European Journal of Medicinal Chemistry 56 (2012) 237–245

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Tormentic acid derivatives: Synthesis and apoptotic activity☆

René Csuk*, Bianka Siewert, Christian Dressel, Renate Schäfer

Bereich Organische Chemie, Martin-Luther Universität Halle-Wittenberg, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany

ARTICLE INFO

Article history:
Received 10 July 2012
Received in revised form
22 August 2012
Accepted 22 August 2012
Available online 31 August 2012

Keywords:
Tormentic acid
Antitumor activity
Apoptosis
Acridine orange/propidium iodide assay
Annexin V
DNA laddering

ABSTRACT

Several derivatives of tormentic acid have been prepared and tested for their antitumor activity. The dichloroacetate **14** is an excellent antitumor active agent acting by an apoptose inducing pathway as demonstrated by OA/PI staining, DNA laddering experiments as well as by an annexin V binding assay.

1. Introduction

Cancer is still one of the leading causes of death. The index of cancer cure is often low and its treatment is still a challenge. Cancer cells hold the ability to evade death, and expressing multidrug resistance is an important draw-back in the chemotherapy of cancer.

Natural products have been used to treat diseases for thousands of years. They still play an important role in development of new drugs. Among them, triterpenes represent a class of most significant compounds. They have been shown to possess a broad variety of medicinal properties. In continuation of our previous studies on betuline, betulinic, glycyrrhetinic and boswellic acid derivatives as antitumor active compounds, we became interested in tormentic acid as a lead compound in the synthesis of antitumor active derivatives.

Common tormentil (bloodroot, *Potentilla erecta*), also known as shepherd's knot, is a low, clump-forming plant growing wild all over northern Europe and all over Asia. Extracts prepared from the dried root have been used to treat bleedings and diarrhea (because of its high content in tannins acting as adstringents) or to dye leather red (because of the presence of phlobaphenes).

As early as 1915 tormentol (tormentoside) [1] was isolated as the β-D-glucopyranosyl ester of tormentic acid the structure of which was established in 1966 [2–4]. Tormentic acid, i.e. (2 *R*, 3 *R*, 19 *R*) 2,3,19-trihydroxy-urs-12-en-28-carboxylic acid (**1**), can be extracted [5–17] from various plants, among them *Myrianthus serratus*, *Perilla frutescens*, *Cotoneaster simsonsii*, *Rubus sieboldii* but also from species of *Potentilla*, e.g. *Potentilla anserina*, *Tormentilla tormentilla* or *P. erecta*.

There are ample examples for the antitumor activity of pentacyclic triterpenes; less is known, however, about the biological activity of **1** and even fewer data have been reported for derivatives of **1**. Thus, **1** is able to inhibit *in vitro* platelet aggregation [18], and the influence of **1** on forming atherosclerotic plaques [19] in mice has been investigated. In addition, **TA** reduced vascular smooth muscle cell proliferation [20] and possesses [21,22] some anti-inflammatory activity. Compound **1** reduced also the viability of human gastric cells [13] by an inhibition [13,23,24] of α- and β-DNA polymerases. Only a weak cytotoxic activity has been established [25,26] for different tumor cell lines; some anticancer activity has been found for **1** for lymphocytic leukemua cells [27]. Interesting to note that **1** shows little toxicity [13] to normal cells, and **1** has been suggested [20] to be developed for the treatment of post-angioplasty re-stenosis. Recently, **1** methyl ester (**2**) has been shown [28] to act as a selective, low micromolar inhibitor of 11β-hydroxysteroid dehydrogenase and to display anti-inflammatory effects [29,30].

☆ Dedicated to Prof. Dr. Rainer Beckert, Friedrich-Schiller Universität Jena, on the occasion of his 60th birthday. Ad multos annos!

* Corresponding author. Tel.: +49 0 345 55 25660; fax: +49 0 345 55 27030.
E-mail address: rene.csuk@chemie.uni-halle.de (R. Csuk).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.08.032

2. Results

2.1. Chemistry

Quite recently, we became aware that small structural modifications, e.g. esterification or acylation of a triterpenoid skeleton [31–38], might result in obtaining compounds of improved cytotoxicity. Thus, **1** was used as an easy accessible starting material for the synthesis of "simple" derivatives.

Treatment of **1** (Scheme 1) with MeI/K_2CO_3 gave the methyl ester **2** [4,28,39,40] in almost quantitative yield. TEMPO oxidation [41,42] of **2** yielded the 2-oxo compound **3** [1] in 83% yield; compound **3** is characterized by the presence of a carbonyl signal in its ^{13}C NMR spectra at $\delta = 211.0$ ppm. This oxidation advances in a regioselective way; no oxidation at position C-3 could be noted. The reason for this regioselectivity might be the steric hindrance at position C-3 because of the presence of the two geminal methyl groups at C-4.

Oxidation of **2** using bis(tri-*n*-butyl-tin)oxide [43,44] in the presence of bromine at 0 °C, however, yielded the 3-oxo compound **4** whose carbonyl group can be found in the ^{13}C NMR spectrum at $\delta = 216.6$ ppm. Using a prolonged reaction time and an excess of oxidizing agent gave the 3-oxo-1,12-diene **5** in 57% yield. Reduction of **3** with sodium borohydride proceeded in a stereoselective way and provided the 2-epi compound **6**; compound **6** represents a 2,3-bis epimer to euscaphic ester **8**; the latter is easily obtained from naturally occurring euscapic acid (**7**) by esterification with diazomethane. As an alternative, reduction of **5** under the same conditions gave a 70% yield of **6**. Compound **8** was oxidized in a regioselective manner to afford **4**.

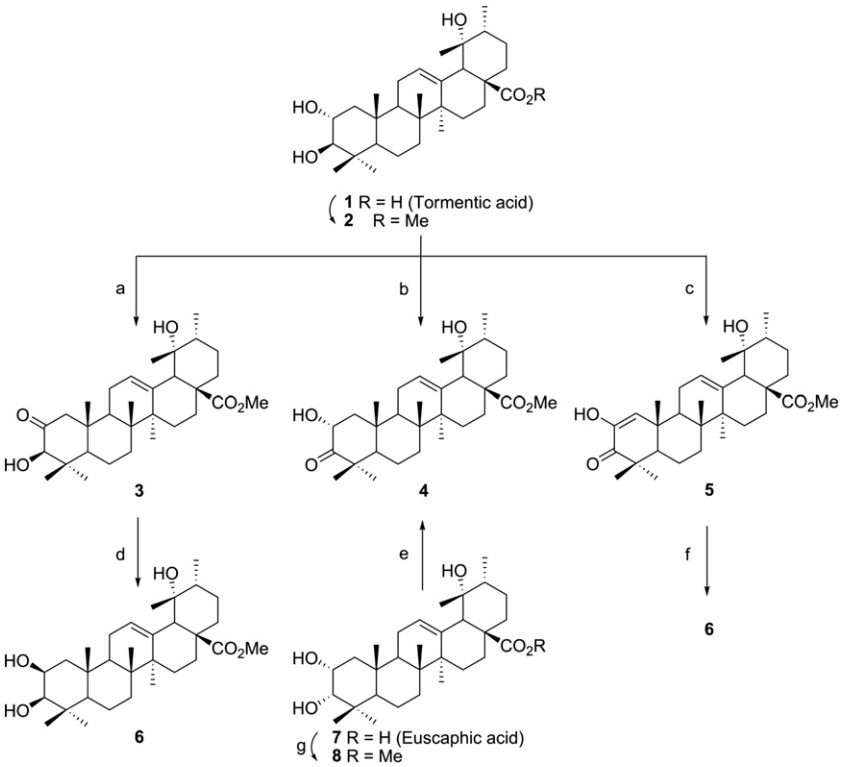
For betulin and betulinic acid, several acylated derivatives showed a higher antitumor activity than their parent compounds [38,45]. Therefore, it seemed of interest to prepare several acylated analogs of **2** and to compare their biological activity with parent **2**.

Acetylation of **1** (Scheme 2) with acetic anhydride in dry pyridine for 24 h yielded 66% of the diacetate **9**. If the reaction was stopped after 3 h, the 2-*O*-acetyl derivative **10** and the 3-*O*-acetyl derivative **11** were isolated in 56% and 21%, respectively. The monoacetates **10** and **11** were previously isolated [25] from *Cecropia lyratiloba*, and shown to be effective inhibiting the viability of a chronic myeloid leukemia blast crisis cell line by inducing apoptosis [2]. Acetylation of **3** under similar conditions provided acetate **12** whereas from compound **4** mono-acetylated **13** was formed. Acylation of **2** with chloroacetyl chloride yielded the 2,3-bis(chloroacetyloxy)-compound **14**, the 2-*O*-chloro acetate **15** and the 3-*O*-chloro acetate **16**.

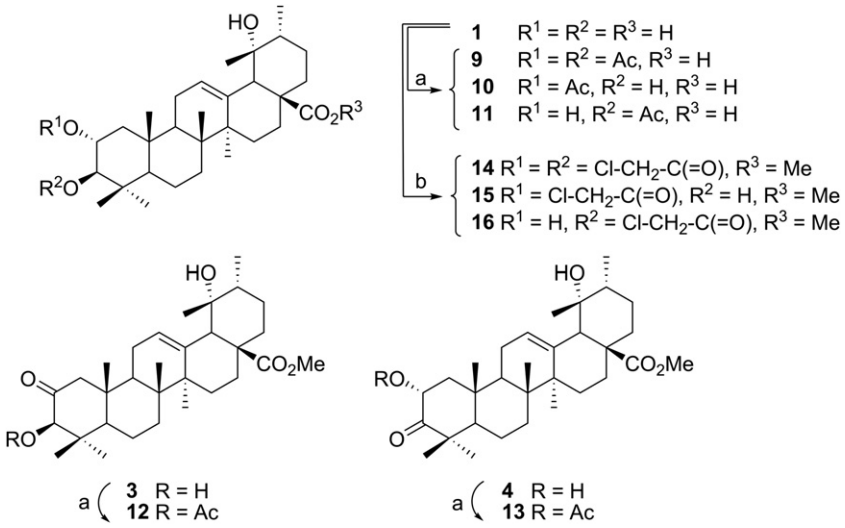
2.2. Biology

Thus, contrary to previous findings with betulinic acid, neither an esterification nor an acetylation resulted in products of significantly increased cytotoxicity (Table 1). Mono- or diacetylated products **9**–**11** showed only moderate cytotoxicity ($IC_{50} > 30$ μmol) for all human tumor cell lines tested. A similar behavior can be found for the mono-acetylated keto compounds **12** [1] and **13** and for the keto compounds **3**, **4** and **5**. An improvement was observed for the mono-chloroacetylated compounds **15** and **16**; a significantly improved cytotoxicity, however, was observed for bis-chloroacetylated **14**.

