize membrane lipids, oxidize proteins and attack mitochondrial DNA [30]. The effect of hederacolchiside E and compound **7** on the intracellular ROS level of PC12 cells was measured using a ROS-sensitive fluorescent probe (DCFH-DA). DCFH-DA can passively enter the cell and react with ROS to produce highly fluorescent dichlorofluorescein (DCF), which is readily detected by flow cytometry. As shown in Fig. 5, after treatment with 40 μM A$\beta_{1\text{-}42}$ for 24 h, the intracellular ROS level of PC12 cells substantially increased to 51.4% relative to the control value (1.12%). When the cells were preconditioned with 10, 50, and 100 μM of **1** or **7**, the A$\beta_{1\text{-}42}$-induced ROS levels were reduced in a dose-dependent manner. The experiments demonstrated that compound **7** significantly reduced ROS levels to 45.0%, 30.7%, and 20.6%, respectively, and showed better ROS-lowering activity than **1** (43.0%, 35.0%, and 29.2%, respectively) in high-dose groups.

3.5. Effects on A$\beta_{1\text{-}42}$-induced malondialdehyde (MDA) in PC12 cells

Lipid peroxidation is one of the earliest recognized effects of



Scheme 3. Synthesis of target compounds **1-8**. Reagents and conditions: (a) 10% Pd-C, H_2, EtOAc, reflux, 2.5 h; (b) TMSOTf, CH_2Cl_2, 4 Å MS, N_2, 0 °C, 30 min; (c) NaOMe, MeOH-CH_2Cl_2 2:1, v/v, rt, 5 h; (d) *p*-TsOH, MeOH-CH_2Cl_2 2:1, v/v, 40 °C.

oxygen toxicity in the cell membranes that can lead to various degradation products such as MDA. This effect has been studied widely as an indicator of lipid peroxidation and a marker of oxidative stress [31]. The *in vitro* MDA expression levels in samples treated with compounds **1** and **7** are displayed in Fig. 6. The results showed that these two compounds significantly decreased the MDA levels in a dose-dependent manner, and compound **7** possessed more potent activity (2.0, 1.6 and 1.2 nmol/mL) than **1** (2.5, 1.9 and 1.3 nmol/mL) at all test concentrations.

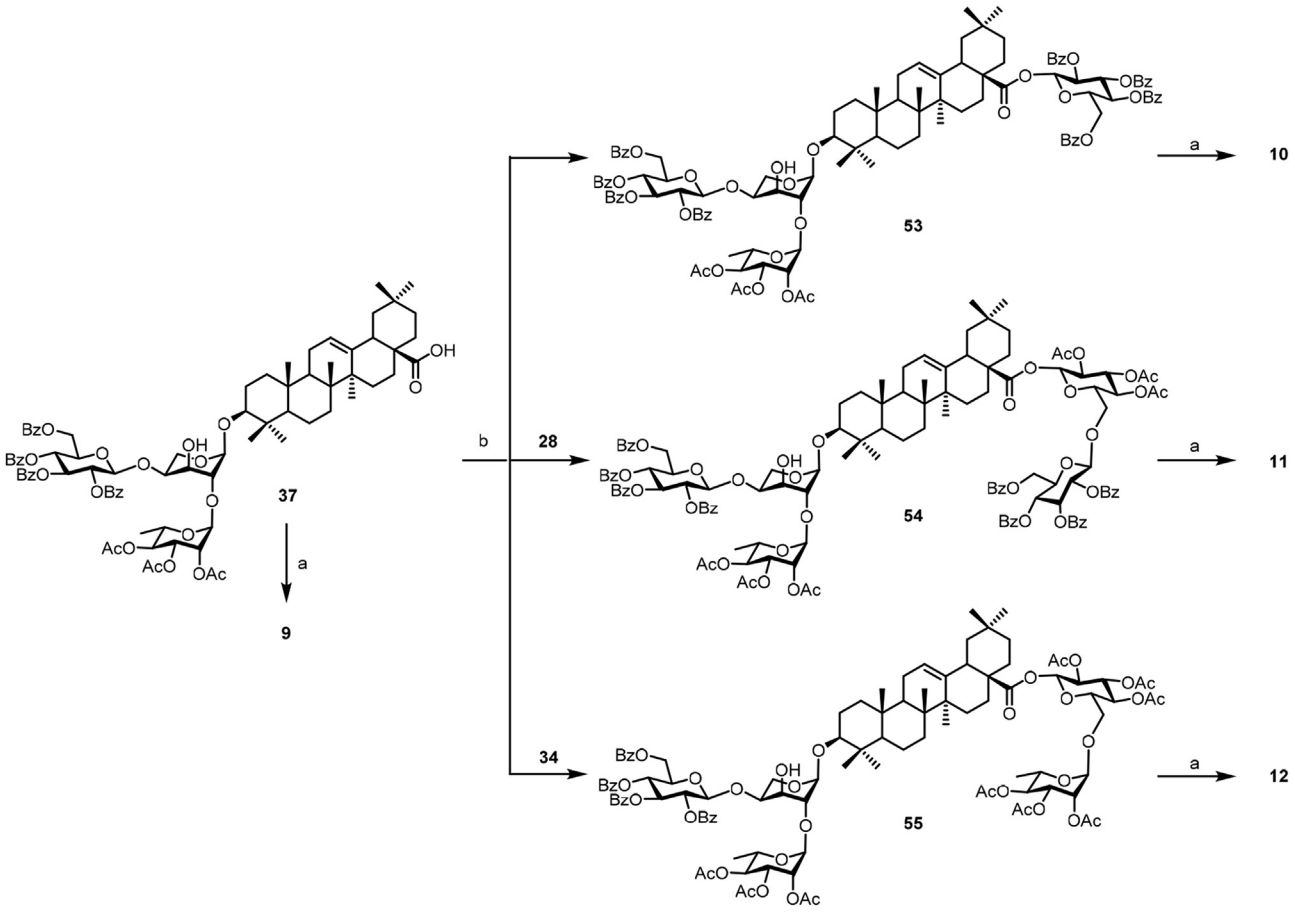
4. Conclusions

Natural saponin hederacolchiside E was first synthesized in a concise and practical way, and its analogs **2-12** were designed and synthesized based on the structure-guided SAR. The neuroprotective effects of these compounds against H_2O_2- and A$\beta_{1\text{-}42}$-induced injury in PC12 cells and the SAR were evaluated and discussed. The twelve compounds showed activity difference in the test and some of them possessed potent neuroprotective effects. Among them, compound **7** showed the best activity against A$\beta_{1\text{-}42}$-induced cell damage, which was noticeably superior to the natural compound **1**. The *in vitro* detection of related indicators in terms of neuroprotection showed that natural saponin **1** and compound **7** could significantly decrease the levels of LDH, ROS and MDA in a concentration-depended manner, indicating that the anti-Aβ activities of these compounds were highly related to their antioxidant activities. In particular, compound **7**, which exhibited a relatively simple chemical structure and potent neuroprotection, could be used as a promising lead for the development of a new series of neuroprotective agents against AD. The pharmacokinetics of these kinds of saponins require further study.

5. Experimental

5.1. Chemistry

All the commercial reagents were used without further purification unless otherwise specified. Solvents were dried and redistilled prior to use in the usual manner. Analytical thin-layer chromatography (TLC) was performed with silica gel HF254. Preparative column chromatography was performed with silica gel H (200-300 mesh). Melting points were measured with a BÜCHI Melting Point B-540. Optical rotations were recorded on an Anton Paar MCP 200 digital polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker ARX 600 MHz spectrometer, using Me_4Si as the internal standard if not otherwise mentioned. *J* values are given in hertz. HR-MS spectra were obtained on a Bruker micro-TOF-Q spectrometer.



Scheme 4. Synthesis of target compound **9-12**. Reagents and conditions: (a) NaOMe, MeOH-CH_2Cl_2 2:1, v/v, rt, 5 h; (b) sugar trichloroacetimidates, TMSOTf, CH_2Cl_2, 4 Å MS, N_2, 0 °C, 30 min.

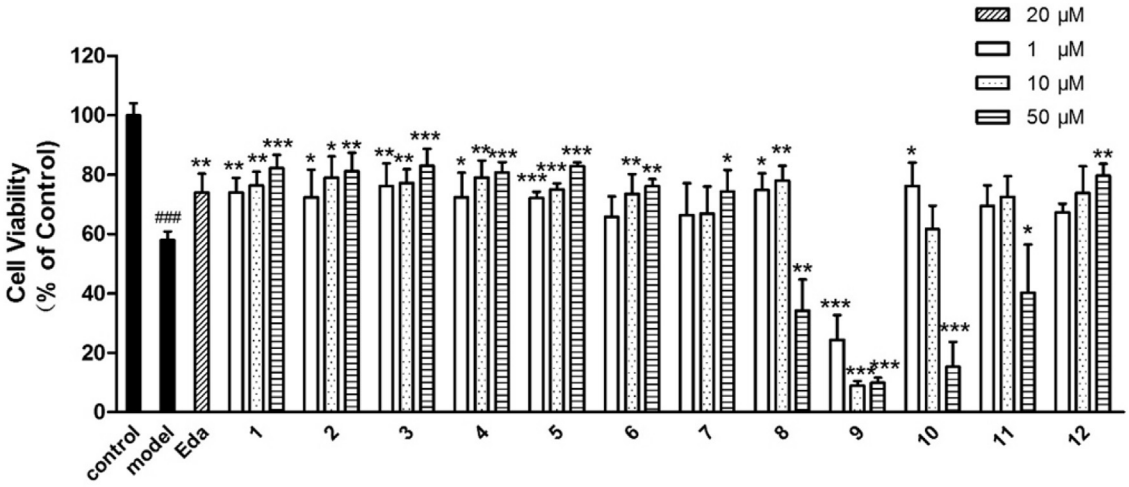


Fig. 3. The neuroprotective effects of compounds **1**–**12** on the H2O2-induced decrease in cell viability in PC12 cells. The cell viability in control was taken as 100%, and the average value of cell viability under H_2O_2 (200 μM) exposure was 57.9 ± 2.9%. Data are expressed as mean ± the standard deviation (SD), $n = 3$. Edaravone (Eda) was used as positive control. $^{###}p < 0.001$ vs the control group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs the H_2O_2-treated group.

*5.1.1. Benzyl Oleanolate 3-O-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**17**)*

A solution of compound **16** (5.03 g, 5.08 mmol) in CH_2Cl_2 – MeOH (1:2, v/v, 50 mL) was added *p*-TsOH (2.62 g, 15.24 mmol), and stirred at rt for 4 h. When TLC (2:1, petroleum ether–EtOAc) showed the deprotection was complete, Et_3N (2 mL) was added to quench the reaction and the mixture was concentrated and purified by a silica gel column chromatography (1.2:1, petroleum

Table 1
The neuroprotective effects of compounds **1-8, 10-12** on the Aβ_{1-42}-induced decrease in cell viability in PC12 cells.

