

Synthesis of Barringtogenol C Derivatives and Their Cytotoxic Activities

Barringtogenol C Türevlerinin Sentezi ve Sitotoksik Aktivitelerinin İncelenmesi

Research Article

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ABSTRACT

A series of barringtogenol C (1) derivatives (2a-c, 3a-c, 4a-c, 5a-d, 6a,b and 7) were synthesized and screened in cytotoxicity assay against various cancer cell lines (HeLa, A549, U87MG, CaCo-2 and MCF-7) together with the normal cell line Vero. Many of the compounds screened have been found to have significantly improved anti-cancer potency in comparison with doxorubicin or barringtogenol C (1). Compound 3a was found to be 26-fold more active than doxorubicin. It was also not toxic to the non-tumoral Vero cells tested under in vitro conditions. In conclusion, compound 3a tested strong cytotoxicity against cancer cells, which could be a promising anti-cancer lead compound for further studies.

Key Words

Styrax officinalis, Triterpene, Barringtogenol C, Cytotoxic activity.

ÖZ

Bir dizi barringtogenol C (1) türevleri (2a-c, 3a-c, 4a-c, 5a-d, 6a,b and 7) sentezlenmiş ve Vero normal hücre hattı ile birlikte çeşitli kanser hücre hatları ile (HeLa, A549, U87MG, CaCo-2 and MCF-7) çalışılmıştır. Doksorubisin ve barringtogenol C ile kıyaslandığında bileşiklerin çoğunun antikanser potansiyelinde anlamlı bir gelişme olduğu görülmüştür. Bileşik 3a'nın doksorubisinden 26 kat daha aktif olduğu bulunmuştur. Ayrıca in vitro koşullar altında test edilen Vero normal hücre hattında toksik değildir. Sonuç olarak bileşik 3a ileri çalışmalar için umut verici antikanser öncü bileşik olabilecek, kanser hücre hatlarına karşı güçlü sitotoksik aktiviteye sahiptir.

Anahtar Kelimeler

Styrax officinalis, Triterpen, Barringtogenol C, Sitotoksik aktivite.

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INTRODUCTION

Saponins are a vast class of natural products whose structural diversity includes a wide array of functional groups. Many compounds of this group are reported to have anti-inflammatory [1], anti-viral [2], anti-tumor [3,4], hepatoprotective [5,6], anti-hyperlipidemic [5,7], anti-obesity [8,9], hypoglycemic [10-12], antihypertensive [13], immunomodulatory [14-16], neuroprotective [17,18], anti-inflammatory [15,19,20], anti-tumor [3,21,22], anti-oxidant [18], wound healing [23,24] and anti-carcinogenic [20,25-27], anti-atherosclerotic [28], cytotoxic and apoptotic activities [16,22,29-32] and chemopreventive [20,33,34] effect.

Recently, saponins have been recognized as potent tumor inhibitors, and they have suddenly become one of the primary targets for new pharmacologically active anti-tumor molecules. Selective cytotoxic activity of oleanolic acid isolated from rhizomes of *Astilbe chinensis* towards the HeLa cancer cell line with a value of $IC_{50} = 6.49 \mu\text{g}/\text{mL}$ was reported by H. X. Sun [35,36]. Derivatives of oleanolic acid, that are formally derived from 2-cyano-3,12-dioxoleana-1,9(11)-dien-28-oic acid (CDDO), showed a range of interesting effects. *In vitro* studies have shown that CDDO induces cell differentiation, growth inhibition, and apoptosis in human leukemia [37,38], osteosarcoma [39] and breast cancer [40] cell lines. Further studies exhibited that the genes (IAP-2, BCL-2, C-MYC, VEGF, MMP-9) related with NF- κ B (nuclear factor kappa B) were suppressed by CDDO. Another study showed that the cytotoxic action of CDDO and its derivatives in glioblastoma and neuroblastoma was through inhibition of NF- κ B [41]. Two of synthetic oleanane derivatives, 2-cyano-3,12-dioxoleana-1,9(11)-dien-28-oic acid (CDDO) and its methyl ester (CDDO-Me) are under evaluation in phase I clinical trials to test for their anti-cancer properties [19,27,35,42].

Barringtonol C (olean-12-ene-3 β ,16 α ,21 β ,22 α ,28 β -pentol) (**1**), an oleanane-type triterpenoid is a prevalent pentacyclic triterpenoid which has been found in various plants in both aglycone and glycoside forms including *Barringtonia racemosa* [43], *Aesculus pavia* [44], *Eryngium yuccifolium* [45], *Careya arborea* [46], *Styrax japonica* [47], *Styrax officinalis* [48,49],

Aesculus hippocastanum [50] and *Antonia ovata* [51]. Several barringtonol C type triterpenoids have shown promising activities against A549 (human non-small cell lung tumor) [52], PC-3 (human prostate) [52], HL-60 (Human promyelocytic leukemia cells) [52], PANC-1 (Human pancreatic carcinoma, epithelial-like cell line) [52], MRC-5 (human fetal lung fibroblast) [52], J-774 (murine monocyte/macrophage) [44], WEHI-164 (murine fibrosarcoma) [44] and KB (human mouth epidermal carcinoma) [51] cell lines.

In the present study, we report the synthesis, characterization, and cytotoxicity of a new series of Barringtonol C (**1**) derivatives against the cell lines of Human lung adenocarcinoma epithelial (A549), human cervix adenocarcinoma (HeLa), human colorectal adenocarcinoma (CaCo-2), human glioblastoma-astrocytoma (U-87 MG) and human breast adenocarcinoma (MCF-7).

RESULTS and DISCUSSION

Chemistry

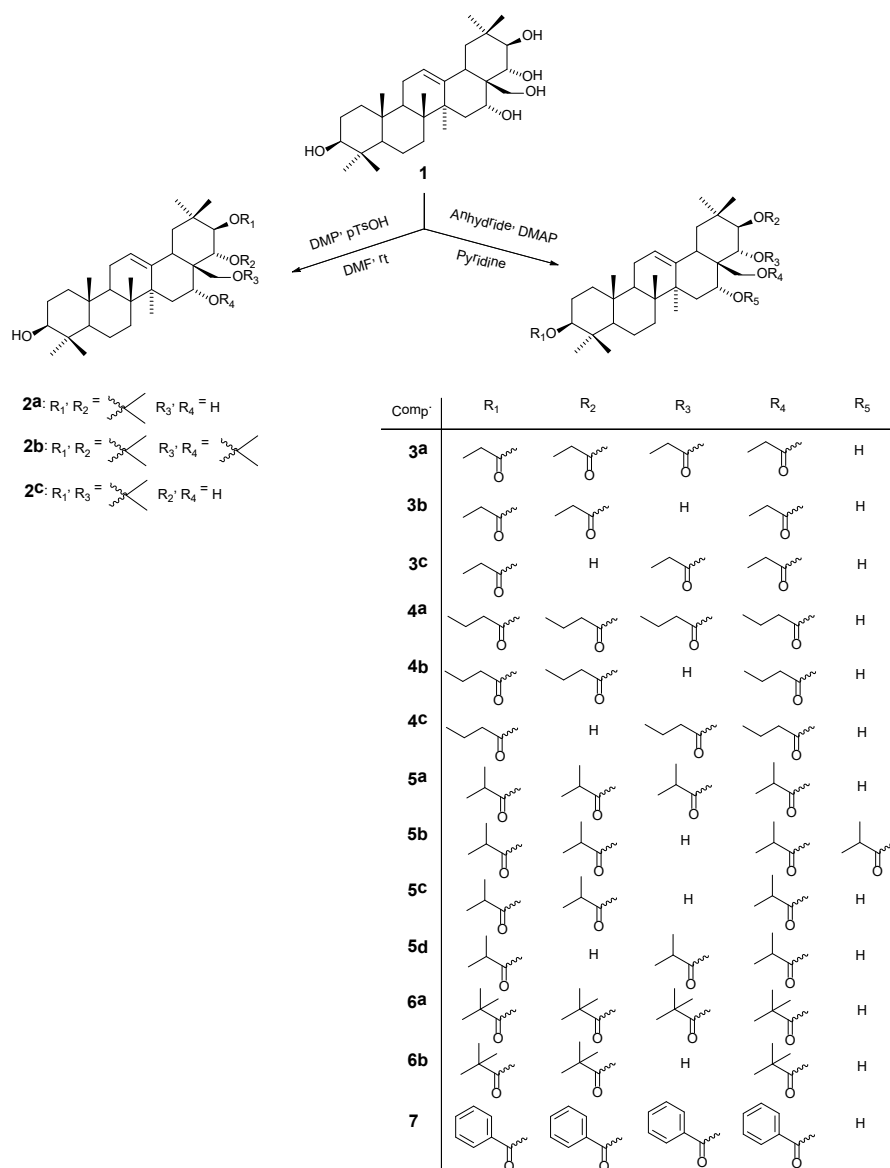
The synthetic pathway in the present work is outlined in Scheme 1. Barringtonol C isolated from the pericarps of fruits of *Styrax officinalis* L. was used as the key starting material.

The reaction of **1** with 2,2-dimethoxypropane (DMP) and *p*-toluenesulfonic acid (*p*TsOH) in DMF at room temperature gave the compounds **2a-c** after 12 h. Compounds **3a-c**, **4a-c**, **5a-d**, **6a,b** and **7** were synthesized by the reaction of **1** with anhydride in the presence of 4-dimethylaminopyridine (DMAP) in dry pyridine at reflux temperature for 4 h.

Cytotoxicity Evaluation

The target compounds **2a-c**, **3a-c**, **4a-c**, **5a-d**, **6a,b** and **7** were subjected to evaluation for their cytotoxic activity against CaCo-2, HeLa, MCF-7, A549, U87MG cancer cells and the normal cell Vero by modified MTT assay [53,54]. Doxorubicin was used as a control drug. The estimated IC_{50} values are listed in Table 1.