Cell death can occur [46,47] either necrotic or programmed by a variety of different forms being known for the latter. Apoptosis is characterized [46] *inter alia* by cell shrinking, membrane blebbing, an enhanced activity of caspases, a translocation of phosphatidylserine and DNA fragmentation. Previous work of Rocha et al. [2,25] and Fogo et al. [20] gave evidence for **1** and several alkynic derivatives thereof for acting by inducing apoptosis. To evaluate the ability of our compounds, tormentic acid methyl ester **2** and the



Scheme 1. a) TEMPO, NaOCl, CH_2Cl_2, 8 h, 63%; b) $[(n\text{Bu})_3\text{Sn}]_2O$, Br_2, 0 °C, 1 min, 62%; c) $[(n\text{Bu})_3\text{Sn}]_2O$, Br_2, 0 °C, 15 min, 57%; d) $NaBH_4$, MeOH, reflux, 1 h, 82%; e) $[(n\text{Bu})_3\text{Sn}]_2O$, Br_2, 0 °C, 1 min, 67%; f) $NaBH_4$, MeOH, reflux, 1 h, 70%; g) CH_2N_2, MeOH, 95%.



Scheme 2. a) Ac_2O, pyridine, CH_2Cl_2, 24 °C, 3–24 h, **9**: 66%, **10**: 56%, **11**: 21%, **12**: 66%, **13**: 68%; b) $ClCH_2COCl$, pyridine, CH_2Cl_2, 24 °C, 4 h, **14**: 26%, **15**: 40%, **16**: 11%.

most active compound of this series, bis-chloroacetyl **14** were tested in more detail.

As indicated above, programmed cell death is characterized [47] by different morphological changes. Thus, living cells (518A2) were stained with acridine orange (AO) and investigated by fluorescence microscopy. AO is an uncharged cationic dye; it binds to nucleic acids. AO and nucleic acids form either monomeric complexes with double stranded nucleic acids (exhibiting a green fluorescence) or dimers with single stranded nucleic acids (emitting orange light). Maslinic acid (MA) is well known for its ability to induce apoptosis in cancer cells and was used as a control [22,27,48–57]. A typical condensation of the chromatin as well as blebbing of the nuclear membrane and a shrinking of the cells was observed. Compound **2**, however, shows only a weak ability to induce cell death for 518A2 cells at a concentration of 30 μM. The condensation of chromatin as well as the blebbing of the nuclear membrane indicates a programmed cell death process. The same phenomenon was observed for 8505C human thyroid carcinoma cell cells. Some red dots were seen during microscopy; these were assigned to proteasomes or are due to lysosomal activity [58].

Additional investigations using an AO/PI exclusion dye assay (Fig. 1) showed that the majority of the dead cells still possess an intact cell membrane. While membrane disruption is a characteristic feature of necrosis [leading to deep red light emission from the propidium iodide (PI)], an intact membrane indicates a programmed cell death since PI – as a double charged molecule – cannot enter the cell as long as the cell membrane is intact [59]. All compounds used in this AO/PI assay induced a controlled cell death hence paralleling previous findings [2,25,50,60] for other triterpenoic acids.

To gain a deeper insight, additional experiments were called for. Phosphatidylserine – a label for cell death – switches from the inner to the outer cell membrane during the cascade of apoptosis [47]. Annexin V, a cellular protein of the annexin group, selectively binds to phosphatidylserine. By a combination of the protein with fluorescine isothiocyanate (FITC) a fluorescence active dye is formed. In this assay 8505C cancer cells emitted green light hence having bound annexin V-FITC and thus indicating that these cells died by apoptosis [61]. The same was true for experiments employing 518A2 cancer cells. Fig. 2 depicts the results from the annexin V-FITC/PI stained cells by FACS-analysis.

Another typical hallmark of apoptosis is an exactly determined cutting of the DNA by endonucleases into multiple 180 bp fragments (and multiples thereof) [62,63]. All tested compounds gave the characteristic DNA ladders. To evaluate the cancer-to-control selectivity of some of our compounds, additional experiments

Table 1
Cytotoxicity (IC_{50} in μmol; SRB assay) for tormentic acid (**1**) and compounds **2**–**16** in a panel of various cancer cell lines [518A2 (melanoma), 8505C (anaplastic thyroid), A253 (head), A2780 (ovarian), A549 (lung), DLD1 (colon), MCF7 (mamma)], non malignant mouse fibroblast (NiH 3T3), and human fibroblast primary culture cells (WW030272). Values were obtained from SRB assays after 96 h of treatment; the values are averaged from at least 5 independent experiments (n.d. not determined).

	518A2	8505C	A253	A2780	A549	DLD-1	MCF7	NiH 3T3	WW030272
1	>30	23.4 ± 0.8	>30	>30	31.0 ± 0.1	31.0 ± 0.2	32.3 ± 2.5	>30	47.7 ± 1.1
2	31.3 ± 3.9	42.0 ± 5.2	17.0 ± 2.0	23.9 ± 4.3	n.d.	37.4 ± 1.0	31.3 ± 3.4	15.6 ± 5.0	47.5 ± 1.1
3	>30	>30	n.d.	17.8 ± 1.9	n.d.	>30	28.7 ± 0.5	19.2 ± 0.6	n.d.
4	8.9 ± 0.5	12.9 ± 0.2	6.8 ± 1.8	4.9 ± 0.4	11.8 ± 0.2	18.0 ± 0.1	9.4 ± 0.5	7.7 ± 2.0	n.d.
5	17.2 ± 2.7	25.0 ± 0.9	>30	>30	>30	>30	26.0 ± 5.4	>30	n.d.
6	>30	>30	15.3 ± 2.0	18.9 ± 3.5	>30	>30	22.6 ± 1.0	>30	n.d.
8	27.7 ± 0.2	29.3 ± 2.5	16.4 ± 12.6	12.8 ± 1.5	30.6 ± 0.4	35.8 ± 1.8	17.8 ± 4.5	>30	23.4 ± 1.7
9	>30	>30	27.3 ± 3.5	17.6 ± 4.9	>30	>30	20.4 ± 3.7	32.3 ± 0.1	n.d.
10	>30	>30	>30	24.3 ± 9.0	>30	>30	25.3 ± 2.6	>30	n.d.
11	>30	>30	>30	28.1 ± 0.9	>30	>30	26.2 ± 0.7	>30	n.d.
12	5.6 ± 1.2	7.0 ± 0.2	6.7 ± 1.1	4.4 ± 0.4	7.8 ± 0.4	13.5 ± 0.4	8.2 ± 0.9	6.7 ± 1.1	n.d.
13	28.9 ± 0.7	>30	18.5 ± 0.4	9.0 ± 1.4	31.3 ± 0.4	28.2 ± 0.5	14.8 ± 0.4	25.1 ± 4.6	n.d.
14	1.1 ± 0.2	1.6 ± 0.7	1.6 ± 0.9	0.8 ± 0.4	1.2 ± 0.5	2.2 ± 0.2	1.5 ± 0.8	1.1 ± 0.1	3.4 ± 1.1
15	4.5 ± 0.4	4.6 ± 0.5	4.6 ± 0.5	2.6 ± 0.3	9.7 ± 0.6	3.7 ± 0.4	2.6 ± 0.3	2.1 ± 0.2	n.d.
16	5.5 ± 0.6	7.5 ± 0.8	4.1 ± 0.4	4.1 ± 0.4	10.0 ± 1.0	6.0 ± 0.6	6.9 ± 0.7	4.2 ± 0.4	n.d.

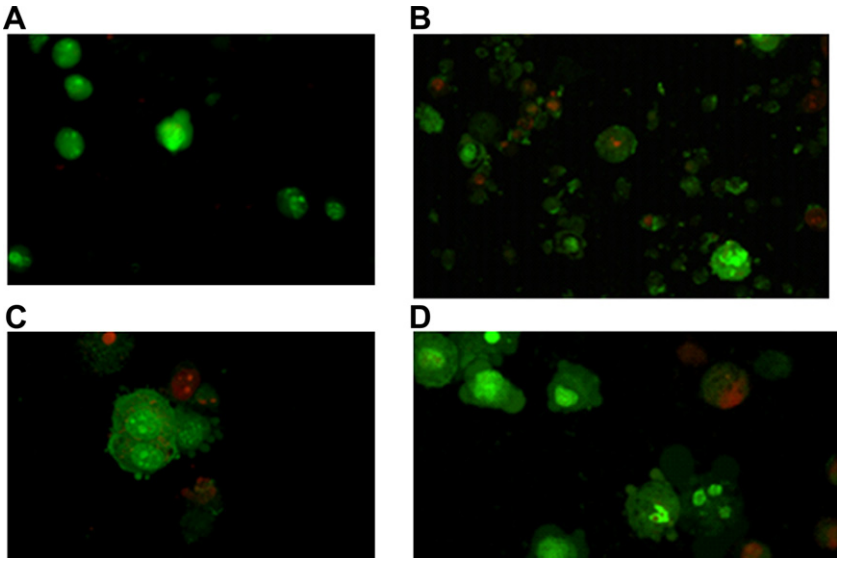


Fig. 1. AO/PI assay of dead 8505C cells. The cells were treated with **MA** (A), tormentic acid (B), **2** (C) and **14** (D); green cells indicate a controlled cell death, deep red cells a necrotic way of exitus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

using human fibroblast primary culture cells WW030272 were used. The results of these experiments are included in the table.

3. Conclusion

In summary, tormentic acid (**1**) as well as its methyl ester **2** are adequate and its dichloroacetate **14** is an excellent antitumor active agent acting by an apoptose inducing pathway as demonstrated by OA/PI staining, DNA laddering experiments as well as by an annexin V binding assay.

4. Experimental

4.1. Biological material

4.1.1. Cell lines and culture conditions

The cell lines 518A2, 8505C, A253, A2780, A549, DLD-1, MCF-7, NiH 3T3 and WW030272 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.

