

Article

Synthesis and Tumor Cytotoxicity of Novel Amide Derivatives of β -Hederin

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Abstract: Thirteen novel triterpenoid saponins, designed as amide derivatives of the natural cytotoxic saponin β -hederin, were synthesized by a stepwise glycosylation strategy. The *in vitro* cytotoxic activity of these compounds was evaluated against five different tumor cell lines. Most of the evaluated compounds showed effective inhibitory activity against at least one tumor cell line at micromolar concentrations. The preliminary structure-activity relationships (SAR) indicate that mide derivatization at C-28 resulted in highly cytotoxic derivatives on specific tumor cell lines, and also resulted in an increase in the antitumor selectivity of β -hederin.

Keywords: triterpenoid saponins; β -hederin; tumor cytotoxicity; synthesis

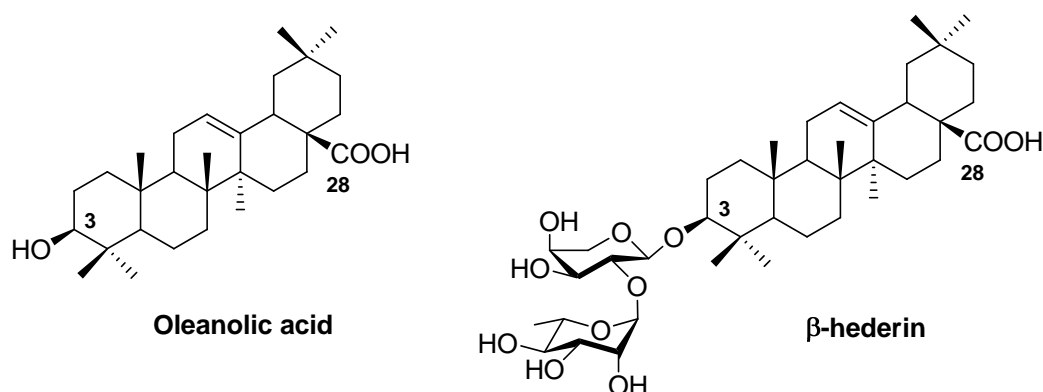
1. Introduction

Oleanolic acid (OA, see Figure 1) is a pentacyclic triterpenoid widely distributed in Nature and possessing various important bioactivities, such as antitumor, anti-HIV, hepatoprotection, and anti-

inflammatory properties[1-3]. OA also serves as an aglycon of many natural saponins, which display significantly higher levels of activity than OA itself. For instance, β -hederin (oleanolic acid 3-*O*- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside, see Figure 1) [4], an OA glycoside that bears a unique disaccharide at C3-OH, shows excellent inhibitory activity against many tumor cells [5,6]. In our previous studies, the chemical synthesis of β -hederin and its glycosylated derivatives was completed, and their antitumor activity was evaluated [7,8].

Recently, some researchers have explored the structure-activity relationships of other pentacyclic triterpenic compounds, such as ursolic acid [9,10] and betulonic acid [11]. Those studies led to similar conclusions that the derivatives with a substitution of amino groups at C-28 often showed stronger cytotoxic antitumor activity. Therefore, we were inspired to investigate the possibility of similar properties in the medicinal chemistry of triterpenoid saponins. The present work describes the initial study of the synthesis of 13 novel amide derivatives of β -hederin and the evaluation of their cytotoxic activity against tumors.

Figure 1. Structures of OA and β -hederin.



2. Results and Discussion

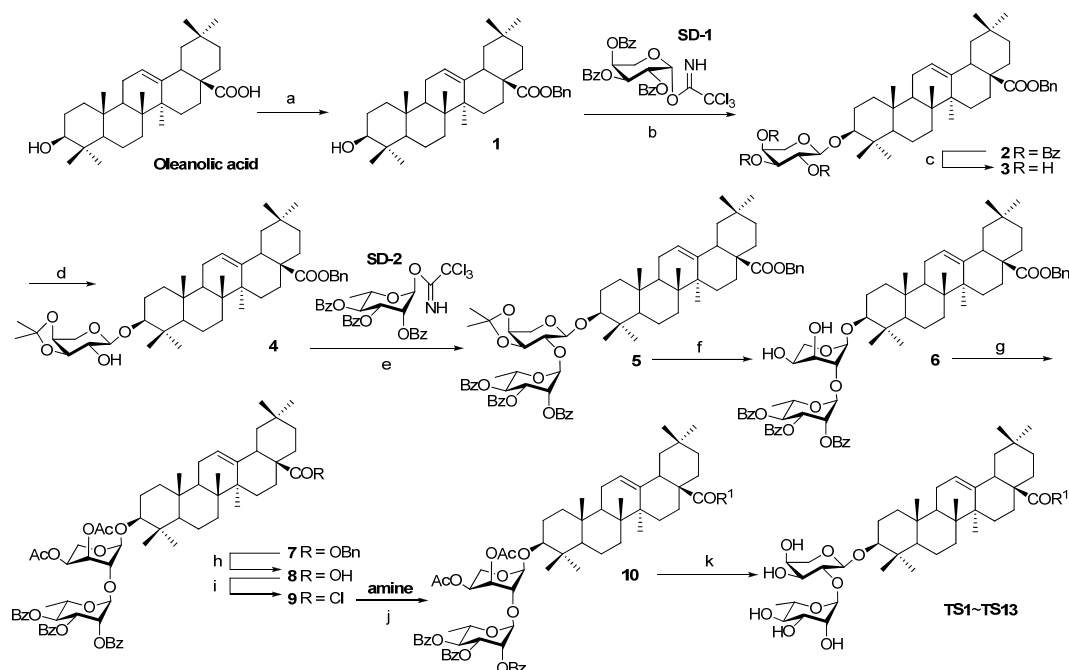
2.1. Synthesis

As far as β -hederin is concerned, the hydroxyl group at C3 is connected with a disaccharide moiety to form the so-called OA 3-glycosides. Therefore, only if the sugar substructure was well protected, can the free carboxyl group at C-28 be modified by various acylations. Based on our previous work [7,8,12], we developed a facile method to prepare the amide derivatives of β -hederin using OA, L-arabinose, L-rhamnose and some commercially available amines as the starting materials.

