Full Paper

Antitumoractive Endoperoxides from Triterpenes

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A series of triterpene endoperoxides was synthesized and screened for antitumor activity in a panel of 15 human cancer cell lines by a sulforhodamine-B (SRB) assay. The compounds induce apoptosis and show excellent antitumor activity.

Keywords: Antitumor-activ / Endoperoxide / Glycyrrhetinic acid / Triterpenes

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Introduction

The most important bioactive compounds of licorice root (*Glycyrrhiza glabra*) are triterpene glycyrrhizin and its aglycon 18β-glycyrrhetinic acid [1]. Both compounds, especially glycyrrhizin, is active against a broad spectrum of viruses, and they both were reported to have anti-inflammatory and antitumor activity [2 – 8].

Qinghaosu [9] (artemisinin), a composition of the traditional Chinese medicine qinghao (*Artemisia annua*) is a spectial triterpene with a unique 1,2,4-trioxane segment and has excellent antimalarial activity, especially for strains resistant to chloroquine [10]. In addition, antitumor properties have been reported for this compound [11]. Recently, endoperoxides from different natural sources were isolated and found to show cytotoxic as well as antifungal activities [12 – 15].

Although the mode of action has been discussed controversially, most probably reactive oxygen species (ROS) are formed by a reductive activation by Fe(II) complexes. Thus, these radicals cause apoptosis by induced oxidative stress; this behavior makes them interesting for cancer therapy [12, 16 – 22].

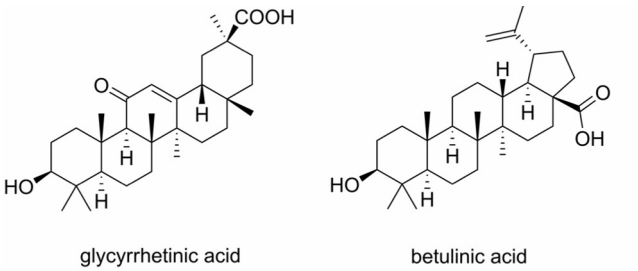

Figure 1. Pharmacological important triterpene acids.

Results and discussion

We set out to synthesize derivatives of pharmacological important triterpenes (Fig. 1) containing peroxide segments. In a first attempt, we planned to synthesize 1,2,4-trioxanes (Scheme 1) from substituted betulinic aldehydes **1** and **2**. Thus, the β-hydroperoxy alcohol **3** [23] was prepared according to established procedures [24]. Subsequent condensation of **3** in the presence of a Lewis-acid catalyst yielded compounds **4** and **5**. Unfortunately, the reaction gave low yields and a mixture of two diastereo-

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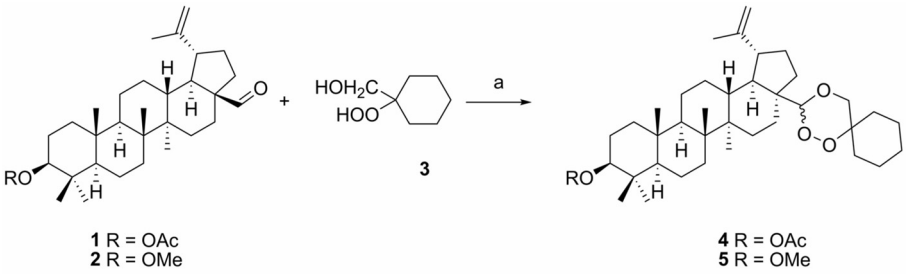
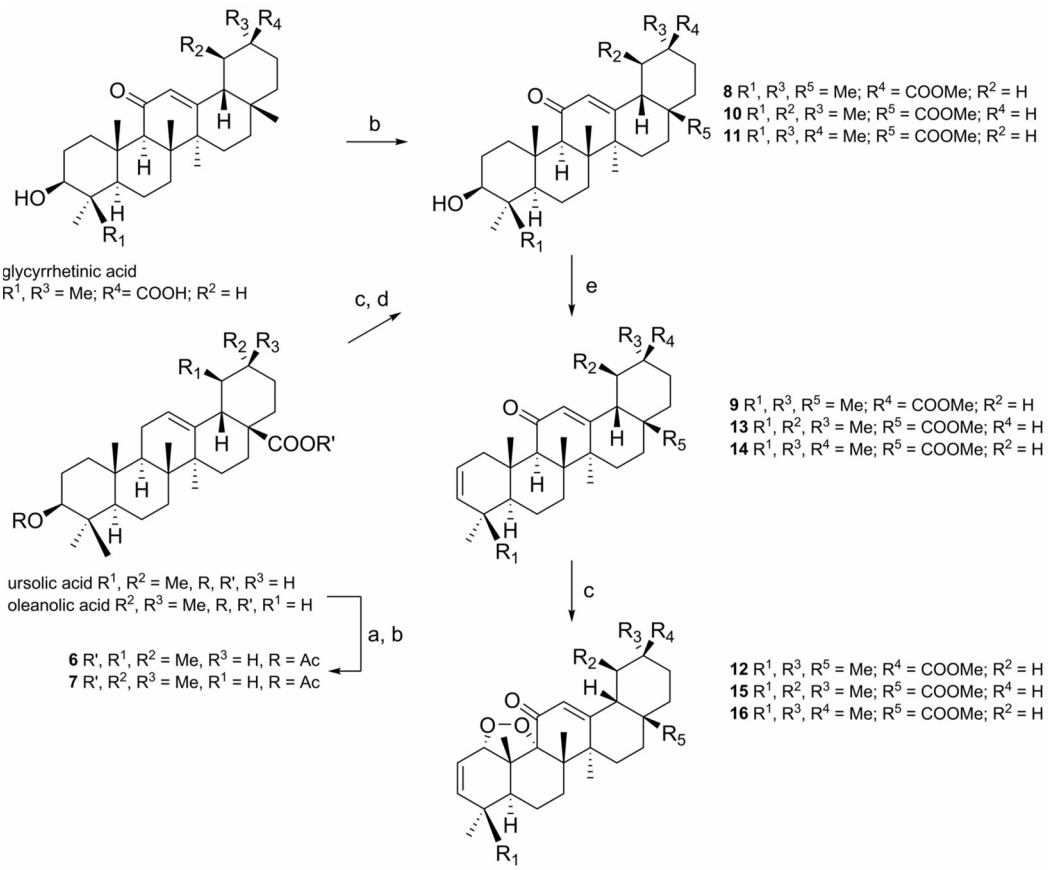
Abbreviations: reactive oxygen species (ROS); sulforhodamine-B (SRB)



Reagents and conditions: a) $BF_3.OEt_2$, CH_2Cl_2, −78°C to 0°C, 5 h, 27–35%.

Scheme 1. Synthesis of peroxides **4** and **5**.



Reagents and conditions: a) Ac_2O, DMAP, TEA, CH_2Cl_2, 0°C, 2 h; b) MeI, K_2CO_3, acetone, 25°C, 24 h; c) $Na_2Cr_2O_7$, *N*-hydroxysuccinimide, AcOH, acetone, 40°C, 72 h; d) NaOMe, MeOH, reflux, 24 h; e) 3,3-dimethylglutarimide, PPh_3, DEAD, THF, 0°C, 12 h.

Scheme 2. Synthesis of the endoperoxides.

meric products (diastereomeric ratio 4:1 to 5:1 as determined by ^{1}H-NMR) was obtained. This mixture of compounds was inseparable under various chromatographic conditions, and the absolute configuration of the major isomer was not determined. Because of these problems and the finding that **4** and **5** showed no significant antitumor activity (vide infra), we stopped the synthesis of these derivatives.

In a second approach, we started from ursolic acid (Scheme 2). Acetylation followed by esterification gave compound **6** in 94% overall yield. In an analoguous manner, oleanolic acid afforded the 3-*O*-acetyl oleanolic acid methylester **7** in 96% yield. Allylic oxidation applying *N*-hydroxysuccinimide/$Na_2Cr_2O_7$ in acetone furnished the 11-keto-derivatives **10** and **11**, respectively. Elimination reaction of **10** under Mitsunobu conditions [25, 26]

Table 1. Results of FACS analysis for Aβ and PrP.