4.1.2. Cytotoxicity assay

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma–Aldrich) 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 (0–30 μM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5%, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96 well plates was thrown away and the cells were fixed with 10% TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a strip washer. The washing was done five times with water using alternate dispensing and aspiration procedures. Afterward the plates were dyed with 100 μl of 0.4% SRB (sulforhodamine B) for about 30 min. The plates were washed

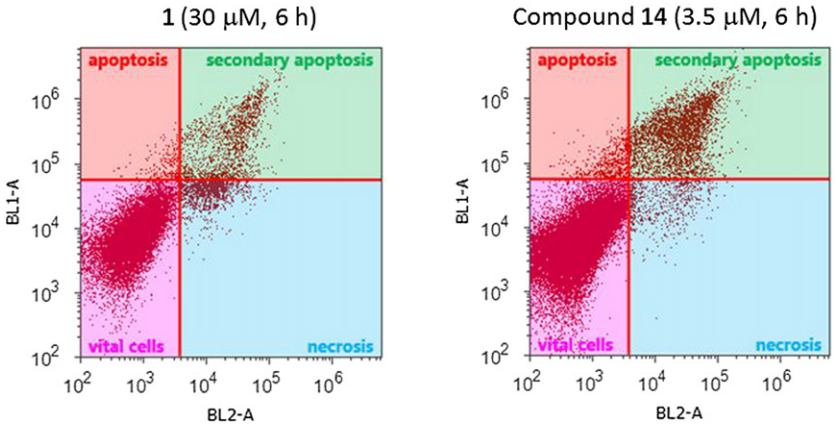


Fig. 2. FACS analysis of 8505C cells after 6 h incubation with **1** (left) or compound **14** (right).

with 1% acetic acid to remove the excess of the dye and allowed to air dry overnight. Tris base solution (100 μl of 10 mM) was 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 estimated from the dose–response curves.

4.1.3. Morphological investigation of living cells

In an eight-well chamber slide (Sigma–Aldrich) 10.000 cells of the human thyroid cancer cell line 8505C or 10.000 cells of the melanoma cell line 518A2 were seeded. After 24 h of incubation, the medium was removed, and the cells were treated with maslinic acid (MA), tormentic acid (**1**) or tormentic acid methyl ester (**2**) (4 ml, 30 μM). On the next day the supernatant medium was removed, and the cells were washed with PBS (w(o, 1 ml)) and stained with acridine orange (5.10^{-6} mol). Visual inspection was performed using a fluorescence microscope (Zeiss Axioskop).

4.1.4. Apoptosis assay of dead cells by AO/PI dye exclusion test and annexin V-FITC

The death of the cells was analyzed employing an OA/PI assay as well as with annexin V-FITC dye using fluorescence microscopy and human cancer cell lines 518A2 and 8505C, respectively. Approx. $1*10^6$ cells were seeded in cell culture flasks (25 cm^2), and the cells were allowed to grow up to 80%. After removing of the used medium, the substance loaded fresh medium was reloaded (or a blank new medium as a control). After 24–48 h, the supernatant medium was collected and centrifuged (300 g, 4 °C), the pellet was gently suspended in phosphate-buffered saline (PBS, 1 ml) and centrifuged again. The PBS was removed, and the pellet again gently suspended in PBS (100 μl). The analysis of the cells was performed using a fluorescence microscope after having mixed the cell suspension (10 μl) with a solution of AO/PI (10 μl). A green fluorescence indicates apoptosis whereas a red colored cell indicates necrosis.

For the investigations using annexin V-FITC, the cells were washed with annexin V binding buffer after the treatment with PBS, then centrifuged and dyed for 15 min using an annexin V staining buffer. Analyses were performed using a fluorescence microscope; green colored cells indicate cells with phosphatidylserine on the outer cell membrane, a phenomenon that is typical for apoptosis.

4.1.5. DNA laddering experiments

Approximately $1*10^6$ cells (518A2 or 8505C) were seeded in cell culture flasks (25 cm^2), and the cells were allowed to grow up to 80%. After removing of the used medium, the substance loaded medium was reloaded (or a blank fresh medium as a control). After 24–48 h, the supernatant medium was collected and centrifuged (300 g, 4 °C). The pellet was gently suspended in phosphate-buffered saline (PBS 1 ml) and centrifuged again. The PBS was removed and lyses buffer (30 μl, 0 °C, 10 min) was added. The cells were incubated for 2 h (37 °C) after treatment with RNAse (10 μl, 0 °C, 10 min) and for 12 h at 50 °C after having been treated with proteine kinase K (10 μl). The extract was mixed with DNA-ladder dye (10 μl) and analyzed by gel electrophoresis (agarose, 150 mV, 2 h).

4.2. General – chemistry

Reagents were bought from commercial suppliers without any further purification. Melting points were measured with a LEICA hot stage microscope and were not corrected. NMR spectra were recorded on VARIAN Gemini 200, Gemini 2000 or Unity 500 spectrometers at 27 °C with trimethylsilane as an internal standard, δ are given in ppm and J in Hz. Mass spectra were taken on a FINNIGAN MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. Elemental analyses were measured on a Foss-Heraeus Vario EL unit. IR spectra were recorded on a Perkin–Elmer FT-IR spectrometer Spectrum 1000, optical rotations on a Perkin–Elmer 341 polarimeter (1 cm micro cell, 25 °C) and UV–vis spectra on a Perkin–Elmer unit, Lambda 14. TLC was performed on silica gel (Merck 5554, detection by UV absorption). Solvents were dried according to usual procedures. The purity of the compounds was checked by HPLC/DAD and found to be >98% for each compound.

4.3. (3 R, 19 R) methyl 3,19-dihydroxy-2-oxo-urs-12-en-28-carboxylate (3)

To a solution of **2** (376 mg, 0.75 mmol) and TEMPO (2 mg, 0.01 mmol) in dichloromethane (20 ml), a solution of KBr (9 mg, 0.075 mmol) and $(n\text{-Bu})_4NBr$ (120 mg, 0.37 mmol), in an aq. solution of $NaHCO_3$ (5%, 3 ml) was added. Under vigorous stirring an aq. solution of NaOCl (1 M, 0.8 ml) was slowly added with 2 h (no further discoloration of the reaction mixture), and stirring was continued for another 6 h. The reaction was quenched by the addition of water (50 ml), and extracted with dichloromethane (4 × 40 ml). The combined organic phases were washed with brine (2 × 30 ml), dried (Na_2SO_4), and the solvent was evaporated. The residue was subjected to chromatography (silica gel, toluene/ethyl acetate/formic acid/*n*-heptane 80:20:3:10) to yield **3** (237 mg, 63%) as a colorless solid; mp 104–106 °C; $[\alpha]_D^{20} = +37.3°$ ($c = 0.47$, $CHCl_3$); R_F = 0.47 (toluene/ethyl acetate/formic acid/*n*-heptane 80:20:3:10); IR (KBr): ν = 3488 br, 2949 s, 1717 s, 1458 m, 1394 m, 1234 m, 1208 m, 1153 m, 1117 m, 1057 m, 1034 m, 970 w, 772 w, 733 w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.28 (dd, $J_{11',12}$ = 3.1, $J_{11'',12}$ = 3.1 Hz, 1 H, H-12), 3.83 (s, 1 H, H-3_{ax}), 3.53 (s, 3 H, H-31), 2.54 (m, 1 H, H-16_{ax}), 2.39 (d, $J_{1eq,1ax}$ = 12.5 Hz, H-1_{eq}), 2.03 (d, $J_{1ax,1eq}$ = 12.5 Hz, 1 H, H-1_{ax}), 1.89 (m, 3 H, H-9, H-11′ and H-11″), 1.67 (m, 1 H, H-22″), 1.66–1.51 (m, 6 H, H-6″, H-7″, H-15_{ax}, H-16_{eq}, H-21″ and H-22′), 1.39 (m, 2 H, H-6′, H-5), 1.37–1.30 (m, 2 H, H-7′, H-20), 1.24 (s, 3 H, H-27), 1.19 (m, 1 H, H-21′), 1.14 (s, 3 H, H-29), 1.13 (s, 3 H, H-23), 0.97 (m, 1 H, H-15_{eq}), 0.87 (d, $J_{20,30}$ = 6.5 Hz, 3 H, H-30), 0.81, 0.63 and 0.62 (each s, 9 H, H-24, H-25, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 211.0 (C2), 178.2 (C28), 138.4 (C13), 128.2 (C12), 82.6 (C3), 73.1 (C19), 54.4 (C5), 53.2 (C18), 53.1 (C1), 51.6 (C31), 47.8 (C17), 47.2 (C9), 45.7 (C4), 43.7 (C10), 41.3 (C14), 41.1 (C20), 40.3 (C8), 37.3 (C22), 32.4 (C7), 29.4 (C23), 28.2 (C15), 27.4 (C29), 25.9 (C21), 25.4 (C16), 24.3 (C27), 23.6 (C11), 21.0 (C6), 18.6, 16.5, 16.1 (C24, C25, C26), 16.2 (C30) ppm; MS (ESI, MeOH): *m*/*z* (%) = 501.4 ($[M+H]^+$, 10), 518.4 ($[M+NH_4]^+$, 10), 523.3 ($[M+Na]^+$, 100), 539.3 ($[M+K]^+$, 19); analysis for $C_{31}H_{48}O_5$ (500.71): C, 74.36; H, 9.66; found: C, 74.21; H, 9.82.

4.4. (2 R, 19 R) methyl 2,19-dihydroxy-3-oxo-urs-12-en-28-carboxylate (4)

From **2**: To a solution of **2** (500 mg, 0.99 mmol) in dry chloroform (20 ml) at 0 °C $[(n\text{-Bu})_3Sn]_2O$ (0.5 ml, 0.99 mmol) and bromine (51 μl, 0.99 mmol) were added. After stirring for 1 min, NEt_3 (0.10 ml) was added, the solvents were removed under diminished pressure, and the residue was subjected to chromatography (silica gel, toluene/ethyl acetate/formic acid/*n*-heptane, 80:20:3:10) to afford **4** (310 mg, 62%) as a colorless solid.