Compd.	Cell Viability (% of Control)		
	10 μM	50 μM	100 μM
1	57.0 ± 2.5	65.8 ± 1.6[b]	70.4 ± 3.0[b]
2	58.3 ± 2.2	59.7 ± 2.6	–[c]
3	61.4 ± 2.1[a]	68.4 ± 3.7[b]	74.6 ± 2.1[b]
4	52.6 ± 1.2	58.6 ± 0.5	–
5	52.7 ± 1.2	55.7 ± 2.1	–
6	58.5 ± 1.1	57.9 ± 1.5	–
7	55.9 ± 2.7	69.9 ± 3.3[b]	79.1 ± 4.2[b]
8	54.3 ± 1.0	60.3 ± 2.4[a]	–
10	14.5 ± 0.3	10.3 ± 1.0	–
11	29.5 ± 0.4	19.8 ± 2.6	–
12	59.3 ± 0.7[a]	63.3 ± 2.0[b]	–
model	55.3 ± 1.5		

The cell viability in control was taken as 100%, and the average value of cell viability under Aβ_{1-42} (40 μM) exposure was 55.3 ± 1.5%. Data are expressed as mean ± SD, $n = 3$.
[a] $p < 0.05$ vs the Aβ_{1-42}-treated group.
[b] $p < 0.01$ vs the Aβ_{1-42}-treated group.
[c] The – means not test.

ether–EtOAc) to give compound **17** (4.20 g, 87%) as a white amorphous solid. R_f = 0.36 (1:1, petroleum ether – EtOAc); Mp 141–143 °C; [α]20 D +9.62 (*c* 2.08, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36–7.28 (m, 5H, Ar–H), 5.30–5.24 (m, 3H), 5.06 (m, 4H), 4.70 (d, *J* = 3.0 Hz, 1H, H-1^{I}), 4.03–3.98 (m, 1H), 3.90 (dt, *J* = 7.9, 4.0 Hz, 1H), 3.88–3.85 (m, 1H), 3.85–3.83 (m, 1H), 3.74 (dd, *J* = 11.7, 8.3 Hz, 1H), 3.59 (dd, *J* = 11.7, 4.3 Hz, 1H), 3.11 (dd, *J* = 11.8, 4.4 Hz, 1H), 2.90 (dd, *J* = 13.7, 4.0 Hz, 1H), 2.14, 2.03, 1.98 (s each, 3H each, 3 × CH_3CO), 1.87–1.20 (m, 18H), 1.18 (d, *J* = 6.3 Hz, 3H, H-6^{II}), 1.10, 0.96, 0.91, 0.89, 0.87, 0.79, 0.59 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.55, 170.19, 170.13, 170.10, 143.83, 136.54, 128.53, 128.51, 128.11, 128.03, 122.55, 102.02, 98.22, 90.40, 76.00, 71.00, 70.60, 69.83, 69.02, 67.09, 66.06, 65.43, 61.07, 55.60, 47.73, 46.85, 46.01, 41.79, 41.49, 39.41, 39.21, 38.64, 36.82, 33.98, 33.22, 32.77, 32.48, 30.82, 28.22, 27.73, 25.97, 25.85, 23.77, 23.52, 23.16, 21.01, 20.91, 20.83, 18.35, 17.45, 16.97, 16.50, 15.44; HRMS(ESI): calcd. for $[M+Na]^+$ $C_{54}H_{78}O_{14}Na$: 973.5284, found 973.5291.

*5.1.2. General procedure for the preparation of intermediates **18-23***

A mixture of **17** (0.70 g, 0.74 mmol), trichloroacetimidate donors (0.89 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (50 mL) wad stirred at 0 °C for 20 min. A solution of TMSOTf (0.22 mL, 0.34 mmol/mL) in dry CH_2Cl_2 was injected to the reaction mixture. The mixture was stirred under these conditions for 30 min, then quenched with Et_3N (0.1 mL). The mixture was the diluted with CH_2Cl_2 and filtered. The filtrate was concentrated and purified by a silica gel column chromatography (2:1 petroleum ether – EtOAc) to afford **18-23**. The ^{1}H-NMR and ^{13}C-NMR data of intermediates **19-23** was shown in supplementary data.

*5.1.3. Benzyl Oleanolate 3-O-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside (**18**)*

Following the general procedure mentioned above to get compound **18** (0.97 g, 86%) as a white foam. R_f = 0.32 (2:1, petroleum ether – EtOAc); Mp 119–121 °C; [α]20 D –92.04 (*c* 2.26, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03–7.27 (m, 25H, Ar–H), 5.92 (t, *J* = 9.7 Hz, 1H), 5.69 (t, *J* = 9.7 Hz, 1H), 5.56 (dd, *J* = 9.8, 7.9 Hz, 1H), 5.30–5.25 (m, 2H), 5.23 (dd, *J* = 3.4, 1.6 Hz, 1H), 5.11–5.00 (m, 4H, $PhCH_2$), 4.97 (d, *J* = 0.9 Hz, 1H, H-1^{II}), 4.68 (dd, *J* = 12.1, 3.2 Hz, 1H), 4.56 (d, *J* = 3.6 Hz, 1H, H-1^{I}), 4.50 (dd, *J* = 12.1, 5.0 Hz, 1H), 4.21 (ddd, *J* = 9.6, 4.7, 3.5 Hz, 1H), 4.06–4.02 (m, 1H), 4.00 (dd, *J* = 10.0, 6.4 Hz, 1H), 3.96 (dt, *J* = 7.2, 3.5 Hz, 1H), 3.80–3.76 (m, 1H), 3.74 (dd, *J* = 5.6, 3.8 Hz, 1H), 3.63 (dd, *J* = 11.9, 3.5 Hz, 1H), 3.03 (dd, *J* = 11.8, 4.4 Hz, 1H), 2.90 (dd, *J* = 13.5, 3.9 Hz, 1H, H-18), 2.13, 2.04, 1.98 (s each, 3H each, 3 × CH_3CO), 1.16 (d, *J* = 6.2 Hz, 3H, H-6^{II}), 1.10, 0.93, 0.92, 0.89, 0.84, 0.74, 0.58 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.57, 170.12, 166.20, 165.90, 165.44, 165.28, 143.79, 136.56, 133.56, 133.36, 133.32, 129.98, 129.90, 129.74, 129.27, 128.93, 128.54, 128.51, 128.42, 128.12, 128.04, 122.65, 102.40, 101.90, 97.89, 90.24, 75.95, 75.92, 72.92, 72.43, 72.21, 71.08, 70.41, 69.83, 69.70, 69.11, 66.83, 66.07, 65.46, 63.12, 60.42, 55.72, 47.74, 46.87, 46.02, 42.12, 41.78, 41.50, 39.42, 39.17, 38.70, 36.80, 33.99, 33.24, 32.78, 32.51, 30.84, 30.27, 29.83, 29.26, 28.22, 27.73, 25.99, 25.85, 23.78, 23.53, 23.49, 23.22, 23.18, 21.03, 20.93, 20.86, 18.35, 17.42, 16.98, 16.49, 15.44, 14.22; HRMS(ESI): calcd. for $[M+Na]^+$ $C_{88}H_{104}O_{23}Na$: 1551.6861, found 1551.6864.

*5.1.4. Phenyl 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranoside (**27**)*

A mixture of compound **26** (0.30 g, 0.75 mmol), perbenzoylated glucopyranosyl trichloroacetimidate (0.67 g, 0.90 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (30 mL) was stirred for 20 min at 0 °C. A solution of TMSOTf (0.22 mL, 0.34 mmol/mL) in dry CH_2Cl_2 was injected to the reaction mixture. The mixture was stirred under these conditions for 30 min, then quenched with Et_3N (0.1 mL). The

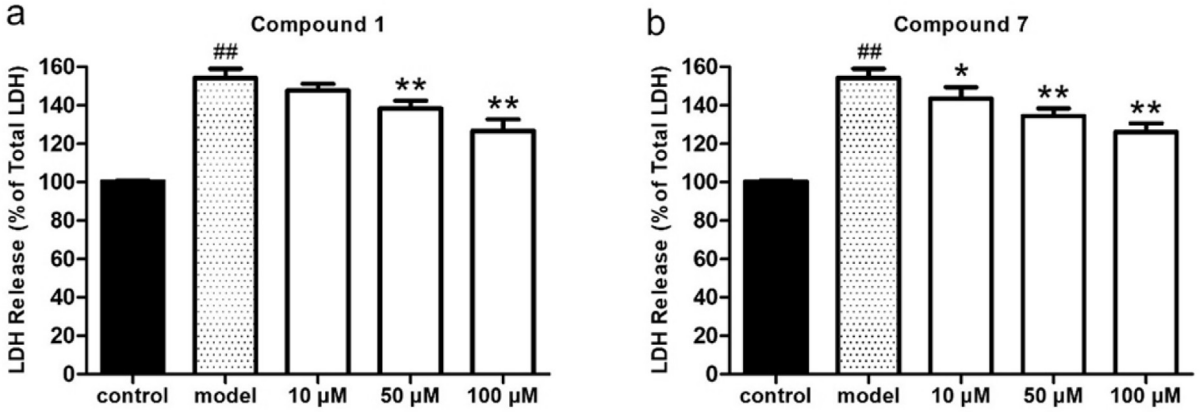


Fig. 4. The effects of compounds **1** (a) and **7** (b) at different concentrations on the LDH leakage in Aβ_{1-42}-treated PC12 cells. PC12 cells were treated with 40 μM Aβ_{1-42} for 24 h in the presence or absence of compounds (10, 50, 100 μM). The results are expressed as mean ± SD, $n = 3$.$^{\#\#}p < 0.01$ as compared with control group. $^{*}p < 0.05$ vs the Aβ_{1-42}-treated group. $^{**}p < 0.01$ vs the Aβ_{1-42}-treated group.