Cell line	BA[b)]	12	15	16
518A2[a)]	11.88	1.20	1.46	1.69
A-431[a)]	15.38	2.70	2.60	3.01
A-253[a)]	11.13	1.79	2.16	2.21
FADU[a)]	10.41	7.79	6.10	7.48
A-549[a)]	14.91	1.28	1.33	1.46
A-2780[a)]	11.01	1.05	0.83	1.05
DLD-1[a)]	17.49	1.79	1.90	2.27
HCT-8[a)]	17.82	1.57	1.72	2.11
HCT-116[a)]	13.26	1.91	2.07	2.27
HAT-29[a)]	16.06	1.68	1.85	2.05
SW-480[a)]	6.41	3.89	3.72	3.94
8505-C[a)]	6.67	4.61	4.01	4.93
SW-1736[a)]	11.62	0.87	0.76	0.89
MCF-7[a)]	14.86	2.90	2.47	2.80
Lipo[a)]	9.73	5.07	4.70	5.41

a) The values for melanoma (518A2), zervic cancer (A431), head and neck tumor (A253, FADU), lung carcinoma (A549), ovarian cancer (A2780), colon cancer (DLD-1, HCT-8, HCT-116, HT-29, SW-480), anaplastic thyroid cancer (8505C, SW-1736), mamma carcinoma (MCF-7), and Liposarcoma were obtained by an SRB-assay after 96 h of treatment and are the average from at least two independent experiments. Variation was ±10%.

b) BA: betulinic acid.

afforded the alkene **13**, whereas from **11** compound **14** was obtained. Finally, reaction of **13** with sodium dichromate in the presence of sodium dichromate furnished the endoperoxide **15**. Similarly, from **14** the peroxide **16** was obtained.

In a third approach, this sequence was applied to glycyrrhetinic acid. Thus, starting from glycyrrhetinic acid, acetylation and esterification gave **8** whose elimination furnished the alkene **9** that was finally transferred into the endoperoxide **12**.

The peroxides were tested for their antitumor activity in a panel of 15 human cancer cell lines using the SRB (sulforhodamine-B) assay. The results from these experiments are summarized in Table 1.

For comparison, well-known antitumor active betulinic acid (BA) was tested under the same conditions. The 1,2,4-trioxanes **4** and **5** showed no inhibition of cell growth at the highest applied concentration of 30 μM even after a prolonged incubation period of 96 h. In contrast, peroxides **12**, **15**, and **16** showed promising results with IC_{50} values at low μM-concentrations. These peroxides showed even better results than betulinic acid. To exclude cytotoxicity from traces of residual chromium compounds, the chromium concentration was determined by ICP-MS and never exceeded 1.77 ppm. To prove that cell death was triggered by apoptosis, a dye-exclusion test was performed. The apoptotic cells have an intact cell membrane and can exclude the dye whereas necrotic cell are colored blue. Another evidence for apoptosis is DNA laddering [27, 28] as observed by gel electrophoresis. The cytotoxicity of the endoperoxides (most likely by an intrinsic mitochondrial type-II-pathway) against tumor cells parallels the behavior of betulinic acid in these assays. Further studies (including the role of caspases) as well as the synthesis of analogues are presently under investigation in our laboratories.

In summary, the peroxides from triterpenes revealed higher activities as compared to betulinic acid. This finding renders them as interesting new lead structures.

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The authors have declared no conflict of interest.

Experimental

Melting points are uncorrected (*Leica* hot stage microscope; Leica Microsystems, Germany), NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000, or Unity 500 (Varian, USA; *d* given in ppm, *J* in Hz, internal Me_4Si), optical rotations were obtained using a Perkin-Elmer 341 polarimeter (1-cm micro cell), IR spectra (film or KBr pellet) on a Perkin-Elmer FT-IR spectrometer Spectrum 1000 (Perkin-Elmer, USA), MS spectra were taken on a Intectra GmbH AMD 402 (electron impact, 70 eV; Intectra GmbH, Harpsted, Germany) or on a Finnigan MAT TSQ 7000 instrument (electrospray, voltage 4.5 kV, sheath-gas nitrogen; Thermo Electron Corporation, Bremen, Germany). Thin layer chromatography was performed on silica gel (Merck 5554, detection by UV absorption; Merck, Darmstadt, Germany). The solvents were dried according to usual procedures.

Chemistry

General procedures

General procedure for the synthesis of 1,2,4-trioxanes GP1

A solution of the corresponding aldehyde (1.0 mmol) and 1-hydroperoxy-1-hydroxymethylcyclohexane (0.29 g, 2.0 mmol, prepared by the method of Tang *et al*. [23]) in THF (20 mL) was cooled to −78°C, then $BF_3 \cdot OEt_2$ (0.1 mL) was added and slowly warmed to room temperature. After stirring overnight, the solution was washed with brine (10 mL), dried over Na_2SO_4, and the solvent was removed *in vacuo*. The residue was purified by column chromatography (silica gel, hexane/ethyl acetate, 95:5).

General procedure for acetylations GP2

To a suspension of the triterpene (10.95 mmol) in CH_2Cl_2 (100 mL), TEA (2.2 g, 21.7 mmol), acetic anhydride (2.8 g, 27.4 mmol)

and DMAP (0.01 g, 0.08 mmol) were added. The mixture was stirred overnight and then quenched by the addition of aq. HCl (2 M, 50 mL). The phases were separated and the aq. phase was extracted with CH_2Cl_2 (100 mL). The combined organic layers were dried over Na_2SO_4, the solvent was removed *in vacuo* and the residue purified by column chromatography (silica gel, hexane/ethyl acetate, 8:2).

General procedure for esterifications GP3

A suspension of the alcohol (11.0 mmol), K_2CO_3 (6.9 g, 44.0 mmol) and methyl iodide (6.2 g, 44.0 mmol) in acetone (100 mL) was stirred for 24 h at r.t. Then the mixture was concentrated *in vacuo* and the residue partitioned between $CHCl_3$ (50 mL) and H_2O (50 mL). The phases were separated and the organic layer was washed with H_2O (50 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The product was used for further reactions as obtained. An analytical sample was obtained by crystallization from ethanol.

General procedure for allylic oxidations GP4

A mixture containing the starting material (0.88 mmol), glacial acetic acid (5 mL), *N*-hydroxysuccinimide (950 mg, 8.25 mmol), and $Na_2Cr_2O_7.2\ H_2O$ (1.04 g, 2.64 mmol) in acetone (50 mL) was stirred for three days at 40°C. The reaction was quenched by addition of aq. sodium disulfite (30 mL). The mixture was extracted with CH_2Cl_2 (2×100 mL), dried over Na_2SO_4, and concentrated *in vacuo*. Purification was achieved by column chromatography (silica gel, hexane/ethyl acetate, 8:2).

General procedure for deacetylations GP5

A mixture of the 3-*O*-acetyl derivative (0.6 mmol) and sodium methoxide (32 mg, 0.6 mmol) in MeOH (30 mL) and THF (15 mL) was heated under reflux until thin layer chromatography revealed the absence of starting material. The solution was concentrated *in vacuo* and the residue partitioned between $CHCl_3$ (50 mL) and H_2O (20 mL). The phases were separated and the organic layer was washed with H_2O (20 mL), dried over Na_2SO_4, and concentrated *in vacuo*.

General procedure for eliminations GP6

To a solution of the alcohol (3.20 mmol), PPh_3 (4.2 g, 16.0 mmol), and 3,3-dimethylglutarimide (2.3 g, 16.0 mmol) in dry THF (20 mL), DEAD (2.8 g, 16.0 mmol) was added dropwise under argon at 0°C. After stirring overnight, the solvent was removed *in vacuo* and the residue purified by column chromatography (silica gel, hexane/ethyl acetate, 9:1).

General procedure for the synthesis of endoperoxides GP7

A mixture of **9**, **13**, or **14** (0.4 mmol), glacial acetic acid (4 mL), *N*-hydroxysuccinimide (546 mg, 4.65 mmol), and $Na_2Cr_2O_7 \cdot 2\ H_2O$ (414 mg, 1.38 mmol) in acetone (20 mL) was stirred for three days at 40°C. The reaction was quenched by the addition of aq. sodium disulfite (20 mL) and aq. $NaHCO_3$ (20 mL). $CHCl_3$ (50 mL) was added to the mixture, and the phases were separated. The aq. layer was extracted with $CHCl_3$ (50 mL) and the combined extracts were concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexane/ethyl acetate, 9:1).