Among all, the compounds **2b**, **3b** and **3c** showed promising cytotoxic activity against five cancer cell lines, whereas compound **2a** exhibited



Scheme 1. Synthetic pathway for barringtonol C derivatives.

cytotoxic activity against four cell lines (CaCo-2, HeLa, MCF-7, and U87MG) at below 100 μM concentration. Compounds 2c, 3a, 5d and 7 showed activity only on one cancer cell line (CaCo-2, U87MG, U87MG and MCF-7), respectively. Remaining derivatives are not active at 100 μM concentration (the highest dose tested). Within these series of compounds, 3a displayed considerable activity against U87MG cell line (IC_{50} = 0.45 μM). While compound 1 is not active on these cell lines, the inhibitory activity of 3a was 26 times more potent than that of doxorubicin (IC_{50} = 11.9 μM). Similarly, compounds 2a (5.28 μM), 2b (6.24 μM), 3b (6.08 μM), and 3c (4.25 μM) exhibited more

potent activity than doxorubicin on the same cell lines. For the MCF-7 cell line, it was found that the most active compound was 2b (IC_{50} = 5.19 μM), followed closely by 7 (IC_{50} = 6.40 μM), were both more active than Doxorubicin. 2b and 7 also exhibited a better cytotoxic profile than compared with compound 1. For the CaCo-2 cell line, the most active compound was 2c (IC_{50} = 14.3 μM) which exhibited a 1.5-fold increase in comparison with doxorubicin and compound 1. Moreover, 2a (IC_{50} = 26.6 μM) and 2b (IC_{50} = 18.8 μM), also showed moderate activity on these cells. While the inhibitory activity of 2b (IC_{50} = 17.0 μM) was 1.2 times more potent than that of doxorubicin, compound

Table 1. Cytotoxic activity of compound 1 derivatives 2a-c, 3a-c, 4a-c, 5a-d, 6a,b and 7 on cell lines after 48 h of exposure.

Cell Lines IC ₅₀ (μM)	Vero	CaCo-2	HeLa	MCF-7	A549	U87MG
Doxorubicin	12.8	20.0	20.0	>50.0	9.94	11.9
1	10.2	22.4	9.79	33.3	-	-
2a	61.3	26.6	60.7	76.3	-	5.28
2b	34.0	18.8	17.0	5.19	34.7	6.24
2c	-	14.3	-	-	-	-
3a	-	-	-	-	-	0.45
3b	-	58.9	34.8	72.9	76.0	6.08
3c	12.5	48.2	22.5	60.3	36.5	4.25
4a	-	-	-	-	-	-
4b	-	-	-	-	-	-
4c	-	-	-	70.6	70.3	64.6
5a	-	-	-	-	-	-
5b	-	-	-	-	-	-
5c	-	-	-	-	-	-
5d	-	-	-	-	-	26.0
6a	-	-	-	-	-	-
6b	-	-	-	-	-	-
7	-	-	-	6.40	-	-

- >100

3c (IC₅₀ = 22.5 μM) showed moderate cytotoxic activity against HeLa cells. Also, for the A549 cell line, compound 2b (IC₅₀ = 34.7 μM) showed some cytotoxic activity despite compound 1 was not exhibited activity on this cells.

In addition, the cytotoxic effect of the compounds **2a-c**, **3a-c**, **4a-c**, **5a-d**, **6a,b** and **7** were also tested on Vero cells and their effect on Vero cells growth is given in Table 1. Only compounds **2a**, **2b** and **3c** were found to be toxic to the growth of normal cells tested under *in vitro* conditions within all compounds examined.

Experimental

General Considerations

The reagents and solvents were purchased from Sigma-Aldrich, Merck and Alfa Aesar and were used as received. Column chromatography was performed on Silica gel 60 (0.063-0.200 mm,

Merck) ¹H NMR (400 MHz) and ¹³C NMR (100 MHz or 150 MHz) spectra were acquired on a Varian AS 400 Mercury or Bruker Avance III HD Ascend 600 ULH spectrometer with CDCl₃ as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) were reported in units (ppm) by assigning TMS resonance in the ¹H spectrum as 0.00 ppm and CDCl₃ resonance in the ¹³C spectrum as 77.0 ppm. All coupling constants (*J* values) were reported in Hertz (Hz). Elemental analyses were performed on a Perkin-Elmer PE 2400 elemental analyzer. FTIR spectra were recorded on a Perkin Elmer Spectrum 100 series. Mass spectra were acquired using a Bruker HCT Ultra Ion Trap Mass Spectrometer.

Plant Material

Pericarps of fruits of *S. officinalis* L. were collected in August 2011 in Kusadasi (Aydın, Turkey). A voucher specimen has been deposited in the herbarium of the Botanic Garden of Ege University.

Extraction

Air-dried powdered pericarps of fruits (500 g) were extracted with MeOH (2×4 L). After filtration, the solvent was removed by rotary evaporation yielding 50 g of extract. The MeOH extract was dissolved in H₂O (350 mL), and successively partitioned with hexane (2×200 mL), EtOAc (2×200 mL) and *n*-BuOH saturated with H₂O (3×200 mL). The *n*-BuOH extract (20 g) was separately dissolved in 2 L MeOH/2N HCl (1:1) and refluxed for 6 h. After cooling, the solution was hydrolysed with 33% KOH at 100°C for 6 h. The reaction mixture was neutralized to pH 7 with 2N HCl and extracted with EtOAc. The mixture was purified by CC using silica gel and CH₂Cl₂:MeOH:H₂O (90:10:1) as eluent to afford barringtonenol C (6 g), which was identified by NMR analyses, and by comparison with literature data [48].

Olean-12-ene-3,16,21,22,28-pentol (1):

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3430 (>OH), 2948 (>CH), 1656 (C=C); ¹H NMR (400 MHz, CDCl₃) δ 5.17 (1H, t, J=3.5 Hz, H-12), 4.24 (1H, br s, H-16), 4.16 (1H, s, H-21), 4.02 (1H, s, H-22), 3.76 (1H, d, J=9.2 Hz, H-28a), 3.58 (1H, d, J=9.2 Hz, H-28b), 2.98 (1H, d, J=8.8, H-3), 2.35 (1H, m, H-19a), 2.30 (1H, m, H-18), 1.80 (2H, m, H-11), 1.79 (1H, m, H-2a), 1.78 (1H, m, H-15a), 1.58 (1H, m, H-2b), 1.56 (1H, m, H-9), 1.54 (1H, m, H-1a), 1.53 (1H, m, H-7a), 1.53 (1H, m, H-6a), 1.42 (1H, m, H-6b), 1.36 (1H, m, H-15b), 1.36 (1H, m, H-7b), 1.33 (3H, s, H-27), 1.04 (1H, m, H-19b), 0.97 (1H, m, H-1b), 0.89 (3H, s, H-29), 0.85 (3H, s, H-30), 0.82 (3H, s, H-23), 0.82 (3H, s, H-25), 0.79 (1H, m, H-5), 0.79 (3H, s, H-26), 0.66 (3H, s, H-24), ¹³C NMR (150 MHz, CDCl₃) δ 143.8 (C-13), 122.5 (C-12), 77.6 (C-21), 77.5 (C-22), 74.8 (C-3), 67.3 (C-28), 66.0 (C-16), 55.6 (C-5), 46.6 (C-9), 46.6 (C-19), 46.0 (C-17), 41.4 (C-8), 40.5 (C-4), 39.9 (C-1), 38.5 (C-14), 37.9 (C-18), 37.0 (C-10), 36.4 (C-20), 33.6 (C-15), 32.9 (C-7), 30.6 (C-29), 28.8 (C-27), 27.6 (C-23), 24.8 (C-11), 23.7 (C-2), 19.4 (C-30), 18.4 (C-6), 17.2 (C-24), 16.7 (C-26), 16.0 (C-25). ESI-MS (pos. ion mode) at *m/z* 513 [M+Na]⁺, *m/z* 1003 [2M+Na]⁺.

General procedures for the preparation of compounds 2a-c

A mixture of **1** (100.0 mg, 0.2 mmol), 2,2-Dimethoxypropane (0.5 ml, 4 mmol) and *p*-Toluenesulfonic acid (2.0 mg, 0.01 mmol) was stirred in DMF (3.0 mL) at rt overnight. Saturated

NaHCO₃ and EtOAc were added and the reaction mixture extracted, washed with water, dried over Na₂SO₄ and the solvent removed under reduced pressure. The residue was chromatographed using a silica gel column with the solvent system Hexane:EtOAc (80:20) to yield **2a** (40mg), **2b** (20 mg) and **2c** (15 mg).