The compounds were synthesized as depicted in Scheme 1. To protect the carboxyl group of OA, benzyl ester **1** was prepared by the combination of OA, BnBr and K_2CO_3 in THF- H_2O with Bu_4NI as a phase transfer catalyst. Compound **1** was glycosylated with perbenzoylated arabinosyl trichloroacetimidate **SD-1** [13] under the promotion of TMSOTf to produce an excellent yield of compound **2**. Debenzoylation of compound **2** in the MeOH solution of NaOMe yielded saponin **3** without affecting the benzyl ester at C-28. Selective protection of 3-OH and 4-OH of the arabinose residue was successfully accomplished using 2,2-dimethoxypropane to furnish compound **4**, which was combined with perbenzoylated rhamnosyl trichloroacetimidate **SD-2** [14] under the same

glycosylation conditions to generate compound **5** with a 79% yield. When the isopropylidene moiety was removed to prepare the intermediate **6**, the $^1\text{C}_4$ conformation of the arabinose residue resulted produced via conformational inversion. Based on the $^1\text{H-NMR}$ spectrum of compound **6**, the $J_{1-2'}$ value of the arabinose residue was changed to 1.3, much smaller than the normal value of the α -arabinosyl conformation (usually not less than 5.0). Moreover, the chemical shift (δ) of anomeric C atom (109.3) is larger than that of the natural product (104.8). From the point of view of the chemical structure, the aglycone and perbenzoylated rhamnose connected with 1-OH and 2-OH of the arabinose residue, respectively, increased steric hindrance to such an extent that a chair inversion of arabinose resulted. Next, the two free hydroxyl groups were shielded by acetylation in pyridine solution to furnish compound **7**, whose benzyl group was easily removed through catalytic hydrogenation to produce compound **8** without affecting the double bond between C-12 and C-13 of OA. Compound **8** was treated with oxalyl chloride to yield compound **9**, which then reacted with the appropriate amino compounds in the presence of Et_3N to generate corresponding compounds **10**. Last, rapid and complete removal of all acyl groups in basic solution yielded the final products **TS1-TS13**. Fortunately, the arabinose ring returned to the normal $^4\text{C}_1$ conformation after the final deprotection, which was confirmed by the $^1\text{H-NMR}$ data.

Scheme 1. Synthesis of amide derivatives of β -hederin.



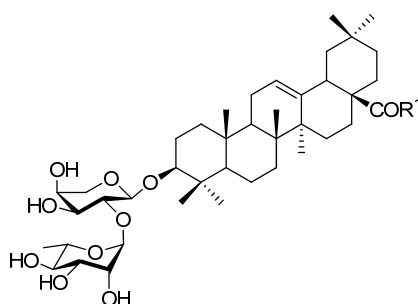
Reagents and conditions: (a) BnBr , Bu_4NI , Et_3N , THF, 98%; (b) TMSOTf, DCM, 4 \AA MS, RT, 97%; (c) NaOMe, DCM-MeOH, RT, 96%; (d) $\text{Me}_2\text{C}(\text{OMe})_2$, *p*-TsOH, acetone, RT, 89%; (e) TMSOTf, DCM, 4 \AA MS, RT, 79%; (f) *p*-TsOH, DCM-MeOH, RT, 98%; (g) Ac_2O , pyridine, RT, 98%; (h) 10% Pd-C, H_2 , EtOAc, reflux, 95% (for 2 steps); (i) $(\text{COCl})_2$, DCM, $0\text{ }^\circ\text{C}$; (j) Et_3N , DCM, RT; (k) NaOMe, DCM-MeOH, RT, 67%–85% (for 3 steps).

2.2. Biological evaluation

The preliminary *in vitro* biological evaluation was performed by a standard MTT assay to investigate the cytotoxicity of these saponins against five tumor cell lines: HeLa (cervical), MCF-7

(breast), HL-60 (leukemia), HT1080 (fibrosarcoma), and HepG2 (liver). The synthetic β -hederin was used as a reference compound. As shown in Table 1, although most of the evaluated compounds were found to be less active than β -hederin, they showed effective inhibitory activity against at least one tumor cell line at micromolar concentrations. It is worth emphasizing that compounds with a substitution of piperazine or methylpiperazine (**TS8** and **TS9**) displayed more potent activity than β -hederin. However, none of the evaluated compounds showed any toxicity towards the HT1080 or HepG2 cell lines within the investigated concentration range. The SAR indicated that the conversion of β -hederin into an amide yielded a moderately active derivative. The modified saponins with the aliphatic amine substructure seemed to display stronger activity than those bearing aromatic amines. Generally, the amide derivatization at C-28 resulted in highly cytotoxic derivatives on specific tumor cell lines, which means the antitumor selectivity of β -hederin was increased.

Table 1. Structures and tumor cytotoxicity of amide derivatives of β -hederin.



Compd.	R ¹	IC ₅₀ (μM)				
		HeLa	MCF-7	HL-60	HT1080	HepG2
TS1		> 100	> 100	14.46	> 50	> 50
TS2		> 100	> 100	12.78	> 200	> 100
TS3		> 100	> 100	11.53	> 100	> 100
TS4		24.14	> 100	> 100	> 200	> 100
TS5		> 100	19.72	> 100	> 100	> 100
TS6		> 100	13.80	> 100	> 50	> 50
TS7		> 100	> 100	15.58	> 50	> 50
TS8		8.80	9.89	> 100	> 50	> 50
TS9		4.74	17.13	> 100	> 100	> 100
TS10		> 100	> 100	18.20	> 100	> 100
TS11		> 200	> 100	> 100	> 100	> 100
TS12		> 100	> 100	16.29	> 100	> 100
TS13		18.99	14.83	> 100	> 50	> 50
β-hederin	-OH	12.47	9.53	8.26	> 50	19.72

3. Experimental

3.1. General

Commercial reagents were used without further purification unless otherwise stated. Solvents were dried and redistilled prior to use in the usual way. Analytical TLC was performed on silica gel HF₂₅₄ plates. Preparative column chromatography (CC) was performed with silica gel H. Melting points were measured with a Büchi B-540 Melting Point apparatus. Optical rotations were measured at the sodium D-line at room temperature (RT) with a Perkin–Elmer 241MC polarimeter. ¹H- and ¹³C-NMR spectra were recorded on a Bruker Avance AV600 MHz spectrometer using Me₄Si as the internal standard. HRMS spectra were recorded on a high resolution ESI-FTICR mass spectrometer.