Compounds

(3RS)-3-[3β-Methoxy-28-norlup-20(29)-en-17β-yl]-1,2,4-trioxaspiro[5.5]undecane **4**

Following GP1 compound **4** (0.22 g, 35%; diastereomeric ratio 4:1) was obtained from 3-*O*-methyl-betulinic aldehyde **1** (0.5 g, 1.1 mmol) as an amorphous colorless solid. $[\alpha]_D = +19.9^\circ$ (c = 2.24, $CHCl_3$); $R_f = 0.74$ (silica gel, hexane/ethyl acetate, 8:2); IR (KBr) ν: 3440*m*, 2937*s*, 2864*m*, 1730*w*, 1641*w*, 1449*m*, 1388*m*, 1372*m*, 1268*w*, 1183*m*, 1136*w*, 1093*m*, 1035*w*, 1013*w* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$; data for the major isomer given) δ: 5.32 (s, 1H, C*H* (28)), 4.71 (d, *J* = 2.1 Hz, 1H, CH_a (30)), 4.55 (dd, *J* = 2.1, 1.7 Hz, 1H, CH_b (30)), 3.45 (dd, *J* = 11.2, 11.2 Hz, 2H, CH_2), 3.32 (s, 1H, OCH_3), 2.71 (ddd, *J* = 11.2, 11.2, 6.2 Hz, 1H, C*H* (19)), 2.61 (dd, *J* = 11.6, 4.6 Hz, 1H, C*H*OCH$_3$ (3)), 2.26 (m, 1H, CH_2), 2.07–1.98 (m, 3H, CH_a (21), CH_a (16), C*H* (13)), 1.96–1.90 (m, 1H, CH_2), 1.80–1.45 (m, 12H, CH_2 (2), CH_a (12), CH_a (15), CH_a (1), $4 \times$ CH_2, C*H* (18), CH_a (6)), 1.65 (s, 3H, CH_3 (29)), 1.44–1.11 (m, 12H, CH_2 (22), CH_2 (11), CH_2 (7), CH_b (6), $3 \times$ CH_2, C*H* (9), CH_b (21)), 1.10–0.95 (m, 4H, CH_b (12), CH_2, CH_b (16), CH_b (15)), 1.04 (s, 3H, CH_3 (25)), 0.94 (s, 3H, CH_3 (27)), 0.93 (s, 3H, CH_3 (23)), 0.86-0.82 (m, 1H, CH_b (1)), 0.81 (s, 6H, 2CH_3 (24 + 26)), 0.64 (d, *J* = 9.5 Hz, 1H, C*H* (5)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$; data for the major isomer given) δ: 150.9 (C20, *C*=CH$_2$), 109.6 (C30, *C*H$_2$=C), 106.2 (C28, *C*H), 88.6 (C3, *C*HOCH$_3$), 77.4 (C$_{quart.}$), 72.9 (*C*H$_2$), 65.9 (C17, *C*$_{quart.}$), 57.5 (O*C*H$_3$), 55.9 (C5, *C*H), 50.4 (C9, *C*H), 49.1 (C18, *C*H), 47.8 (C19, *C*H), 42.5 (C14, *C*$_{quart.}$), 41.0 (C8, *C*$_{quart.}$), 38.8 (C1, *C*H$_2$), 38.6 (C4, *C*$_{quart.}$), 37.3 (C13, *C*H), 37.2 (C10, *C*$_{quart.}$), 34.3 (*C*H$_2$), 34.2 (C7, *C*H$_2$), 33.1 (C16, *C*H$_2$), 32.1 (C22, *C*H$_2$), 30.8 (C21, *C*H$_2$), 30.2 (CH$_2$), 28.0 (C15, *C*H$_2$), 28.0 (C23, *C*H$_3$), 25.9 (*C*H$_2$), 25.5 (C12, *C*H$_2$), 22.3 (C2, *C*H$_2$), 21.5 (*C*H$_2$), 21.0 (*C*H$_2$), 20.8 (C11, *C*H$_2$), 19.0 CC29, *C*H$_3$), 18.2 (C6, *C*H$_2$), 16.1 (C24, *C*H$_3$), 16.0 (C26, *C*H$_3$), 15.9 (C25, *C*H$_3$), 14.8 (C27,*C*H$_3$) ppm; MS (ESI, MeOH) *m*/*z*: 605.2 $[M + Na]^+$ (20%), 1187.2 $[2\ M + Na]^+$ (100%). Anal. calcd. for $C_{38}H_{62}O_4$ (582.90): C, 78.30; H, 10.72; found: C, 77.90; H, 10.46.

(3RS)-3-[3β-Acetoxy-28-norlup-20(29)-en-17β-yl]-1,2,4-trioxaspiro[5.5]undecane **5**

Following GP1 compound **5** (0.17 g, 27%; diastereomeric ratio 5:1) was obtained from 3-*O*-acetylbetulinic aldehyde **2** (0.50 g, 1.03 mmol) as an amorphous colorless solid. $[a]_D = +21.3^\circ$ (c = 5.2, $CHCl_3$); $R_f = 0.64$ (silica gel, hexane/ethyl acetate, 8:2); IR (KBr) ν: 3446*m*, 2938*s*, 2864*m*, 1734*s*, 1641*w*, 1449*m*, 1369*m*, 1245*m*, 1171*w*, 1136*w*, 1092*m*, 1030*m* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$; data for the major isomer given) δ: 5.32 (s, 1H,C*H* (28)), 4.71 (d, *J* = 2.5 Hz, 1H, CH_a (30)), 4.55 (dd, *J* = 1.2, 1.2 Hz, 1H, CH_b (30)), 3.45 (dd, *J* = 11.2, 11.2 Hz, 2H, CH_2), 4.45 (dd, *J* = 10.4, 5.8 Hz, 1H, C*H*OAc (3)), 2.71 (ddd, *J* = 11.2, 11.2, 6.2 Hz, 1H, C*H* (19)), 2.26 (m, 1H, CH_2), 2.08–1.98 (m, 2H, CH_a (16), C*H* (13), CH_a (21)), 2.02 (s, 3H, Ac), 1.96–1.88 (m, 1H, CH_2), 1.71–1.45 (m, 12H, CH_a (12), CH_a (15), CH_a (1), CH_2 (2), C*H* (18), $4 \times$ CH_2, CH$_a$ (6), CH$_a$ (11)), 1.65 (s, 3H, CH_3 (29)), 1.44–1.18 (m, 11H, CH_2 (22), CH_b (11), CH_b (6), CH_2 (7), $3 \times$ CH_2, C*H* (9), CH_b (21)), 1.10–0.97 (m, 5H, CH_b (12), CH_2, CH_b (16), CH_b (15), CH_b (1)), 1.05 (s, 3H, CH_3 (25)), 0.96 (s, 3H, CH_3 (27)), 0.84 (s, 6H, $2 \times$ CH_3 (24 + 26)), 0.82 (s, 3H, CH_3 (23)), 0.77 (d, *J* = 9.1 Hz, 1H, C*H* (5)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$; data for the major isomer given) δ: 170.9 (*C*=O), 150.8 (C20, *C*=CH$_2$), 109.7 (C30, *C*H$_2$=C), 106.3 (C28, *C*H), 80.9 (C3, *C*HOAc), 77.5 (*C*$_{quart.}$), 72.9 (*C*H$_2$), 65.9 (C17, *C*$_{quart.}$), 55.4 (C5, *C*H), 50.5 (C9, *C*H), 48.0 (C18, *C*H), 47.8 (C19, *C*H), 42.7 (C14, *C*$_{quart.}$), 42.5 (C8, *C*$_{quart.}$), 38.3 (C1, *C*H$_2$), 37.8 (C4, *C*$_{quart.}$), 37.3 (C13, *C*H), 37.0 (C10, *C*$_{quart.}$), 34.3 (*C*H$_2$), 34.2 (C7, *C*H$_2$), 33.1 (C16, *C*H$_2$), 32.1 (C22, *C*H$_2$), 30.7 (C21, *C*H$_2$), 30.2

(CH_2), 28.0 (C15, CH_2), 27.9 (C23, CH_3), 25.6 (C12, CH_2), 23.7 (C2, CH_2), 21.5 (CH_2), 21.3 (Ac), 21.0 (CH_2), 20.8 (C11, CH_2), 19.0 (C29, CH_3), 18.1 (C6, CH_2), 16.5 (C24, CH_3), 16.5 (C26, CH_3), 16.1 (C25, CH_3), 15.0 (C27, CH_3) ppm; MS (ESI, MeOH) *m/z*: 633.2 $[M + Na]^+$ (30%), 1243.2 $[2\ M + Na]^+$ (100%). Anal. calcd. for $C_{39}H_{62}O_5$ (610.91): C, 76.68; H, 10.23; found: C, 76.23; H, 9.97.