From **8**: Analogous synthesis starting from **8** (673 mg, 0.99 mmol) gave **4** (452 mg, 67%) as a colorless solid; mp 114–117 °C; $[\alpha]_D^{20} = +31.3°$ (c = 0.42, $CHCl_3$); R_F = 0.46 (toluene/ethyl acetate/formic acid/*n*-heptane, 80:20:3:10); IR (KBr): ν = 3483 br, 2935 s, 1720 s, 1458 m, 1390 m, 1263 m, 1232 m, 1207 m, 1192 m, 1153 s, 1094 m, 1056 m, 961 w, 866 w, 771 w cm^{-1}; ^1H NMR

(500 MHz, $CDCl_3$): $\delta = 5.32$ (dd, 1 H, $J_{12,\,11'} = 3.5$, $J_{12,\,11''} = 3.5$ Hz, H-12), 4.51 (dd, 1 H, $J_{2ax,\,1ax} = 12.5$, $J_{2ax,\,1eq} = 6.5$ Hz, 1 H, H-2_{ax}), 3.58 (s, 3 H, H-31), 2.57 (s, 1 H, H-18), 2.51 (m, 1 H, H-16_{ax}), 2.40 (dd, $J_{1eq,\,1ax} = 12.5$, $J_{1eq,\,2ax} = 6.5$ Hz, 1 H, H-1_{eq}), 2.04–2.01 (m, 2 H, H-11′, H-11″), 1.72–1.28 (m, 12 H, H-6′, H-6″, H-7′, H-7″, H-9, H-15_{ax}, H-16_{eq}, H-20, H-21′, H-21″, H-22′, H-22″), 1.25 (s, 3 H, H-25), 1.22 (s, 3 H, H-27), 1.20 (s, 3 H, H-29), 1.16 (s, 3 H, H-24), 1.16–1.13 (m, 2 H, H-1_{ax}, H-5), 1.11 (s, 3 H, H-23), 1.01 (m, 1 H, H-15_{eq}), 0.93 (d, $J_{30,\,20} = 7.0$, 3 H, H-30), 0.73 (s, 3 H, H-26) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 216.6$ (C3), 178.3 (C28), 138.3 (C13), 128.5 (C12), 73.1 (C19), 69.1 (C2), 57.6 (C5), 53.1 (C18), 51.6 (C31), 49.5 (C1), 47.8, 47.7 (C4, C17), 46.9 (C9), 41.2 (C14), 41.1 (C20), 40.0 (C8), 37.6 (C10), 37.3 (C22), 32.4 (C7), 28.2 (C15), 27.4 (C29), 25.9 (C21), 25.4 (C16), 24.7 (C23), 24.4 (C27), 23.8 (C11), 21.6 (C24), 19.2 (C6), 16.8 (C26), 16.1 (C30), 15.9 (C25) ppm; MS (ESI, MeOH): m/z (%) = 501.4 ($[M + H]^+$, 116), 518.4 ($[M + NH_4]^+$, 10), 523.3 ($[M + Na]^+$, 100), 539.3 ($[M + K]^+$, 17); analysis for $C_{31}H_{48}O_5$ (500.71): C, 74.36; H, 9.66; found: C, 74.25; H, 9.81.

4.5. (19 R) methyl 2,19-dihydroxyursa-3-oxo-1,12-dien-28-carboxylate (**5**)

Following the procedure as described above (15 min reaction time), from **2** (214 mg, 0.43 mmol), $[(n\text{-}Bu)_3Sn]_2O$ (0.44 ml, 0.86 mmol) and bromine (44 μl, 86 mmol), compound **5** (121 mg, 57%) was obtained as a white solid; mp 115–118 °C; $[\alpha]_D^{20} = +62.8°$ ($c = 0.47$, $CHCl_3$); $R_F = 0.65$ (toluene/ethyl acetate/formic acid/*n*-heptane 80:20:3:10); IR (KBr): $\nu = 3436$ br, 2933 s, 2876 s, 1725 s, 1669 s, 1648 m, 1458 m, 1404 m, 1383 s, 1238 s, 1208 s, 1152 s, 1091 m, 1054 m, 1034 m, 970 w, 930 w, 865 w, 786 w, 772 w, 753 w, 538 w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 6.34$ (s, 1 H, H-1), 5.93 (s, 1 H, OH), 5.40 (dd, 1 H, $J_{12,\,11'} = 3.5$, $J_{12,\,11''} = 3.5$ Hz, H-12), 3.61 (s, 3 H, H-31), 2.62 (s, 1 H, H-18), 2.52 (m, 1 H, H-16_{ax}), 2.21 (dd, 1 H, $J_{11'',\,9} = 6.6$, $J_{11'',\,12} = 3.5$ Hz, H-11″), 2.13 (dd, 1 H, $J_{11',\,9} = 11.2$, $J_{11',\,12} = 3.5$ Hz, H-11′), 1.96 (dd, 1 H, $J_{9,\,11'} = 11.2$, $J_{9,\,11''} = 6.6$ Hz, H-9), 1.76–1.52 (m, 9 H, H-5, H-6′, H-6″, H-7″, H-15_{ax}, H-16_{eq}, H-21″, H-22′, H-22″), 1.43–1.37 (m, 2 H, H-7′, H-20), 1.26 (s, 3 H, H-27), 1.24 (m, 1 H, H-21′), 1.22, 1.21, 1.12 (each s, 12 H, H-23, H-24, H-25, H-29), 1.04 (m, 1 H, H-15_{eq}), 0.94 (d, 3 H, $J_{30,\,20} = 7.0$ Hz, H-30), 0.77 (s, 3 H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 201.1$ (C3), 178.3 (C28), 143.6 (C2), 138.6 (C13), 128.4 (C12), 128.1 (C1), 73.1 (C19), 53.8 (C5), 53.3 (C18), 51.6 (C31), 47.9 (C17), 43.9 (C4), 42.6 (C9), 41.6 (C10), 42.0 (C20), 40.6 (C14), 38.4 (C8), 37.3 (C22), 32.6 (C7), 28.2 (C15), 27.4 (C29), 27.1 (C23), 26.0 (C21), 25.4 (C16), 24.5 (C27), 23.6 (C11), 21.8, 19.4 (C24, C25), 18.7 (C6), 17.1 (C26) 16.0 (C30) ppm; MS (ESI, MeOH): m/z (%) = 499.7 ($[M + H]^+$, 13), 521.5 ($[M + Na]^+$, 100), 537.4 ($[M + K]^+$, 75), 553.2 ($[M + MeOH]^+$, 98), 569.3 ($[M + K + MeOH]^+$, 34); analysis for $C_{31}H_{46}O_5$ (498.69): C, 74.66; H, 9.29; found: C, 74.53; H, 9.38.

4.6. (2 S, 3 R, 19 R) methyl 2,3,19-trihydroxyurs-12-en-28-carboxylate (**6**)

From **3**: To a solution of $NaBH_4$ (53 mg, 1.41 mmol) in MeOH (2 ml), a solution of **3** (235 mg, 0.47 mmol) was added drop-wise and heated under reflux for 1 h. After quenching with an aqueous solution of NH_4Cl (satd., 5 ml), dilution with water (20 ml), the mixture was extracted with ethyl acetate (3 × 20 ml), the combined extracts were washed (2 × 20 ml) and dried (Na_2SO_4). The solvent was removed and the residue subjected to chromatography (silica gel, *n*-pentane/ethyl acetate, 2:1) to afford **6** (193 mg, 82%) as a colorless solid.

From **5**: In analogous manner from **5** (80 mg, 0.16 mmol) compound **6** (56 mg, 70%) was obtained as a colorless solid; mp 106–107 °C; $[\alpha]_D^{20} = +50.7°$ ($c = 0.6$, $CHCl_3$) (lit.: [3] 16.3°); $R_F = 0.26$ (toluene/ethyl acetate/formic acid/*n*-heptane, 80:20:3:10); IR (KBr): $\nu = 3510$ br, 2929 s, 1721 s, 1648 w, 1458 m, 1380 m, 1368 m, 1322 m, 1262 m, 1229 m, 1208 m, 1152 s, 1114 m, 1095 m, 1050 m, 1030 m, 1000 m, 973 w, 932 w, 900 w, 867 w, 806 w, 787 w, 772 w, 706 w, 684 w, 654 w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.35$ (dd, 1 H, $J_{12,\,11'} = 3.4$, $J_{12,\,11''} = 3.4$ Hz, H-12), 4.07 (ddd, 1 H, $J_{2eq,\,3ax} = 4.0$, $J_{2eq,\,1ax} = 3.6$, $J_{2eq,\,1eq} = 2.8$ Hz, H-2_{eq}), 3.58 (s, 3 H, H-31), 3.20 (d, 1 H, $J_{3ax,\,2eq} = 4.0$ Hz, H-3_{ax}), 2.58 (s, 1 H, H-18), 2.48 (m, 1 H, H-16_{ax}), 2.08 (dd, 1 H, $J_{1eq,\,1ax} = 14.5$, $J_{1eq,\,2eq} = 2.8$ Hz, H-1_{eq}), 2.02–1.99 (m, 2 H, H-11′, H-11″), 1.72–1.47 (m, 9 H, H-6′, H-6″, H-7″, H-9, H-15_{ax}, H-16_{eq}, H-21″, H-22′, H-22″), 1.39 (m, 1 H, H-20), 1.29 (m, 1 H, H-21′), 1.26 (m, 1 H, H-7′), 1.23 (s, 3 H, H-27), 1.21 (s, 3 H, H-25), 1.19 (s, 3 H, H-29), 1.14 (dd, 1 H, $J_{1ax,\,1eq} = 14.5$, $J_{1ax,\,2eq} = 3.6$ Hz, H-1_{ax}), 1.01 (m, 1 H, H-15_{eq}), 0.99 and 0.98 (each s, 6 H, H-23, H-24), 0.92 (d, 3 H, $J_{30,\,20} = 6.6$ Hz, H-30), 0.81 (m, 1 H, H-5), 0.68 (s, 3 H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 125 MHz): $\delta = 178.3$ (C28), 138.1 (C13), 129.3 (C12), 78.5 (C3), 73.2 (C19), 71.1 (C2), 55.1 (C5), 53.2 (C18), 51.6 (C31), 47.9 (C17), 47.6 (C9), 44.0 (C1), 41.3 (C14), 41.1 (C20), 40.0 (C8), 38.1 (C4), 37.4 (C22), 36.7 (C10), 32.7 (C7), 29.7 (C23), 28.1 (C15), 27.4 (C29), 26.0 (C21), 25.5 (C16), 24.6 (C27), 23.7 (C11), 18.2 (C6), 17.3 (C24), 16.6 (C26), 16.2 (C25), 16.1 (C30) ppm; MS (ESI, MeOH): m/z (%) = 525.5 ($[M + Na]^+$, 100), 556.9 ($[M + MeOH]^+$, 21); analysis for $C_{31}H_{50}O_5$ (502.73): C, 74.06; H, 10.02; found: C, 73.96; H, 10.14.

4.7. Euscaphic acid methyl ester (**8**)

From the esterification of euscaphic acid (**7**) with diazomethane; mp: 120–122 °C (lit.: [40] 130–132 °C; [64] 122–124 °C; [65] 140 °C); $[\alpha]_D^{20} = +31.6°$ ($c = 0.46$, $CHCl_3$) (lit: +31° [66]); $R_F = 0.19$ (toluene/ethyl acetate/formic acid/*n*-heptane, 80:20:3:10); MS (ESI, MeOH): m/z (%) = 503.4 ($[M + H]^+$, 10), 520.3 ($[M + NH_4]^+$, 6), 525.5 ($[M + Na]^+$, 100), 556.9 ($[M + MeOH]^+$, 46).