3.2. Benzyl oleanolate 3-O-3,4-O-isopropylidene- α -L-arabinopyranoside (**4**)

A suspension of OA (1.00 g, 2.2 mmol), BnBr (0.42 mL, 3.5 mmol), K₂CO₃ (0.60 g, 4.4 mmol) and Bu₄NI (0.08 g, 0.22 mmol) in 40:1 THF–H₂O (41 mL) was stirred overnight at RT. The mixture was then filtered, and the filtrate was concentrated under vacuum and purified by silica gel column chromatography (8:1 petroleum ether–EtOAc) to give oleanolic acid benzyl ester **1** (1.17 g, 98%) as a white amorphous solid. Compound **1** (1.00 g, 1.83 mmol), trichloroacetimidate **SD-1** (1.27 g, 2.10 mmol) and powdered 4Å molecular sieves (MS, 500 mg) were mixed in dry DCM (25 mL) and stirred at RT for 20 min. A dry DCM solution (2.0 mL) of TMSOTf (0.02 mL, 0.01 mmol) was then added dropwise and the mixture was stirred for approximately 1.5 h until the reagents were completely consumed. The mixture was neutralized with Et₃N (0.20 mL) and filtered. The filtrate was concentrated and purified by CC (8:1 petroleum ether–EtOAc) to generate compound **2** (1.87 g, 97%) as a white foam. A fresh solution of NaOMe in MeOH (1.0 mol/L, 1.70 mL) was added to a solution of **2** (1.50 g, 1.50 mmol) in 1:2 DCM–MeOH (40 mL). The mixture was stirred at RT for 2 h, neutralized with Dowex H⁺ resin, filtered, the filtrate was concentrated, and the residue subjected to CC (EtOAc) to yield saponin **3** (991 mg, 96%) as a white amorphous solid. Me₂C(OMe)₂ (0.31 mL, 2.50 mmol) and *p*-TsOH (17.2 mg) was added to a solution of **3** (679 mg, 1.00 mmol) in dry acetone (10 mL). The mixture was stirred for 4 h before Et₃N (0.20 mL) was added. The solution was concentrated and purified by CC (6:1 petroleum ether–EtOAc) to generate afford compound **4** (634 mg, 89%) as a white foam. $[\alpha]_D^{25} +45.0$ (*c* 1.60, CHCl₃); ¹H-NMR (CDCl₃): δ 7.34 (m, 5H, Ar-H), 5.28 (t, *J* = 3.0 Hz, 1H, H-12), 5.07 (dd, *J* = 18.7, 12.6 Hz, 2H, PhCH₂), 4.22–4.17 (m, 3H, H-1', H-4', H-5'-1), 4.06 (dd, *J* = 7.8, 6.1 Hz, 1H, H-3'), 3.75 (dd, *J* = 13.9, 3.5 Hz, 1H, H-5'-2), 3.63 (dd, *J* = 7.8, 7.8 Hz, 1H, H-2'), 3.12 (dd, *J* = 11.5, 4.6 Hz, 1H, H-3), 2.91 (dd, *J* = 13.8, 3.3 Hz, 1H, H-18), 2.30 (br s, 1H, OH), 1.54, 1.36 (s each, 3H each, O-(CH₃)₂C-O), 1.11, 0.98, 0.92, 0.89, 0.88, 0.82, 0.60 (s each, 3H each, 7×Me); HRMS: calcd for C₃₈H₅₉O₇ (M–Bn): 627.4261; found: *m/z* 627.4255.

3.3. Benzyl oleanolate 3-O-2,3,4-tri-O-benzoyl- α -L-rhamnopyranosyl-(1→2)-3,4-O-isopropylidene- α -L-arabinopyranoside (**5**)

A mixture of compound **4** (560 mg, 0.78 mmol), trichloroacetimidate **SD-2** (630 mg, 1.00 mmol) and powdered 4Å MS (300 mg) in dry DCM (10 mL) was stirred at RT for 20 min. A dry DCM

solution of TMSOTf (0.005 mmol) was added dropwise, and the mixture was stirred for 2 h, followed by the addition of Et₃N (0.20 mL) and filtration. The filtrate was concentrated and subjected to CC (8:1 petroleum ether–EtOAc) to furnish disaccharide saponin **5** (730 mg, 79%) as a white foam. $[\alpha]_D^{25} +96.7$ (*c* 2.58, CHCl₃); ¹H-NMR (CDCl₃): δ 8.12–7.21 (m, 20H, Ar-H), 5.87 (dd, *J* = 10.2, 3.3 Hz, 1H, H-3"), 5.76 (s, 1H, H-1"), 5.65 (m, 2H, H-2", H-4"), 5.30 (t, *J* = 3.0 Hz, 1H, H-12), 5.07 (dd, *J* = 22.4, 12.6 Hz, 2H, PhCH₂), 4.53 (m, 1H, H-5"), 4.47 (d, *J* = 3.0 Hz, 1H, H-1'), 4.25 (m, 2H, H-3', H-4'), 4.17 (m, 1H, H-5'-1), 3.90 (dd, *J* = 3.0, 3.0 Hz, 1H, H-2'), 3.79 (m, 1H, H-5'-2), 3.17 (dd, *J* = 11.3, 4.1 Hz, 1H, H-3), 2.92 (m, 1H, H-18), 1.55, 1.35 (s each, 3H each, O-(CH₃)₂C-O), 1.34 (d, *J* = 6.1 Hz, 3H, H-6"), 1.14, 0.95, 0.93, 0.92, 0.90, 0.89, 0.64 (s each, 3H each, 7×Me); HRMS: calcd for C₆₅H₈₁O₁₄ (M-Bn): 1085.5626; found: *m/z* 1085.5619.

3.4. Benzyl oleanolate 3-*O*-2,3,4-tri-*O*-benzoyl- α -*L*-rhamnopyranosyl-(1→2)- α -*L*-arabinopyranoside (**6**)

Compound **5** (705 mg, 0.60 mmol) was dissolved in DCM–MeOH (1:2, 40 mL), and then *p*-TsOH (78 mg) was added. The solution was stirred at RT for 3 h, after which Et₃N (0.40 mL) was added, and the mixture was concentrated and purified by CC (2:1 petroleum ether–EtOAc) to yield compound **6** (666 mg, 98%) as a white amorphous solid. $[\alpha]_D^{25} +77.3$ (*c* 2.27, CHCl₃); ¹H-NMR (CDCl₃): δ 8.10–7.23 (m, 20H, Ar-H), 5.84 (dd, *J* = 10.2, 3.1 Hz, 1H, H-3"), 5.65 (m, 2H, H-2", H-4"), 5.36 (s, 1H, H-1"), 5.29 (br s, 1H, H-12), 5.07 (dd, *J* = 18.7, 12.6 Hz, 2H, PhCH₂), 4.81 (d, *J* = 1.3 Hz, 1H, H-1'), 4.34 (m, 1H, H-5"), 4.11–3.98 (m, 3H, H-2', H-4', OH), 3.82 (m, 1H, H-5'-1), 3.67 (m, 1H, H-5'-2), 3.45 (d, *J* = 7.9 Hz, 1H, H-3'), 3.18 (dd, *J* = 11.0, 3.2 Hz, 1H, H-3), 2.91 (m, 1H, H-18), 2.52 (br s, 1H, OH), 1.34 (d, *J* = 6.0 Hz, 3H, H-6"), 1.12, 1.05, 0.92, 0.89, 0.88, 0.84, 0.61 (s each, 3H each, 7×Me); HRMS: calcd for C₆₂H₇₇O₁₄ (M-Bn): 1045.5313; found: *m/z* 1045.5307.