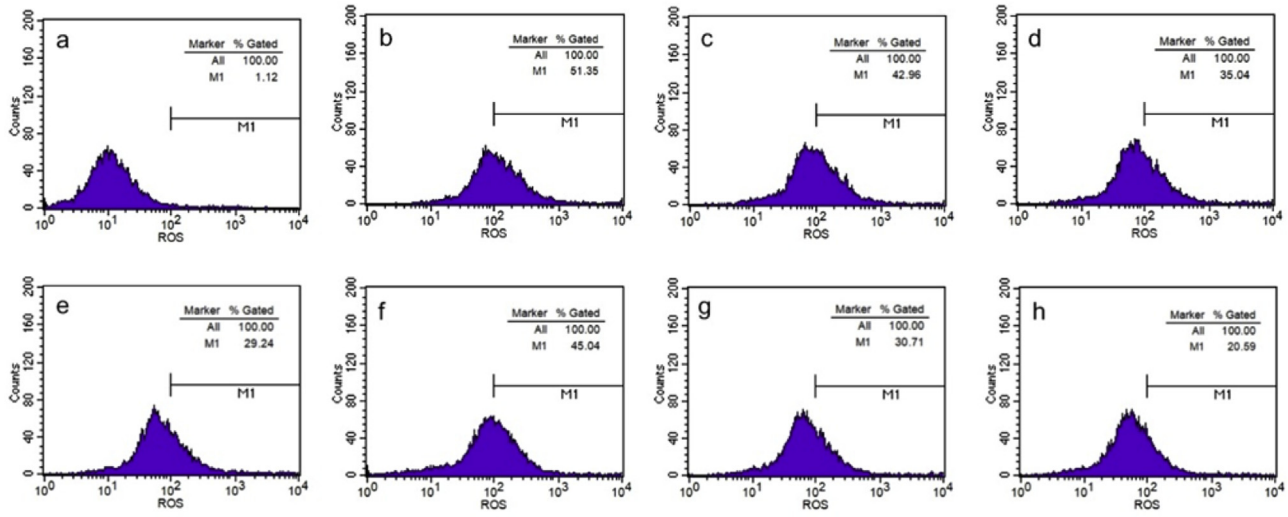


Fig. 5. The effects of compounds **1** and **7** on A$\beta_{1\text{-}42}$-induced ROS level in PC12 cells as measured by flow cytometry. The figure shows representative flow cytometric histograms. (a) Control; (b) 40 μM A$\beta_{1\text{-}42}$ alone; (c) 10 μM compound **1**+40 μM A$\beta_{1\text{-}42}$; (d) 50 μM compound **1**+40 μM A$\beta_{1\text{-}42}$; (e) 100 μM compound **1**+40 μM A$\beta_{1\text{-}42}$; (f) 10 μM compound **7**+40 μM A$\beta_{1\text{-}42}$; (g) 50 μM compound **7**+40 μM A$\beta_{1\text{-}42}$; (h) 100 μM compound **7**+40 μM A$\beta_{1\text{-}42}$.

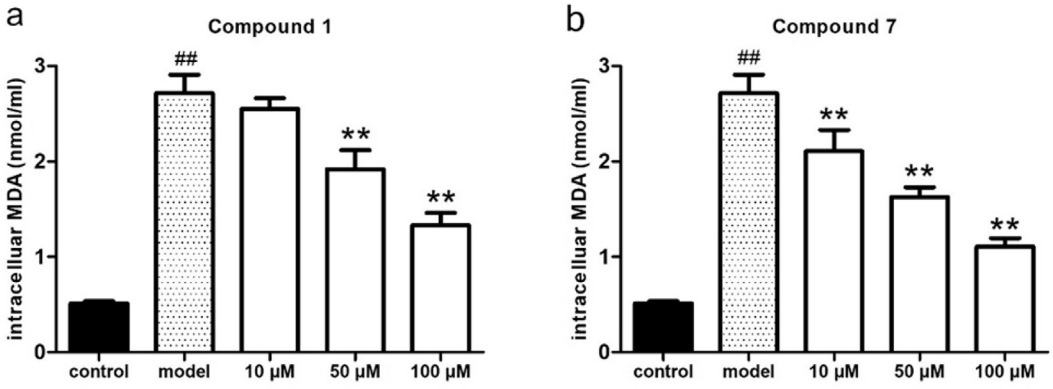


Fig. 6. The effects of compounds **1** (a) and **7** (b) at different concentrations on the MDA levels in A$\beta_{1\text{-}42}$-treated PC12 cells. PC12 cells were treated with 40 μM of A$\beta_{1\text{-}42}$ for 24 h in the presence or absence of compounds (10, 50, 100 μM). The results are expressed as mean ± SD, $n = 3$. $^{\#\#}p < 0.01$ as compared with control group. $^{**}p < 0.01$ vs the A$\beta_{1\text{-}42}$-treated group.

mixture was the diluted with CH_2Cl_2 and filtered. The residue was purified by a silica gel column chromatography (2.5:1 petroleum ether − EtOAc) to give compound **27** (0.61 g, 83%) as a white foam. $R_f = 0.27$ (2:1, petroleum ether − EtOAc); Mp 100–103 °C; [α]20 D −1.06 (*c* 1.89, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, $J = 7.2$ Hz, 2H), 7.92 (d, $J = 7.2$ Hz, 2H), 7.89 (d, $J = 7.2$ Hz, 2H), 7.83 (d, $J = 7.2$ Hz, 2H), 7.56 (t, $J = 7.4$ Hz, 1H), 7.51 (t, $J = 7.4$ Hz, 1H), 7.47 (t, $J = 7.4$ Hz, 1H), 7.46–7.42 (m, 5H), 7.38–7.35 (m, 5H), 7.33 (t, $J = 7.7$ Hz, 2H), 7.29 (t, $J = 7.8$ Hz, 2H), 5.83 (t, $J = 9.6$ Hz, 1H), 5.63 (t, $J = 9.7$ Hz, 1H), 5.49 (dd, $J = 9.7$, 7.9 Hz, 1H), 5.13 (t, $J = 9.3$ Hz, 1H), 4.92 (d, $J = 7.9$ Hz, 1H), 4.85 (t, $J = 9.7$ Hz, 1H), 4.78 (t, $J = 9.8$ Hz, 1H), 4.66–4.59 (m, 2H), 4.46 (dd, $J = 12.1$, 5.4 Hz, 1H), 4.08–4.03 (m, 1H), 3.83 (dd, $J = 11.8$, 2.1 Hz, 1H), 3.80–3.75 (m, 1H), 3.69 (ddd, $J = 9.6$, 7.2, 2.1 Hz, 1H), 2.05 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.05, 169.57, 169.24, 166.11, 165.78, 165.23, 165.16, 133.48, 133.22, 132.91, 131.64, 129.84, 129.84, 129.80, 129.77, 129.58, 129.19, 128.87, 128.82, 128.52, 128.48, 128.45, 128.34, 128.32, 100.86, 85.41, 77.93, 73.83, 72.92, 72.39, 71.80, 70.00, 69.70, 68.84, 68.02, 63.02, 20.74, 20.57, 20.50; HRMS(ESI): calcd. for $[M+Na]^+$ $C_{52}H_{48}O_{17}SNa$: 999.2504, found 999.2484.

*5.1.5. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-acetyl-β-D-glucopyranosyl trichloroacetimidate (**28**)*

A solution of compound **27** (0.54 g, 0.55 mmol) in acetone-H_2O (9:1, 50 mL) was added TCCA (0.13 g, 0.55 mmol) slowly at rt. The solution was stirred for 1 h and then acetone was removed *in vacuum*. The reaction mixture was dissolved with CH_2Cl_2 and successively washed with saturated $NaHCO_3$ and followed by brine, then dried over Na_2SO_4. $R_f = 0.35$ (1:1, petroleum ether − EtOAc). A mixture of crude solid, CCl_3CN (0.22 mL, 2.20 mmol) and DBU (0.01 mL, 0.07 mmol) in CH_2Cl_2 (30 mL) was strried at rt for 1 h. After completion of the reaction based on TLC, the solvent was removed *in vacuum* and the resulting residue was purified by a silica gel column chromatography (2:1 petroleum ether–EtOAc) to give compound **28** (0.47 g, 83% over two steps) as a white foam. $R_f = 0.25$ (2:1, petroleum ether − EtOAc); Mp 107–109 °C; [α]20 D +35.54 (*c* 1.21, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.43 (s, 1H), 8.02 (d, $J = 7.1$ Hz, 2H), 7.95 (d, $J = 7.2$ Hz, 2H), 7.89 (d, $J = 7.2$ Hz, 2H), 7.81 (d, $J = 7.2$ Hz, 2H), 7.55 (t, $J = 7.4$ Hz, 1H), 7.52–7.47 (m, 2H), 7.42 (dd, $J = 15.0$, 7.5 Hz, 4H), 7.37 (t, $J = 7.8$ Hz, 1H), 7.33 (t, $J = 7.7$ Hz, 2H), 7.27 (t, $J = 7.7$, 2H), 6.39 (d, $J = 3.6$ Hz, 1H), 5.87 (t, $J = 9.6$ Hz, 1H), 5.65 (t, $J = 9.7$ Hz, 1H), 5.51–5.46 (m, 2H), 4.96 (t,

J = 4.2, 1H), 4.94–4.91 (m, 2H), 4.63 (dd, J = 12.1, 3.2 Hz, 1H), 4.49 (dd, J = 12.1, 5.2 Hz, 1H), 4.19–4.12 (m, 2H), 3.96 (dd, J = 11.6, 2.0 Hz, 1H), 3.70 (dd, J = 11.6, 6.1 Hz, 1H), 1.97 (s, 6H), 1.96 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.88, 169.74, 169.53, 166.12, 165.78, 165.20, 165.07, 160.61, 133.44, 133.21, 133.15, 133.13, 129.93, 129.85, 129.76, 129.63, 129.30, 128.83, 128.81, 128.44, 128.41, 128.31, 128.29, 100.78, 92.62, 90.63, 72.87, 72.34, 71.65, 71.38, 69.78, 69.72, 68.49, 67.40, 63.06, 20.64, 20.52, 20.41; HRMS(ESI): calcd. for $[M+Na]^+$ $C_{48}H_{44}O_{18}NCl_3Na$: 1050.1516, found 1050.1492.

*5.1.6. 2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-O-acetyl-6-O-benzoyl-β-D- glucopyranosyl trichloroacetimidate (**34**)*

Crude **34** was prepared from **33** (1.51 g, 2.06 mmol) by the same procedure as for **28**, and purified by a silica gel column chromatography (3:1 petroleum ether–EtOAc) to give compound **34** (1.32 g, 81% over two steps) as a white foam. R_f = 0.41 (1:1, petroleum ether – EtOAc); Mp 82–84 °C; [α]20 D +23.78 (*c* 2.08, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.66 (s, 1H, NH), 8.04 (d, J = 7.4 Hz, 2H, Ph), 7.58 (t, J = 7.4 Hz, 1H, Ph), 7.45 (t, J = 7.7 Hz, 2H, Ph), 6.51 (d, J = 3.7 Hz, 1H, H-1^{V}), 5.62 (t, J = 9.7 Hz, 1H), 5.26 (dd, J = 10.1, 3.2 Hz, 1H), 5.19–5.15 (m, 1H), 5.09–5.00 (m, 2H), 4.92 (d, J = 1.5 Hz, 1H, H-1^{VI}), 4.80 (dd, J = 12.5, 1.4 Hz, 1H), 4.54 (dd, J = 12.5, 3.7 Hz, 1H), 4.28 (d, J = 8.9 Hz, 1H), 3.99 (t, J = 9.7 Hz, 1H), 3.92 (dq, J = 12.4, 6.1 Hz, 1H), 2.09, 2.08, 2.05, 2.01, 1.99 (s each, 3H each, 5 × CH_3CO), 1.18 (d, J = 6.2 Hz, 3H, H-6^{VI}). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.17, 170.06, 170.02, 169.83, 165.90, 161.02, 133.28,129.94, 129.82, 128.51, 99.58, 93.11, 90.87, 76.76, 71.31, 70.90, 70.78, 70.35, 70.15, 68.66, 67.99, 62.02, 21.22, 20.95, 20.91, 20.83, 20.58, 17.39; HRMS(ESI): calcd. for $[M+Na]^+$ $C_{31}H_{36}O_{16}NCl_3Na$: 806.0992, 808.0962, found 806.0954, 808.0943.