Olean-12-ene-3,16,21,22,28-pentol, cyclic 21,22-(1-methylethylidene acetal) (2a):

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3435 (>OH), 2950 (>CH), 1650 (C=C); ¹H NMR (400 MHz, CDCl₃) δ 5.29 (1H, t, J=3.5 Hz, H-12), 4.77 (1H, br s, H-16), 4.16 (1H, d, J=10.0 Hz, H-21), 3.74 (1H, d, J=10.0 Hz, H-22), 3.56 (1H, d, J=12.0 Hz, H-28a), 3.35 (1H, d, J=12.0 Hz, H-28b), 3.23 (1H, dd, J=8.8, 6.4 Hz, H-3), 2.50 (1H, m, H-19a), 2.10 (1H, m, H-18), 1.85 (2H, m, H-11), 1.60 (1H, m, H-2a), 1.59 (1H, m, H-15a), 1.58 (1H, m, H-2b), 1.56 (1H, m, H-9), 1.54 (1H, m, H-1a), 1.53 (1H, m, H-7a), 1.53 (1H, m, H-6a), 1.48, 1.45 (C(CH₃)₂), 1.42 (1H, m, H-6b), 1.40 (3H, s, H-27), 1.36 (1H, m, H-15b), 1.36 (1H, m, H-7b), 1.04 (1H, m, H-19b), 0.97 (1H, m, H-1b), 1.02 (3H, s, H-29), 0.99 (3H, s, H-30), 0.99 (3H, s, H-23), 0.87 (3H, s, H-26), 0.82 (3H, s, H-25), 0.79 (1H, m, H-5), 0.78 (3H, s, H-24), ¹³C NMR (150 MHz, CDCl₃) δ 143.8 (C-13), 122.5 (C-12), 100.9 (C(CH₃)₂), 78.4 (C-3), 78.1 (C-21), 72.3 (C-22), 70.5 (C-28), 67.2 (C-16), 54.5 (C-5), 46.7 (C-19), 46.1 (C-9), 46.0 (C-17), 41.4 (C-8), 40.5 (C-4), 39.9 (C-1), 38.5 (C-14), 37.9 (C-18), 37.0 (C-10), 36.4 (C-20), 33.6 (C-15), 32.9 (C-7), 30.6 (C-29), 29.2, 29.2 (C(CH₃)₂), 27.4 (C-27), 27.6 (C-23), 24.8 (C-11), 23.7 (C-2), 19.4 (C-30), 18.4 (C-6), 17.1 (C-26), 15.8 (C-24), 15.7 (C-25). ESI-MS (pos. ion mode) at *m/z* 553 [M+Na]⁺, *m/z* 1083 [2M+Na]⁺. Anal. (Found: C, 70.35; H, 9.23. Calc. For C₃₃H₅₄O₅: C, 70.56; H, 9.30%).

Olean-12-ene-3,16,21,22,28-pentol, cyclic 21,22,16,28-bis(1-methylethylidene acetal) (2b):

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3435 (>OH), 2950 (>CH), 1650 (C=C); ¹H NMR (400 MHz, CDCl₃) δ 5.35 (1H, t, J=3.5 Hz, H-12), 4.16 (1H, br s, H-16), 4.08 (1H, d, J=10.0 Hz, H-21), 3.95 (1H, d, J=10.0 Hz, H-22), 3.20 (1H, dd, J=8.8, 6.4 Hz, H-3), 3.14 (1H, d, J=8.8 Hz, H-28a), 3.04 (1H, d, J=8.8 Hz, H-28b), 2.50 (1H, m, H-19a), 2.11 (1H, m, H-18), 1.86 (2H, m, H-11), 1.62 (1H, m, H-2a), 1.60 (1H, m, H-15a), 1.58 (1H, m, H-2b), 1.56 (1H, m, H-9), 1.54 (1H, m, H-1a),

1.52 (1H, m, H-7a), 1.51 (1H, m, H-6a), 1.42 (1H, m, H-6b), 1.40 (3H, s, H-27), 1.36 (1H, m, H-15b), 1.36 (1H, m, H-7b), 1.25, 1.25, 1.22, 1.22 ($2 \times \text{C}(\text{CH}_3)_2$), 1.04 (1H, m, H-19b), 0.97 (1H, m, H-1b), 1.02 (3H, s, H-29), 0.99 (3H, s, H-30), 0.99 (3H, s, H-23), 0.87 (3H, s, H-26), 0.82 (3H, s, H-25), 0.79 (1H, m, H-5), 0.78 (3H, s, H-24), ^{13}C NMR (150 MHz, CDCl_3) δ 141.1 (C-13), 124.7 (C-12), 108.5, 100.2 ($2 \times \text{C}(\text{CH}_3)_2$), 83.3 (C-21), 79.3 (C-3), 78.0 (C-22), 70.8 (C-28), 63.7 (C-16), 54.5 (C-5), 46.8 (C-19), 46.1 (C-9), 46.0 (C-17), 41.4 (C-8), 40.4 (C-4), 39.9 (C-1), 38.6 (C-14), 37.9 (C-18), 37.0 (C-10), 36.4 (C-20), 33.5 (C-15), 32.9 (C-7), 29.8 (C-29), 28.4 (C-27), 27.8, 27.8, 27.7, 27.7 ($2 \times \text{C}(\text{CH}_3)_2$), 27.6 (C-23), 24.8 (C-11), 23.9 (C-30), 23.7 (C-2), 18.4 (C-6), 17.4 (C-26), 17.3 (C-24), 16.0 (C-25). ESI-MS (pos. ione mode) at m/z 593 $[\text{M}+\text{Na}]^+$. Anal. (Found: C, 75.51; H, 14.12. Calc. For $\text{C}_{36}\text{H}_{58}\text{O}_5$: C, 75.75; H, 14.01%)

Olean-12-ene-3,16,21,22,28-pentol, cyclic 21,28-(1-methylethylidene acetal) (2c):

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3435 ($>\text{OH}$), 2950 ($>\text{CH}$), 1650 (C=C); ^1H NMR (400 MHz, CDCl_3) δ 5.29 (1H, t, $J=3.5$ Hz, H-12), 4.58 (1H, br s, H-16), 4.29 (1H, d, $J=10.0$ Hz, H-21), 3.55 (1H, d, $J=10.0$ Hz, H-22), 3.50 (1H, d, $J=11.6$ Hz, H-28a), 3.26 (1H, d, $J=11.6$ Hz, H-28b), 3.23 (1H, dd, $J=10.0, 6.0$ Hz, H-3), 2.40 (1H, m, H-19a), 2.10 (1H, m, H-18), 1.85 (2H, m, H-11), 1.60 (1H, m, H-2a), 1.59 (1H, m, H-15a), 1.58 (1H, m, H-2b), 1.56 (1H, m, H-9), 1.54 (1H, m, H-1a), 1.52 (1H, m, H-7a), 1.53 (1H, m, H-6a), 1.42 (1H, m, H-6b), 1.40 (3H, s, H-27), 1.36 (1H, m, H-15b), 1.36 (1H, m, H-7b), 1.25, 1.25 ($\text{C}(\text{CH}_3)_2$), 1.04 (1H, m, H-19b), 0.97 (1H, m, H-1b), 1.02 (3H, s, H-29), 0.99 (3H, s, H-30), 0.99 (3H, s, H-23), 0.87 (3H, s, H-26), 0.82 (3H, s, H-25), 0.79 (1H, m, H-5), 0.78 (3H, s, H-24), ^{13}C NMR (150 MHz, CDCl_3) δ 140.6 (C-13), 124.6 (C-12), 109.2 ($\text{C}(\text{CH}_3)_2$), 82.9 (C-21), 82.5 (C-22), 79.1 (C-3), 72.5 (C-28), 67.9 (C-16), 55.4 (C-5), 46.7 (C-19), 46.1 (C-9), 46.0 (C-17), 41.4 (C-8), 40.5 (C-4), 39.9 (C-1), 38.5 (C-14), 37.9 (C-18), 37.0 (C-10), 36.4 (C-20), 33.6 (C-15), 32.9 (C-7), 30.6 (C-29), 28.3 (C-27), 27.6 (C-23), 27.3 ($\text{C}(\text{CH}_3)_2$), 24.8 (C-11), 23.7 (C-2), 19.4 (C-30), 18.4 (C-6), 17.5 (C-24), 17.1 (C-26), 15.8 (C-25). ESI-MS (pos. ione mode) at m/z 553 $[\text{M}+\text{Na}]^+$, m/z 1083 $[2\text{M}+\text{Na}]^+$. Anal. (Found: C, 70.32; H, 9.41. Calc. For $\text{C}_{33}\text{H}_{54}\text{O}_5$: C, 70.56; H, 9.30%).

General Procedures for the Preparation of Compounds 3a-c

A solution of 1 (100.0 mg, 0.2 mmol), propionic anhydride (0.128 μl , 1 mmol), and DMAP (12.0 mg, 0.099 mmol) in dry pyridine (5 mL) was stirred at 120°C for 4 h. The reaction mixture was neutralized with 5% HCl (100 ml) and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column with the solvent system Hexane: EtOAc (80:20) to yield 3a (30mg), 3b (20 mg) and 3c (20 mg).

Olean-12-ene-3,16,21,22,28-pentol, 3,21,22,28-tetrapropanoate (3a):