3.5. Oleanolic acid 3-*O*-2,3,4-tri-*O*-benzoyl- α -*L*-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl- α -*L*-arabinopyranoside (**8**)

A solution of compound **6** (600 mg, 0.53 mmol) and Ac₂O (0.25 mL, 2.65 mmol) in dry pyridine (5 mL) was stirred at RT overnight. The solvent was evaporated in a vacuum, and the resulting residue was dissolved in DCM (20 mL), washed with water (15 mL × 3), and dried over MgSO₄. The mixture was filtered, and the filtrate was concentrated to produce crude compound **7** as a white amorphous solid, which was then dissolved in dry EtOAc (20 mL). After adding in 100 mg of 10% Pd–C, the solution was refluxed and bubbled up with H₂ (25 mL/min) for 5 h. Pd–C was removed through filtration, and the filtrate was concentrated to dryness, which was purified by CC (3:1 petroleum ether–EtOAc) to generate compound **8** (569 mg, 95%, for the 2 steps) as a white amorphous solid. $[\alpha]_D^{25} +57.2$ (*c* 1.68, CHCl₃); ¹H-NMR (CDCl₃): δ 8.17–7.27 (m, 15H, Ar-H), 5.85 (dd, *J* = 10.2, 3.3 Hz, 1H, H-3"), 5.57 (s, 1H, H-1"), 5.65 (m, 2H, H-2", H-4"), 5.28 (t, *J* = 3.0 Hz, 1H, H-12), 4.77 (d, *J* = 3.0 Hz, 1H, H-1'), 4.53 (m, 1H, H-5"), 4.25–4.21 (m, 2H, H-3', H-4'), 4.18 (m, 1H, H-5'-1), 3.93 (m, 1H, H-2'), 3.71 (m, 1H, H-5'-2), 3.16 (dd, *J* = 11.3, 4.1 Hz, 1H, H-3), 2.94 (m, 1H, H-18), 1.94 (s, 6H, 2×CH₃CO), 1.37 (d, *J* = 6.0 Hz, 3H, H-6"), 1.19, 1.15, 1.06, 0.90, 0.89, 0.83, 0.78 (s each, 3H each, 7×Me); HRMS: calcd for C₆₂H₇₇O₁₄ (M+Na): 1155.5603; found: *m/z* 1155.5597.

3.6. General procedure for the synthesis of oleanolic amide 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranosides

Oxalyl chloride (0.37 mL, 4.40 mmol) was added dropwise to a solution of compound **8** (500 mg, 0.44 mmol) in redistilled DCM (10 mL). The mixture was stirred at RT for 6 h. Then, the solvents were co-evaporated with toluene for complete removal of the excess oxalyl chloride to yield compound **9** as a yellow foam. A solution of compound **9** in redistilled DCM (10 mL) was treated with the corresponding amine (0.80 mmol) and 5 drops of Et₃N and stirred for 4 h under nitrogen. The mixture was washed with satd aq NaCl (10 mL \times 3), dried over MgSO₄, and concentrated *in vacuo* to yield the crude product **10** which was dissolved in dry 1:2 DCM–MeOH (12 mL) and treated with a fresh solution of NaOMe in MeOH (1.0 mol/L, 1.00 mL). The solution was stirred at RT for 2 h, neutralized with Dowex H⁺ resin to pH 7, and filtered. The filtrate was concentrated and subjected to CC (20:10:1 CHCl₃–MeOH–H₂O) to give the target saponins in yields of 67%–85% (for the 3 steps).

3.6.1. N-isopropyleoleanolic amide 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside (**TS1**)

White powder, m.p. 176.4–178.3 °C; $[\alpha]_D^{25}$ +11.5 (*c* 0.13, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 6.92 (d, 1H, *J* = 7.2 Hz, *NH*), 6.12 (s, 1H, H-1"), 5.43 (br s, 1H, H-12), 4.90 (d, 1H, *J* = 5.4 Hz, H-1'), 4.72 (m, 1H, H-2"), 4.62–4.54 (m, 3H, H-2', H-3", H-5"), 4.34–4.27 (m, 5H, H-3', H-4', H-5'-1, H-4", *NCH*(CH₃)₂), 3.82 (m, 1H, H-5'-2), 3.25 (dd, 1H, *J* = 11.3, 4.1 Hz, H-3), 3.01 (br d, 1H, *J* = 13.8 Hz, H-18), 1.63 (d, 3H, *J* = 6.5 Hz, H-6"), 1.26, 1.20, 1.75, 1.16, 1.08, 0.96, 0.92, 0.91, 0.86 (s each, 3H each, 9 \times Me); ¹³C-NMR(pyridine-*d*₅): δ 176.5 (C-28), 144.9 (C-13), 122.6 (C-12), 104.9 (C-1'), 101.7 (C-1"), 88.7 (C-3), 75.8 (C-2'), 74.1, 74.1 (C-3', C-4"), 72.6, 72.4 (C-2", C-3"), 69.9 (C-5"), 68.9 (C-4'), 64.9 (C-5'), 55.8 (C-5), 47.9 (C-9), 46.7 (C-17), 46.1 (C-19), 42.2 (C-14), 41.8 (C-18), 41.4 (C-NH), 39.8 (C-4), 39.5 (C-8), 38.9 (C-1), 37.0 (C-10), 34.4 (C-21), 33.8 (C-29), 33.2 (C-7), 33.1 (C-22), 30.8 (C-20), 28.0, 27.8 (C-15, C-23), 26.5 (C-2), 26.0 (C-27), 23.7, 23.7, 23.5 (C-30, C-11, C-16), 22.9, 22.3 (CH(CH₃)₂), 18.6, 18.6 (C-6, C-6"), 17.3 (C-26), 17.0 (C-24), 15.7 (C-25); HRMS: calcd for C₄₄H₇₃NO₁₀ (M+Na): 798.5232; found: m/z 798.5227.

3.6.2. N-tert-butyleoleanolic amide 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside (**TS2**)

White powder, m.p. 187.5–189.3 °C; $[\alpha]_D^{25}$ +7.5 (*c* 0.24, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 7.15 (d, 1H, *J* = 7.0 Hz, *NH*), 6.12 (s, 1H, H-1"), 5.43 (br s, 1H, H-12), 4.89 (d, 1H, *J* = 5.3 Hz, H-1'), 4.73 (m, 1H, H-2"), 4.64–4.54 (m, 3H, H-2', H-3", H-5"), 4.33–4.27 (m, 4H, H-3', H-4', H-5'-1, H-4"), 3.81 (m, 1H, H-5'-2), 3.24 (dd, 1H, *J* = 11.5 Hz, 4.0, H-3), 2.87 (br d, 1H, *J* = 13.8 Hz, H-18), 1.62 (d, 3H, *J* = 6.1 Hz, H-6"), 1.44 (s, 9H, *t*-Bu), 1.25, 1.17, 1.10, 0.98, 0.91, 0.89, 0.88 (s each, 3H each, 7 \times Me); ¹³C-NMR (pyridine-*d*₅): δ 176.8 (C-28), 145.0 (C-13), 122.7 (C-12), 104.9 (C-1'), 101.7 (C-1"), 88.7 (C-3), 75.9 (C-2'), 74.1, 73.9 (C-3', C-4"), 72.6, 72.4 (C-2", C-3"), 69.9 (C-5"), 68.7 (C-4'), 64.8 (C-5'), 55.9 (C-5), 50.7 (C-NH), 47.9 (C-9), 47.0 (C-17), 46.8 (C-19), 42.4 (C-14), 42.3 (C-18), 39.8 (C-4), 39.5 (C-8), 39.0 (C-1), 37.0 (C-10), 34.5 (C-21), 33.5 (C-29), 33.2 (C-7), 33.0 (C-22), 30.8 (C-20), 28.8 (C(CH₃)₃), 28.1, 27.8 (C-15, C-23), 26.6 (C-2), 25.8 (C-27), 23.9, 23.9, 23.7 (C-30, C-11, C-16), 18.6, 18.6 (C-6", C-6), 18.0 (C-26), 17.0 (C-24), 15.7 (C-25); HRMS: calcd for C₄₅H₇₅NO₁₀ (M+Na): 812.5289; found: m/z 812.5285.