*5.1.7. Phenyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-O-acetyl-6-O-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranoside (**35**)*

Crude **35** was prepared from **34** (1.34 g, 1.71 mmol) with **26** by the same procedure as for **27**, and purified by a silica gel column chromatography (3:2 petroleum ether – EtOAc) to give compound **35** (1.52 g, 87%) as a white foam. R_f = 0.23 (1.5:1, petroleum ether – EtOAc); Mp 102–104 °C; [α]20 D −44.34 (*c* 1.06, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03 (dd, J = 8.2, 1.1 Hz, 2H, Ph), 7.56 (t, J = 7.4 Hz, 1H, Ph), 7.47–7.40 (m, 4H, Ph), 7.34–7.28 (m, 3H, Ph), 5.24–5.16 (m, 3H), 5.13 (dd, J = 3.1, 2.1 Hz, 1H), 5.04 (t, J = 10.0 Hz, 1H), 4.94–4.90 (m, 1H), 4.89 (d, J = 1.8 Hz, 1H), 4.88–4.79 (m, 3H), 4.69 (d, J = 10.1 Hz, 1H), 4.58 (d, J = 7.9 Hz, 1H), 4.45 (dd, J = 12.3, 4.3 Hz, 1H), 3.91 (t, J = 9.3 Hz, 1H), 3.88–3.82 (m, 2H), 3.74–3.69 (m, 1H), 3.66 (ddd, J = 9.7, 4.1, 1.9 Hz, 1H), 3.61 (dd, J = 11.3, 7.3 Hz, 1H), 2.07, 2.06, 2.06, 2.03, 1.97, 1.96, 1.95, 1.95 (s each, 3H each, 8 × CH_3CO), 1.16 (d, J = 6.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.30, 170.23, 170.14, 170.04, 169.98, 169.66, 169.40, 165.91, 133.28, 132.27, 132.24, 129.94, 129.83, 129.31, 128.57, 128.30, 100.52, 99.21, 85.71, 77.47, 74.11, 74.03, 73.02, 72.04, 70.78, 70.15, 70.06, 68.82, 68.65, 68.36, 67.93, 62.32, 21.17, 20.94, 20.91, 20.87, 20.83, 20.79, 20.71, 20.65, 17.35; HRMS(ESI): calcd. for $[M+Na]^+$ $C_{47}H_{56}O_{23}S_1Na$: 1043.2825, found 1043.2833.

*5.1.8. 2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-O-acetyl-6-O-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-acetyl-β-D-glucopyranosyl trichloroacetimidate (**36**)*

Crude **36** was prepared from **35** (1.52 g, 1.50 mmol) by the same procedure as for **34**, and purified by a silica gel column chromatography (1.5:1 petroleum ether – EtOAc) to give compound **36** (1.33 g, 83%) as a white foam. R_f = 0.47 (1:1, petroleum ether – EtOAc); Mp 100–102 °C; [α]20 D +15.43 (*c* 1.88, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.66 (s, 1H, NH), 8.03 (d, J = 7.9 Hz, 2H, Ph), 7.56 (t, J = 7.2 Hz, 1H, Ph), 7.45 (t, J = 7.6 Hz, 2H, Ph), 6.50 (d, J = 3.6 Hz, 1H, H-1^{IV}), 5.51 (t, J = 9.8 Hz, 1H), 5.22–5.18 (m, 2H), 5.11 (s, 1H), 5.06–4.99 (m, 3H), 4.87–4.83 (m, 2H), 4.77 (d, J = 11.3 Hz, 1H), 4.58 (d, J = 7.9 Hz, 1H), 4.47 (dd, J = 12.3, 4.1 Hz, 1H), 4.15–4.11 (m, 1H), 3.91 (t, J = 9.6 Hz, 2H), 3.87–3.82 (m, 1H), 3.73 (d, J = 9.4 Hz, 1H), 3.54 (dd, J = 11.2, 5.2 Hz, 1H), 2.05, 2.05, 2.04, 2.02, 2.00, 1.99, 1.99, 1.96 (s each, 3H each, 8 × CH_3CO), 1.14 (d, J = 6.2 Hz, 3H, H-6^{VI}); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.27, 170.14, 170.12, 170.03, 170.00, 169.90, 169.48, 160.85, 133.26, 129.93, 129.85, 128.53, 100.38, 99.14, 92.89, 90.83, 76.85, 74.01, 73.01, 71.83, 70.78, 70.11, 70.07, 69.84, 68.63, 68.24, 67.87, 67.22, 62.45, 21.13, 20.90, 20.80, 20.68, 20.55, 17.33; HRMS(ESI): calcd. for $[M+Na]^+$ $C_{43}H_{52}Cl_3NO_{24}Na$: 1094.1837, found 1094.1832.

*5.1.9. General procedure for the preparation of intermediates **37-42***

A sustention of **18** (or **19-23**) (0.50 mmol) and 10% Pd-C (0.2 g) in EtOAc (50 mL) was refluxed and bubbled up with H_2 (70 mL/min) for 3.5 h. After completion of the reaction based on TLC, Pd-C was removed through filtration and the filtrate was concentrated to dryness and purified by a silica gel column chromatography (1.5:1 petroleum ether – EtOAc) to afford **37-42**. The ^{1}H-NMR and ^{13}C-NMR data of intermediates **38-42** was shown in supplementary data.

*5.1.10. Oleanolic acid 3-O-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside (**37**)*

Following the general procedure mentioned above to get compound **37** (0.65 g, 91%) as a white foam. R_f = 0.19 (2:1, petroleum ether – EtOAc); Mp 175–178 °C; [α]20 D +27.27 (*c* 1.98, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.03–7.28 (m, 20H, Ar–H), 5.91 (t, J = 9.7 Hz, 1H), 5.69 (t, J = 9.7 Hz, 1H), 5.56 (dd, J = 9.7, 8.0 Hz, 1H), 5.30–5.25 (m, 2H), 5.23 (dd, J = 3.3, 1.5 Hz, 1H), 5.04 (dd, J = 19.7, 9.0 Hz, 2H), 4.94 (br s, 1H, H-1^{II}), 4.68 (dd, J = 12.1, 3.1 Hz, 1H), 4.59 (d, J = 3.1 Hz, 1H, H-1^{I}), 4.50 (dd, J = 12.1, 4.9 Hz, 1H), 4.23–4.18 (m, 1H), 4.05–3.95 (m, 3H), 3.82–3.77 (m, 1H), 3.77–3.73 (m, 1H), 3.64 (dd, J = 11.5, 3.3 Hz, 1H), 3.04 (dd, J = 11.6, 4.4 Hz, 1H), 2.81 (dd, J = 13.6, 3.8 Hz, 1H), 2.13, 2.03, 1.98 (s each, 3H each, 3 × CH_3CO), 1.16 (d, J = 6.2 Hz, 3H, H-6^{II}), 1.12, 0.93, 0.92, 0.90, 0.87, 0.74, 0.74 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 183.11, 170.12, 166.21, 165.90, 165.44, 165.29, 143.66, 133.56, 133.34, 133.22, 129.99, 129.91, 129.75, 129.31, 128.99, 128.95, 128.94, 128.54, 128.52, 128.42, 122.75, 102.28, 101.86, 97.99, 90.23, 76.07, 75.75, 72.95, 72.42, 72.20, 71.08, 70.15, 69.85, 69.71, 69.12, 66.89, 63.13, 60.14, 55.71, 47.78, 46.67, 46.05, 41.76, 41.11, 39.41, 39.20, 38.69, 36.86, 33.96, 33.20, 32.74, 32.52, 30.80, 29.84, 28.22, 27.80, 26.01, 25.84, 23.71, 23.54, 23.13, 21.03, 20.93, 20.87, 18.34, 17.43, 17.09, 16.46, 15.46; HRMS(ESI): calcd. for $[M+Na]^+$ $C_{81}H_{98}O_{23}Na$: 1461.6391, found 1461.6398.

*5.1.11. General procedure for the preparation of compounds **1-6***

A mixture of compounds **37** (or **38-42**) (0.45 mmol), **36** (0.58 g, 0.54 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (30 mL) were stirred under an atmosphere of N_2 for 30 min at 0 °C. A solution of TMSOTf (0.13 mL, 0.34 mmol/mL) in dry CH_2Cl_2 was injected. The mixture was stirred under these conditions for 30 min, then quenched with Et_3N (0.1 mL), and concentrated to dryness to afford crude intermediates **42-48**. The resulting residue was dissolved in dry MeOH-CH_2Cl_2 (2:1, v/v, 30 mL), to which a freshly prepared solution of NaOMe in MeOH solution (1.0 mol/L, 0.45 mL) was added. The solution was stirred at rt for 5 h and then neutralized with Dowex H^+ resin to pH 7 and filtered. The filtrate was

concentrated and purified on a Sephadex LH-20 column using MeOH as the eluent to afford compound **1-6**.