4.8. (2 R, 3 R, 19 R) 2,3-Bis(acetyloxy)-19-hydroxyurs-12-en-28-carboxylic acid (**9**)

Acetylation of **1** (150 mg, 0.31 mmol) in dry pyridine (6 ml) with acetic anhydride for 12 h at 24 °C yielded after usual work-up and re-crystallization from toluene **9** as a colorless solid; mp 178–180 °C (lit.: [55] 186–189 °C); $[\alpha]_D^{20} = +5.8°$ ($c = 0.51$, $CHCl_3$) (lit.: +12° [25]; +6° [67]); $R_F = 0.45$ (toluene/ethyl acetate/formic acid/*n*-heptane, 80:20:3:10); IR (KBr): $\nu = 3433$ br, 2937 s, 1743 s, 1456 m, 1369 s, 1252 s, 1154 m, 1109 w, 1033 m, 965 m, 932 w, 866 w, 759 w, 642 w, 598 w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.31$ (dd, 1 H, $J_{12,\,11'} = 3.2$, $J_{12,\,11''} = 3.2$ Hz, H-12), 5.08 (ddd, 1 H, $J_{2ax,\,1ax} = 11.1$, $J_{2ax,\,3ax} = 10.3$, $J_{2ax,\,1eq} = 4.7$ Hz, H-2_{ax}), 4.73 (d, 1 H, $J_{3ax,\,2ax} = 10.3$ Hz, H-3_{ax}), 2.52 (m, 2 H, H-18, H-16_{ax}), 2.03 (s, 3 H, H-32 or H-34), 2.02 (m, 1 H, H-1_{eq}), 1.96 (m, 5 H, H-32 or H-34 and H-11′, H-11″), 1.79–1.47 (m, 8 H, H-6″, H-7″, H-9, H-15_{ax}, H-16_{eq}, H-21″, H-22′, H-22″), 1.42–1.35 (m, 2 H, H-6′, H-20), 1.32–1.27 (m, 2 H, H-7′, H-21′), 1.23 (s, 3 H, H-27), 1.18 (s, 3 H, H-29), 1.09 (m, 1 H, H-1_{ax}), 1.04 (s, 3 H, H-25), 0.99 (m, 1 H, H-15_{eq}), 0.96 (m, 1 H, H-5), 0.93 (d, 3 H, $J_{30,\,20} = 6.6$ Hz, H-30), 0.88 (s, 6 H, H-23, H-24), 0.70 (s, 3 H, H-26) ppm; ^{13}C NMR (125 MHZ, $CDCl_3$): $\delta = 184.2$ (C28), 170.8, 170.6 (C31, C33), 138.0 (C13), 128.8 (C12), 80.6 (C3), 73.0 (C19), 70.0 (C2), 54.7 (C5), 52.8 (C18), 47.7 (C17), 47.1 (C9), 43.9 (C1), 41.1 (C14), 41.0 (C20), 39.9 (C8), 39.3 (C4), 38.1 (C10), 37.4 (C22), 32.4 (C7), 28.4 (C23), 28.1 (C15), 27.3 (C29), 25.9 (C21), 25.2 (C16), 24.4 (C27), 23.7 (C11), 22.0, 20.9 (C32, C34), 18.2 (C6), 17.6 (C24), 16.9 (C26), 16.3 (C25), 16.1 (C30) ppm; MS (ESI, MeOH): m/z (%) = 571.4 ($[M - H]^-$, 100), 616.9 ($[M + HCO_2]^-$, 27); analysis for $C_{34}H_{52}O_7$ (572.77): C, 71.30; H, 9.15; found: C, 71.18; H, 9.23.

4.9. (2 R, 3 R, 19 R) 2-acetyloxy-3,19-dihydroxyurs-12-en-28-carboxylic acid (**10**) and (2 R, 3 R, 19 R) 3-acetyloxy-2,19-dihydroxyurs-12-en-28-carboxylic acid (**11**)

Acetylation of **1** (300 mg, 0.61 mmol) in dichloromethane (30 ml) containing dry pyridine (2 ml) with acetic anhydride (1 ml) for 3 h at 24 °C followed by usual aqueous work-up and chromatography (silica gel, *n*-pentane/ethyl acetate/ethanol, 17:10:1) afforded **10** (182 mg, 56%) and **11** (67 mg, 21%).

Data for **10**: colorless solid; mp 171–174 °C; $[\alpha]_D^{20} = +4.7°$ ($c = 0.51$, $CHCl_3$); $R_F = 0.23$ (toluene/ethyl acetate/formic acid/*n*-heptane, 80:20:3:10); IR (KBr): $\nu = 3510$ br, 2937 s, 1724 s, 1457 m, 1369 m, 1255 s, 1155 m, 1095 m, 1031 m, 961 m, 933 w, 865 w, 766 w, 660 w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.30$ (dd, 1 H, $J_{12,\ 11'} = 3.2$, $J_{12,\ 11''} = 3.2$ Hz, H-12), 4.92 (ddd, 1 H, $J_{2ax,\ 1ax} = 10.9$, $J_{2ax,\ 3ax} = 10.0$, $J_{2ax,\ 1eq} = 4.4$ Hz, H-2_{ax}), 3.18 (d, 1 H, $J_{3ax,\ 2ax} = 10.0$ Hz, H-3_{ax}), 2.51 (s, 1 H, H-18), 2.45 (m, 1 H, H-16_{ax}), 2.04 (s, 3 H, H-33), 1.99 (m, 1 H, H-1_{eq}), 1.99–1.94 (m, 2 H, H-11′, H-11″), 1.78–1.45 (m, 8 H, H-6″, H-7″, H-9, H-15_{ax}, H-16_{eq}, H-21″, H-22′, H-22″), 1.40–1.26 (m, 3 H, H-7′, H-20, H-21′), 1.22 (s, 3 H, H-27), 1.17 (s, 3 H, H-29), 1.03, 1.01 (each s, 6 H, H-23, H-25), 1.00 (m, 1 H, H-15_{eq}), 0.96 (m, 1 H, H-1_{ax}), 0.92 (d, 3 H, $J_{30,\ 20} = 6.6$ Hz, H-30), 0.86 (m, 1 H, H-5), 0.83 (s, 3 H, H-24), 0.69 (s, 3 H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 184.1$ (C28), 171.6 (C31), 138.0 (C13), 129.0 (C12), 80.8 (C3), 73.3 (C2), 73.1 (C19), 55.0 (C5), 52.8 (C18), 47.7 (C17), 47.1 (C9), 43.7 (C1), 41.1 (C14), 41.0 (C20), 40.0 (C8), 39.7 (C4), 38.3 (C10), 37.4 (C22), 32.5 (C7), 28.5 (C23), 28.1 (C15), 27.3 (C29), 25.9 (C21), 25.3 (C16), 24.5 (C27), 23.7 (C11), 21.3 (C32), 18.3 (C6), 17.0, 16.6, 16.3 (C24, C25, C26), 16.1 (C30) ppm; MS (ESI, MeOH): m/z (%) = 529.7 ($[M - H]^-$, 100), 575.3 ($[M + HCO_2]^-$, 13); analysis for $C_{32}H_{50}O_6$ (530.74): C, 72.42; H, 9.50; found: C, 72.36; H, 9.58.

Data for **11**: mp 190–192 °C; $[\alpha]_D^{20} = +0.99°$ ($c = 0.41$, MeOH); $R_F = 0.20$ (toluene/ethyl acetate/formic acid/*n*-heptane, 80:20:3:10); (KBr): $\nu = 3576$ m, 3432 br, 2930 s, 1737 s, 1689 s, 1461 m, 1369 s, 1253 s, 1158 m, 1104 m, 1049 m, 1031 m, 1005 m, 959 m, 934 w, 907 w, 868 w, 769 w, 650 w, 562 w cm^{-1}; ^1H NMR (500 MHz, CD_3OD): $\delta = 5.29$ (dd, 1 H, $J_{12,\ 11'} = 3.3$, $J_{12,\ 11''} = 3.3$ Hz, H-12), 4.51 (d, 1 H, $J_{3ax,\ 2ax} = 9.9$ Hz, H-3_{ax}), 3.76 (ddd, 1 H, $J_{2ax,\ 1ax} = 10.9$, $J_{2ax,\ 3ax} = 9.9$, $J_{2ax,\ 1eq} = 4.4$ Hz, H-2_{ax}), 2.58 (ddd, 1 H, $J_{16ax,\ 16eq} = 14.3$, $J_{16ax,\ 15ax} = 12.8$, $J_{16ax,\ 15eq} = 4.3$ Hz, H-16_{ax}), 2.50 (s, 1 H, H-18), 2.09 (s, 3 H, H-32), 2.04–1.97 (m, 3 H, H-1_{eq}, H-11′, H-11″), 1.83–1.41 (m, 9 H, H-6″, H-7″, H-9, H-15_{ax}, H-16_{eq}, H-20, H-21″, H-22′, H-22″), 1.35 (s, 3 H, H-27), 1.33–1.30 (m, 3 H, H-6′, H-7′, H-21′), 1.19 (s, 3 H, H-29), 1.03 (s, 3 H, H-23), 1.01–0.96 (m, 3 H, H-1_{ax}, H-5, H-15_{eq}), 0.93 (d, 3 H, $J_{30,\ 20} = 6.7$ Hz, H-30), 0.88, 0.87 (each s, 6 H, H-24, H-25), 0.80 (s, 3 H, H-26) ppm; ^{13}C NMR (125 MHz, CD_3OD): $\delta = 180.8$ (C28), 171.9 (C31), 138.7 (C13), 127.7 (C12), 84.4 (C3), 72.1 (C19), 66.1 (C2), 55.0 (C5), 53.6 (C18), 48.1 (C17), 47.1 (C9), 46.9 (C1), 41.7 (C20), 41.2 (C14), 39.7 (C8), 38.9 (C4), 37.7 (C10), 37.6 (C22), 32.6 (C7), 28.1 (C15), 27.7 (C24), 25.9 (C21), 25.6 (C29), 25.2 (C16), 23.4 (C27), 23.3 (C11), 19.7 (C32), 18.1 (C6), 16.7 (C25), 16.0 (C26), 15.6 (C23), 15.2 (C30) ppm; MS (ESI, MeOH): m/z (%) = 529.7 ($[M - H]^-$, 100), 575.3 ($[M + HCO_2]^-$, 15); analysis for $C_{32}H_{50}O_6$ (530.74): C, 72.41; H, 9.50; found: C, 72.31; H, 9.66.