3.6.3. N-cyclohexyloleanolic amide 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside (TS3)

White powder, m.p. 201.5–203.7 °C; $[\alpha]_D^{25}$ +27.1 (*c* 0.30, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 6.71 (d, 1H, *J* = 7.5 Hz, *NH*), 6.14 (s, 1H, H-1''), 5.46 (br s, 1H, H-12), 4.90 (d, 1H, *J* = 5.3 Hz, H-1'), 4.74 (m, 1H, H-2''), 4.65–4.55 (m, 3H, H-2', H-3'', H-5''), 4.33–4.28 (m, 4H, H-3', H-4', H-5'-1, H-4''), 4.06 (m, 1H, *NCH*), 3.82 (m, 1H, H-5'-2), 3.25 (dd, 1H, *J* = 11.3, 3.8 Hz, H-3), 3.09 (br d, 1H, *J* = 9.8 Hz, H-18), 2.12–1.78 (m, 10H, hexanyl), 1.62 (d, 3H, *J* = 6.1 Hz, H-6''), 1.27, 1.18, 1.09, 0.98, 0.95, 0.93, 0.89 (s each, 3H each, 7 \times Me); ¹³C-NMR (pyridine-*d*₅): δ 176.5 (C-28), 145.0 (C-13), 122.7 (C-12), 104.9 (C-1'), 101.7 (C-1''), 88.8 (C-3), 75.9 (C-2'), 74.1, 73.9 (C-3', C-4''), 72.5, 72.4 (C-2'', C-3''), 69.9 (C-5''), 68.8 (C-4'), 64.8 (C-5'), 55.9 (C-5), 48.6 (C-NH), 48.0 (C-9), 46.8 (C-17), 46.3 (C-19), 42.3 (C-14), 41.9 (C-18), 39.8 (C-4), 39.5 (C-8), 38.9 (C-1), 37.0 (C-10), 34.5 (C-21), 33.6 (C-29), 33.2 (C-7), 33.0 (C-22), 30.9 (C-20), 28.1, 27.9 (C-15, C-23), 26.5 (C-2), 26.0 (C-27), 25.9, 25.6, 25.5 (hexanyl), 23.8, 23.8, 23.7 (C-30, C-11, C-16), 18.6, 18.6 (C-6'', C-6), 17.8 (C-26), 17.0 (C-24), 15.6 (C-25); HRMS: calcd for C₄₇H₇₇NO₁₀ (M+Na): 838.5547; found: *m/z* 838.5544.

3.6.4. N-propyloleanolic amide 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside (TS4)

White powder, m.p. 178.6–181.0 °C; $[\alpha]_D^{25}$ +17.7 (*c* 0.27, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 7.31 (m, 1H, *NH*), 6.15 (s, 1H, H-1''), 5.39 (br s, 1H, H-12), 4.88 (d, 1H, *J* = 5.4 Hz, H-1'), 4.73 (m, 1H, H-2''), 4.63–4.55 (m, 3H, H-2', H-3'', H-5''), 4.34–4.27 (m, 4H, H-3', H-4', H-5'-1, H-4''), 3.80 (m, 1H, H-5'-2), 3.48–3.42 (m, 1H, *NCH*₂CH₂CH₃), 3.27–3.21 (m, 2H, H-3, *NCH*₂CH₂CH₃), 3.07 (br d, 1H, *J* = 13.0 Hz, H-18), 1.61 (d, 3H, *J* = 5.4 Hz, H-6''), 1.58–1.54 (m, 2H, *NCH*₂CH₂CH₃), 1.24, 1.15, 1.07, 0.91, 0.90, 0.89, 0.86 (s each, 3H each, 7 \times Me), 0.84–0.81 (t, 3H, *NCH*₂CH₂CH₃); ¹³C-NMR (pyridine-*d*₅): δ 177.4 (C-28), 144.9 (C-13), 122.7 (C-12), 104.9 (C-1'), 101.7 (C-1''), 88.7 (C-3), 75.8 (C-2'), 74.1, 74.0 (C-3', C-4''), 72.6, 72.4 (C-2'', C-3''), 69.9 (C-5''), 68.8 (C-4'), 64.8 (C-5'), 55.8 (C-5), 47.9 (C-9), 46.8 (C-17), 46.4 (C-19), 42.2, 41.9, 41.6 (*NCH*₂CH₂CH₃, C-14, C-18), 39.7 (C-4), 39.5 (C-8), 38.8 (C-1), 37.0 (C-10), 34.4 (C-21), 33.8 (C-29), 33.2 (C-7), 33.0 (C-22), 30.9 (C-20), 28.0, 27.8 (C-15, C-23), 26.5 (C-2), 26.1 (C-27), 23.8 (C-30), 23.7 (C-11), 23.7 (C-16), 23.4 (*NCH*₂CH₂CH₃), 18.6, 18.5 (C-6, C-6''), 17.5 (C-26), 17.0 (C-24), 15.6 (C-25), 11.8 (*NCH*₂CH₂CH₃); HRMS: calcd for C₄₄H₇₃NO₁₀ (M+Na): 798.5234; found: *m/z* 798.5230.

3.6.5. N-(2-hydroxyethyl)oleanolic amide 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside (TS5)

White powder, m.p. 184.6–187.3 °C; $[\alpha]_D^{25}$ +0.3 (*c* 0.30, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 7.51 (m, 1H, *NH*), 6.13 (s, 1H, H-1''), 5.42 (br s, 1H, H-12), 4.90 (d, 1H, *J* = 5.4 Hz, H-1'), 4.74 (m, 1H, H-2''), 4.62–4.54 (m, 3H, H-2', H-3'', H-5''), 4.32–4.28 (m, 4H, H-3', H-4', H-5'-1, H-4''), 4.02–4.00 (m, 2H, *NCH*₂CH₂OH), 3.89–3.87 (m, 1H, *NCH*₂CH₂OH), 3.86–3.82 (m, 1H, H-5'-2), 3.65–3.59 (m, 1H, *NCH*₂CH₂OH), 3.26 (dd, 1H, *J* = 11.1, 4.1 Hz, H-3), 3.06 (br d, 1H, *J* = 12.8 Hz, H-18), 1.62 (d, 3H, *J* = 6.6 Hz, H-6''), 1.26, 1.22, 1.07, 0.96, 0.91, 0.90, 0.86 (s each, 3H each, 7 \times Me); ¹³C-NMR (pyridine-*d*₅): δ 178.1 (C-28), 144.7 (C-13), 122.9 (C-12), 104.9 (C-1'), 101.7 (C-1''), 88.7 (C-3), 75.8 (C-2'), 74.0, 74.0 (C-3', C-4''), 72.6, 72.4 (C-2'', C-3''), 69.8 (C-5''), 68.8 (C-4'), 64.9 (C-5'), 61.4 (*NCH*₂CH₂OH), 55.8 (C-5), 47.9 (C-9), 46.7 (C-17), 46.4 (C-19), 43.0 (*NCH*₂CH₂OH), 42.1, 41.9 (C-