*5.1.12. 3-O-[α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl]oleanolic acid 28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester (**1**)*

Following the general procedure mentioned above to get compound **1** (0.52 g, 84% over two steps) as a white foam. R_f = 0.24 (4:1:5, *n*-BuOH-AcOH-H_2O); Mp 226–228 °C; [α]20 D −21.74 (*c* 1.15, CH_3OH); ^{1}H NMR (600 MHz, C_5D_5N) δ 8.40–6.45 (m, 16H. OH), 6.27 (d, *J* = 8.1 Hz, 1H, H-1^{IV}), 6.20 (br s, 1H, H-1^{II}), 5.93 (d, *J* = 9.0 Hz, 1H, OH), 5.88 (br s, 1H, H-1^{VI}), 5.42 (br s, 1H, H-12), 5.16 (d, *J* = 7.8 Hz, 1H, H-1^{III}), 4.99 (d-like, *J* = 6.0 Hz, 1H, H-1^{V}), 4.78 (d, *J* = 6.0 Hz, 1H, H-1^{I}), 4.75 (s, 1H), 4.71–4.62 (m, 4H), 4.57 (dd, *J* = 9.1, 2.5 Hz, 1H), 4.58–4.52 (m, 2H), 4.47–4.38 (m, 3H), 4.38–4.25 (m, 8H), 4.25–4.20 (m, 3H), 4.19–4.09 (m, 4H), 4.06 (t, *J* = 8.4 Hz, 1H), 3.96 (t, *J* = 8.4 Hz, 1H), 3.93–3.90 (m, 1H), 3.82 (d, *J* = 10.8 Hz, 1H), 3.68 (d, *J* = 9.4 Hz, 1H), 3.26–3.16 (m, 2H), 2.32 (td, *J* = 13.6, 2.6 Hz, 1H), 2.11–2.00 (m, 2H), 1.99–1.74 (m, 7H), 1.72 (d, *J* = 6.1 Hz, 3H, H-6^{VI}), 1.66 (d, *J* = 6.1 Hz, 3H, H-6^{II}), 1.25, 1.18, 1.13, 1.11, 0.90, 0.90, 0.89 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, C_5D_5N) δ 176.53 (C-28), 144.13 (C-13), 122.88 (C-12), 106.42 (C-1^{III}), 104.95 (C-1^{I}), 104.90 (C-1^{V}), 102.76 (C-1^{VI}), 101.80 (C-1^{II}), 95.67 (C-1^{IV}), 88.69, 79.67, 78.81, 78.76, 78.56, 78.23, 78.08, 77.19, 76.52, 76.35, 75.50, 75.37, 74.09, 74.02, 73.90, 72.79, 72.60, 72.51, 72.32, 71.27, 70.87, 70.32, 69.84, 69.22, 64.53, 62.55, 61.29, 56.00, 48.07, 47.05, 46.24, 42.16, 41.68, 39.90, 39.50, 38.93, 37.03, 34.01, 33.14, 32.56, 30.77, 28.29, 28.07, 26.63, 26.07, 23.81, 23.70, 23.38, 18.67, 18.55, 17.51, 17.04, 15.68; HRMS(ESI): calcd. for [M+Na]$^+$ $C_{65}H_{106}O_{30}Na$: 1389.6661, found 1389.6677.

*5.1.13. 3-O-[α-L-rhamnopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→4)]-α-L-arabinopyranosyl]oleanolic acid 28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester (**2**)*

Following the general procedure mentioned above to get compound **2** (0.49 g, 82% over two steps) as a white foam. R_f = 0.24 (4:1:5, *n*-BuOH-AcOH-H_2O); Mp 230–233 °C; [α]20 D −51.49 (*c* 1.34, CH_3OH); ^{1}H NMR (600 MHz, C_5D_5N) δ 8.09 (s, 1H, OH), 7.54–7.52 (m, 2H, OH), 7.44 (d, *J* = 4.3 Hz, 1H, OH), 7.36 (d, *J* = 4.8 Hz, 1H, OH), 6.86 (d, *J* = 3.0 Hz, 2H, OH), 6.83 (d, *J* = 4.4 Hz, 1H, OH), 6.81–6.77 (m, 1H, OH), 6.76 (d, *J* = 4.1 Hz, 1H, OH), 6.71 (d, *J* = 4.5 Hz, 1H, OH), 6.67 (d, *J* = 3.6 Hz, 1H, OH), 6.54–6.51 (m, 3H, OH), 6.27 (d, *J* = 8.1 Hz, 1H, H-1^{IV}), 6.16 (br s, 1H, H-1^{II}), 5.90 (br s, 1H, H-1^{VI}), 5.73 (d, *J* = 8.8 Hz, 1H, OH), 5.41 (s, 1H, H-12), 5.04–5.00 (m, 2H, H-1^{V}), 4.93 (d, *J* = 7.4 Hz, 1H, H-1^{III}), 4.81 (d, *J* = 5.8 Hz, 1H, H-1^{I}), 4.75 (br s, 1H), 4.71–4.69 (m, 2H), 4.67–4.63 (m, 2H), 4.60–4.57 (m, 1H), 4.53–4.43 (m, 4H), 4.40–4.31 (m, 4H), 4.31–4.25 (m, 4H), 4.25–4.20 (m, 2H), 4.20–4.14 (m, 2H), 4.14–4.08 (m, 3H), 3.99–3.95 (m, 8.4 Hz, 1H), 3.87 (d, *J* = 11.0 Hz, 1H), 3.72–3.67 (m, 2H), 3.25–3.18 (m, 2H), 2.32 (t, *J* = 12.6 Hz, 1H), 1.73 (d, *J* = 6.1 Hz, 3H, H-6^{VI}), 1.65 (d, *J* = 6.0 Hz, 3H, H-6^{II}), 1.25, 1.19, 1.13, 1.10, 0.90, 0.90, 0.88 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, C_5D_5N) δ 176.53 (C-28), 144.08 (C-13), 122.88 (C-12), 106.91 (C-1^{III}), 104.93 (C-1^{I}), 104.88 (C-1^{V}), 102.74 (C-1^{VI}), 101.74 (C-1^{II}), 95.66 (C-1^{IV}), 88.70, 79.13, 78.74, 78.18, 78.06, 77.17, 76.50, 76.33, 75.36, 74.78, 74.05, 74.01, 73.95, 73.88, 72.78, 72.58, 72.50, 72.31, 70.82, 70.31, 69.82, 69.79, 69.18, 67.47, 64.53, 61.25, 55.99, 49.68, 48.06, 47.03, 46.21, 42.13, 41.66, 39.88, 39.50, 38.92, 37.02, 33.99, 33.14, 32.53, 30.76, 29.98, 28.27, 28.06, 26.63, 26.06, 23.79, 23.68, 23.36, 18.65, 18.55, 17.50, 17.02, 15.67; HRMS(ESI): calcd. for [M+Na]$^+$ $C_{64}H_{104}O_{29}Na$: 1359.6555, found 1359.6563.

*5.1.14. 3-O-[α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-α-L-arabinopyranosyl]oleanolic acid 28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester (**3**)*

Following the general procedure mentioned above to get compound **3** (0.51 g, 84% over for two steps) as a white foam. R_f = 0.24 (4:1:5, *n*-BuOH-AcOH-H_2O); Mp 211–214 °C; [α]20 D −36.76 (*c* 1.36, CH_3OH); ^{1}H NMR (600 MHz, C_5D_5N) δ 7.54–7.36 (m, 4H, OH), 6.89–6.29 (m, 12H, OH), 6.27 (d, *J* = 8.1 Hz, 1H, H-1^{IV}), 6.03 (br s, 1H, H-1^{II}), 5.90 (br s, 1H, H-1^{VI}), 5.77 (br s, 1H, H-1^{III}), 5.41 (s, 1H, H-12), 5.03–5.00 (m, 2H, H-1^{V}), 4.92 (d, *J* = 4.3 Hz, 1H, H-1^{I}), 4.76 (s, 1H), 4.71–4.69 (m, 2H), 4.65 (d, *J* = 4.3 Hz, 1H), 4.63–4.56 (m, 3H), 4.55–4.50 (m, 2H), 4.48–4.45 (m, 2H), 4.43–4.37 (m, 3H), 4.37–4.32 (m, 4H), 4.31–4.20 (m, 3H), 4.20–4.05 (m, 5H), 3.97 (t, *J* = 6.9 Hz, 1H), 3.89 (d, *J* = 10.1 Hz, 1H), 3.68 (d, *J* = 9.4 Hz, 1H), 3.62 (d, *J* = 4.2 Hz, 1H), 3.20 (t, *J* = 16.7 Hz, 2H), 2.32 (t, *J* = 12.5 Hz, 1H), 1.73 (d, *J* = 6.1 Hz, 3H, H-6^{VI}), 1.69 (d, *J* = 6.0 Hz, 3H, H-6^{II}), 1.64 (d, *J* = 6.1 Hz, 3H), 1.26, 1.17, 1.11, 1.09, 0.90, 0.90, 0.87 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, C_5D_5N) δ 176.52 (C-28), 144.09 (C-13), 122.85 (C-12), 104.85 (C-1^{I}), 104.81 (C-1^{V}), 102.73 (C-1^{VI}), 102.52 (C-1^{III}), 101.98 (C-1^{II}), 95.63 (C-1^{IV}), 89.01, 78.72, 78.17, 78.04, 77.16, 76.49, 76.32, 75.34, 74.94, 74.02, 73.86, 72.76, 72.57, 72.29, 72.16, 72.01, 70.80, 70.30, 70.27, 70.12, 69.16, 62.90, 61.24, 55.88, 49.68, 48.04, 47.03, 46.21, 42.13, 41.65, 39.87, 39.48, 38.86, 37.00, 33.98, 33.14, 32.52, 30.75, 29.97, 28.26, 28.03, 26.50, 26.05, 23.78, 23.68, 23.36, 18.71, 18.56, 18.54, 17.47, 16.90, 15.62; HRMS(ESI): calcd. for [M+Na]$^+$ $C_{65}H_{106}O_{29}Na$: 1373.6712, found 1373.6672.

*5.1.15. 3-O-[α-L-rhamnopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→4)]-α-L-arabinopyranosyl]oleanolic acid 28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester (**4**)*

Following the general procedure mentioned above to get compound **4** (0.52 g, 84% over two steps) as a white foam. R_f = 0.24 (4:1:5, *n*-BuOH-AcOH-H_2O); Mp 228–231 °C; [α]20 D −43.52 (*c* 2.16, CH_3OH); ^{1}H NMR (600 MHz, C_5D_5N) δ 8.18 (d, *J* = 3.0 Hz, 1H, OH), 7.55–7.32 (m, 4H, OH), 6.88–6.66 (m, 8H, OH), 6.54–6.51 (m, 3H, OH), 6.27 (d, *J* = 8.1 Hz, 1H, H-1^{IV}), 6.17 (br s, 1H, H-1^{II}), 5.90 (br s, 1H, H-1^{VI}), 5.81 (d, *J* = 8.9 Hz, 1H, OH), 5.42 (s, 1H, H-12), 5.08 (d-like, *J* = 8.9 Hz, 1H, H-1^{III}), 5.03–5.01 (m, 2H, H-1^{V}), 4.78 (d, *J* = 5.9 Hz, 1H, H-1^{I}), 4.74 (s, 1H), 4.71–4.69 (m, 2H), 4.68–4.61 (m, 2H), 4.61–4.52 (m, 3H), 4.51–4.48 (m, 2H), 4.47–4.43 (m, 3H), 4.39–4.31 (m, 4H), 4.30–4.28 (m, 2H), 4.26–4.18 (m, 3H), 4.18–4.10 (m, 5H), 4.07 (t, *J* = 5.8 Hz, 1H), 3.97 (td, *J* = 8.4, 4.6 Hz, 1H), 3.83 (d, *J* = 11.1 Hz, 1H), 3.68 (d, *J* = 9.5 Hz, 1H), 3.25–3.16 (m, 2H), 2.32 (t, *J* = 11.7 Hz, 1H), 1.73 (d, *J* = 6.2 Hz, 3H, H-6^{VI}), 1.65 (d, *J* = 6.1 Hz, 3H, H-6^{II}), 1.25, 1.18, 1.14, 1.11, 0.90, 0.90, 0.89 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, C_5D_5N) δ 176.53 (C-28), 144.11 (C-13), 122.89 (C-12), 107.06 (C-1^{III}), 104.92 (C-1^{I}, C-1^{V}), 102.77 (C-1^{VI}), 101.74 (C-1^{II}), 95.68 (C-1^{IV}), 88.68, 79.56, 78.78, 78.21, 78.09, 77.24, 77.20, 76.53, 76.36, 75.45, 75.38, 74.09, 74.04, 73.90, 73.05, 72.80, 72.61, 72.52, 72.33, 70.87, 70.36, 70.33, 69.81, 69.23, 64.62, 62.36, 61.28, 56.00, 49.69, 48.06, 47.05, 46.23, 42.15, 41.68, 39.89, 39.50, 38.92, 37.03, 34.01, 33.15, 32.56, 30.77, 30.00, 28.29, 28.07, 26.64, 26.07, 23.81, 23.70, 23.38, 18.67, 18.56, 17.52, 17.04, 15.68; HRMS(ESI): calcd. for [M+Na]$^+$ $C_{65}H_{106}O_{30}Na$: 1389.6661, found 1389.6717.