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3430 ($>\text{OH}$), 2948 ($>\text{CH}$), 1730 (C=O), 1656 (C=C); ^1H NMR (400 MHz, CDCl_3) δ 5.56 (1H, d, $J=10.4$ Hz, H-21), 5.46 (1H, d, $J=10.4$ Hz, H-22), 5.35 (1H, t, $J=3.5$ Hz, H-12), 4.51 (1H, dd, $J=8.8, 6.4$ Hz, H-3), 4.16 (1H, br s, H-16), 3.68 (1H, d, $J=11.2$ Hz, H-28a), 3.61 (1H, d, $J=11.2$ Hz, H-28b), 2.45 (1H, m, H-19a), 2.32, 2.31, 2.30, 2.29 ($4 \times \text{OOCCH}_2\text{CH}_3$, q, $J=7.5$ Hz), 2.28 (1H, m, H-18), 1.90 (2H, m, H-11), 1.85 (1H, m, H-2a), 1.82 (1H, m, H-15a), 1.68 (1H, m, H-2b), 1.66 (1H, m, H-9), 1.62 (1H, m, H-1a), 1.62 (1H, m, H-7a), 1.56 (1H, m, H-6a), 1.42 (1H, m, H-6b), 1.41 (3H, s, H-27), 1.36 (1H, m, H-15b), 1.36 (1H, m, H-7b), 1.16, 1.15, 1.14, 1.13 ($4 \times \text{OOCCH}_2\text{CH}_3$, t, $J=7.5$ Hz), 1.05 (3H, s, H-23), 1.04 (1H, m, H-19b), 0.97 (3H, s, H-25), 0.97 (1H, m, H-1b), 0.93 (3H, s, H-29), 0.93 (3H, s, H-26), 0.90 (3H, s, H-30), 0.85 (3H, s, H-24), 0.79 (1H, m, H-5), ^{13}C NMR (150 MHz, CDCl_3) δ 174.4, 174.4, 174.1, 173.6 ($4 \times \text{C}=\text{O}$), 140.8 (C-13), 124.9 (C-12), 80.6 (C-3), 78.4 (C-21), 73.5 (C-22), 69.0 (C-28), 66.3 (C-16), 46.7 (C-5), 46.6 (C-9), 46.6 (C-19), 46.0 (C-17), 41.4 (C-8), 40.5 (C-4), 39.9 (C-1), 38.5 (C-14), 37.9 (C-18), 37.0 (C-10), 36.4 (C-20), 33.6 (C-15), 32.9 (C-7), 29.0 (C-29), 28.3 (C-27), 28.3 (C-23), 27.9, 27.8, 27.7, 27.1 ($4 \times \text{OOCCH}_2\text{CH}_3$), 24.8 (C-11), 23.7 (C-2), 19.6 (C-30), 18.4 (C-6), 17.0 (C-24), 16.9 (C-26), 15.8 (C-25), 9.5, 9.5, 9.4, 9.3 ($4 \times \text{OOCCH}_2\text{CH}_3$). ESI-MS (pos. ione mode) m/z 737 $[\text{M}+\text{Na}]^+$, MS/MS m/z 663 $[\text{M}+\text{Na}-\text{CH}_3\text{CH}_2\text{COOH}]^+$, 589 $[\text{M}+\text{Na}-2(\text{CH}_3\text{CH}_2\text{COOH})]^+$, 515 $[\text{M}+\text{Na}-3(\text{CH}_3\text{CH}_2\text{COOH})]^+$ and 441 $[\text{M}+\text{Na}-4(\text{CH}_3\text{CH}_2\text{COOH})]^+$. Anal. (Found: C, 70.25; H, 9.40. Calc. For $\text{C}_{42}\text{H}_{66}\text{O}_9$: C, 70.56; H, 9.30%).

Olean-12-ene-3,16,21,22,28-pentol, 3,21,28-tripropanoate (3b):

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3433 (ν_{OH}), 2948 (ν_{CH}), 1730 (C=O), 1655 (C=C); ^1H NMR (400 MHz, CDCl_3) δ 5.38 (1H, d, $J=10.0$ Hz, H-21), 5.35 (1H, t, $J=3.5$ Hz, H-12), 4.51 (1H, dd, $J=8.8, 6.4$ Hz, H-3), 4.27 (1H, br s, H-16), 3.89 (1H, d, $J=10.0$ Hz, H-22), 3.89 (1H, d, $J=10.8$ Hz, H-28a), 3.80 (1H, d, $J=10.8$ Hz, H-28b), 2.43 (1H, m, H-19a), 2.39, 2.38, 2.37, ($3\times\text{OOCCH}_2\text{CH}_3$, q, $J=7.5$ Hz), 2.26 (1H, m, H-18), 1.93 (2H, m, H-11), 1.84 (1H, m, H-2a), 1.80 (1H, m, H-15a), 1.67 (1H, m, H-2b), 1.64 (1H, m, H-9), 1.63 (1H, m, H-1a), 1.60 (1H, m, H-7a), 1.57 (1H, m, H-6a), 1.43 (1H, m, H-6b), 1.40 (3H, s, H-27), 1.37 (1H, m, H-15b), 1.36 (1H, m, H-7b), 1.19, 1.18, 1.16 ($3\times\text{OOCCH}_2\text{CH}_3$, t, $J=7.5$ Hz), 1.04 (3H, s, H-23), 1.03 (1H, m, H-19b), 0.98 (3H, s, H-25), 0.97 (1H, m, H-1b), 0.94 (3H, s, H-29), 0.93 (3H, s, H-26), 0.91 (3H, s, H-30), 0.84 (3H, s, H-24), 0.82 (1H, m, H5), ^{13}C NMR (150 MHz, CDCl_3) 176.1, 174.5, 174.3 ($3\times\text{C}=\text{O}$), 141.1 (C-13), 124.6 (C-12), 81.3 (C-21), 80.6 (C-3), 73.2 (C-22), 68.4 (C-28), 66.8 (C-16), 55.4 (C-5), 46.9 (C-17), 46.7 (C-19), 46.5 (C-9), 41.4 (C-8), 40.0 (C-4), 39.9 (C-1), 38.5 (C-14), 38.0 (C-18), 37.0 (C-10), 36.4 (C-20), 34.3 (C-15), 32.9 (C-7), 29.2 (C-29), 28.3 (C-23), 28.2 (C-27), 28.1, 27.8, 27.1 ($3\times\text{OOCCH}_2\text{CH}_3$), 24.8 (C-11), 23.8 (C-2), 19.5 (C-30), 18.4 (C-6), 17.1 (C-24), 16.9 (C-26), 15.8 (C-25), 9.5, 9.5, 9.4 ($3\times\text{OOCCH}_2\text{CH}_3$). ESI-MS (pos. ione mode) m/z 681 $[\text{M}+\text{Na}]^+$, MS/MS m/z 607 $[\text{M}+\text{Na}-\text{CH}_3\text{CH}_2\text{COOH}]^+$, 533 $[\text{M}+\text{Na}-2(\text{CH}_3\text{CH}_2\text{COOH})]^+$, 459 $[\text{M}+\text{Na}-3(\text{CH}_3\text{CH}_2\text{COOH})]^+$. Anal. (Found: C, 71.35; H, 9.35. Calc. For $\text{C}_{39}\text{H}_{62}\text{O}_8$: C, 71.09; H, 9.48%).

Olean-12-ene-3,16,21,22,28-pentol, 3,22,28-tripropanoate (3c):

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3430 (ν_{OH}), 2948 (ν_{CH}), 1732 (C=O), 1656 (C=C); ^1H NMR (400 MHz, CDCl_3) δ 5.27 (1H, d, $J=9.6$ Hz, H-22), 5.35 (1H, t, $J=3.5$ Hz, H-12), 4.50 (1H, dd, $J=8.8, 6.4$ Hz, H-3), 4.18 (1H, br s, H-16), 4.01 (1H, d, $J=9.6$ Hz, H-21), 3.70 (2H, s, H-28), 2.44 (1H, m, H-19a), 2.38, 2.37, 2.35, ($3\times\text{OOCCH}_2\text{CH}_3$, q, $J=7.5$ Hz), 2.25 (1H, m, H-18), 1.93 (2H, m, H-11), 1.83 (1H, m, H-2a), 1.81 (1H, m, H-15a), 1.66 (1H, m, H-2b), 1.64 (1H, m, H-9), 1.62 (1H, m, H-1a), 1.61 (1H, m, H-7a), 1.56 (1H, m, H-6a), 1.44 (1H, m, H-6b), 1.41 (3H, s, H-27), 1.37 (1H, m, H-15b), 1.35 (1H, m, H-7b), 1.19, 1.18, 1.18 ($3\times\text{OOCCH}_2\text{CH}_3$, t, $J=7.5$ Hz), 1.04 (3H, s,

H-23), 1.02 (1H, m, H-19b), 0.99 (3H, s, H-25), 0.97 (1H, m, H-1b), 0.95 (3H, s, H-29), 0.93 (3H, s, H-26), 0.92 (3H, s, H-30), 0.84 (3H, s, H-24), 0.82 (1H, m, H5), ^{13}C NMR (150 MHz, CDCl_3) 175.3, 174.4, 174.1 ($3\times\text{C}=\text{O}$), 140.0 (C-13), 124.8 (C-12), 80.7 (C-3), 78.3 (C-22), 76.4 (C-21), 69.4 (C-28), 66.3 (C-16), 55.5 (C-5), 46.9 (C-17), 46.8 (C-19), 46.0 (C-9), 41.3 (C-8), 40.6 (C-4), 39.9 (C-1), 38.5 (C-4), 38.0 (C-18), 37.0 (C-10), 36.4 (C-20), 33.9 (C-15), 32.9 (C-7), 29.2 (C-29), 28.2, 28.2, 28.0 ($3\times\text{OOCCH}_2\text{CH}_3$), 27.8 (C-23), 27.1 (C-27), 24.8 (C-11), 23.7 (C-2), 18.3 (C-6), 18.3 (C-30), 17.0 (C-24), 16.9 (C-26), 15.8 (C-25), 9.5, 9.4, 9.2 ($3\times\text{OOCCH}_2\text{CH}_3$). ESI-MS (pos. ione mode) m/z 681 $[\text{M}+\text{Na}]^+$, MS/MS m/z 607 $[\text{M}+\text{Na}-\text{CH}_3\text{CH}_2\text{COOH}]^+$, 533 $[\text{M}+\text{Na}-2(\text{CH}_3\text{CH}_2\text{COOH})]^+$, 459 $[\text{M}+\text{Na}-3(\text{CH}_3\text{CH}_2\text{COOH})]^+$. Anal. (Found: C, 71.41; H, 9.30. Calc. For $\text{C}_{39}\text{H}_{62}\text{O}_8$: C, 71.09; H, 9.48%).

General Procedures for the Preparation of Compounds 4a-c

A solution of 1 (100.0 mg, 0.2 mmol), butyric anhydride (0.245 μl , 1.5 mmol), and DMAP (12.0 mg, 0.099 mmol) in dry pyridine (5 mL) was stirred at 120°C for 4 h. The reaction mixture was neutralized with 5% HCl (100 ml) and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column with the solvent system Hexane: EtOAc (80:20) to yield 8 (32mg), 9 (20 mg) and 10 (18 mg).