14, C-18), 39.7 (C-4), 39.5 (C-8), 38.8 (C-1), 36.9 (C-10), 34.3 (C-21), 33.6 (C-29), 33.1 (C-7), 32.8 (C-22), 30.9 (C-20), 28.0, 27.8 (C-15, C-23), 26.5, 26.1 (C-2, C-27), 23.8, 23.7, 23.7 (C-30, C-11, C-16), 18.6, 18.5 (C-6, C-6''), 17.3, 17.0 (C-26, C-24), 15.5 (C-25); HRMS: calcd for C₄₃H₇₁NO₁₁ (M+Na): 800.5027; found: m/z 800.5022.

3.6.6. N,N-diethyleleanolic amide 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside (TS6)

White powder, m.p. 159.4–161.2 °C; $[\alpha]_D^{25}$ -20.0 (*c* 0.14, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 6.17 (s, 1H, H-1''), 5.43 (br s, 1H, H-12), 4.90 (d, 1H, *J* = 5.4 Hz, H-1'), 4.75 (m, 1H, H-2''), 4.64–4.56 (m, 3H, H-2', H-3'', H-5''), 4.32–4.27 (m, 4H, H-3', H-4', H-5'-1, H-4''), 3.83 (m, 1H, H-5'-2), 3.41–3.26 (m, 6H, H-3, H-18, N(CH₂CH₃)₂), 1.63 (d, 3H, *J* = 6.6 Hz, H-6''), 1.27, 1.18, 1.14, 1.14, 1.09, 0.96, 0.93, 0.93, 0.88 (s each, 3H each, 9 \times Me); ¹³C-NMR (pyridine-*d*₅): δ 175.0 (C-28), 145.6 (C-13), 121.8 (C-12), 105.0 (C-1'), 101.8 (C-1''), 88.9 (C-3), 76.0 (C-2'), 74.2, 74.0 (C-3', C-4''), 72.7, 72.5 (C-2'', C-3''), 70.0 (C-5''), 68.9 (C-4'), 64.9 (C-5'), 56.1 (C-5), 48.2 (C-9), 47.8 (C-17), 47.2 (C-19), 44.2 (NCH₂CH₃), 42.3, 42.3 (C-14, C-18), 39.8 (C-4), 39.6 (C-8), 38.9 (C-1), 37.2 (C-10), 34.5 (C-21), 33.6 (C-29), 33.3, 33.3 (C-7, C-22), 30.6 (C-20), 30.4 (NCH₂CH₃), 28.6, 28.2 (C-15, C-23), 26.7 (C-2), 26.0 (C-27), 24.3, 23.9, 22.9 (C-30, C-11, C-16), 18.6 (C-6, C-6''), 17.7 (C-26), 17.2 (C-24), 15.8 (C-25), 13.7, 13.7 (N(CH₂CH₃)₂); HRMS: calcd for C₄₅H₇₅NO₁₀ (M+Na): 812.5289; found: m/z 812.5281.

3.6.7. 1-(piperidin-1-yl)ole-28-one 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside (TS7)

White powder, m.p. 174.0–176.3 °C; $[\alpha]_D^{25}$ -12.5 (*c* 0.12, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 6.17 (s, 1H, H-1''), 5.42 (br s, 1H, H-12), 4.89 (d, 1H, *J* = 5.4 Hz, H-1'), 4.75 (m, 1H, H-2''), 4.64–4.56 (m, 3H, H-2', H-3'', H-5''), 4.32–4.27 (m, 4H, H-3', H-4', H-5'-1, H-4''), 3.81 (d, 1H, *J* = 11.4 Hz, H-5'-2), 3.60–3.54 (m, 4H, H-piperidin-2,6), 3.40 (dd, 1H, *J* = 11.9, 4.2 Hz, H-3), 3.25 (br d, 1H, *J* = 13.0 Hz, H-18), 1.50 (m, 6H, H-piperidin-3,4,5), 1.62 (d, 3H, *J* = 6.6 Hz, H-6''), 1.27, 1.18, 1.09, 0.95, 0.94, 0.94, 0.88 (s each, 3H each, 7 \times Me); ¹³C-NMR (pyridine-*d*₅): δ 174.5 (C-28), 145.5 (C-13), 121.8 (C-12), 105.0 (C-1'), 101.8 (C-1''), 88.8 (C-3), 75.9 (C-2'), 74.1, 73.9 (C-3', C-4''), 72.6, 72.5 (C-2'', C-3''), 69.9 (C-5''), 68.8 (C-4'), 64.8 (C-5'), 56.1 (C-5), 48.1, 47.6, 46.9, 46.7 (C-9, C-piperidine, C-17, C-19), 44.2 (C-piperidine), 42.3, 42.3 (C-14, C-18), 39.6 (C-4), 39.5 (C-8), 38.9 (C-1), 37.1 (C-10), 34.3 (C-21), 33.4 (C-29), 33.2, 33.2 (C-7, C-22), 30.6 (C-20), 30.1 (C-piperidine), 28.4, 28.1 (C-15, C-23), 26.5, 26.5 (C-2, C-27), 26.2, 25.1 (C-piperidine), 24.2, 23.8, 22.9 (C-30, C-11, C-16), 18.6 (C-6, C-6''), 17.3, 17.1 (C-26, C-24), 15.7 (C-25); HRMS: calcd for C₄₆H₇₅NO₁₀ (M+Na): 824.5389; found: m/z 824.5386.

3.6.8. 1-(piperazin-1-yl)ole-28-one 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside (TS8)

White powder, m.p. 230.3–231.1 °C; $[\alpha]_D^{25}$ -19.4 (*c* 0.32, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 6.17 (s, 1H, H-1''), 5.41 (br s, 1H, H-12), 4.90 (d, 1H, *J* = 5.4 Hz, H-1'), 4.75 (m, 1H, H-2''), 4.65–4.56 (m, 3H, H-2', H-3'', H-5''), 4.33–4.28 (m, 4H, H-3', H-4', H-5'-1, H-4''), 4.28–4.20 (m, 4H, H-piperazine-2,6), 3.82 (d, 1H, *J* = 11.4 Hz, H-5'-2), 3.43–3.77 (m, 5H, H-3, H-piperazine-3,5), 3.25 (br d, 1H, *J* = 13.3 Hz, H-18), 1.63 (d, 3H, *J* = 6.0 Hz, H-6''), 1.24, 1.19, 1.11, 0.94, 0.93, 0.88, 0.85 (s each, 3H each, 7 \times Me); ¹³C-NMR (pyridine-*d*₅): δ 175.3 (C-28), 145.0 (C-13), 122.0 (C-12), 104.9 (C-1'), 101.7 (C-1''), 88.7 (C-3), 75.9 (C-2'), 74.1, 74.0 (C-3', C-4''), 72.6, 72.4 (C-2'', C-3''), 69.9 (C-5''), 68.8 (C-4'),