*5.1.16. 3-O-[α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-α-L-arabinopyranosyl]oleanolic acid 28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester (**5**)*

Following the general procedure mentioned above to get compound **5** (0.51 g, 84% over two steps) as a white foam. R_f = 0.24 (4:1:5, *n*-BuOH-AcOH-H_2O); Mp 228–232 °C; [α]20 D −33.55 (*c* 1.52, CH_3OH); ^{1}H NMR (600 MHz, C_5D_5N) δ 8.34 (d, *J* = 3.8 Hz, 1H,

OH), 7.54–7.34 (m, 5H, OH), 7.20 (d, J = 4.5 Hz, 1H, OH), 6.85–6.54 (m, 8H, OH), 6.27 (d, J = 8.1 Hz, 1H, H-1^{IV}), 6.19 (br s, 1H, H-1^{II}), 5.89 (br s, 1H, H-1^{VI}), 5.80 (d, J = 9.0 Hz, 1H, OH), 5.41 (s, 1H, H-12), 5.03–4.99 (m, 3H, H-1^{III}, H-1^{V}), 4.81 (d, J = 5.8 Hz, 1H, H-1^{I}), 4.76 (s, 1H), 4.72–4.68 (m, 2H), 4.68–4.62 (m, 2H), 4.61–4.56 (m, 1H), 4.54–4.51 (m, 1H), 4.50–4.44 (m, 2H), 4.38–4.31 (m, 6H), 4.30–4.28 (m, 1H), 4.25–4.20 (m, 3H), 4.20–4.15 (m, 2H), 4.15–4.08 (m, 4H), 4.03 (td, J = 8.4, 4.1 Hz, 1H), 3.97 (td, J = 8.1, 4.4 Hz, 1H), 3.89 (d, J = 10.7 Hz, 1H), 3.69–3.63 (m, 2H), 3.21 (ddd, J = 16.2, 12.3, 3.3 Hz, 2H), 2.32 (t, J = 11.5 Hz, 1H), 1.73 (d, J = 6.2 Hz, 3H, H-6^{VI}), 1.66 (d, J = 6.1 Hz, 3H, H-6^{II}), 1.25, 1.18, 1.12, 1.10, 0.90, 0.89, 0.88 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, C_5D_5N) δ 176.56 (C-28), 144.13 (C-13), 122.92 (C-12), 106.96 (C-1^{III}), 104.94 (C-1^{I}, C-1^{V}), 102.80 (C-1^{VI}), 101.84 (C-1^{II}), 95.70 (C-1^{IV}), 88.73, 79.33, 78.80, 78.66, 78.23, 78.11, 77.23, 76.55, 76.37, 75.41, 75.24, 74.11, 74.06, 73.93, 72.83, 72.64, 72.55, 72.37, 71.01, 70.89, 70.36, 69.90, 69.25, 67.47, 64.55, 61.31, 56.02, 49.72, 48.09, 47.08, 46.25, 42.18, 41.71, 39.92, 39.53, 38.96, 37.05, 34.03, 33.18, 32.58, 30.80, 28.32, 28.11, 26.64, 26.10, 23.83, 23.72, 23.41, 18.70, 18.59, 17.54, 17.07, 15.69; HRMS(ESI): calcd. for $[M+Na]^+$ $C_{64}H_{104}O_{29}Na$: 1359.6555, found 1359.6449.

5.1.17. 3-O-[α-L-rhamnopyranosyl-(1→2)-[β-D-ribofuranosyl-(1→4)]-α-L-arabinopyranosyl]oleanolic acid 28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester (**6**)

Following the general procedure mentioned above to get compound **6** (0.47 g, 79% over two steps) as a white foam. R_f = 0.24 (4:1:5, n-BuOH-AcOH-H_2O); Mp 220–223 °C; [α]20 D −72.32 (c 1.12, CH_3OH); ^{1}H NMR (600 MHz, C_5D_5N) δ 7.53–7.30 (m, 4H, OH), 7.00–6.47 (m, 11H), 6.27 (d, J = 7.9 Hz, 1H, H-1^{IV}), 6.17 (s, 1H, OH), 6.09 (s, 1H, H-1^{II}), 5.90 (s, 1H, H-1^{VI}), 5.65 (d, J = 2.4 Hz, 1H, H-1^{III}), 5.41 (s, 1H, H-12), 5.01 (d, J = 7.6 Hz, 2H, H-1^{V}), 4.87 (d, J = 3.4 Hz, 1H, H-1^{I}), 4.74 (s, 1H), 4.71–4.68 (m, 2H), 4.67–4.60 (m, 2H), 4.59 (d, J = 9.1 Hz, 1H), 4.54–4.51 (m, 1H), 4.50–4.43 (m, 3H), 4.41–4.28 (m, 7H), 4.27–4.06 (m, 11H), 3.99–3.95 (m, 1H), 3.88 (d, J = 10.2 Hz, 1H), 3.68 (d, J = 8.6 Hz, 1H), 3.22 (ddd, J = 15.3, 11.5, 1.9 Hz, 2H), 2.32 (t, J = 13.7 Hz, 1H), 1.73 (d, J = 5.6 Hz, 3H, H-6^{VI}), 1.67 (d, J = 5.5 Hz, 3H, H-6^{II}), 1.25, 1.19, 1.11, 1.11, 0.90, 0.90, 0.89 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, C_5D_5N) δ 176.53 (C-28), 144.11 (C-13), 122.87 (C-12), 104.91 (C-1^{I}, C-1^{V}), 103.57 (C-1^{III}), 102.77 (C-1^{VI}), 101.97 (C-1^{II}), 95.67 (C-1^{IV}), 88.85, 78.77, 78.19, 78.08, 77.20, 77.02, 76.52, 76.36, 75.38, 74.04, 73.90, 72.88, 72.81, 72.61, 72.55, 72.52, 72.30, 70.87, 70.33, 70.08, 70.03, 69.22, 65.33, 61.28, 55.95, 48.07, 47.05, 46.23, 42.15, 41.68, 39.89, 39.51, 38.92, 37.03, 34.00, 33.15, 32.55, 30.77, 30.00, 28.29, 28.07, 26.60, 26.07, 23.80, 23.69, 23.38, 18.68, 18.57, 17.50, 16.96, 15.66; HRMS(ESI): calcd. for $[M+Na]^+$ $C_{64}H_{104}O_{29}Na$: 1359.6555, found 1359.6423.

5.1.18. 3-O-α-L-arabinopyranosyl-oleanolic acid 28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester (**7**)

Compound **7** was synthesized from **49** (0.41 g, 0.45 mmol) by the same procedure as for compound **1-6** and purified on a Sephadex LH-20 column using MeOH as the eluent. (0.42 g, 89% over two steps), white foam; R_f = 0.36 (4:1:5, n-BuOH-AcOH-H_2O); Mp 186–189 °C; [α]20 D −27.50 (c 1.12, CH_3OH); ^{1}H NMR (600 MHz, C_5D_5N) δ 7.51 (d, J = 5.6 Hz, 1H, OH), 7.49 (d, J = 4.5 Hz, 1H, OH), 7.39 (d, J = 4.5 Hz, 1H, OH), 7.33 (d, J = 5.0 Hz, 1H, OH), 6.97 (d, J = 4.9 Hz, 1H, OH), 6.82 (d, J = 4.3 Hz, 1H, OH), 6.79 (d, J = 5.0 Hz, 1H, OH), 6.74 (dd, J = 7.4, 5.3 Hz, 1H, OH), 6.66 (d, J = 4.6 Hz, 1H, OH), 6.58 (d, J = 5.7 Hz, 1H, OH), 6.50 (d, J = 6.4 Hz, 1H, OH), 6.31 (d, J = 4.0 Hz, 1H, OH), 6.27 (d, J = 8.1 Hz, 1H, H-1^{IV}), 5.89 (s, 1H, H-1^{VI}), 5.42 (s, 1H, H-12), 5.00 (d, J = 5.4 Hz, 1H, H-1^{V}), 4.78 (d, J = 7.1 Hz, 1H, H-1^{I}), 4.70–4.68 (m, 2H), 4.60–4.55 (m, 1H), 4.47–4.44 (m, 2H), 4.38–4.31 (m, 5H), 4.26–4.20 (m, 2H), 4.19–4.14 (m, 3H), 4.13–4.09 (m, 2H), 3.96 (td, J = 8.5, 4.5 Hz, 1H), 3.85 (d, J = 10.9 Hz, 1H), 3.68 (d, J = 9.5 Hz, 1H), 3.35 (dd, J = 11.7, 4.2 Hz, 1H), 3.20 (dd, J = 13.6, 3.8 Hz, 1H), 1.72 (d, J = 6.1 Hz, 3H, H-6^{VI}), 1.28, 1.25, 1.11, 0.98, 0.90, 0.90, 0.90 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, C_5D_5N) δ 176.52 (C-28), 144.12 (C-13), 122.90 (C-12), 107.47 (C-1^{I}), 104.87 (C-1^{V}), 102.76 (C-1^{VI}), 95.65 (C-1^{IV}), 88.70, 78.75, 78.33, 78.05, 77.16, 76.53, 75.35, 74.65, 74.01, 73.88, 72.93, 72.77, 72.57, 70.92, 70.32, 69.52, 69.25, 66.74, 61.33, 55.91, 49.68, 48.10, 47.06, 46.25, 42.15, 41.70, 39.93, 39.57, 38.84, 37.06, 34.02, 33.14, 32.56, 30.77, 28.30, 28.25, 26.65, 26.08, 23.83, 23.72, 23.38, 18.52, 17.53, 16.94, 15.63; HRMS(ESI): calcd. for $[M+H]^+$ $C_{53}H_{87}O_{21}$: 1059.5734, found 1059.5745.