3β-Acetylursolic acid methyl ester **6**

Following GP2 and GP3 compound **6** (1.05 g, 94%) was obtained from ursolic acid (1.00 g, 2.19 mmol) as a colorless solid; m.p.: 243 – 246°C (lit.: 243 – 245°C [33]); $[\alpha]_D$ = +65.6° (c = 4.5; $CHCl_3$); R_F = 0.75 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr) ν: 2942*s*, 2868*m*, 1734*s*, 1458*m*, 1385*m*, 1371*m*, 1312*w*, 1244*s*, 1202*m*, 1187*m*, 1167*w*, 1150*w*, 1114*w*, 1073*w*, 1027*m*, 1006*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) *d*: 5.23 (s, 1H, C*H* (12)), 4.48 (dd, *J* = 10.6, 5.5 Hz, 1H, C*H* (3)), 3.59 (*s*, 3H, OMe), 2.17 (d, *J* = 11.3 Hz, 1H, C*H* (18)), 2.03 (s, 3H, Ac), 1.98 (ddd, *J* = 13.5, 13.4, 4.3 Hz, 1H, CH_a (16)), 1.89 (dd, *J* = 8.7, 3.4 Hz, 2H, CH_2 (11)), 1.85 (ddd, *J* = 13.6, 13.5, 4.3 Hz, 1H, CH_a (15)), 1.69 – 1.55 (m, 6H, CH_2 (22), CH_a (1), CH_b (16), CH_2 (2)), 1.54 – 1.43 (m, 4H, C*H* (9), CH_a (6), CH_a (21), CH_a (7)), 1.38-1.26 (m, 4H, C*H* (19), CH_b (6), CH_b (7), CH_b (21)), 1.10 – 1.02 (m, 2H, CH_b (15), CH_b (1)), 1.06 (s, 3H, CH_3 (27)), 0.99 – 0.95 (m, 1H, C*H* (20)), 0.93 (s, 3H, CH_3 (25)), 0.93 (d, *J* = 5.0 Hz, 3H, CH_3 (30)), 0.87 – 0.83 (m, 1H, C*H* (5)), 0.86 (s, 3H, CH_3 (23)), 0.85 (d, *J* = 5.4 Hz, 3H, CH_3 (29)), 0.84 (s, 3H, CH_3 (26)), 0.73 (s, 3H, CH_3 (24)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 178.0 (C28, *C*=O), 170.9 (Ac, *C*=O), 138.1 (C13, *C*=CH), 125.4 (C12, H*C*=C), 80.9 (C3, H*C*-O), 55.3 (C5, *C*H), 52.8 (C18, *C*H), 51.4 (OMe, CH_3), 48.0 (C17, $C_{quart.}$), 47.5 (C9, *C*H), 41.9 (C14, $C_{quart.}$), 39.5 (C8, $C_{quart.}$), 39.0 (C19, *C*H), 38.8 (C20, *C*H), 38.3 (C1, CH_2), 37.6 (C4, $C_{quart.}$), 36.8 (C10, CH_2), 36.6 (C22, $C_{quart.}$), 32.9 (C7, CH_2), 30.6 (C21, CH_2), 28.0 (C23, CH_3), 28.0 (C15, CH_2), 24.2 (C16, CH_2), 23.5 (C27, CH_3), 23.5 (C2, CH_2), 23.3 (C11, CH_2), 21.3 (Ac, CH_3), 21.1 (C30, CH_3), 18.2 (C6, CH_2), 17.0 (C29, CH_3), 17.0 (C24, CH_3), 16.8 (C26, CH_3), 15.5 (C25, CH_3) ppm; MS (ESI, MeOH) *m/z*: 513.2 $[M + H]^+$ (40%), 535.5 $[M + Na]^+$ (100%). Anal. calcd. for $C_{33}H_{52}O_4$ (512.76): C, 77.30; H, 10.22; found: C, 77.18; H, 10.16.

3β-Acetyloleanolic acid methyl ester **7**

Following GP2 and GP3 compound **7** (1.06 g, 96%) was obtained from oleanolic acid (1.00 g, 2.19 mmol) as a colorless solid; m.p.: 219-221°C (lit.: 223 – 225°C [34]); $[\alpha]_D$ = +69.3° (c = 3.8; $CHCl_3$); R_F = 0.75 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr) ν: 2939*s*, 1730*s*, 1471*m*, 1386*m*, 1364*m*, 1267*m*, 1240*s*, 1176*w*, 1163*w*, 1123*w*, 1096*w*, 1022*m*, 983*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.26 (s, 1H, C*H* (12)), 4.47 (ddd, *J* = 8.4, 7.7, 1.7 Hz, 1H, C*H* (3)), 3.60 (s, 3H, OMe), 2.82 (d, *J* = 13.7 Hz, 1H, C*H* (18)), 2.02 (s, 3H, Ac), 1.95 (ddd, *J* = 14.6, 14.5, 3.4 Hz, 1H, CH_a (16)), 1.88 – 1.84 (m, 2H, CH_2 (11)), 1.69 – 1.23 (m, 14H, C*H* (9), CH_a (1), CH_a (19), CH_2 (6), CH_2 (7), CH_a (15), CH_a (21), CH_2 (22), CH_b (16), CH_2 (2)), 1.20-1.10 (m, 2H, CH_b (19), CH_b (21)), 1.11 (s, 3H, CH_3 (27)), 1.06-1.00 (m, 2H, CH_b (15), CH_b (1)), 0.91 (s, 3H, CH_3 (25)), 0.91 (s, 3H, CH_3 (30)), 0.88 (s, 3H, CH_3 (29)), 0.86 – 0.79 (m, 1H, C*H* (5)), 0.85 (s, 3H, CH_3 (23)), 0.83 (s, 3H, CH_3 (26)), 0.71 (s, 3H, CH_3 (24)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 178.2 (C28, *C*=O), 170.9 (Ac, *C*=O), 143.8 (C13, *C*=CH), 122.2 (C12, H*C*=C), 80.9 (C3, H*C*-O), 55.3 (C5, *C*H), 51.5 (OMe, CH_3), 47.5 (C9, *C*H), 46.7 (C17, $C_{quart.}$), 45.8 (C19, CH_2), 41.6 (C14, $C_{quart.}$), 41.3 (C18, *C*H), 39.3 (C8, $C_{quart.}$), 38.1 (C1, CH_2), 37.7 (C4, $C_{quart.}$), 36.9 (C10, $C_{quart.}$), 33.7 (C21, CH_2), 33.1 (C29, CH_3), 32.5 (C7, CH_2), 32.3 (C22, CH_2), 30.6 (C20, $C_{quart.}$), 28.0 (C23, CH_3), 27.6 (C15, CH_2), 25.9 (C27, CH_3), 23.6 (C30, CH_3), 23.3 (C11, CH_2), 23.3 (C2, CH_2), 23.0 (C16, CH_2), 21.3 (Ac, CH_3), 18.2 (C6, CH_2), 16.8 (C24, CH_3), 16.6 (C26, CH_3), 15.3 (C25, CH_3) ppm; MS (ESI, MeOH) *m/z*: 535.5 $[M + Na]^+$ (100%). Anal. calcd. for $C_{33}H_{52}O_4$ (512.76): C, 77.30; H, 10.22; found: C, 77.06; H, 10.12.

18-Glycyrrhetinic acid methyl ester **8**

Following GP3 compound **8** (5.1 g, quant.) was obtained from 18β-glycyrrhetinic acid as a colorless solid; m.p.: 254 – 258°C (lit. 252 – 254°C [35]); $[\alpha]_D$ = +141.2° (c = 4.8, $CHCl_3$); R_f = 0.48 (silica gel, hexane/ethyl acetate; 7:3); IR (KBr) ν: 3614*br*, 2970*s*, 2955*s*, 2875*m*, 1726*s*, 1659*s*, 1466*m*, 1450*m*, 1364*w*, 1216*m*, 1189*m*, 1136*w*, 1085*w*, 1040*w*, 992*w* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.65 (s, 1H, C*H* (12)), 3.67 (s, 3H, OCH_3), 3.21 (dd, *J* = 10.8, 5.7 Hz, 1H, CH_2 (3)), 2.78 (ddd, *J* = 13.3, 3.3, 3.3 Hz, 1H, CH_2 (1)), 2.32 (s, 1H, C*H* (9)), 2.06 (dd, *J* = 13.3, 3.8 Hz, 1H, C*H* (18)), 2.00 (m, 1H, CH_2 (15)), 1.95 (m, 1H, CH_2 (21)), 1.90 (dd, *J* = 13.8, 3.9 Hz, 1H, CH_2 (19)), 1.83 (ddd, *J* = 14.3, 5.2, 5.2 Hz, 1H, CH_2 (16)), 1.65 (m, 1H, CH_2 (2)), 1.62 (m, 1H, CH_2 (7)), 1.58 (m, 1H, CH_2 (2)), 1.57 (m, 1H, CH_2 (19)), 1.57 (m, 1H, CH_2 (6)), 1.43 (m, 1H, CH_2 (6)), 1.38 (m, 1H, CH_2 (7)), 1.36 (m, 1H, CH_2 (22)), 1.34 (s, 3H, CH_3 (27)), 1.30 (m, 1H, CH_2 (22)), 1.28 (m, 1H, CH_2 (21)), 1.17 (m, 1H, CH_2 (16)), 1.13 (s, 3H, CH_3 (28)), 1.12 (s, 3H, CH_3 (25)), 1.11 (s, 3H, CH_3 (26)), 1.00 (m, 1H, CH_2 (15)), 0.99 (s, 3H, CH_3 (23)), 0.96 (m, 1H, CH_2 (1)), 0.79 (s, 3H, CH_3 (24)), 0.79 (s, 3H, CH_3 (29)), 0.68 (m, 1H, C*H* (5)); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 200.0 (C11, C=O), 176.8 (C30, C=O), 169.0 (C13, $C_{quart.}$), 128.6 (C12), 78.8 (C3), 61.9 (C9), 55.1 (C5), 51.8 (OCH_3), 48.5 (C18), 45.5 (C14, $C_{quart.}$), 44.1 (C20, $C_{quart.}$), 43.3 (C8, $C_{quart.}$), 41.2 (C19), 39.2 (C1), 39.2 (C4, $C_{quart.}$), 37.8 (C22), 37.2 (C10, $C_{quart.}$), 32.9 (C7), 31.9 (C17, $C_{quart.}$), 31.2 (C21), 28.6 (C29), 28.4 (C23), 28.2 (C28), 27.4 (C2), 26.6 (C16), 26.6 (C15), 23.5 (C27), 18.8 (C26), 17.6 (C6), 16.4 (C25), 15.6 (C24); MS (ESI, MeOH) *m/z*: 485.5 $[M + H]^+$ (55%), 507.5 $[M + Na]^+$ (12%), 539.1 $[M + MeOH + Na]^+$ (100%). Anal. calcd. for $C_{31}H_{48}O_4$ (484.71): C, 76.82; H, 9.98; found: C, 76.55; H, 10.11.