64.9 (C-5'), 56.0 (C-5), 48.1 (C-9), 47.6, 46.7 (C-17, C-19), 44.4, 44.0, 44.0 (C-piperazine), 42.2, 42.2 (C-14, C-18), 39.5, 39.5, 38.9 (C-4, C-8, C-1), 37.1 (C-10), 34.1 (C-21), 33.1, 33.1, 33.1 (C-7, C-29, C-22), 30.5 (C-20), 30.2 (C-piperazine), 28.3, 28.1 (C-15, C-23), 26.5 (C-2), 26.1 (C-27), 24.1, 23.7, 22.7 (C-30, C-11, C-16), 18.6 (C-6, C-6''), 17.1, 17.0 (C-26, C-24), 15.6 (C-25); HRMS: calcd for C₄₅H₇₄N₂O₁₀ (M+Na): 825.5343; found: m/z 825.5336.

3.6.9. 1-(4-methylpiperazin-1-yl)ole-28-one 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside (TS9)

White powder, m.p. 190.2–192.7 °C; $[\alpha]_D^{25}$ -17.5 (*c* 0.16, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 6.20 (s, 1H, H-1''), 5.42 (br s, 1H, H-12), 4.89 (d, 1H, *J* = 5.4 Hz, H-1'), 4.77 (m, 1H, H-2''), 4.66–4.58 (m, 3H, H-2', H-3'', H-5''), 4.34–4.28 (m, 4H, H-3', H-4', H-5'-1, H-4''), 3.83–3.74 (m, 5H, H-5'-2, H-piperazine-2,6), 3.40 (dd, 1H, *J* = 11.7, 4.0 Hz, H-3), 3.25 (br d, 1H, *J* = 13.1 Hz, H-18), 2.33 (m, 4H, H-piperazine-3,5), 2.17 (s, 3H, NCH₃), 1.63 (d, 3H, *J* = 6.6 Hz, H-6''), 1.26, 1.19, 1.10, 0.95, 0.94, 0.93, 0.88 (s each, 3H each, 7 \times Me); ¹³C-NMR (pyridine-*d*₅): δ 174.8 (C-28), 145.4 (C-13), 121.8 (C-12), 104.9 (C-1'), 101.8(C-1''), 88.8 (C-3), 75.9 (C-2'), 74.1, 73.9 (C-3', C-4''), 72.6, 72.4 (C-2'', C-3''), 69.9 (C-5''), 68.7(C-4'), 64.8 (C-5'), 56.0 (C-5), 55.6 (C-piperazine), 48.1 (C-9), 47.6, 46.7 (C-17, C-19), 46.0, 45.7, 44.1 (C-piperazine), 42.2, 42.2 (C-14, C-18), 39.6, 39.5, 38.9 (C-4, C-8, C-1), 37.1 (C-10), 34.2 (C-21), 33.4 (C-29), 33.2, 33.2 (C-7, C-22), 30.6 (C-20), 30.2 (NCH₃), 28.3, 28.1 (C-15,C-23), 26.5 (C-2), 26.2 (C-27), 24.2, 23.8, 22.8 (C-30, C-11, C-16), 18.6 (C-6, C-6''), 17.3, 17.0 (C-26, C-24), 15.7 (C-25); HRMS: calcd for C₄₆H₇₆N₂O₁₀ (M+Na): 839.5500; found: m/z 839.5497.

3.6.10. 1-morpholinoole-28-one 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside (TS10)

White powder, m.p. 201.7–204.1 °C; $[\alpha]_D^{25}$ -10.1 (*c* 0.10, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 6.16 (s, 1H, H-1''), 5.40 (br s, 1H, H-12), 4.88 (d, 1H, *J* = 4.9 Hz, H-1'), 4.73 (m, 1H, H-2''), 4.64–4.55 (m, 3H, H-2', H-3'', H-5''), 4.32–4.26 (m, 4H, H-3', H-4', H-5'-1, H-4''), 3.84–3.68 (m, 9H, H-5'-2, H-morpholine), 3.38 (dd, 1H, *J* = 11.5, 4.0 Hz, H-3), 3.24 (br d, 1H, *J* = 7.9 Hz, H-18), 1.62 (d, 3H, *J* = 5.7 Hz, H-6''), 1.25, 1.18, 1.09, 0.94, 0.93, 0.89, 0.88 (s each, 3H each, 7 \times Me); ¹³C-NMR (pyridine-*d*₅): δ 175.0 (C-28), 145.2 (C-13), 122.0 (C-12), 105.0 (C-1'), 101.8 (C-1''), 88.8 (C-3), 75.9 (C-2'), 74.1, 74.0 (C-3', C-4''), 72.6, 72.5 (C-2'', C-3''), 69.9 (C-5''), 68.8 (C-4'), 67.2 (C-morpholine), 64.9 (C-5'), 56.0 (C-5), 48.1 (C-9), 47.6 (C-morpholine), 46.6, 46.4 (C-17, C-19), 44.1 (C-morpholine), 42.2 (C-14, C-18), 39.6, 39.5 (C-4, C-8), 38.9 (C-1), 37.1 (C-10), 34.2 (C-21), 33.3 (C-29), 33.2 (C-7, C-22), 30.6 (C-20), 30.0 (C-morpholine), 28.2, 28.1 (C-15, C-23), 26.6 (C-2), 26.2 (C-27), 24.2 (C-30), 23.8, 22.7 (C-11, C-16), 18.6 (C-6, C-6''), 17.2, 17.0 (C-26, C-24), 15.7 (C-25); HRMS: calcd for C₄₅H₇₃NO₁₁ (M+Na): 826.5184; found: m/z 826.5180.

3.6.11. N-phenyloleanolic amide 3-O- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside (TS11)

White powder, m.p. 156.7–158.3 °C; $[\alpha]_D^{25}$ -11.9 (*c* 0.15, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 7.76–7.29 (m, 5H, Ar-H), 6.13 (s, 1H, H-1''), 5.55 (br s, 1H, H-12), 4.88 (d, 1H, *J* = 5.4 Hz, H-1'), 4.73 (m, 1H, H-2''), 4.60–4.52 (m, 3H, H-2', H-3'', H-5''), 4.32–4.28 (m, 4H, H-3', H-4', H-5'-1, H-4''), 3.80 (d, 1H, *J* = 11.1 Hz, H-5'-2), 3.22 (br d, 1H, *J* = 10.8 Hz, H-3), 3.02 (br d, 1H, *J* = 11.6 Hz, H-18), 1.60