4.10. (3 R, 19 R) methyl 3-acetyloxy-19-hydroxy-2-oxo-urs-12-en-28-carboxylate (**12**)

To a solution of **3** (100 mg, 0.20 mmol) in dry pyridine (4 ml) acetic anhydride (8 ml) was slowly added and stirring at 24 °C was continued for 24 h. The reaction mixture was poured into ice-cold water, and the precipitate was filtered off. Re-crystallization from methanol yielded **12** (72 mg, 66%) as a colorless solid; mp 218–220 °C; $[\alpha]_D^{20} = +72.2°$ ($c = 0.67$, $CHCl_3$); $R_F = 0.56$ (toluene/ethyl acetate/formic acid/*n*-heptane, 80:20:3:10); IR (KBr): $\nu = 3511$ br, 2935 s, 1721 s, 1458 m, 1396 m, 1371 m, 1292 m 1236 s, 1151 m, 1096 w, 1053 m, 1034 m, 1009 m, 969 w, 930 w, 866 w, 772 w, 691 w, 499 w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.28$ (dd, 1 H, $J_{12,\ 11'} = 2.5$, $J_{12,\ 11''} = 2.5$ Hz, H-12), 4.88 (s, 1 H, H-3_{ax}), 3.53 (s, 3 H, H-31), 2.54 (s, 1 H, H-18), 2.46 (m, 1 H, H-16_{ax}), 2.34 (d, 1 H, $J_{1eq,\ 1ax} = 12.2$ Hz, H-1_{eq}), 2.12 (d, 1 H, $J_{1ax,\ 1eq} = 12.2$ Hz, H-1_{ax}), 2.11 (s, 3 H, H-33), 1.88–1.85 (m, 3 H, H-9, H-11′, H-11″), 1.68–1.51 (m, 7 H, H-6′, H-6″, H-15_{ax}, H-16_{eq}, H-21″, H-22′, H-22″), 1.46 (m, 1 H, H-5), 1.37–1.30 (m, 3 H, H-7′, H-7″, H-20), 1.24 (s, 3 H, H-27), 1.21 (m, 1 H, H-21′), 1.15 (s, 3 H, H-29), 1.04 (s, 3 H, H-23), 0.99 (m, 1 H, H-15_{eq}), 0.88 (d, 3 H, $J_{30,\ 20} = 6.7$ Hz, H-30), 0.84, 0.79, 0.62 (each s, 9 H, H-24, H-25, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 204.2$ (C2), 178.2 (C28), 170.5 (C32), 138.3 (C13), 128.2 (C12), 84.1 (C3), 73.1 (C19), 55.2 (C5), 53.9 (C1), 53.2 (C18), 51.6 (C31), 47.9 (C17), 47.1 (C9), 43.6, 43.1 (C4, C10), 41.3 (C14), 41.1 (C20), 40.3 (C8), 37.3 (C22), 32.4 (C7), 29.0 (C23), 28.2 (C15), 27.4 (C29), 26.0 (C21), 25.4 (C16), 24.3 (C27), 23.6 (C11), 20.6 (C33) 18.6 (C6), 17.5, 16.1, 15.9 (C24, C25, C26), 16.2 (C30); MS (ESI, MeOH): m/z (%) = 543.4 ($[M + H]^+$, 39), 560.6 ($[M + NH_4]^+$, 38), 565.5 ($[M + Na]^+$, 100), 581.4 ($[M + K]^+$, 18), 597.0 ($[M + Na + MeOH]^+$, 25); analysis for $C_{33}H_{50}O_6$ (542.75): C, 73.03; H, 9.29; found: C, 72.87; H, 9.41.

4.11. (2 R, 19 R) methyl 2-acetyloxy-19-hydroxy-3-oxo-urs-12-en-28-carboxylate (**13**)

Following the procedure given for **12**, from **4** (100 mg, 0.20 mmol), pyridine (4 ml) and acetic anhydride (8 ml) **13** (74 mg, 68%) was obtained as a colorless solid; mp 101–104 °C; $[\alpha]_D^{20} = +39.3°$ ($c = 0.46$, $CHCl_3$); $R_F = 0.61$ (toluene/ethyl acetate/formic acid/*n*-heptane, 80:20:3:10); IR KBr: $\nu = 3545$ br, 2936 s, 2878 m, 1724 s, 1458 m, 1371 s, 1235 s, 1152 s, 1093 m, 1032 m, 1011 m, 960 m, 931 w, 906 w, 866 w, 804 w, 772 w, 704 w, 655 w, 601 w, 485 w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.59$ (dd, 1 H, $J_{2ax,\ 1ax} = 13.3$ Hz, $J_{2ax,\ 1eq} = 6.5$ Hz, H-2_{ax}), 5.33 (dd, 1 H, $J_{12,\ 11'} = 3.3$, $J_{12,\ 11''} = 3.3$ Hz, H-12), 3.59 (s, 3 H, H-31), 2.58 (s, 1 H, H-18), 2.49 (m, 1 H, H-16_{ax}), 2.19 (dd, 1 H, $J_{1eq,\ 1ax} = 12.5$ Hz, $J_{1eq,\ 2ax} = 6.5$ Hz, H-1_{eq}), 2.12 (s, 3 H, H-33), 2.02 (m, 2 H, H-11′, H-11″), 1.73–1.50 (m, 8 H, H-6″, H-7″, H-9, H-15_{ax}, H-16_{eq}, H-21″, H-22′, H-22″), 1.41–1.32 (m, 5 H, H-1_{ax}, H-6′, H-7′, H-20, H-21′), 1.27 (s, 3 H, H-25), 1.22 (s, 3 H, H-27), 1.18 (s, 3 H, H-29), 1.15 (m, 1 H, H-5), 1.13 (s, 3 H, H-24), 1.10 (s, 3 H, H-23), 1.02 (m, 1 H, H-15_{eq}), 0.92 (d, 3 H, $J_{30,\ 20} = 6.7$ Hz, H-30), 0.73 (s, 3 H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 209.3$ (C3), 178.3 (C28), 170.2 (C32), 138.5 (C13), 128.3 (C12), 73.1 (C19), 71.7 (C2) 57.0 (C5), 53.2 (C18), 51.6 (C31), 48.7 (C4), 47.8 (C17), 46.9 (C9), 45.6 (C1), 41.3 (C20), 41.1 (C14), 40.0 (C8), 37.8 (C10), 37.3 (C22), 32.4 (C7), 28.2 (C15), 27.4 (C29), 25.9 (C16), 25.4 (C21), 24.8 (C23), 24.4 (C27), 23.8 (C11), 21.3 (C24), 20.7 (C33), 19.2 (C6), 16.8 (C26), 16.0 (C30), 15.8 (C25) ppm; MS (ESI, MeOH): m/z (%) = 543.3 ($[M + H]^+$ 34), 560.4 ($[M + NH_4]^+$, 42), 565.5 ($[M + Na]^+$, 100), 581.4 ($[M + K]^+$, 43), 597.1 ($[M + Na + MeOH]^+$, 27); analysis for $C_{33}H_{50}O_6$ (542.75): C, 73.02; H, 9.29; found: C, 72.88; H, 9.31.

4.12. (2 R, 3 R, 19 R) methyl 2,3-bis(chloroacetyloxy)-19-hydroxy urs-12-en-28-carboxylate (**14**), (2 R, 3 R, 19 R) methyl 2-chloro acetyloxy-3,19-dihydroxyurs-12-en-28-carboxylate (**15**), and (2 R, 3 R, 19 R) methyl 3-chloroacetyloxy-2,19-dihydroxyurs-12-en-28-carboxylate (**16**)

Compound **2** (1.03 g, 2.05 mmol) was acylated at 24 °C for 4 h with chloroacetyl chloride (282 mg, 2.5 mmol) and pyridine (0.2 ml) in dry dichloromethane (30 ml). After usual aqueous work-up and chromatography (silica gel, toluene/ethyl acetate/formic acid/*n*-heptane, 80/20/3/10), compounds **14** (341 mg, 26%), **15** (463 mg, 40%) and **16** (124 mg, 11%) were obtained.

Data for **14**: colorless solid; mp 92–93 °C; $[\alpha]_D^{20} = -8.11°$ ($c = 0.39$, $CHCl_3$); $R_F = 0.77$ (toluene/ethyl acetate/formic acid/*n*-heptane, 80/20/3/10); IR (KBr): $\nu = 3568$ br, 2948 s, 2878 m, 1736 s, 1457 m, 1412 m, 1397 m, 1370 m, 1310 s, 1262 s, 1168 s, 1071 w, 1023 m, 1001 m, 968 m, 928 m, 865 w, 791 m, 772 w, 696 w, 656 w, 587 w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.32$ (dd, 1 H, $J_{12,\ 11'} = 3.5$, $J_{12,\ 11''} = 3.5$ Hz, H-12), 5.17 (ddd, 1 H, $J_{2ax,\ 1ax} = 11.4$, $J_{2ax,\ 3ax} = 10.3$, $J_{2ax,\ 1eq} = 4.7$ Hz, 1 H, H-2_{ax}), 4.84 (d, 1 H, $J_{3ax,\ 2ax} = 10.3$ Hz, H-3_{ax}), 4.02, 3.93 (each s, 4 H, H-33, H-35), 3.58 (s, 3 H, H-31), 2.57 (s, 1 H, H-18), 2.49 (m, 1 H, H-16_{ax}), 2.07 (dd, 1 H, $J_{1eq,\ 1ax} = 12.3$, $J_{1eq,\ 2ax} = 4.7$, H-1_{eq}), 1.98–1.95 (m, H, H-11′, H-11″), 1.73–1.51 (m, 8 H, H-6″, H-7″, H-9, H-15_{ax}, H-16_{eq}, H-21″, H-22′, H-22″), 1.45–1.26 (m, 4 H, H-6′, H-7′, H-20, H-21′), 1.23 (s, 3 H, H-27), 1.18 (s, 3 H, H-29), 1.13 (m, 1 H, H-1_{ax}), 1.05 (s, 3 H, H-25), 1.02–0.98 (m, 2 H, H-5, H-15_{eq}), 0.92 (d, 3 H, $J_{30,\ 20} = 6.4$, H-30), 0.92 (s, 6 H, H-23, H-24), 0.67 (s, 3 H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 178.2$ (C28), 167.2, 166.9 (C32, C34), 138.3 (C13), 128.4 (C12), 82.3 (C3), 73.1 (C19), 72.2 (C2), 54.7 (C5), 53.1 (C18), 51.6 (C31), 47.8 (C17), 47.1 (C9), 43.6 (C1), 41.2 (C14), 41.1 (C20), 40.8 (C33, C35), 39.9 (C8), 39.6 (C4), 38.2 (C10), 37.3 (C22), 32.5 (C7), 28.3 (C23), 28.1 (C15), 27.4 (C29), 26.0 (C21), 25.4 (C16), 24.4 (C27), 23.7 (C11), 18.2 (C6), 17.5 (C24), 16.6 (C26), 16.3 (C25), 16.1 (C30) ppm; MS (ESI, MeOH): m/z (%) = 672.3 ($[M + NH_4]^+$, 14), 677.4 ($[M + Na]^+$, 100); analysis for $C_{35}H_{52}Cl_2O_7$ (655.69): C, 64.11; H, 7.99; found: C, 64.00; H, 8.09.