Methyl 11-oxo-olean-2,12-dien-30-oate **9**

Following GP6 compound **9** (1.51 g, quant.) was obtained from **8** (1.55 g, 3.20 mmol) as a colorless solid; m.p.: 182-186°C; $[\alpha]_D$ = +204.6° (c = 3.42, $CHCl_3$); R_f = 0.32 (silica gel, $CHCl_3$/Et_2O, 8:2); IR (KBr) ν: 3436*m*, 2957*s*, 2868*m*, 1723*s*, 1728*s*, 1655*s*, 1616*m*, 1465*m*, 1386*m*, 1360*m*, 1320*m*, 1279*m*, 1260*m*, 1218*m*, 1156*m*, 1089*m*, 1048*w*, 1029*w* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.66 (s, 1H, C*H* (12)), 5.40 (ddd, *J* = 10.0, 5.8, 1.7 Hz, 1H, CH (2)), 5.34 (dd, *J* = 10.0, 2.1 Hz, 1H, C*H* (3)), 3.67 (s, 3H, OCH_3), 3.02 (dd, *J* = 17.4, 5.8 Hz, 1H, C*H* (1)), 2.39 (s, 1H, C*H* (9)), 2.07 (dd, *J* = 13.7, 4.2 Hz, 1H, C*H* (18)), 2.00 (ddd, *J* = 13.7, 13.7, 5.0 Hz, 1H, CH_2 (15)), 1.96 (m, 1H, CH_2 (21)), 1.90 (m, 1H, CH_2 (19)), 1.85 (ddd, *J* = 13.7, 13.7, 5.0 Hz, 1H, CH_2 (16)), 1.68 (m, 1H, CH_2 (7)), 1.64 (m, 1H, CH_2 (1)), 1.60 (m, 1H, CH_2 (19)), 1.53 (m, 1H, CH_2 (6)), 1.46 (m, 1H, CH_2 (6)), 1.40 (m, 1H, CH_2 (7)), 1.37 (m, 1H, CH_2 (22)), 1.34 (s, 3H, CH_3 (27)), 1.32-1.27 (m, 2H, CH_2 (21), CH_2 (22)), 1.22 (m, 1H, CH_2 (16)), 1.14 (s, 3H, CH_3 (25)), 1.13 (s, 3H, CH_3 (26)), 1.12 (s, 3H, CH_3 (28)), 1.08 (m, 1H, C*H* (5)), 0.99 (m, 1H, CH_2 (15)), 0.94 (s, 3H, CH_3 (23)), 0.89 (s, 3H, CH_3 (24)), 0.80 (s, 3H, CH_3 (29)); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 200.1 (C11, C=O), 176.9 (C30, C=O), 169.4 (C13, $C_{quart.}$), 137.0 (C3), 128.6 (C12), 121.9 (C2), 60.5 (C9), 51.8 (C5), 51.7 (OCH_3), 48.4 (C18, $C_{quart.}$), 45.3 (C14, $C_{quart.}$), 44.0 (C20, $C_{quart.}$), 43.2 (C8, $C_{quart.}$), 41.5 (C1), 41.2 (C19), 37.8 (C22), 36.2 (C4, $C_{quart.}$), 34.3 (C10, $C_{quart.}$), 31.9 (C7), 31.9 (C23), 31.8 (C17, $C_{quart.}$), 31.1 (C21), 28.5 (C29), 28.3 (C28), 26.5 (C16), 26.5 (C15), 23.3 (C27), 23.0 (C24), 18.7 (C6), 18.3 (C26), 16.1 (C25); MS (ESI, MeOH) *m/z*: 467.6 $[M + H]^+$ (48%), 489.4 $[M + Na]^+$ (8%), 521.0 $[M + Na + MeOH]^+$ (100%). Anal. calcd. for $C_{31}H_{46}O_5$ (498.69): C, 79.78; H, 9.93; found: C, 79.68; H, 9.86.

11-Oxo-ursolic acid methyl ester ***10***

Following GP4 and GP5 compound **10** (0.57 g, 62%) was obtained from compound **6** (1.0 g, 1.9 mmol) as a colorless solid; m.p.: 132–135°C; $[\alpha]_D$ = +87.0° (c = 3.0; $CHCl_3$); R_F = 0.34 ($CHCl_3$/Et_2O, 95:5); IR (KBr) ν: 2946*s*, 2870*m*, 1726*s*, 1661*s*, 1456*m*, 1387*m*, 1313*w*, 1273*w*, 1246*m*, 1200*m*, 1182*m*, 1146*w*, 1103*w*, 1041*m*, 995*w* cm^{-1}; UV-VIS (methanol) λ_{max} (log *e*): 269 nm (4.04);^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.58 (s, 1H, C*H* (12)), 3.58 (s, 3H, OMe), 3.20 (dd, *J* = 11.0, 5.2 Hz, 1H, C*H* (3)), 2.76 (d, *J* = 13.4 Hz, 1H, CH_a (1)), 2.39 (d, *J* = 11.3 Hz, 1H, C*H* (18)), 2.28 (s, 1H, C*H* (9)), 2.06 (ddd, *J* = 11.9, 10.8, 4.3 Hz, 1H, CH_a (16)), 1.80–1.71 (m, 3H, CH_b (16), CH_a (7), CH_a (15)), 1.66–1.50 (m, 7H, CH_2 (2), CH_2 (22), CH_2 (6), CH_a (21)), 1.42-1.20 (m, 4H, C*H* (19), CH_b (15), CH_b (7), CH_b (21)), 1.28 (s, 3H, CH_3 (27)), 1.10 (s, 3H, CH_3 (25)), 1.06–0.96 (m, 2H, CH_b (1), C*H* (20)), 0.97 (s, 3H, CH_3 (23)), 0.94 (d, *J* = 6.3 Hz, 3H, CH_3 (30)), 0.89 (s, 3H, CH_3 (26)), 0.84 (d, *J* = 6.4 Hz, 3H, CH_3 (29)), 0.77 (s, 3H, CH_3 (24)), 0.65 (d, *J* = 11.3 Hz, 1H, C*H* (5)) ppm; ^{13}C-NMR (125MHz, $CDCl_3$) δ: 199.8 (C11, *C*=O), 177.2 (C28, *C*=O), 162.8 (C13, *C*=CH), 130.6 (C12, H*C*=C), 78.8 (C3, *C*-O), 61.4 (C9, *C*H), 54.9 (C5, *C*H), 52.7 (C18, *C*H), 51.8 (OMe, *C*H_3), 47.6 (C17, $C_{quart.}$), 44.6 (C8, $C_{quart.}$), 43.7 (C14, $C_{quart.}$), 39.1 (C4, $C_{quart.}$), 39.1 (C1, *C*H_2), 38.6 (C19, *C*H), 38.5 (C20, *C*H), 37.1 (C10, $C_{quart.}$), 35.9 (C22, *C*H_2), 33.0 (C7, *C*H_2), 30.3 (C21, *C*H_2), 28.3 (C15, *C*H_2), 28.1 (C23, *C*H_3), 27.2 (C2, *C*H_2), 23.9 (C16, *C*H_2), 21.0 (C27, *C*H_3), 20.9 (C30, *C*H_3), 18.8 (C26, *C*H_3), 17.4 (C6, *C*H_2),17.0 (C29, *C*H_3), 16.2 (C25, *C*H_3), 15.6 (C24, *C*H_3) ppm; MS (ESI, MeOH) *m/z*: 485.5 $[M + H]^+$ (100%), 507.5 $[M + Na]^+$ (50%). Anal. calcd. for $C_{31}H_{48}O_4$ (484.71): C, 76.82; H, 9.98; found: C, 76.39; H, 9.82.