5.1.19. Oleanolic acid 3-O-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4-O-isopropylidene-α-L-arabinopyranoside (**51**)

A suspension of **16** (0.60 g, 0.61 mmol) and 10% Pd-C (0.18 g) in EtOAc (30 mL) was refluxed and bubbled up with H_2 (70 mL/min) for 1.5 h. Pd-C was removed through filtration and the filtrate was concentrated to dryness and purified by a silica gel column chromatography (2:1 petroleum ether − EtOAc) to afford **51** (0.47 g, 87%) as a white foam. R_f = 0.34 (2:1, petroleum ether − EtOAc); Mp 144–148 °C; [α]20 D −24.75 (c 1.01, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.27 (s, 1H), 5.36–5.31 (m, 3H), 5.29–5.27 (m, 2H), 5.05 (t, J = 9.9 Hz, 1H), 4.36 (d, J = 7.5 Hz, 1H), 4.22–4.18 (m, 2H), 4.17–4.14 (m, 1H), 4.11 (dd, J = 10.4, 2.7 Hz, 1H), 3.78–3.73 (m, 2H), 3.10 (dd, J = 11.8, 4.3 Hz, 1H), 2.82 (dd, J = 13.7, 3.9 Hz, 1H), 2.15 (s, 3H), 2.02 (s, 3H), 1.96 (s, 3H), 1.93–1.54 (m, 14H), 1.53 (s, 3H), 1.33 (s, 3H), 1.20 (d, J = 6.2 Hz, 3H), 1.13 (s, 3H), 1.06 (s, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.82 (s, 3H), 0.74 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 183.11, 170.17, 169.99, 169.94, 143.55, 122.66, 110.38, 103.14, 95.25, 89.05, 79.11, 75.20, 73.35, 71.28, 69.63, 69.09, 66.23, 62.61, 55.88, 47.67, 46.55, 45.88, 41.67, 41.09, 39.33, 39.08, 38.74, 36.77, 33.83, 33.07, 32.66, 32.46, 30.68, 28.05, 27.78, 27.64, 26.10, 25.92, 23.58, 23.42, 22.95, 20.97, 20.81, 20.72, 18.23, 17.36, 16.92, 16.35, 15.40; HRMS(ESI): calcd. for $[M+Na]^+$ $C_{50}H_{76}O_{14}Na$: 923.5127, found 923.5116.

5.1.20. 3-O-[2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4-O-isopropylidene-α-L-arabinopyranoside]oleanolic acid 28-O-2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2,3-di-O-acetyl-6-O-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-acetyl-β-D-glucopyranosyl ester (**52**)

A mixture of compounds **51** (0.47 g, 0.53 mmol), **36** (0.68 g, 0.64 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (30 mL) were stirred under an atmosphere of N_2 for 30 min at 0 °C. A solution of TMSOTf (0.16 mL, 0.34 mmol/mL) in dry CH_2Cl_2 was injected. The mixture was stirred under these conditions for 30 min, then quenched with Et_3N (0.1 mL), and concentrated to dryness to afford crude intermediates **52**. The residue was purified by a silica gel column chromatography (5:2 petroleum ether − EtOAc) to give compound **52** (0.83 g, 87%) as a white foam. R_f = 0.3 (3:2, petroleum ether − EtOAc); Mp 154–156 °C; [α]20 D −21.67 (c 2.18, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, J = 7.2 Hz, 2H), 7.58 (t, J = 7.4 Hz, 1H), 7.47 (t, J = 7.7 Hz, 2H), 5.54 (d, J = 8.3 Hz, 1H), 5.36–5.34 (m, 2H), 5.31 (dd, J = 3.5, 1.7 Hz, 1H), 5.30–5.29 (m, 1H), 5.22–5.17 (m, 3H), 5.13–5.09 (m, 2H), 5.05 (t, J = 8.1 Hz, 1H), 5.02 (t, J = 8.0 Hz, 1H), 4.95 (t, J = 9.7 Hz, 1H), 4.87–4.82 (m, 2H), 4.77 (d, J = 10.6 Hz, 1H), 4.55 (d, J = 7.9 Hz, 1H), 4.48 (dd, J = 12.3, 4.3 Hz, 1H), 4.36 (d, J = 7.5 Hz, 1H), 4.22–4.18 (m, 2H), 4.18–4.14 (m, 1H), 4.14–4.10 (m, 1H), 3.90 (t, J = 9.3 Hz, 1H), 3.87–3.82 (m, 2H), 3.79–3.73 (m, 3H), 3.69 (ddd, J = 9.7, 4.0, 1.9 Hz, 1H), 3.56 (dd, J = 11.5, 5.6 Hz, 1H), 3.09 (dd, J = 11.7, 4.3 Hz, 1H), 2.78 (dd, J = 13.5, 3.8 Hz, 1H), 2.15 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 6H), 1.96 (s, 3H), 1.92–1.56 (m, 11H), 1.53

(s, 3H), 1.51–1.35 (m, 4H), 1.33 (s, 3H), 1.32–1.24 (m, 5H), 1.20 (d, J = 6.3 Hz, 3H), 1.15 (d, J = 6.2 Hz, 3H), 1.11 (s, 3H), 1.05 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.83 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.27, 170.16, 170.13, 170.07, 169.99, 169.92, 169.85, 169.38, 168.90, 165.79, 142.86, 133.13, 132.33, 130.91, 129.81, 129.77, 128.85, 128.44, 122.85, 110.36, 103.18, 100.28, 99.08, 95.15, 91.47, 88.99, 79.15, 75.02, 73.99, 73.83, 73.39, 72.91, 72.87, 71.80, 71.27, 70.70, 69.99, 69.60, 69.07, 68.80, 68.52, 67.76, 67.60, 66.19, 65.56, 62.66, 62.36, 55.84, 53.43, 47.62, 46.72, 45.81, 41.72, 41.06, 39.30, 39.07, 38.75, 36.76, 33.72, 32.95, 31.69, 30.56, 29.70, 28.01, 27.85, 27.78, 25.57, 23.49, 23.41, 22.83, 21.03, 20.98, 20.79, 20.72, 20.69, 20.65, 20.59, 20.54, 19.19, 18.19, 17.35, 17.21, 16.99, 16.40, 15.47, 13.73; HRMS(ESI): calcd. for $[M+H]^+$ $C_{91}H_{127}O_{37}$: 1811.8051, found 1811.8015.

*5.1.21. 3-O-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] oleanolic acid 28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester (**8**)*

P-TsOH (0.15 g, 0.90 mmol) was added to a solution of **52** (0.81 g, 0.45 mmol) in MeOH-CH_2Cl_2 (2:1, v/v, 30 mL). The mixture was stirred at 40 °C for 6 h followed by addition of NaOMe in MeOH solution (1.0 mol/L, 1.35 mL). The mixture was stirred at rt for 5 h and then neutralized with Dowex H^+ resin to pH 7 and filtered. The filtrate was concentrated and purified on a Sephadex LH-20 column using MeOH as the eluent to afford compound **8** (0.43 g, 79% over two steps) as a white foam. R_f = 0.34 (4:1:5, *n*-BuOH-AcOH-H_2O); Mp 168–172 °C; [α]20 D −21.37 (*c* 1.17, CH_3OH); ^{1}H NMR (600 MHz, C_5D_5N) δ 6.28 (d, J = 8.2 Hz, 1H, H-1^{IV}), 6.20 (s, 1H, H-1^{II}), 5.91 (s, 1H, H-1^{VI}), 5.42 (s, 1H, H-12), 5.04–5.01 (m, 3H, H-1^{V}), 4.91 (d, J = 5.4 Hz, 1H, H-1^{I}), 4.79 (d, J = 1.8 Hz, 1H), 4.73–4.65 (m, 3H), 4.65–4.57 (m, 3H), 4.47 (t, J = 9.3 Hz, 1H), 4.40–4.27 (m, 7H), 4.26–4.10 (m, 6H), 3.97 (t, J = 8.4 Hz, 1H), 3.85 (d, J = 9.9 Hz, 1H), 3.68 (d, J = 9.4 Hz, 1H), 3.26 (dd, J = 11.6, 4.1 Hz, 1H), 3.19 (dd, J = 13.9, 3.8 Hz, 1H), 2.33 (t, J = 12.6 Hz, 1H), 1.73 (d, J = 6.2 Hz, 3H, H-6^{VI}), 1.65 (d, J = 6.1 Hz, 3H, H-6^{II}), 1.26, 1.18, 1.11, 1.10, 0.91, 0.90, 0.89 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, C_5D_5N) δ 176.53 (C-28), 144.12 (C-13), 122.85 (C-12), 104.83 (C-1^{V}), 104.76 (C-1^{I}), 102.73 (C-1^{VI}), 101.73 (C-1^{II}), 95.65 (C-1^{IV}), 88.78, 78.72, 78.30, 78.05, 77.14, 76.51, 75.94, 75.33, 74.05, 73.98, 73.87, 73.68, 72.74, 72.57, 72.55, 72.36, 70.89, 70.30, 69.89, 69.23, 68.59, 64.58, 61.31, 55.93, 48.07, 47.06, 46.24, 42.16, 41.68, 39.90, 39.49, 38.93, 37.04, 34.01, 33.13, 32.55, 30.76, 28.27, 28.11, 26.51, 26.06, 23.80, 23.70, 23.38, 18.56, 18.51, 17.51, 16.98, 15.64; HRMS(ESI): calcd. for $[M+Na]^+$ $C_{59}H_{96}O_{25}Na$: 1227.6133, found 1227.6101.

*5.1.22. General procedure for the preparation of compounds **10-12***

A mixture of compounds **37** (0.6 g, 0.41 mmol), trichloroacetimidate donors (perbenzoylated glucosyl trichloroacetimidate, **28** or **34**), (0.49 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (30 mL) were stirred under an atmosphere of N_2 for 30 min at 0 °C. A solution of TMSOTf (0.12 mL, 0.34 mmol/mL) in dry CH_2Cl_2 was injected. The mixture was stirred under these conditions for 30 min, then quenched with Et_3N (0.1 mL), and concentrated to dryness to afford crude intermediates **53-55**. The resulting residue was dissolved in dry MeOH-CH_2Cl_2 (2:1, v/v, 30 mL), and a newly prepared solution of NaOMe in MeOH (1.0 mol/L, 0.41 mL) was added. The solution was stirred at rt for 5 h and then neutralized with Dowex H^+ resin to pH 7 and filtered. The filtrate was concentrated and purified on a Sephadex LH-20 column using MeOH as the eluent to afford compound **10-12**.