(m, 3H, H-6"), 1.26, 1.14, 1.02, 0.96, 0.94, 0.77, 0.75 (s each, 3H each, 7×Me); ¹³C-NMR (pyridine-*d*₅): δ 175.8 (C-28), 143.2 (C-13), 138.4, 127.4, 127.3, 127.0, 123.8, 123.7 (6×Ar-C), 122.5 (C-12), 104.2 (C-1'), 101.1 (C-1"), 88.1 (C-3), 75.2 (C-2'), 73.4, 73.2 (C-3', C-4"), 72.0, 71.8 (C-2", C-3"), 69.3 (C-5"), 68.0 (C-4'), 64.0 (C-5'), 55.1 (C-5), 47.5, 47.2, 46.1 (C-9, C-17, C-19), 41.9 (C-14), 41.5 (C-18), 39.1 (C-4), 38.8 (C-8), 38.2 (C-1), 36.3 (C-21), 32.7 (C-29), 32.4, 32.1 (C-7, C-22), 30.2 (C-20), 27.4 (C-15), 27.1 (C-23), 25.8 (C-2), 25.5 (C-27), 23.6 (C-30), 23.2, 23.0 (C-11, C-16), 17.9, 17.8 (C-6, C-6"), 16.4, 16.3 (C-24, C-26), 14.9 (C-25); HRMS: calcd for C₄₇H₇₁NO₁₀ (M+Na): 832.5078; found: m/z 832.5072.

3.6.12. N-(2-hydroxyphenyl)oleanolic amide 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (TS12)

White powder, m.p. 191.3–194.0 °C; [α]_D²⁵ -28.6 (*c* 0.21, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 12.27 (br s, 1H, Ph-OH), 9.07 (m, 1H, Ar-H), 8.98 (m, 1H, Ar-H), 7.13 (m, 1H, Ar-H), 7.00 (m, 1H, Ar-H), 6.14 (s, 1H, H-1"), 5.67 (br s, 1H, H-12), 4.87 (d, 1H, *J* = 5.3 Hz, H-1'), 4.72 (m, 1H, H-2"), 4.63–4.52 (m, 3H, H-2', H-3", H-5"), 4.32–4.25 (m, 4H, H-3', H-4', H-5'-1, H-4"), 3.80 (br d, 1H, *J* = 9.6 Hz, H-5'-2), 3.20 (dd, 1H, *J* = 11.6, 4.1 Hz, H-3), 3.07 (br d, 1H, *J* = 13.2 Hz, H-18), 1.60 (d, 3H, *J* = 6.1 Hz, H-6"), 1.26, 1.13, 1.02, 0.91, 0.89, 0.82, 0.70 (s each, 3H each, 7×Me); ¹³C-NMR (pyridine-*d*₅): δ 175.7 (C-28), 147.2 (OH-Ph-C), 143.3 (C-13), 128.2 (Ar-C), 123.6, 123.4 (Ar-C, C-12), 119.9 (Ar-C), 119.3 (Ar-C), 114.8 (Ar-C), 104.2 (C-1'), 101.1 (C-1"), 88.1 (C-3), 75.2 (C-2'), 73.4, 73.2 (C-3', C-4"), 71.9, 71.8 (C-2", C-3"), 69.2 (C-5"), 68.0 (C-4'), 64.1 (C-5'), 55.1 (C-5), 47.4, 47.3, 46.3 (C-9, C-17, C-19), 42.0 (C-14), 41.6 (C-18), 39.1 (C-4), 38.8 (C-8), 38.3 (C-1), 36.2 (C-10), 33.8 (C-21), 32.8, 32.4, 32.2 (C-7, C-22, C-29), 30.2 (C-20), 27.4 (C-15), 27.2 (C-23), 25.9 (C-2), 25.4 (C-27), 23.8, 23.3, 23.0 (C-11, C-16, C-30), 17.9, 17.8 (C-6, C-6"), 16.3, 16.2 (C-24, C-26), 14.9 (C-25); HRMS: calcd for C₄₇H₇₁NO₁₁ (M+Na): 848.5027; found: m/z 848.5019.

3.6.13. N-benzyleoleanolic amide 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (TS13)

White powder, m.p. 168.3–171.1 °C; [α]_D²⁵ -9.5 (*c* 0.19, CH₃OH); ¹H-NMR (pyridine-*d*₅): δ 8.02–8.00 (m, 1H, NH), 7.45 (m, 2H, Ar-H), 7.31 (m, 2H, Ar-H), 7.22 (m, 1H, Ar-H), 6.17 (s, 1H, H-1"), 5.37 (br s, 1H, H-12), 4.86 (d, 1H, *J* = 5.4 Hz, H-1'), 4.77 (m, 2H, H-2", NHCH₂Ph), 4.63–4.55 (m, 4H, H-2', H-3", H-5", NHCH₂Ph), 4.31–4.25 (m, 4H, H-3', H-4', H-5'-1, H-4"), 3.80 (m, 1H, H-5'-2), 3.22 (dd, 1H, *J* = 11.5, 4.1 Hz, H-3), 3.14 (br d, 1H, *J* = 13.9 Hz, H-18), 1.61 (d, 3H, *J* = 6.6 Hz, H-6"), 1.23, 1.19, 1.07, 0.88, 0.88, 0.87, 0.85 (s each, 3H each, 7×Me); ¹³C-NMR (pyridine-*d*₅): δ 177.5 (C-28), 144.8 (C-13), 140.9, 128.8, 128.8, 128.1, 128.1, 127.1 (Ar-C), 122.8 (C-12), 104.9 (C-1'), 101.8 (C-1"), 88.7 (C-3), 75.9 (C-2'), 74.1, 74.0 (C-3', C-4"), 72.6, 72.4 (C-2", C-3"), 69.9 (C-5"), 68.8 (C-4'), 64.8 (C-5'), 55.8 (C-5), 47.9 (C-9), 46.9, 46.5 (C-17, C-19), 43.5 (CH₂Ph), 42.1, 41.9 (C-14, C-18), 39.7 (C-4), 39.5 (C-8), 38.8 (C-1), 37.0 (C-10), 34.4 (C-21), 33.8 (C-7), 33.2 (C-29), 33.0 (C-22), 30.9 (C-20), 28.0, 27.9 (C-15, C-23), 26.5 (C-2), 26.1 (C-27), 23.7 (C-30), 23.7 (C-11), 23.7 (C-16), 18.6, 18.5 (C-6, C-6"), 17.4 (C-26), 17.0 (C-24), 15.6 (C-25); HRMS: calcd for C₄₈H₇₃NO₁₀ (M+Na): 846.5234; found: m/z 846.5229.

4. Conclusions

In summary, 13 novel amide derivatives of β -hederin were synthesized and evaluated *in vitro* for tumor cytotoxicity. The results from the antitumor screening showed that compounds **TS8** and **TS9** possessed potent antitumor activity against HeLa and MCF-7 cell lines. The preliminary structure-activity relationships indicated that the conversion of the C-28 carboxylic acid group of β -hederin into an amide derivative often resulted in a loss of broad spectrum antitumor activity, but also resulted in an increase in the antitumor selectivity. To provide more clarity about the structure-activity relationship, further studies on additional systematic structural variations are underway.

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Sample Availability: Samples are available from the authors.

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