11-Oxo-oleanolic acid methyl ester ***11***

Following GP4 and GP5 compound **11** (0.61 g, 67%) was obtained from compound **7** (1.0 g, 1.9 mmol) as a colorless solid; m.p.: 181–188°C; $[\alpha]_D$ = +82° (c = 1.4; $CHCl_3$); R_F = 0.35 ($CHCl_3$/Et_2O, 95:5); IR (KBr) ν: 2949*s*, 2866*s*, 1724*s*, 1661*s*, 1466*m*, 1387*m*, 1365*w*, 1330*w*, 1304*w*, 1261*m*, 1227*w*, 1209*m*, 1189*m*, 1162*m*, 1125*w*, 1089*w*, 1039*m*, 1013*w*, 994*w* cm^{-1}; UV-VIS (methanol) λ_{max} (log *e*): 269 nm (4.03); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.61 (s, 1H, C*H* (12)), 3.61 (s, 3H, OMe), 3.20 (dd, *J* = 10.8, 1H, 5.5 Hz, C*H* (3)), 2.98 (dd, *J* = 13.8, 3.7 Hz, 1H, C*H* (18)), 2.80 (d, *J* = 13.4 Hz, 1H, CH_a (1)), 2.30 (s, 1H, C*H* (9)), 2.02 (ddd, *J* = 13.8, 13.8, 4.0 Hz, 1H, CH_a (16)), 1.75–1.52 (m, 9H, CH_a (19), CH_b (16), CH_a (7), CH_a (15), CH_2 (22), CH_2 (2), CH_a (6)), 1.42–1.13 (m, 6H, CH_b (19), CH_b (7), CH_2 (21), CH_b (15), CH_b (6)), 1.34 (s, 3H, CH_3 (27)), 1.08 (s, 3H, CH_3 (25)), 1.00–0.95 (m, 1H, CH_b (1)), 0.97 (s, 3H, CH_3 (23)), 0.92 (s, 3H, CH_3 (30)), 0.91 (s, 3H, CH_3 (29)), 0.89 (s, 3H, CH_3 (26)), 0.78 (s, 3H, CH_3 (24)), 0.66 (d, *J* = 11.3 Hz, 1H, C*H* (5)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 200.3 (C11, *C*=O), 177.4 (C28, *C*=O), 168.6 (C13, *C*=CH), 127.9 (C12, H*C*=C), 78.7 (C3, H*C*-O), 61.7 (C9, *C*H), 55.0 (C5, *C*H), 51.8 (OMe, *C*H_3), 46.2 (C17, $C_{quart.}$), 45.0 (C8, $C_{quart.}$), 44.2 (C19, *C*H_2), 43.4 (C14, $C_{quart.}$), 41.5 (C18, *C*H), 39.1 (C1, *C*H_2), 38.8 (C4, $C_{quart.}$), 37.2 (C10, $C_{quart.}$), 33.7 (C21, *C*H_2), 32.9 (C7, *C*H_2), 32.8 (C29, *C*H_3), 31.6 (C22, *C*H_2), 30.6 (C20, $C_{quart.}$), 28.1 (C23, *C*H_3), 27.7 (C15, *C*H_2), 27.3 (C2, *C*H_2), 23.5 (C27, *C*H_3), 23.4 (C30, *C*H_3), 22.9 (C16, *C*H_2), 18.9 (C26, *C*H_3), 17.4 (C6, *C*H_2), 16.1 (C25, *C*H_3), 15.5 (C24, *C*H_3) ppm; MS (ESI, MeOH) *m/z*: 485.6 $[M + H]^+$ (100%), 507.5 $[M + Na]^+$ (35%). Anal. calcd. for $C_{31}H_{48}O_4$ (484.71): C, 76.82; H, 9.98; found: C, 76.54; H, 9.85.

Methyl 2,3-dihydro-1a,9a-peroxo-11-olean-12-en-30-oate ***12***

Following GP7 compound **12** (0.1 g, 53%) was obtained from **9** (186 mg, 0.4 mmol) as a colorless solid; m.p.: 151-155°C; $[\alpha]_D$ = +48.1° (c = 3.08, $CHCl_3$); R_f = 0.22 (silica gel, hexane/ethyl acetate, 9:1); IR (KBr) ν: 3441*m*, 2957*s*, 1732*s*, 1662*s*, 1614*m*, 1460*m*, 1388*m*, 1361*m*, 1315*m*, 1282*m*, 1258*m*, 1218*m*, 1190*m*, 1161*m*, 1088*m*, 1066*w*, 1019*w* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.80 (d, *J* = 9.2 Hz, 1H, C*H*(3)), 5.77 (s, 1H, C*H*(12)), 5.49 (dd, *J* = 10.0, 5.4 Hz, 1H, C*H*(2)), 4.90 (d, *J* = 5.4 Hz, 1H, C*H* (1)), 3.67 (s, 3H, OCH_3), 2.10–1.94 (m, 6H, CH_2 (15), C*H* (5), CH_2 (19), CH_2 (7), CH_2 (21), C*H* (18)), 1.77 (ddd, *J* = 13.6, 13.6, 4.6 Hz, 1H, CH_2 (16)), 1.69-1.60 (m, 2H, CH_2 (19), CH_2 (6)), 1.50 (ddd, *J* = 12.9, 12.9, 2.9 Hz, 1H, CH_2 (6)), 1.40 (m, 3H, CH_2 (7), CH_2 (22)), 1.37 (s, 3H, CH_3 (27)), 1.34 (s, 3H, CH_3 (25)), 1.29 (m, 1H, CH_2 (21)), 1.24 (s, 3H, CH_3 (26)), 1.22 (m, 1H, CH_2 (16)), 1.13 (s, 3H, CH_3 (28)), 1.05 (s, 3H, CH_3 (23)), 0.98 (m, 1H, CH_2 (15)), 0.94 (s, 3H, CH_3 (24)), 0.88 (s, 3H, CH_3 (29)); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 193.8 (C11, C=O), 177.0 (C30, C=O), 169.1 (C13, $C_{quart.}$), 144.6 (C3), 126.4 (C12), 117.6 (C2), 90.6 (C9, $C_{quart.}$), 80.3 (C1), 51.7 (OCH_3), 49.7 (C5), 46.6 (C14, $C_{quart.}$), 44.3 (C8, $C_{quart.}$), 44.0 (C20, $C_{quart.}$), 42.9 (C18), 40.6 (C19), 37.7 (C22), 35.3 (C4, $C_{quart.}$), 32.6 (C10, $C_{quart.}$), 31.2 (C23), 31.2 (C21), 31.2 (C17, $C_{quart.}$), 29.8 (C7), 28.6 (C29), 28.3 (C28), 27.9 (C16), 26.1 (C15), 26.4 (C27), 21.7 (C24), 20.2 (C26), 17.6 (C6), 16.0 (C25); MS (ESI, MeOH) *m/z*: 497.4 $[M + H]^+$ (55%), 519.4 $[M + Na]^+$ (100%), 550.9 $[M + Na + MeOH]^+$ (66%). Anal. calcd. for $C_{31}H_{44}O_5$ (496.68): C, 74.96; H, 8.93; found: C, 74.66; H, 8.76.