Olean-12-ene-3,16,21,22,28-pentol, 3,21,22,28-tetrabutanoate (4a):

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3432 (ν_{OH}), 2950 (ν_{CH}), 1725 (C=O), 1656 (C=C); ^1H NMR (400 MHz, CDCl_3) δ 5.56 (1H, d, $J=10.0$ Hz, H-21), 5.48 (1H, d, $J=10.0$ Hz, H-22), 5.34 (1H, t, $J=3.5$ Hz, H-12), 4.53 (1H, dd, $J=9.6, 6.4$ Hz, H-3), 4.13 (1H, br s, H-16), 3.70 (1H, d, $J=10.8$ Hz, H-28a), 3.55 (1H, d, $J=10.8$ Hz, H-28b), 2.45 (1H, m, H-19a), 2.32, 2.31, 2.29, 2.28 ($4\times\text{OOCCH}_2\text{CH}_2\text{CH}_3$, t, $J=7.5$ Hz), 2.27 (1H, m, H-18), 1.90 (2H, m, H-11), 1.86 (1H, m, H-2a), 1.82 (1H, m, H-15a), 1.67 (1H, m, H-2b), 1.65 (1H, m, H-9), 1.62 (1H, m, H-1a), 1.63 (1H, m, H-7a), 1.62, 1.62, 1.61, 1.60 ($4\times\text{OOCCH}_2\text{CH}_2\text{CH}_3$, m), 1.56 (1H, m, H-6a), 1.42 (1H, m, H-6b), 1.40 (3H, s, H-27), 1.36 (1H, m, H-15b), 1.35 (1H, m, H-7b), 1.05 (3H, s, H-23), 1.04 (1H, m, H-19b), 0.97 (3H, s, H-25), 0.97 (1H, m, H-1b),

0.93 (3H, s, H-29), 0.93 (3H, s, H-26), 0.90 (3H, s, H-30), 0.89, 0.88, 0.87, 0.87 (4×OOCCH₂CH₂CH₃, t, J=7.5 Hz), 0.85 (3H, s, H-24), 0.79 (1H, m, H5), ¹³C NMR (150 MHz, CDCl₃) δ 173.7, 173.6, 173.3, 172.8 (4×C=O), 140.8 (C-13), 124.9 (C-12), 80.6 (C-3), 78.3 (C-21), 73.2 (C-22), 69.1 (C-28), 65.9 (C-16), 55.4 (C-5), 46.6 (C-9), 46.6 (C-19), 46.0 (C-17), 41.4 (C-8), 40.4 (C-4), 39.9 (C-1), 38.5 (C-14), 37.9 (C-18), 37.0 (C-10), 36.9, 36.6, 36.5, 36.5 (4×OOCCH₂CH₂CH₃), 36.4 (C-20), 33.7 (C-15), 32.9 (C-7), 29.1 (C-29), 28.3 (C-27), 28.3 (C-23), 24.8 (C-11), 23.7 (C-2), 19.6 (C-30), 18.8, 18.7, 18.7, 18.5 (4×OOCCH₂CH₂CH₃), 18.4 (C-6), 17.0 (C-24), 16.9 (C-26), 15.8 (C-25), 13.9, 13.9, 13.8, 13.8 (4×OOCCH₂CH₂CH₃). ESI-MS (pos. ione mode) at m/z 793 [M+Na]⁺. Anal. (Found: C, 71.40; H, 9.75. Calc. For C₄₆H₇₄O₉: C, 71.65; H, 9.67%).

Olean-12-ene-3,16,21,22,28-pentol, 3,21,28-tributanoate (4b):

Amorphous white solid; IR ν^{KBr}_{max} cm⁻¹: 3432 (>OH), 2948 (>CH), 1725 (C=O), 1656 (C=C); ¹H NMR (400 MHz, CDCl₃) δ 5.39 (1H, d, J=10.0 Hz, H-21), 5.35 (1H, t, J=3.5 Hz, H-12), 4.50 (1H, dd, J=8.8, 6.4 Hz, H-3), 4.30 (1H, br s, H-16), 3.89 (1H, d, J=10.0 Hz, H-22), 3.89 (1H, d, J=10.8 Hz, H-28a), 3.80 (1H, d, J=10.8 Hz, H-28b), 2.42 (1H, m, H-19a), 2.33, 2.32, 2.30 (3×OOCCH₂CH₂CH₃, t, J=7.5 Hz), 2.25 (1H, m, H-18), 1.93 (2H, m, H-11), 1.85 (1H, m, H-2a), 1.81 (1H, m, H-15a), 1.67 (1H, m, H-2b), 1.63 (1H, m, H-9), 1.62, 1.62, 1.61 (3×OOCCH₂CH₂CH₃, m), 1.61 (1H, m, H-1a), 1.60 (1H, m, H-7a), 1.57 (1H, m, H-6a), 1.43 (1H, m, H-6b), 1.40 (3H, s, H-27), 1.36 (1H, m, H-15b), 1.36 (1H, m, H-7b), 1.04 (3H, s, H-23), 1.03 (1H, m, H-19b), 0.98 (3H, s, H-25), 0.97 (1H, m, H-1b), 0.94 (3H, s, H-29), 0.93 (3H, s, H-26), 0.91 (3H, s, H-30), 0.89, 0.88, 0.87 (3×OOCCH₂CH₂CH₃, t, J=7.5 Hz), 0.84 (3H, s, H-24), 0.82 (1H, m, H5), ¹³C NMR (150 MHz, CDCl₃) 175.4, 173.7, 173.5 (3×C=O), 141.2 (C-13), 124.4 (C-12), 81.3 (C-21), 80.6 (C-3), 73.1 (C-22), 68.4 (C-28), 66.5 (C-16), 55.4 (C-5), 46.8 (C-17), 46.7 (C-19), 46.6 (C-9), 41.3 (C-8), 40.1 (C-4), 39.9 (C-1), 38.5 (C-14), 38.0 (C-18), 37.0 (C-10), 36.9, 36.6, 36.5 (3×OOCCH₂CH₂CH₃), 36.5 (C-20), 34.4 (C-15), 32.9 (C-7), 29.1 (C-29), 28.3 (C-23), 28.2 (C-27), 28.2, 27.8, 27.1 (3×OOCCH₂CH₃), 24.8 (C-11), 23.8 (C-2), 19.5 (C-30), 18.8, 18.7, 18.7 (3×OOCCH₂CH₂CH₃), 18.4 (C-6), 17.1 (C-24), 16.9 (C-26), 15.8 (C-25), 13.9, 13.9, 13.8 (3×OOCCH₂CH₂CH₃). ESI-MS (pos. ione mode) at m/z 723 [M+Na]⁺. Anal. (Found: C, 71.65; H, 9.60. Calc. For C₄₂H₆₈O₈: C, 71.96; H, 9.77%).

Olean-12-ene-3,16,21,22,28-pentol, 3,22,28-tributanoate (4c):

Amorphous white solid; IR ν^{KBr}_{max} cm⁻¹: 3430 (>OH), 2948 (>CH), 1725 (C=O), 1656 (C=C); ¹H NMR (400 MHz, CDCl₃) δ 5.28 (1H, d, J=10.0 Hz, H-22), 5.34 (1H, t, J=3.5 Hz, H-12), 4.50 (1H, dd, J=8.8, 6.4 Hz, H-3), 4.14 (1H, br s, H-16), 4.00 (1H, d, J=10.0 Hz, H-21), 3.68 (1H, d, J=11.2 Hz, H-28a), 3.62 (1H, d, J=11.2 Hz, H-28b), 2.42 (1H, m, H-19a), 2.33, 2.31, 2.30 (3×OOCCH₂CH₂CH₃, t, J=7.5 Hz), 2.24 (1H, m, H-18), 1.92 (2H, m, H-11), 1.86 (1H, m, H-2a), 1.81 (1H, m, H-15a), 1.66 (1H, m, H-2b), 1.63 (1H, m, H-9), 1.63, 1.62, 1.61 (3×OOCCH₂CH₂CH₃, m), 1.61 (1H, m, H-1a), 1.60 (1H, m, H-7a), 1.58 (1H, m, H-6a), 1.43 (1H, m, H-6b), 1.41 (3H, s, H-27), 1.37 (1H, m, H-15b), 1.35 (1H, m, H-7b), 1.04 (3H, s, H-23), 1.03 (1H, m, H-19b), 0.99 (3H, s, H-25), 0.97 (1H, m, H-1b), 0.95 (3H, s, H-29), 0.93 (3H, s, H-26), 0.91 (3H, s, H-30), 0.90, 0.88, 0.87 (3×OOCCH₂CH₂CH₃, t, J=7.5 Hz), 0.86 (3H, s, H-24), 0.84 (1H, m, H5), ¹³C NMR (150 MHz, CDCl₃) 175.4, 173.7, 173.5 (3×C=O), 141.2 (C-13), 124.4 (C-12), 81.3 (C-21), 80.6 (C-3), 73.1 (C-22), 68.4 (C-28), 66.5 (C-16), 55.4 (C-5), 46.8 (C-17), 46.7 (C-19), 46.6 (C-9), 41.3 (C-8), 40.1 (C-4), 39.9 (C-1), 38.5 (C-14), 38.0 (C-18), 37.0 (C-10), 36.9, 36.6, 36.5 (3×OOCCH₂CH₂CH₃), 36.5 (C-20), 34.4 (C-15), 32.9 (C-7), 29.1 (C-29), 28.3 (C-23), 28.2 (C-27), 28.2, 27.8, 27.1 (3×OOCCH₂CH₃), 24.8 (C-11), 23.8 (C-2), 19.5 (C-30), 18.8, 18.7, 18.7 (3×OOCCH₂CH₂CH₃), 18.4 (C-6), 17.1 (C-24), 16.9 (C-26), 15.8 (C-25), 13.9, 13.9, 13.8 (3×OOCCH₂CH₂CH₃). ESI-MS (pos. ione mode) at m/z 723 [M+Na]⁺. Anal. (Found: C, 71.60; H, 9.85. Calc. For C₄₂H₆₈O₈: C, 71.96; H, 9.77%).