*5.1.23. 3-O-[α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl]oleanolic acid 28-O-β-D-glucopyranosyl] ester (**10**)*

Following the general procedure mentioned above to get compound **10** (0.38 g, 88% over two steps) as a white foam. R_f = 0.36 (4:1:5, *n*-BuOH-AcOH-H_2O); Mp 217–220 °C; [α]20 D −5.16 (*c* 1.55, CH_3OH); ^{1}H NMR (600 MHz, C_5D_5N) δ 8.50 (s, 1H, OH), 7.57–7.29 (m, 5H, OH), 6.84–6.48 (m, 5H, OH), 6.37 (d, J = 8.2 Hz, 1H, H-1^{IV}), 6.23 (s, 1H, H-1^{II}), 5.99 (d, J = 9.0 Hz, 1H, OH), 5.45 (s, 1H, H-12), 5.18 (d, J = 7.8 Hz, 1H, H-1^{III}), 4.80–4.72 (m, 2H, H-1^{I}), 4.70–4.61 (m, 2H), 4.58–4.48 (m, 3H), 4.46–4.41 (m, 4H), 4.37–4.19 (m, 7H), 4.08–4.06 (m, 2H), 3.96–3.89 (m, 1H), 3.82 (d, J = 11.1 Hz, 1H), 3.26–3.14 (m, 2H), 2.37 (t, J = 12.8 Hz, 1H), 1.67 (d, J = 6.1 Hz, 3H, H-6^{II}), 1.27, 1.19, 1.13, 1.12, 0.93, 0.90, 0.87 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, C_5D_5N) δ 176.45 (C-28), 144.12 (C-13), 122.88 (C-12), 106.47 (C-1^{III}), 104.98 (C-1^{I}), 101.82 (C-1^{II}), 95.78 (C-1^{IV}), 88.64, 79.78, 79.40, 78.95, 78.84, 78.58, 76.36, 75.51, 74.18, 74.09, 72.52, 72.33, 71.23, 71.06, 69.84, 64.61, 62.52, 62.17, 55.97, 48.05, 47.01, 46.19, 42.15, 41.75, 39.90, 39.49, 38.91, 37.01, 34.00, 33.16, 32.55, 30.79, 28.25, 28.06, 26.64, 26.09, 23.81, 23.67, 23.42, 18.69, 18.53, 17.48, 17.04, 15.65; HRMS(ESI): calcd. for $[M+H]^+$ $C_{53}H_{87}O_{21}$: 1059.5734, found 1059.5739.

*5.1.24. 3-O-[α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl]oleanolic acid 28-O-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester (**11**)*

Following the general procedure mentioned above to get compound **11** (0.43 g, 86% over two steps) as a white foam. R_f = 0.32 (4:1:5, *n*-BuOH-AcOH-H_2O); Mp 215–217 °C; [α]20 D −16.67 (*c* 1.50, CH_3OH); ^{1}H NMR (600 MHz, C_5D_5N) δ 8.48 (s, 1H, OH), 7.56–7.29 (m, 5H, OH), 6.83–6.45 (m, 4H, OH), 6.30 (d, J = 8.1 Hz, 1H, H-1^{IV}), 6.22 (s, 1H, H-1^{II}), 5.98 (d, J = 8.0 Hz, 1H, OH), 5.43 (s, 1H, H-12), 5.18 (d, J = 7.9 Hz, 1H, H-1^{III}), 5.06 (d, J = 7.8 Hz, 1H, H-1^{V}), 4.78 (d, J = 5.9 Hz, 1H, H-1^{I}), 4.76–4.74 (m, 2H), 4.70–4.60 (m, 2H), 4.57–4.47 (m, 3H), 4.45–4.37 (m, 5H), 4.34 (t, J = 9.5 Hz, 1H), 4.32–4.20 (m, 7H), 4.18–4.13 (m, 2H), 4.09–4.02 (m, 2H), 3.94–3.90 (m, 2H), 3.83 (d, J = 11.0 Hz, 1H), 3.27–3.17 (m, 2H), 2.33 (t, J = 11.9 Hz, 1H), 1.67 (d, J = 6.1 Hz, 3H, H-6^{II}), 1.26, 1.18, 1.13, 1.12, 0.90, 0.89, 0.89 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, C_5D_5N) δ 176.55 (C-28), 144.15 (C-13), 122.87 (C-12), 106.44 (C-1^{III}), 105.35 (C-1^{V}), 104.96 (C-1^{I}), 101.83 (C-1^{II}), 95.71 (C-1^{IV}), 88.67, 79.72, 78.84, 78.79, 78.58, 78.52, 78.42, 78.02, 76.37, 75.51, 75.19, 74.09, 73.92, 72.52, 72.33, 71.48, 71.24, 70.90, 69.85, 69.38, 64.54, 62.60, 62.52, 55.98, 48.06, 47.05, 46.24, 42.16, 41.69, 39.89, 39.49, 38.90, 37.02, 33.98, 33.14, 32.54, 30.78, 30.00, 28.28, 28.06, 26.63, 26.08, 23.81, 23.68, 23.39, 18.69, 18.54, 17.51, 17.04, 15.70; HRMS(ESI): calcd. for $[M+H]^+$ $C_{59}H_{97}O_{26}$: 1221.6263, found 1221.6290.

*5.1.25. 3-O-[α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl]oleanolic acid 28-O-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl] ester (**12**)*

Following the general procedure mentioned above to get compound **12** (0.42 g, 86% over two steps) as a white foam. R_f = 0.34 (4:1:5, *n*-BuOH-AcOH-H_2O); Mp 162–165 °C; [α]20 D −37.50 (*c* 1.44, CH_3OH); ^{1}H NMR (600 MHz, C_5D_5N) δ 6.28 (d, J = 8.2 Hz, 1H, H-1^{IV}), 6.22 (s, 1H, H-1^{II}), 5.95 (s, 1H, H-1^{VI}), 5.43 (s, 1H, H-12), 5.18 (d, J = 7.8 Hz, 1H, H-1^{III}), 5.02–4.97 (m, 2H), 4.79 (d, J = 6.0 Hz, 1H, H-1^{I}), 4.77 (s, 1H), 4.73–4.64 (m, 4H), 4.60–4.52 (m, 5H), 4.44–4.33 (m, 5H), 4.32–4.20 (m, 7H), 4.20–4.09 (m, 3H), 4.07 (t, J = 8.5 Hz, 1H), 3.95–3.91 (m, 1H), 3.84–3.83 (m, 1H), 3.21 (t, J = 12.5 Hz, 2H), 2.32 (t, J = 12.5 Hz, 1H), 1.72 (d, J = 6.2 Hz, 3H, H-6^{VI}), 1.67 (d, J = 6.1 Hz, 3H, H-6^{II}), 1.26, 1.19, 1.13, 1.09, 0.93, 0.90, 0.86 (s each, 3H each, 7 × CH_3); ^{13}C NMR (150 MHz, C_5D_5N) δ 176.40 (C-28), 144.05 (C-13), 122.91 (C-12), 106.29 (C-1^{III}), 104.86 (C-1^{I}), 102.68 (C-1^{VI}), 101.77 (C-1^{II}), 95.41 (C-1^{IV}), 88.69, 79.45, 78.71, 78.49, 77.89, 77.08, 76.41, 75.46, 74.23, 74.03, 73.94 73.89, 73.83, 72.73, 72.66, 72.54, 72.47, 72.26, 71.31, 70.41, 69.83, 65.64, 64.39, 62.55, 61.02, 55.98, 48.05, 47.01, 46.18, 42.15, 41.74, 39.90, 39.48, 38.93, 37.02, 34.00,

33.12, 32.54, 30.76, 28.24, 28.09, 26.59, 26.07, 23.79, 23.64, 23.37, 18.61, 18.52, 17.44, 17.00, 15.62; HRMS(ESI): calcd. for $[M+H]^+$ $C_{59}H_{97}O_{25}$: 1205.6313, found 1205.6294.

5.2. Biology

5.2.1. Cell culture and treatments

PC12 cells were purchased from Jiangsu Keygen Biotech Company, China, grown in Roswell Park Memorial Institute-1640 (Gibico), supplemented with 15% fetal bovine serum and 1% antibiotic mixture comprising penicilline and streptomycin (100 U/mL of penicillin and 100 μg/mL of streptomycin), in a humidified atmosphere at 37 °C with 5% CO_2. Cells were sub-cultured every 2 days and in culture for 8 days before being used for experiments.

5.2.2. Cell viability assay

Cells were trypsinized with 0.25% trypsin, counted, and seeded in 96-well culture plates (1×10^4 cells/well). After 12 h of incubation, the cells were pretreated with compounds in different concentrations for 12 h before incubation in medium containing H_2O_2 (200 μM) or $A\beta_{1\text{-}42}$ (40 μM). After 12 h of treatment, 20 μL of MTT (5 mg/mL) was added. After incubation for 4 h the supernatant was discarded, and DMSO (150 μL/well) was added. The 96-well plate was vibrated on a microvibrator for 10 min, and the optical density at 490 nm was measured using a microplate reader.

5.2.3. LDH release assay

The amount of LDH release was determined by using an assay kit according to the manufacturer's protocol. At the end of drug treatment, the supernatant and cell lysates were transferred to 96-well plates and incubated with 1 mg/ml NADH in pyruvate substrate solution for 15 min at 37 °C. After additional incubation for 15 min with 2,4-dinitrophenylhydrazine at 37 °C, the reaction was stopped by adding 0.4 mol/L NaOH. The changes in absorbance were determined at 450 nm by using a microplate reader. LDH leakage was expressed as the percentage (%) of the total LDH activity (LDH in the supernatant + LDH in the cell lysate), according to the equation: %LDH released = (LDH activity in the medium/total LDH activity) × 100%.

5.2.4. Measurement of intracellular ROS level

ROS level was measured by using DCFH-DA method [29]. After the treatment, PC12 cells were washed with PBS and incubated with DCFH-DA at a final concentration of 10 μmol/L for 20 min at 37 °C in darkness. Then the cells were washed with PBS three times. The fluorescence intensity was measured by using a flow cytometer (BD FACSCallibur 488/530 nm).

5.2.5. Measurement of MDA level

The MDA content was determined using the 2-thiobarbituric acid (TBA) method [32]. After the treatment, two volumes of 2-thiobarbituric acid reagent (0.375% TBA, 15% trichloroacetic acid, and 0.1 mM EDTA) were added to the cell samples and boiled at 95 °C for 40 min. After cooling and centrifugation at 3000 × g for 10 min, the absorbance of each supernatant was measured at 532 nm by using a microplate reader.

5.2.6. Statistical analysis

All samples were cultured in triplicate. The data are expressed as the means ± SD of three assays. Statistical analysis was performed using one-way analysis of variance (ANOVA) with Dunnett's test. $P < 0.05$ was considered significant.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2017.11.040.

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