Methyl 11-oxo-urs-2,12-dien-28-oate ***13***

Following GP6 compound **14** (1.42 g, 93%) was obtained from **10** (1.58 g, 3.26 mmol) as a colorless solid; m.p.: 179–187°C; $[\alpha]_D$ = +141° (c = 1.8; $CHCl_3$); R_f = 0.76 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr) ν: 2948*s*, 2857*s*, 1726*s*, 1667*s*, 1620*m*, 1459*m*, 1426*w*, 1378*w*, 1362*w*, 1318*w*, 1286*w*, 1274*m*, 1246*m*, 1224*m*, 1203*s*, 1147*m*, 1113*w*, 1084*w*, 1035*w*, 1001*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.62 (s, 1H, C*H* (12)), 5.42 (dd, *J* = 10.1, 5.8 Hz, 1H, C*H* (2)), 5.35 (dd, *J* = 10.1, 2.4 Hz, 1H, C*H* (3)), 3.60 (s, 3H, OMe), 3.02 (dd, *J* = 17.4, 6.1 Hz, 1H, CH_a (1)), 2.42 (d, *J* = 11.0 Hz, 1H, C*H* (18)), 2.35 (s, 1H, C*H* (9)), 2.07 (ddd, *J* = 15.0, 14.7, 4.6 Hz, 1H, CH_a (16)), 1.82-1.72 (m, 3H, CH_a (22), CH_b (16), CH_a (15)), 1.68–1.49 (m, 5H, CH_b (22), CH_a (21), CH_a (6), CH_b (1), CH_a (7)), 1.46-1.20 (m, 5H, CH_b (6), C*H* (19), CH_b (15), CH_b (7), CH_b (21)), 1.28 (s, 3H, CH_3 (27)), 1.14 (s, 3H, CH_3 (25)), 1.10–1.01 (m, 2H, C*H* (20), C*H* (5)), 0.95 (d, *J* = 8.2 Hz, 3H, CH_3 (30)), 0.94 (s, 3H, CH_3 (23)), 0.92 (s, 3H, CH_3 (26)), 0.89 (s, 3H, CH_3 (24)), 0.86 (d, *J* = 6.4 Hz, 3H, CH_3 (29)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) *d*: 199.7 (C11, *C*=O), 177.2 (C28, *C*=O), 162.9 (C13, *C*=CH), 137.0 (C3, H*C*=CH), 130.8 (C12, H*C*=C), 121.9 (C2, H*C*=CH), 60.1 (C9, *C*H), 52.7 (C18, *C*H), 51.9 (C5, *C*H), 51.8 (OMe, *C*H_3), 47.7 (C17, $C_{quart.}$), 44.6 (C14, $C_{quart.}$), 43.8 (C8, $C_{quart.}$), 41.5 (C1, *C*H_2), 38.7 (C19, *C*H), 38.6 (C20, *C*H), 36.2 (C4, $C_{quart.}$), 35.9 (C22, *C*H_2), 34.3 (C10, $C_{quart.}$), 32.2 (C7, *C*H_2), 31.9 (C23, *C*H_3), 30.3 (C21, *C*H_2), 28.4 (C15, *C*H_2), 23.9 (C16, *C*H_2), 22.8 (C26, *C*H_3), 21.0 (C27, *C*H_3), 20.9 (C30, *C*H_3), 18.6 (C6, *C*H_2), 18.5 (C24, *C*H_3), 17.1 (C29, *C*H_3), 16.0 (C25, *C*H_3) ppm; MS (ESI, MeOH) *m/z*: 467.5 $[M + H]^+$ (100%), 489.5 $[M + Na]^+$ (30%). Anal. calcd. for $C_{31}H_{46}O_3$ (466.70): C, 79.78; H, 9.93; found: C, 79.58; H, 9.84.

Methyl 11-oxo-olean-2,12-dien-28-oate ***14***

Following GP6 compound **14** (1.5 g, 96%) was obtained from **11** (1.58 g, 3.26 mmol) as a colorless solid; m.p.: 177–183°C; $[\alpha]_D$ = +147° (c = 1.5; $CHCl_3$); R_f = 0.75 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr) ν: 2951*s*, 2867*m*, 1729*s*, 1660*s*, 1463*m*, 1385*m*, 1361*w*, 1329*w*, 1307*w*, 1262*w*, 1228*w*, 1210*m*, 1166*m*, 1127*w*, 1081*w*, 1014*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.64 (s, 1H, C*H* (12)), 5.41 (m, 1H, C*H* (2)), 5.34 (dd, *J* = 9.9, 2.6 Hz, 1H, C*H* (3)), 3.62 (s, 3H, OMe), 3.00 (dd, *J* = 13.9, 3.7 Hz, 1H, C*H* (18)), 3.05 (dd, *J* =

17.8, 5.9 Hz, 1H, C*H*$_a$ (1)), 2.37 (s, 1H, C*H* (9)), 2.03 (ddd, *J* = 13.9, 13.6, 4.0 Hz, 1H, C*H*$_a$ (16)), 1.76-1.50 (m, 8H, C*H*$_a$ (19), C*H*$_b$ (1), C*H*$_b$ (16), C*H*$_a$ (7), C*H*$_a$ (15), C*H*$_2$ (22), C*H*$_a$ (6)), 1.46 – 1.32 (m, 3H, C*H*$_b$ (7), C*H*$_a$ (21), C*H*$_b$ (6)), 1.34 (s, 3H, C*H*$_3$ (27)), 1.27 – 1.17 (m, 3H, C*H*$_b$ (21), C*H*$_b$ (15), C*H*$_b$ (19)), 1.14 – 1.07 (m, 1H, C*H* (5)), 1.11 (s, 3H, C*H*$_3$ (25)), 0.94 (s, 3H, C*H*$_3$ (23)), 0.93 (s, 3H, C*H*$_3$ (29)), 0.93 (s, 3H, C*H*$_3$ (30)), 0.91 (s, 3H, C*H*$_3$ (24)), 0.89 (s, 3H, C*H*$_3$ (26)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 200.2 (C11, *C*=O), 177.5 (C28, *C*=O), 168.8 (C13, *C*=CH), 137.0 (C3, H*C*=CH), 127.9 (C12, H*C*=C), 121.9 (C2, H*C*=CH), 60.5 (C9, *C*H), 51.8 (C5, *C*H), 51.8 (OMe, *C*H$_3$), 46.2 (C17, *C*$_{quart.}$), 44.9 (C14, *C*$_{quart.}$), 44.3 (C19, *C*H), 43.5 (C8, *C*$_{quart.}$), 41.6 (C18, *C*H), 41.5 (C1, *C*H$_2$), 36.3 (C4, *C*$_{quart.}$), 34.3 (C10, *C*$_{quart.}$), 33.7 (C21, *C*H$_2$), 32.8 (C29, *C*H$_3$), 32.0 (C7, *C*H$_2$), 31.8 (C22, *C*H$_2$), 31.6 (C23, *C*H$_3$), 30.6 (C20, *C*$_{quart.}$), 27.7 (C15, *C*H$_2$), 23.5 (C27, *C*H$_3$), 23.4 (C30, *C*H$_3$), 23.0 (C16, *C*H$_2$), 23.0 (C26, *C*H$_3$), 18.6 (C6, *C*H$_2$), 18.5 (C24, *C*H$_3$), 15.9 (C25, *C*H$_3$) ppm; MS (ESI, MeOH) *m/z*: 467.5 $[M + H]^+$ (100%). Anal. calcd. for $C_{31}H_{46}O_3$ (466.70): C, 79.78; H, 9.93; found: C, 79.61; H, 9.86.