General Procedures for the Preparation of Compounds 5a-d.

A solution of 1 (100.0 mg, 0.2 mmol), isobutyric anhydride (249 μl, 1.5 mmol), and DMAP (12.0 mg, 0.099 mmol) in dry pyridine (5 ml) was stirred at 120°C for 4 h. The reaction mixture was neutralized with 5% HCl (100 ml) and extracted with EtOAc. The organic layer was dried over anhydrous Na₂SO₄ and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column with the solvent system Hexane: EtOAc (80:20) to yield 5a (25mg), 5b (15 mg), 5c (15 mg) and 5d (15 mg).

**Olean-12-ene-3,16,21,22,28-pentol,
3,21,22,28-tetra(2-methylpropanoate) (5a):**

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3432 (ν_{OH}), 2945 (ν_{CH}), 1728 (C=O), 1655 (C=C); ^1H NMR (400 MHz, CDCl_3) δ 5.55 (1H, d, $J=10.4$ Hz, H-21), 5.53 (1H, d, $J=10.4$ Hz, H-22), 5.34 (1H, t, $J=3.5$ Hz, H-12), 4.50 (1H, dd, $J=9.6, 6.4$ Hz, H-3), 4.10 (1H, br s, H-16), 3.72 (1H, d, $J=10.8$ Hz, H-28a), 3.43 (1H, d, $J=10.8$ Hz, H-28b), 2.54, 2.53, 2.53, 2.52 ($4 \times \text{OOCCH}(\text{CH}_3)_2$, m), 2.45 (1H, m, H-19a), 2.27 (1H, m, H-18), 1.91 (2H, m, H-11), 1.87 (1H, m, H-2a), 1.82 (1H, m, H-15a), 1.67 (1H, m, H-2b), 1.65 (1H, m, H-9), 1.65 (1H, m, H-1a), 1.63 (1H, m, H-7a), 1.56 (1H, m, H-6a), 1.43 (1H, m, H-6b), 1.40 (3H, s, H-27), 1.37 (1H, m, H-15b), 1.35 (1H, m, H-7b), 1.17, 1.16, 1.15, 1.14 ($4 \times \text{OOCCH}(\text{CH}_3)_2$, d, $J=6.8$ Hz), 1.04 (3H, s, H-23), 1.03 (1H, m, H-19b), 0.97 (3H, s, H-25), 0.96 (1H, m, H-1b), 0.93 (3H, s, H-29), 0.92 (3H, s, H-26), 0.90 (3H, s, H-30), 0.82 (3H, s, H-24), 0.81 (1H, m, H5), ^{13}C NMR (150 MHz, CDCl_3) δ 177.1, 176.9, 176.8, 176.0 ($4 \times \text{C}=\text{O}$), 140.8 (C-13), 124.7 (C-12), 80.4 (C-3), 78.1 (C-21), 72.4 (C-22), 69.3 (C-28), 65.1 (C-16), 55.4 (C-5), 46.7 (C-9), 46.6 (C-19), 46.1 (C-17), 41.5 (C-8), 40.3 (C-4), 39.8 (C-1), 38.5 (C-14), 37.8 (C-18), 37.0 (C-10), 36.4 (C-20), 34.8, 34.6, 34.5, 34.3 ($4 \times \text{OOCCH}(\text{CH}_3)_2$), 33.7 (C-15), 32.8 (C-7), 29.1 (C-29), 28.2 (C-27), 28.1 (C-23), 24.8 (C-11), 23.6 (C-2), 19.6 (C-30), 19.1, 18.8, 18.7, 18.5 ($4 \times \text{OOCCH}(\text{CH}_3)_2$), 18.4 (C-6), 17.0 (C-24), 16.9 (C-26), 15.9 (C-25). ESI-MS (pos. ione mode) at m/z 793 $[\text{M}+\text{Na}]^+$. Anal. (Found: C, 71.80; H, 9.80. Calc. For $\text{C}_{46}\text{H}_{74}\text{O}_9$: C, 71.65; H, 9.67%).

**Olean-12-ene-3,16,21,22,28-pentol,
3,16,21,28-tetra(2-methylpropanoate) (5b):**

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3430 (ν_{OH}), 2950 (ν_{CH}), 1728 (C=O), 1655 (C=C); ^1H NMR (400 MHz, CDCl_3) δ 5.40 (1H, br s, H-16), 5.33 (1H, t, $J=3.5$ Hz, H-12), 5.10 (1H, d, $J=10.0$ Hz, H-21), 4.50 (1H, dd, $J=9.6, 6.4$ Hz, H-3), 3.89 (1H, d, $J=10.0$ Hz, H-22), 3.80 (1H, d, $J=10.8$ Hz, H-28a), 3.70 (1H, d, $J=10.8$ Hz, H-28b), 2.55, 2.54, 2.53, 2.52 ($4 \times \text{OOCCH}(\text{CH}_3)_2$, m), 2.46 (1H, m, H-19a), 2.28 (1H, m, H-18), 1.91 (2H, m, H-11), 1.87 (1H, m, H-2a), 1.83 (1H, m, H-15a), 1.68 (1H, m, H-2b), 1.65 (1H, m, H-9), 1.64 (1H, m, H-1a), 1.63 (1H, m, H-7a), 1.57 (1H, m, H-6a), 1.43 (1H, m, H-6b), 1.41 (3H, s, H-27), 1.37 (1H, m, H-15b), 1.35 (1H, m, H-7b), 1.16, 1.15, 1.14, 1.13 ($4 \times \text{OOCCH}(\text{CH}_3)_2$, d, $J=6.8$ Hz), 1.04 (3H, s, H-23), 1.02 (1H, m, H-19b), 0.97 (3H, s, H-25), 0.96 (1H, m, H-1b), 0.93 (3H, s,

H-29), 0.92 (3H, s, H-26), 0.90 (3H, s, H-30), 0.87 (3H, s, H-24), 0.85 (1H, m, H5), ^{13}C NMR (150 MHz, CDCl_3) δ 178.2, 177.0, 176.7, 176.6 ($4 \times \text{C}=\text{O}$), 140.1 (C-13), 125.4 (C-12), 80.4 (C-3), 79.6 (C-21), 72.4 (C-22), 70.2 (C-28), 66.8 (C-16), 55.3 (C-5), 46.8 (C-9), 46.5 (C-19), 46.1 (C-17), 41.4 (C-8), 40.2 (C-4), 39.8 (C-1), 38.6 (C-14), 37.7 (C-18), 37.1 (C-10), 36.4 (C-20), 34.7, 34.6, 34.5, 34.3 ($4 \times \text{OOCCH}(\text{CH}_3)_2$), 33.7 (C-15), 32.7 (C-7), 29.1 (C-29), 28.1 (C-27), 28.0 (C-23), 24.8 (C-11), 23.6 (C-2), 19.7 (C-30), 19.0, 18.8, 18.6, 18.5 ($4 \times \text{OOCCH}(\text{CH}_3)_2$), 18.4 (C-6), 17.1 (C-24), 17.0 (C-26), 15.8 (C-25). ESI-MS (pos. ione mode) at m/z 793 $[\text{M}+\text{Na}]^+$. Anal. (Found: C, 71.40; H, 9.75. Calc. For $\text{C}_{46}\text{H}_{74}\text{O}_9$: C, 71.65; H, 9.67%).

**Olean-12-ene-3,16,21,22,28-pentol,
3,21,28-tris(2-methylpropanoate) (5c):**

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3430 (ν_{OH}), 2948 (ν_{CH}), 1728 (C=O), 1656 (C=C); ^1H NMR (400 MHz, CDCl_3) δ 5.41 (1H, d, $J=10.0$ Hz, H-21), 5.33 (1H, t, $J=3.5$ Hz, H-12), 4.50 (1H, dd, $J=9.6, 6.4$ Hz, H-3), 4.15 (1H, br s, H-16), 3.86 (1H, d, $J=10.0$ Hz, H-22), 3.86 (1H, d, $J=11.2$ Hz, H-28a), 3.77 (1H, d, $J=11.2$ Hz, H-28b), 2.60, 2.58, 2.57 ($3 \times \text{OOCCH}(\text{CH}_3)_2$, m), 2.44 (1H, m, H-19a), 2.28 (1H, m, H-18), 1.90 (2H, m, H-11), 1.88 (1H, m, H-2a), 1.83 (1H, m, H-15a), 1.68 (1H, m, H-2b), 1.65 (1H, m, H-9), 1.64 (1H, m, H-1a), 1.63 (1H, m, H-7a), 1.55 (1H, m, H-6a), 1.43 (1H, m, H-6b), 1.41 (3H, s, H-27), 1.37 (1H, m, H-15b), 1.35 (1H, m, H-7b), 1.22, 1.21, 1.20 ($3 \times \text{OOCCH}(\text{CH}_3)_2$, d, $J=6.8$ Hz), 1.04 (3H, s, H-23), 1.03 (1H, m, H-19b), 0.97 (3H, s, H-25), 0.96 (1H, m, H-1b), 0.93 (3H, s, H-29), 0.91 (3H, s, H-26), 0.90 (3H, s, H-30), 0.83 (3H, s, H-24), 0.82 (1H, m, H5), ^{13}C NMR (150 MHz, CDCl_3) δ 178.7, 177.0, 176.9 ($3 \times \text{C}=\text{O}$), 141.2 (C-13), 124.4 (C-12), 80.4 (C-3), 81.2 (C-21), 73.0 (C-22), 68.4 (C-28), 66.5 (C-16), 55.4 (C-5), 46.8 (C-9), 46.6 (C-19), 46.0 (C-17), 41.5 (C-8), 40.2 (C-4), 39.7 (C-1), 38.5 (C-14), 37.6 (C-18), 37.0 (C-10), 36.3 (C-20), 34.7, 34.6, 34.5, ($3 \times \text{OOCCH}(\text{CH}_3)_2$), 33.6 (C-15), 32.8 (C-7), 29.1 (C-29), 28.2 (C-27), 28.0 (C-23), 24.7 (C-11), 23.5 (C-2), 19.6 (C-30), 19.1, 18.8, 18.7 ($3 \times \text{OOCCH}(\text{CH}_3)_2$), 18.3 (C-6), 17.0 (C-24), 16.9 (C-26), 16.0 (C-25). ESI-MS (pos. ione mode) at m/z 723 $[\text{M}+\text{Na}]^+$. Anal. (Found: C, 71.80; H, 9.65. Calc. For $\text{C}_{42}\text{H}_{68}\text{O}_8$: C, 71.96; H, 9.77%).