Data for **15**: colorless solid; mp 93–95 °C; $[\alpha]_D^{20} = -3.42°$ ($c = 0.50$, $CHCl_3$); $R_F = 0.46$ (toluene/ethyl acetate/formic acid/*n*-heptane, 80/20/3/10); IR (KBr): $\nu = 3528$ s, 2947 s, 2877 s, 1727 s, 1457 m, 1380 m, 1310 s, 1263 m, 1231 m, 1192 s, 1168 s, 1074 m, 1046 m, 1033 m, 1015 m, 964 m, 865 w, 791 w, 772 w, 661 w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) $\delta = 5.34$ (dd, 1 H, $J_{12,\ 11'} = 3.5$, $J_{12,\ 11''} = 3.5$ Hz, H-12), 5.03 (ddd, 1 H, $J_{2ax,\ 1ax} = 11.5$, $J_{2ax,\ 3ax} = 10.0$, $J_{2ax,\ 1eq} = 4.5$ Hz, H-2_{ax}), 4.06 (s, 2 H, H-33), 3.60 (s, 3 H, H-31), 3.25 (d, 1 H, $J_{3ax,\ 2ax} = 10.0$ Hz, 1 H, H-3_{ax}), 2.59 (s, 1 H, H-18), 2.50 (m, 1 H, H-16_{ax}), 2.03 (dd, 1 H, $J_{1eq,\ 1ax} = 12.2$, $J_{1eq,\ 2ax} = 4.5$ Hz, H-1_{eq}), 1.99–1.96 (m, 2 H, H-11′, H-11″), 1.74–1.48 (m, 8 H, H-6″, H-7″, H-9, H-15_{ax}, H-16_{eq}, H-21″, H-22′, H-22″), 1.45–1.39 (m, 2 H, H-6′, H-20), 1.33 (m, 1 H, H-7′), 1.31 (m, 1 H, H-21′), 1.25 (s, 3 H, H-27), 1.19 (s, 3 H, H-29), 1.06, 1.03 (each s, 6 H, H-23, H-25), 1.02 (m, 1 H, H-1_{ax}), 0.99 (m, 1 H, H-15_{eq}), 0.93 (d, 3 H, $J_{30,\ 20} = 6.7$ Hz, H-30), 0.89 (m, 1 H, H-5), 0.87 (s, 3 H, H-24), 0.68 (s, 3 H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 178.3$ (C28), 167.5 (C32), 138.2 (C13), 128.7 (C12), 80.5 (C3), 75.6 (C2), 73.1 (C19), 55.0 (C5), 53.2 (C18), 51.6 (C31), 47.9 (C17), 47.1 (C9), 43.4 (C1), 41.2 (C14), 41.1 (C20, C33), 39.9 (C4, C8), 38.3 (C10), 37.3 (C22), 32.6 (C7), 28.5 (C23), 28.1 (C15), 27.4 (C29), 26.0 (C21), 25.4 (C16), 24.5 (C27), 23.7 (C11), 18.4 (C6), 16.6, 16.2 (C24, C25, C26), 16.1 (C30) ppm; MS (ESI, MeOH): m/z (%) = 601.4 ($[M + Na]^+$, 100); analysis for $C_{33}H_{51}ClO_6$ (579.21): C, 68.43; H, 8.88; found: C, 68.32; H, 9.03.

Data for **16**: colorless solid; mp 108–113 °C; $[\alpha]_D^{20} = +13.82°$ ($c = 0.47$, $CHCl_3$); $R_F = 0.33$ (toluene/ethyl acetate/formic acid/*n*-heptane, 80/20/3/10); IR (KBr): $\nu = 3528$ br, 2946 s, 2878 s, 1725 s, 1669 m, 1456 m, 1380 m, 1311 s, 1263 s, 1230 s, 1192 s, 1152 s, 1095 m, 1043 m, 1016 m, 988 m, 969 m, 929 m, 866 w, 788 w, 772 w, 698 w, 659 w, 465 w cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.34$ (dd, 1 H, $J_{12,\ 11'} = 3.5$, $J_{12,\ 11''} = 3.5$ Hz, H-12), 4.58 (d, 1 H, $J_{3ax,\ 2ax} = 10.0$ Hz, H-3_{ax}), 4.14 (s, 2 H, H-33), 3.82 (ddd, 1 H, $J_{2ax,\ 1ax} = 11.5$, $J_{2ax,\ 3ax} = 10.0$, $J_{2ax,\ 1eq} = 4.4$ Hz, H-2_{ax}), 3.59 (s, 3 H, H-31), 2.59 (s, 1 H, H-18), 2.51 (m, 1 H, H-16_{ax}), 2.07 (dd, 1 H, $J_{1eq,\ 1ax} = 12.6$, $J_{1eq,\ 2ax} = 4.4$, 1 H, H-1_{eq}), 2.02–1.98 (m, 2 H, H-11′, H-11″), 1.74–1.48 (m, 8 H, H-6″, H-7″, H-9, H-15_{ax}, H-16_{eq}, H-21″, H-22′, H-22″), 1.43–1.38 (m, 2 H, H-6′, H-20), 1.33 (m, 1 H, H-7′), 1.26 (s, 3 H, H-27), 1.24 (m, 1 H, H-21′), 1.21 (s, 3 H, H-29), 1.06–1.01 (m, 3 H, H-1_{ax}, H-5, H-15_{ax}), 0.99, 0.91, 0.89 (each s, 9 H, H 23, H-24, H-25), 0.93 (d, 3 H, $J_{30,\ 20} = 6.7$ Hz, H-30), 0.68 (s, 3 H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 178.3$ (C28), 168.3 (C32), 138.2 (C13), 128.7 (C12), 87.1 (C3), 73.2 (C19), 67.3 (C2), 55.0 (C5), 53.2 (C18), 51.6 (C31), 47.8 (C17), 47.6 (C1), 47.1 (C9), 41.2 (C14), 41.1 (C20, C33), 39.9 (C8), 39.4 (C4), 38.1 (C10), 37.3 (C22), 32.5 (C7), 28.5 (C23), 28.1 (C15), 27.3 (C29), 25.9 (C21), 25.4 (C16), 24.4 (C27), 23.7 (C11), 18.3 (C6), 17.5, 16.6, 16.4 (C24, C25, C26), 16.1 (C30) ppm; MS (ESI, MeOH): m/z (%) = 601.4 ($[M + Na]^+$, 100); analysis for $C_{33}H_{51}ClO_6$ (579.21): C, 68.43; H, 8.88; found: C, 68.27; H, 8.99.

Acknowledgments

We like to thank Dr. G. Kaluderovic for many helpful discussions and Dr. St. Schwarz for performing some preliminary biological testing. Many thanks are due to Dr. R. Kluge for the measurement of the MS spectra, to Dr. D. Ströhl for recording the NMR spectra, and to Dr. Th. Müller, Dept. Haematology/Oncology (Univ. Halle), for providing the cell lines. Support by "Gründerwerkstatt – Biowissenschaften" is gratefully acknowledged.

References

[1] M.S. Kemp, P.J. Holloway, R.S. Burden, 3β,19α-Dihydroxy-2-oxours-12-en-28-oic acid: a pentacyclic triterpene induced in the wood of *Malus pumila* Mill. infected with *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar., and a constituent of the cuticular wax of apple fruits, J. Chem. Res. Synop. (1985) 154–155.
[2] G.d.G. Rocha, M. Simoes, R.R. Oliveira, M.A.C. Kaplan, C.R. Gattass, 3β-acetyl tormentic acid induces apoptosis of resistant leukemia cells independently of P-gp/ABCB1 activity or expression, Invest. New Drugs 30 (2012) 105–113.
[3] G. Delgado, J. Hernandez, R. Pereda-Miranda, Triterpenoid acids from *Cunila lythrifolia*, Phytochemistry 28 (1989) 1483–1485.
[4] P. Potier, B. Das, A.M. Bui, M.M. Janot, A. Pourrat, H. Pourrat, Structure of tormentic acid, a pentacyclic triterpene acid isolated from the roots of *Potentilla tormentilla*, Bull. Soc. Chim. Fr. (1966) 3458–3465.
[5] D. Lontsi, N.F. Ngounou, A.L. Tapondjou, B.L. Sondengam, B. Bodo, M.-T. Martin, An E-ring γ-lactone pentacyclic triterpene from *Myrianthus serratus*, Phytochemistry 49 (1998) 2473–2476.
[6] A.L. Tapondjou, N.F. Ngounou, D. Lontsi, B. Sondengam, M.-T. Martin, B. Bodo, Pentacyclic triterpenes from *Myrianthus liberecus*, Phytochemistry 40 (1995) 1761–1764.
[7] T. Akihisa, S. Kamo, T. Uchiyama, H. Akazawa, N. Banno, Y. Taguchi, K. Yasukawa, Cytotoxic activity of *Perilla frutescens* var. *japonica* leaf extract is due to high concentrations of oleanolic and ursolic acids, J. Nat. Med. 60 (2006) 331–333.
[8] N. Banno, T. Akihisa, H. Tokuda, K. Yasukawa, H. Higashihara, M. Ukiya, K. Watanabe, Y. Kimura, J.-i. Hasegawa, H. Nishino, Triterpene acids from the leaves of *Perilla frutescens* and their anti-inflammatory and antitumor-promoting effects, Biosci. Biotechnol. Biochem. 68 (2004) 85–90.
[9] J.H. Chen, Z.H. Xia, R.X. Tan, High-performance liquid chromatographic analysis of bioactive triterpenes in *Perilla frutescens*, J. Pharm. Biomed. Anal. 32 (2003) 1175–1179.
[10] F. Matsuyama, T. Yoshida, T. Hatano, S. Taniguchi, Cell Culture for Producing Triterpenes. WO 2005063227A1 20050714 (2005).
[11] E. Palme, A.R. Bilia, I. Morelli, Flavonols and isoflavones from *Cotoneaster simonsii*, Phytochemistry 42 (1996) 903–905.
[12] H. Tabata, Y. Kato, Y. Naohara, Method for Producing Extract Containing Triterpenes or Flavonoids, and Composition Containing the Same. JP 2011026265 A20110210 (2011).
[13] C. Murakami, K. Ishijima, M. Hirota, K. Sakaguchi, H. Yoshida, Y. Mizushina, Novel anti-inflammatory compounds from *Rubus sieboldii*, triterpenoids, are inhibitors of mammalian DNA polymerases, Biochim. Biophys. Acta Protein Struct. Mol. Enzymol. 1596 (2002) 193–200.
[14] L. Chu, L. Wang, Z. Zhang, H. Gao, J. Huang, B. Sun, L. Wu, Studies on the chemical constituents of *Potentilla anserine* L, Mod. Chin. Med. 10 (2008) 10–12.
[15] P. Liu, H. Duan, Q. Pan, Y. Zhang, Z. Yao, Triterpenes from herb of *Potentilla chinensis*, China J. Chin. Mater. Med. 31 (2006) 1875–1879.
[16] H. Luo, X. Zhao, Method for Extracting Total Triterpenoids from *Potentilla* Plant. CN101172131 A20080507 (2008).
[17] X. Zhao, H. Luo, Medical Application of Total Triterpene Extract of *Potentilla* Plant to Prepare the Medicine for Treating and Preventing Type II Diabetes Mellitus And/or Hyperlipemia. CN101172109 A20080507 (2008).
[18] J.L. Jin, Y.Y. Lee, J.E. Heo, S. Lee, J.M. Kim, H.S. Yun-Choi, Anti-platelet pentacyclic triterpenoids from leaves of *Campsis grandiflora*, Arch. Pharm. Res. 27 (2004) 376–380.
[19] Q. Zhang, Z. Chang, Q. Wang, Ursane triterpenoids inhibit atherosclerosis and xanthoma in LDL receptor knockout mice, Cardiovasc. Drugs Ther. 20 (2006) 349–357.