Methyl 2,3-dihydro-1a,9a-peroxo-11-oxo-urs-12-en-28-oate ***15***

Following GP7 compound **15** (130 mg, 48%) was obtained from **13** (250 mg, 0.54 mmol) as a colorless solid; m.p.: 165-170°C; $[\alpha]_D$ = −7.5° (c = 1.9; $CHCl_3$); R_f = 0.65 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr) ν: 2954*s*, 1725*s*, 1671*s*, 1459*m*, 1383*w*, 1275*w*, 1246*w*, 1224*w*, 1204*m* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.80 (d, *J* = 9.9 Hz, 1H, C*H* (3)), 5.75 (s, 1H, C*H* (12)), 5.48 (dd, *J* = 9.9, 5.2 Hz, 1H, C*H* (2)), 4.89 (d, *J* = 5.2 Hz, 1H, C*H* (1)), 3.59 (s, 3H, OMe), 2.44(d, *J* = 11.4 Hz, 1H, C*H* (18)), 2.10 (ddd, *J* = 12.9, 11.4, 4.4 Hz, 1H, C*H*$_a$ (16)), 2.00 – 1.92 (m, 2H, C*H* (5), C*H*$_a$ (15)), 1.80-1.69 (m, 3H, C*H*$_b$ (16), C*H*$_a$ (7), C*H*$_a$ (22)), 1.66 – 1.49 (m, 3H, C*H*$_a$ (6), C*H*$_a$ (21), C*H*$_b$ (22)), 1.47 – 1.40 (m, 2H, C*H* (19), C*H*$_b$ (6)), 1.37 – 1.27 (m, 9H, C*H*$_b$ (21), C*H*$_b$ (15), C*H*$_b$ (7)), 1.34 (s, 3H, C*H*$_3$ (27)), 1.33 (s, 3H, C*H*$_3$ (25)), 1.05 – 1.01 (m, 1H, C*H* (20)), 1.04 (s, 3H, C*H*$_3$ (23)), 1.03 (s, 3H, C*H*$_3$ (26)), 0.94 (d, *J* = 6.6 Hz, 3H, C*H*$_3$ (30)), 0.93 (s, 3H, C*H*$_3$ (24)), 0.91 (d, *J* = 6.5 Hz, 3H, C*H*$_3$ (29)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 193.5 (C11, *C*=O), 177.2 (C28, *C*=O), 163.7 (C13, *C*=CH), 144.7 (C3, H*C*=CH), 128.6 (C12, H*C*=C), 117.6 (C2, H*C*=CH), 90.2 (C9, *C*H), 80.2 (C1, *C*-O), 55.2 (C17, *C*$_{quart.}$), 54.1 (C18, *C*H), 51.8 (OMe, *C*H$_3$), 48.5 (C8, *C*$_{quart.}$), 46.0 (C10, *C*$_{quart.}$), 44.8 (C14, *C*$_{quart.}$), 43.0 (C5, *C*H), 39.0 (C19, *C*H), 38.6 (C20, *C*H), 35.9 (C22, *C*H$_2$), 35.2 (C4, *C*$_{quart.}$), 31.2 (C23, *C*H$_3$), 30.4 (C21, *C*H$_2$), 30.1 (C15, *C*H$_2$), 29.9 (C7, *C*H$_2$), 24.3 (C27, *C*H$_3$), 23.6 (C16, *C*H$_2$), 21.7 (C30, *C*H$_3$), 20.7 (C26, *C*H$_3$), 20.7 (C24, *C*H$_3$), 17.5 (C6, *C*H$_2$), 17.1 (C29, *C*H$_3$), 15.5 (C25, *C*H$_3$) ppm; MS (ESI, MeOH) *m/z*: 497.4 $[M + H]^+$ (30%), 519.5 $[M + Na]^+$ (100%). Anal. for $C_{31}H_{44}O_5$ (496.68): C, 74.96; H, 8.93; found: C, 74.74; H, 8.90.

Methyl 2,3-dihydro-1a,9a-peroxo-11-oxo-olean-12-en-28-oate ***16***

Following GP7 compound **16** (120 mg, 45%) was obtained from **14** (250 mg, 0.54 mmol) as a colorless solid; m.p.: 160-165°C; $[\alpha]_D$ = −19° (c = 1.7; $CHCl_3$); R_f = 0.61 (silica gel, hexane/ethyl acetate, 8:2); IR (KBr) ν: 2946*s*, 1726*s*, 1662*s*, 1459*m*, 1386*m*, 1364*w*, 1320*w*, 1280*w*, 1260*m*, 1227*w*, 1190*m*, 1176*m*, 1161*m*, 1129*w*, 1084*w*, 1068*w*, 1037*w*, 1013*w* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.81 (d, *J* = 9.8 Hz, 1H, C*H* (3)), 5.79 (s, 1H, C*H* (12)), 5.50 (dd, *J* = 10.1, 5.2 Hz, 1H, C*H* (2)), 4.95 (d, *J* = 5.2 Hz, 1H, C*H* (1)), 3.61 (s, 3H, OMe), 3.02 (dd, *J* = 13.7, 3.6 Hz, 1H, C*H* (18)), 2.09 (ddd, *J* = 13.7, 13.4, 4.0 Hz, 1H, C*H*$_a$ (16)), 1.99 – 1.91 (m, 2H, C*H* (5), C*H*$_a$ (15)), 1.75 – 1.50 (m, 6H, C*H*$_a$ (19), C*H*$_a$ (7), C*H*$_2$ (22), C*H*$_a$ (6), C*H*$_b$ (16)), 1.46 (ddd, *J* = 13.1, 12.8, 3.1 Hz, 1H, C*H*$_b$ (6)), 1.40 – 1.22 (m, 5H, C*H*$_2$ (21), C*H*$_b$ (15), C*H*$_b$ (19), C*H*$_b$ (7)), 1.38 (s, 3H, C*H*$_3$ (27)), 1.31 (s, 3H, C*H*$_3$ (25)), 1.05 (*s*, 3H, C*H*$_3$ (26)), 1.05 (s, 3H, C*H*$_3$ (23)), 0.94 (s, 3H, C*H*$_3$ (24)), 0.93 (s, 3H, C*H*$_3$ (29)), 0.93 (s, 3H, C*H*$_3$ (30)) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 193.6 (C11, *C*=O), 177.5 (C28, *C*=O), 168.7 (C13, *C*=CH), 144.6 (C3, H*C*=CH), 126.0 (C12, H*C*=C), 117.6 (C2, H*C*=CH), 90.5 (C9, *C*H), 80.3 (C1, H*C*-O), 55.4 (C17, *C*$_{quart.}$), 51.9 (OMe, *C*H$_3$), 46.9 (C10, *C*$_{quart.}$), 46.3 (C14, *C*$_{quart.}$), 44.5 (C8, *C*$_{quart.}$), 43.6 (C19, *C*H), 42.9 (C18, *C*H), 42.8 (C5, *C*H), 35.3 (C4, *C*$_{quart.}$), 33.7 (C21, *C*H$_2$), 32.8 (C29, *C*H$_3$), 31.5 (C22, *C*H$_2$), 31.2 (C23, *C*H$_3$), 30.6 (C20, *C*$_{quart.}$), 29.8 (C15, *C*H$_2$), 29.2 (C7, *C*H$_2$), 26.6 (C27, *C*H$_3$), 23.3 (C30, *C*H$_3$), 22.6 (C16, *C*H$_2$), 21.7 (C24, *C*H$_3$), 20.7 (C26, *C*H$_3$), 17.5 (C6, *C*H$_2$), 15.5 (C25, *C*H$_3$) ppm; MS (ESI, MeOH) *m/z*: 497.4 $[M + H]^+$ (25%), 519.6 $[M + Na]^+$ (100%). Anal. calcd. for $C_{31}H_{44}O_5$ (496.68): C, 74.96; H, 8.93; found: C, 74.68; H, 8.92.

Biology

Cell lines and culture conditions

The cell lines 518A2, 8505C, A253, A2780, A431, A549, DLD-1, FaDu, HCT-116, HCT-8, HT-29, LIPO, MCF-7, SW1736, SW480 were included in this study.

Cultures were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10% heat-inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin / streptomycin (PAA Laboratories) at 37°C in a humidified atmosphere of 5% CO_2/95% air.

Cytotoxicity assay

The cytotoxic activities of our compounds were evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich, Germany) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the compounds (0 to 30 μM) for 96 h. The final concentration of DMSO or DMF as a solvent never exceeded 0.5%, which was shown to be non-toxic to the cells. The percentages of surviving cells compared to untreated controls were determined 96 h after the beginning of drug exposure. After 96 h of treatment, the supernatant medium from the 96-well plates was discarded and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to stand at 4°C. After fixation, the cells were washed in a strip washer four times with water using alternate dispensing and aspiration procedures. The plates were dyed with 100 μL of 0.4% SRB for about 20 min. After dying, the plates were washed with 1% acetic acid to remove the dye and allowed to air dry overnight. Then, 100 μL of 10 mM Tris base solution were added to each well and absorbance was measured at 570 nm using a 96-well plate reader (Tecan Spectra, Crailsheim, Germany). The IC_{50} was calculated from the semi-logarithmic dose-response curves.

Dye-exclusion test

Apoptotic cell death was analyzed by trypan blue dye (Sigma Aldrich) on A431 and A2780 cell lines. The cell culture flasks with 70% to 80% confluence were treated with IC 90 doses of the compounds for 24 h. After treatment, the supernatant medium with floating cells was collected and centrifuged to collect the dead and apoptotic cells. The cell pellet was suspended in serum-free media. Equal amounts of cell suspension and trypan blue were mixed and analyzed by microscope. The viable cells exclude the dye and are colorless and cells, with destroyed cell

membrane are turning blue. When there were more colorless than colored cells, death is characterized as apoptotic.

DNA fragmentation assay

Determination of apoptotic cell death was performed by DNA gel electrophoresis. Briefly, A431 and A2780 were treated with respective IC 90 doses of the compounds for 24 h. Floating cells (induced by drug exposure) were collected, washed with PBS and lysed with lysis buffer (100 mM Tris-HCL pH 8.0; 20 mM EDTA; 0.8% SDS) (all from Sigma Aldrich). Then, they are treated with RNAse A at 37°C for 2 h and proteinase K at 50°C (both from Roche Diagnostics Chemical Company, Mannheim, Germany). DNA laddering was observed by running the samples on 2% agarose gel followed by ethidium bromide (Sigma Aldrich) staining.

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