**Olean-12-ene-3,16,21,22,28-pentol,
3,22,28-tris(2-methylpropanoate) (5d):**

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3430 (ν_{OH}), 2948 (ν_{CH}), 1728 (C=O), 1655 (C=C); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.33 (1H, t, $J=3.5$ Hz, H-12), δ 5.27 (1H, d, $J=10.0$ Hz, H-22), 4.50 (1H, dd, $J=9.6, 6.4$ Hz, H-3), 4.20 (1H, br s, H-16), 4.05 (1H, d, $J=10.0$ Hz, H-21), 3.70 (1H, d, $J=11.6$ Hz, H-28a), 3.50 (1H, d, $J=11.6$ Hz, H-28b), 2.60, 2.59, 2.57 ($3\times\text{OOCCH}(\text{CH}_3)_2$, m), 2.45 (1H, m, H-19a), 2.29 (1H, m, H-18), 1.91 (2H, m, H-11), 1.87 (1H, m, H-2a), 1.83 (1H, m, H-15a), 1.67 (1H, m, H-2b), 1.65 (1H, m, H-9), 1.64 (1H, m, H-1a), 1.62 (1H, m, H-7a), 1.55 (1H, m, H-6a), 1.44 (1H, m, H-6b), 1.41 (3H, s, H-27), 1.38 (1H, m, H-15b), 1.35 (1H, m, H-7b), 1.23, 1.21, 1.20 ($3\times\text{OOCCH}(\text{CH}_3)_2$, d, $J=6.8$ Hz), 1.04 (3H, s, H-23), 1.03 (1H, m, H-19b), 0.98 (3H, s, H-25), 0.96 (1H, m, H-1b), 0.92 (3H, s, H-29), 0.91 (3H, s, H-26), 0.90 (3H, s, H-30), 0.82 (3H, s, H-24), 0.81 (1H, m, H5), $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 178.0, 177.7, 176.8 ($3\times\text{C}=\text{O}$), 140.0 (C-13), 125.0 (C-12), 80.4 (C-3), 78.4 (C-21), 75.5 (C-22), 69.5 (C-28), 65.2 (C-16), 55.4 (C-5), 46.8 (C-9), 46.7 (C-19), 46.0 (C-17), 41.4 (C-8), 40.1 (C-4), 39.7 (C-1), 38.5 (C-14), 37.5 (C-18), 37.0 (C-10), 36.2 (C-20), 34.8, 34.7, 34.5, ($3\times\text{OOCCH}(\text{CH}_3)_2$), 33.5 (C-15), 32.8 (C-7), 29.0 (C-29), 28.2 (C-27), 28.0 (C-23), 24.6 (C-11), 23.6 (C-2), 19.7 (C-30), 19.0, 18.8, 18.7 ($3\times\text{OOCCH}(\text{CH}_3)_2$), 18.2 (C-6), 17.1 (C-24), 16.9 (C-26), 16.0 (C-25). ESI-MS (pos. ione mode) at m/z 723 $[\text{M}+\text{Na}]^+$. Anal. (Found: C, 71.75; H, 9.55. Calc. For $\text{C}_{42}\text{H}_{68}\text{O}_8$: C, 71.96; H, 9.77%).

**General Procedures for the Preparation of
Compounds 6a,b.**

A solution of 1 (100.0 mg, 0.2 mmol), pivalic anhydride (304 μl , 1.5 mmol), and DMAP (12.0 mg, 0.099 mmol) in dry pyridine (5 mL) was stirred at 120°C for 4 h. The reaction mixture was neutralized with 5% HCl (100 ml) and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column with the solvent system Hexane: EtOAc (80:20) to yield 6a (35mg) and 6b (20 mg).

**Olean-12-ene-3,16,21,22,28-pentol,
3,21,22,28-tetra(2,2-dimethylpropanoate)
(6a):**

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3435 (ν_{OH}),

2950 (ν_{CH}), 1735 (C=O), 1656 (C=C); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.57 (1H, d, $J=10.4$ Hz, H-21), 5.53 (1H, d, $J=10.4$ Hz, H-22), 5.34 (1H, t, $J=3.5$ Hz, H-12), 4.44 (1H, dd, $J=9.6, 6.4$ Hz, H-3), 4.06 (1H, br s, H-16), 3.72 (1H, d, $J=10.8$ Hz, H-28a), 3.24 (1H, d, $J=10.8$ Hz, H-28b), 2.44 (1H, m, H-19a), 2.28 (1H, m, H-18), 1.90 (2H, m, H-11), 1.86 (1H, m, H-2a), 1.82 (1H, m, H-15a), 1.66 (1H, m, H-2b), 1.65 (1H, m, H-9), 1.65 (1H, m, H-1a), 1.63 (1H, m, H-7a), 1.56 (1H, m, H-6a), 1.44 (1H, m, H-6b), 1.40 (3H, s, H-27), 1.36 (1H, m, H-15b), 1.35 (1H, m, H-7b), 1.18, 1.17, 1.16, 1.14 ($4\times\text{OOC}(\text{CH}_3)_3$, s), 1.03 (3H, s, H-23), 1.03 (1H, m, H-19b), 0.98 (3H, s, H-25), 0.97 (1H, m, H-1b), 0.93 (3H, s, H-29), 0.91 (3H, s, H-26), 0.90 (3H, s, H-30), 0.84 (3H, s, H-24), 0.82 (1H, m, H5), $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 178.4, 178.3, 178.1, 177.1 ($4\times\text{C}=\text{O}$), 140.6 (C-13), 124.7 (C-12), 80.4 (C-3), 78.1 (C-21), 71.4 (C-22), 69.7 (C-28), 64.0 (C-16), 55.4 (C-5), 46.9 (C-9), 46.7 (C-19), 46.5 (C-17), 41.2 (C-8), 40.0 (C-4), 39.9 (C-1), 39.4, 39.3, 39.2, 39.1 ($4\times\text{OOC}(\text{CH}_3)_3$), 38.5 (C-14), 38.1 (C-18), 37.1 (C-10), 36.4 (C-20), 34.2 (C-15), 32.9 (C-7), 29.1 (C-29), 28.4 (C-27), 28.3 (C-23), 27.7, 27.6, 27.6, 27.5 ($4\times\text{OOC}(\text{CH}_3)_3$), 24.8 (C-11), 23.8 (C-2), 19.7 (C-30), 18.4 (C-6), 17.0 (C-24), 17.0 (C-26), 15.6 (C-25). ESI-MS (pos. ione mode) m/z 849 $[\text{M}+\text{Na}]^+$, MS/MS m/z z 747 $[\text{M}+\text{Na}-(\text{CH}_3)_3\text{CCOOH}]^+$, 645 $[\text{M}+\text{Na}-2((\text{CH}_3)_3\text{CCOOH})]^+$, 543 $[\text{M}+\text{Na}-3((\text{CH}_3)_3\text{CCOOH})]^+$, 441 $[\text{M}+\text{Na}-4((\text{CH}_3)_3\text{CCOOH})]^+$. Anal. (Found: C, 72.80; H, 10.01. Calc. For $\text{C}_{50}\text{H}_{82}\text{O}_9$: C, 72.60; H, 9.99%).

**Olean-12-ene-3,16,21,22,28-pentol,
3,21,28-tris(2,2-dimethylpropanoate) (6b):**

Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3430 (ν_{OH}), 2948 (ν_{CH}), 1734 (C=O), 1656 (C=C); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.38 (1H, d, $J=10.0$ Hz, H-21), 5.34 (1H, t, $J=3.5$ Hz, H-12), 4.46 (1H, dd, $J=9.6, 6.4$ Hz, H-3), 4.23 (1H, br s, H-16), 3.86 (1H, d, $J=10.0$ Hz, H-22), 3.86 (1H, d, $J=11.2$ Hz, H-28a), 3.77 (1H, d, $J=11.2$ Hz, H-28b), 2.45 (1H, m, H-19a), 2.27 (1H, m, H-18), 1.90 (2H, m, H-11), 1.85 (1H, m, H-2a), 1.82 (1H, m, H-15a), 1.65 (1H, m, H-2b), 1.64 (1H, m, H-9), 1.64 (1H, m, H-1a), 1.63 (1H, m, H-7a), 1.56 (1H, m, H-6a), 1.45 (1H, m, H-6b), 1.41 (3H, s, H-27), 1.37 (1H, m, H-15b), 1.35 (1H, m, H-7b), 1.22, 1.21, 1.20 ($3\times\text{OOC}(\text{CH}_3)_3$, s), 1.03 (3H, s, H-23), 1.02 (1H, m, H-19b), 0.98 (3H, s, H-25), 0.97 (1H, m, H-1b), 0.93 (3H, s, H-29), 0.91 (3H, s, H-26), 0.90 (3H, s, H-30), 0.83 (3H, s, H-24), 0.82 (1H, m, H5), $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 179.8, 178.1, 178.1 ($3\times\text{C}=\text{O}$), 140.6

(C-13), 124.6 (C-12), 80.9 (C-21), 80.1 (C-3), 72.9 (C-22), 68.3 (C-28), 66.2 (C-16), 55.4 (C-5), 46.8 (C-9), 46.7 (C-19), 46.4 (C-17), 41.2 (C-8), 40.1 (C-4), 39.9 (C-1), 39.4, 39.3, 39.2 ($3 \times \text{OCC}(\text{CH}_3)_3$), 38.4 (C-14), 38.1 (C-18), 37.0 (C-10), 36.4 (C-20), 34.1 (C-15), 32.9 (C-7), 29.0 (C-29), 28.4 (C-27), 28.2 (C-23), 27.7, 27.6, 27.6 ($4 \times \text{OCC}(\text{CH}_3)_3$), 24.7 (C-11), 23.8 (C-2), 19.6 (C-30), 18.4 (C-6), 17.1 (C-24), 17.0 (C-26), 15.7 (C-25). ESI-MS (pos. ione mode) m/z 765 [M+Na]⁺, MS/MS m/z z 663 [M+Na-(CH₃)₃CCOOH]⁺, 561 [M+Na-2((CH₃)₃CCOOH)]⁺, 459 [M+Na-3((CH₃)₃CCOOH)]⁺. Anal. (Found: C, 72.60; H, 10.10. Calc. For C₄₅H₇₄O₈: C, 72.74; H, 10.04%).

Olean-12-ene-3,16,21,22,28-pentol, 3,21,22,28-tetrabenzoate (7):

A solution of 1 (100.0 mg, 0.2 mmol), benzoic anhydride (339 mg, 1.5 mmol), and DMAP (12.0 mg, 0.099 mmol) in dry pyridine (5 mL) was stirred at 120°C for 4 h. The reaction mixture was neutralized with 5% HCl (100 ml) and extracted with EtOAc. The organic layer was dried over anhydrous Na₂SO₄ and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column with the solvent system Hexane: EtOAc (80:20) to yield 7 (35mg). Amorphous white solid; IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3433 (>OH), 2945 (>CH), 1716 (C=O), 1655 (C=C); ¹H NMR (400 MHz, CDCl₃) δ 8.06, 7.86, 7.86, 7.76 (4×H-2',6'), 7.30, 7.27, 7.27, 7.25 (4×H-3',5'), 7.45, 7.44, 7.44, 7.43 (4×H-4'), 6.10 (1H, d, J=10.4 Hz, H-21), 6.00 (1H, d, J=10.4 Hz, H-22), 5.33 (1H, t, J=3.5 Hz, H-12), 4.75 (1H, dd, J=9.5, 6.5 Hz, H-3), 4.54 (1H, br s, H-16), 4.19 (1H, d, J=11.2 Hz, H-28a), 4.01 (1H, d, J=11.2 Hz, H-28b), 2.45 (1H, m, H-19a), 2.27 (1H, m, H-18), 1.91 (2H, m, H-11), 1.86 (1H, m, H-2a), 1.83 (1H, m, H-15a), 1.66 (1H, m, H-2b), 1.65 (1H, m, H-9), 1.65 (1H, m, H-1a), 1.62 (1H, m, H-7a), 1.56 (1H, m, H-6a), 1.44 (1H, m, H-6b), 1.40 (3H, s, H-27), 1.37 (1H, m, H-15b), 1.35 (1H, m, H-7b), 1.03 (3H, s, H-23), 1.02 (1H, m, H-19b), 0.99 (3H, s, H-25), 0.98 (1H, m, H-1b), 0.92 (3H, s, H-29), 0.91 (3H, s, H-26), 0.90 (3H, s, H-30), 0.85 (3H, s, H-24), 0.82 (1H, m, H5), ¹³C NMR (150 MHz, CDCl₃) δ 175.1, 174.3, 174.1, 173.7 (4×C=O), 140.6 (C-13), 130.4, 129.8, 129.7, 129.7 (4×C-4'), 128.6, 128.6, 128.5, 128.5 (4×C-1'), 128.3, 128.3, 128.2, 128.2 (4×C-2',6'), 128.1, 128.1, 128.0, 128.0 (4×C-3',5'), 124.8 (C-12), 81.4 (C-3), 78.8 (C-21), 72.1 (C-22), 68.1 (C-28), 64.7 (C-16), 55.4 (C-5), 46.9 (C-9), 46.7 (C-19),

46.4 (C-17), 41.2 (C-8), 40.0 (C-4), 39.9 (C-1), 38.5 (C-14), 38.1 (C-18), 37.0 (C-10), 36.4 (C-20), 34.2 (C-15), 32.8 (C-7), 29.1 (C-29), 28.5 (C-27), 28.3 (C-23), 24.9 (C-11), 23.9 (C-2), 19.8 (C-30), 18.5 (C-6), 17.3 (C-24), 17.0 (C-26), 15.8 (C-25). ESI-MS (pos. ione mode) m/z 929 [M+Na]⁺, MS/MS m/z 807 [M+Na-C₆H₅COOH]⁺, 685 [M+Na-2(C₆H₅COOH)]⁺, 563 [M+Na-3(C₆H₅COOH)]⁺, 441 [M+Na-4(C₆H₅COOH)]⁺. Anal. (Found: C, 76.51; H, 7.35. Calc. For C₅₈H₆₆O₉: C, 76.79; H, 7.33%).

Cell Culture and in vitro Cytotoxicity Assay

Human lung adenocarcinoma epithelial (A549), human cervix adenocarcinoma (HeLa), human colorectal adenocarcinoma (CaCo-2), human glioblastoma-astrocytoma (U-87 MG), human breast adenocarcinoma (MCF-7) cells were used as cancer cell lines. Kidney epithelial cells from an African green monkey (Vero) were used as a non-cancerous cell line. Cell lines were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Germany). All the cells were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL of penicillin and 100 µg/mL of streptomycin (Biochrom AG, Berlin, Germany). The cells were incubated at 37°C in a humidified atmosphere of 5% CO₂.

Cytotoxicity of compounds were determined by following the general procedure based on cell viability using a modified MTT [3-(4, 5- Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide] [53,54] which measures the activity of mitochondrial reductase of viable cells colorimetrically. The assay is based on cleavage of MTT that forms formazan crystals. This cleavage appears in living cells with succinate-dehydrogenase. Adding dimethyl sulfoxide to wells helps formazan crystals to be dissolved. The optical density (OD) was measured at 570 nm (with a reference at 690 nm) by U.V. visible spectrophotometer (Thermo Scientific, USA) in triplicates. All cell lines were cultivated for 24 h in 96-well microplates with an initial concentration of 1×10⁵ cells/ml. Then, the cultured cells were treated with different doses of 0-100 µM and incubated for 48 h at 37°C. Doxorubicin, a chemotherapeutic agent, was used as a positive cytotoxic control drug. Percentages of surviving cells in each culture were determined after treat-

ment of venom. The % viability was determined as formulated below:

$$\text{Viable cells (\%)} = \left[\frac{\text{(The absorbance of the treated cells)} - \text{(the absorbance of the blank)}}{\text{(The absorbance of the control)} - \text{(the absorbance of the blank)}} \right] \times 100$$

Determination of IC₅₀

Inhibition of growth 50% (IC₅₀), which is the concentration of compounds causing 50% inhibition in cell growth compared to untreated controls, calculated by using OD values of controls and compounds doses as described previously. Cytotoxicity was expressed as an increase of the mean percentage of cytotoxicity relative to the unexposed control \pm standard deviation (SD). Control values were set at 0% cytotoxicity. IC₅₀ was calculated by fitting the data to a sigmoidal curve and using a four parameters logistic model and presented as an average of three independent measurements. The IC₅₀ values were reported at 95% confidence interval and calculation was performed using GraphPad Prism software (San Diego, USA). The values of the blank wells were subtracted from each well of treated and control cells and inhibition of growth 50% was calculated in comparison with untreated controls.

CONCLUSIONS

In conclusion, 16 ester derivatives of barringtogenol C (**1**) have been synthesized and evaluated for the growth inhibition of various cancer cells such as CaCo-2, HeLa, MCF-7, A549 and U87MG cancer cell lines together with normal cells Vero. Several compounds have been found to have better or comparable anti-cancer potency in comparison with doxorubicin or barringtogenol C (**1**). Moreover, the cytotoxicity of the synthesized compounds appears to be selective on non-tumoral Vero cells, which tolerated substantially higher doses of these compounds than tumor cells. Among these, compounds **2a**, **2b** bearing an isopropylidene group at C21, C22 and C21, C28 showed an enhanced cytotoxic activity against the U87MG and CaCo-2 cell lines, respectively. However, the addition of isopropylidene moiety at C21, C22, C16 and C28, (**2b**) increased the activity of **1** on MCF-7, A549 and U87MG cell lines. While esterification of

hydroxyl groups at C3, C21, C22 and C28 positions with propionic acid (**3a**) produced a considerable increase in the activity of **1** on U87MG cell lines, the addition of propanoate moiety at C3, C21 and C28 (**3b**) or C3, C22 and C28 (**3c**) positions led to an increase in cytotoxicity of U87MG and A549 cell lines. Regarding compound **4c**, the results showed that substitution of C3, C22 and C28 hydroxyl groups with butanoic acid moiety increased activity against to U87MG and A549 cell lines. In case of compound **5d**, isobutanoate groups at C3, C22 and C28 positions increased cytotoxicity against to U87MG cell lines. Nevertheless, the addition of benzoate moiety at C3, C21 and C28 positions (**7**) resulted in increased activity of **1** on U87MG cell lines.

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