[20] A.S. Fogo, E. Antonioli, J.B. Calixto, A.H. Campos, Tormentic acid reduces vascular smooth muscle cell proliferation and survival, Eur. J. Pharmacol. 615 (2009) 50–54.
[21] N. Zeng, Y. Shen, L.-Z. Li, W.-H. Jiao, P.-Y. Gao, S.-J. Song, W.-S. Chen, H.-W. Lin, Anti-inflammatory triterpenes from the leaves of *Rosa laevigata*, J. Nat. Prod. 74 (2011) 732–738.
[22] H. Gao, L. Wu, M. Kuroyanagi, K. Harada, N. Kawahara, T. Nakane, K. Umehara, A. Hirasawa, Y. Nakamura, Antitumor-promoting constituents from *Chaenomeles sinensis* Koehne and their activities in JB6 mouse epidermal cells, Chem. Pharm. Bull. 51 (2003) 1318–1321.
[23] Y. Mizushina, M. Hirota, C. Murakami, T. Ishidoh, S. Kamisuki, N. Shimazaki, M. Takemura, M. Perpelescu, M. Suzuki, H. Yoshida, F. Sugawara, O. Koiwai, K. Sakaguchi, Some anti-chronic inflammatory compounds are DNA polymerase λ-specific inhibitors, Biochem. Pharmacol. 66 (2003) 1935–1944.
[24] Y. Mizushina, F. Sugawara, K. Sakaguchi, H. Yoshida, DNA polymerase λ inhibitors, anticancer agents, and antiinflammatory agents containing phenols, and method for screening of anticancer agents or antiinflammatory agents by testing of inhibitory activity against DNA polymerase λ. JP 2005029571 A 20050203 (2005).
[25] G.d.G. Rocha, M. Simoes, K.A. Lucio, R.R. Oliveira, K.M.A. Coelho, C.R. Gattass, Natural triterpenoids from *Cecropia lyratiloba* are cytotoxic to both sensitive and multidrug resistant leukemia cell lines, Bioorg. Med. Chem. 15 (2007) 7355–7360.
[26] J.-J. Cheng, L.-J. Zhang, H.-L. Cheng, C.-T. Chiou, I.J. Lee, Y.-H. Kuo, Cytotoxic hexacyclic triterpene acids from *Euscaphis japonica*, J. Nat. Prod. 73 (2010) 1655–1658.
[27] A. Numata, P. Yang, C. Takahashi, R. Fujiki, M. Nabae, E. Fujita, Cytotoxic triterpenes from a Chinese medicine, Goreishi, Chem. Pharm. Bull. 37 (1989) 648–651.
[28] J.M. Rollinger, D.V. Kratschmar, D. Schuster, P.H. Pfisterer, C. Gumy, E.M. Aubry, S. Brandstoetter, H. Stuppner, G. Wolber, A. Odermatt, 11β-Hydroxysteroid dehydrogenase 1 inhibiting constituents from *Eriobotrya japonica* revealed by bioactivity-guided isolation and computational approaches, Bioorg. Med. Chem. 18 (2010) 1507–1515.
[29] H.-J. An, I.-T. Kim, H.-J. Park, H.-M. Kim, J.-H. Choi, K.-T. Lee, Tormentic acid, a triterpenoid saponin, isolated from *Rosa rugosa*, inhibited LPS-induced iNOS, COX-2, and TNF-α expression through inactivation of the nuclear factor-κb pathway in RAW 264.7 macrophages, Int. Immunopharmacol. 11 (2011) 504–510.
[30] C.-T. Chang, S.-S. Huang, S.-S. Lin, S. Amagaya, H.-y. Ho, W.-C. Hou, P.-H. Shie, J.-B. Wu, G.-J. Huang, Anti-inflammatory activities of tormentic acid from suspension cells of *Eriobotrya japonica* ex vivo and in vivo, Food Chem. 127 (2011) 1131–1137.
[31] R. Csuk, A. Barthel, R. Kluge, D. Ströhl, Synthesis, cytotoxicity and liposome preparation of 28-acetylenic betulin derivatives, Bioorg. Med. Chem. 18 (2010) 7252–7259.
[32] R. Csuk, A. Barthel, R. Kluge, D. Ströhl, H. Kommera, R. Paschke, Synthesis and biological evaluation of antitumour-active betulin derivatives, Bioorg. Med. Chem. 18 (2010) 1344–1355.
[33] R. Csuk, A. Barthel, S. Schwarz, H. Kommera, R. Paschke, Synthesis and biological evaluation of antitumor-active gamma-butyrolactone substituted betulin derivatives, Bioorg. Med. Chem. 18 (2010) 2549–2558.
[34] R. Csuk, A. Barthel, R. Sczepek, B. Siewert, S. Schwarz, Synthesis, encapsulation and antitumor activity of new betulin derivatives, Arch. Pharm. 344 (2011) 37–49.
[35] R. Csuk, S. Schwarz, R. Kluge, D. Ströhl, Improvement of the cytotoxicity and tumor selectivity of glycyrrhetinic acid by derivatization with bifunctional aminoacids, Arch. Pharm. 344 (2011) 505–513.
[36] R. Csuk, S. Schwarz, B. Siewert, R. Kluge, D. Ströhl, Synthesis and antitumor activity of ring A-modified glycyrrhetinic acid derivatives, Z. Naturforsch. B 66 (2011) 521–532.
[37] S. Schwarz, R. Csuk, Synthesis and antitumour activity of glycyrrhetinic acid derivatives, Bioorg. Med. Chem. 18 (2010) 7458–7474.
[38] H. Kommera, G.N. Kaluderovic, J. Kalbitz, B. Dräger, R. Paschke, Small structural changes of pentacyclic lupane type triterpenoid derivatives lead to significant differences in their anticancer properties, Eur. J. Med. Chem. 45 (2010) 3346–3353.
[39] D. Lontsi, B.L. Sondengam, M.T. Martin, B. Bodo, Cecropioic acid, a pentacyclic triterpene from *Musanga cecropioides*, Phytochemistry 48 (1998) 171–174.
[40] C.M. Ojinnaka, J.I. Okogun, D.A. Okorie, Triterpene acids from *Myrianthus arboreus*, Phytochemistry 19 (1980) 2482–2483.
[41] Z.-j. Ma, Z.-j. Zhao, Studies on chemical constituents from stem barks of *Fraxinus paxiana*, China J. Chin. Mater. Med. 33 (2008) 1990–1993.
[42] Z. Hong, W. Chen, J. Zhao, Z. Wu, J. Zhou, T. Li, J. Hu, Hepatoprotective effects of *Rubus aleaefolius* Poir. and identification of its active constituents, J. Ethnopharmacol. 129 (2010) 267–272.
[43] Y. Tsuda, M. Hanajima, K. Yoshimoto, Utilization of sugars in organic-synthesis .13. Regioselective oxidation of a β-L-arabinopyranoside via cyclic tin intermediate – facile synthesis of 4-amino-4-deoxy-l-arabinose, an amino-sugar found in lipopolysaccharides of some *Salmonella* R-mutant strains, Chem. Pharm. Bull. 31 (1983) 3778–3780.
[44] Y. Ueno, M. Okawara, Oxidation using distannoxane .1. Selective oxidation of alcohols, Tetrahedron Lett. (1976) 4597–4600.
[45] H. Kommera, G.N. Kaluderovic, J. Kalbitz, R. Paschke, Synthesis and anticancer activity of novel betulinic acid and betulin derivatives, Arch. Pharm. 343 (2010) 449–457.
[46] Z. Darzynkiewicz, G. Juan, X. Li, W. Gorczyca, T. Murakami, F. Traganos, Cytometry in cell necrobiology: analysis of apoptosis and accidental cell death (necrosis), Cytometry 27 (1997) 1–20.
[47] D. Wlodkowic, J. Skommer, Z. Darzynkiewicz, Cytometry in cell necrobiology revisited. Recent advances and new vistas, Cytometry A 77A (2010) 591–606.
[48] M.E. Juan, U. Wenzel, V. Ruiz-Gutierrez, H. Daniel, J.M. Planas, Olive fruit extracts inhibit proliferation and induce apoptosis in HT-29 human colon cancer cells, J. Nutr. 136 (2006) 2553–2557.
[49] F.J. Reyes, J.J. Centelles, J.A. Lupianez, M. Cascante, (2α,3β)-2,3-dihydroxyolean-12-en-28-oic acid, a new natural triterpene from *Olea europea*, induces caspase dependent apoptosis selectively in colon adenocarcinoma cells, FEBS Lett. 580 (2006) 6302–6310.
[50] F.J. Reyes-Zurita, G. Pachon-Pena, D. Lizarraga, E.E. Rufino-Palomares, M. Cascante, J.A. Lupianez, The natural triterpene maslinic acid induces apoptosis in HT29 colon cancer cells by a JNK-p53-dependent mechanism, BMC Cancer 11 (2011) 154.
[51] F.J. Reyes-Zurita, E.E. Rufino-Palomares, J.A. Lupianez, M. Cascante, Maslinic acid, a natural triterpene from *Olea europaea* L., induces apoptosis in HT29 human colon-cancer cells via the mitochondrial apoptotic pathway, Cancer Lett. 273 (2009) 44–54.
[52] C. Li, Z. Yang, C. Zhai, W. Qiu, D. Li, Z. Yi, L. Wang, J. Tang, M. Qian, J. Luo, M. Liu, Maslinic acid potentiates the anti-tumor activity of tumor necrosis factor alpha by inhibiting NF-kappaB signaling pathway, Mol. Cancer 9 (2010) 73.
[53] C.R. Pungitore, J.M. Padron, L.G. Leon, C. Garcia, G.M. Ciuffo, V.S. Martin, C.E. Tonn, Inhibition of DNA topoisomerase I and growth inhibition of human cancer cell lines by an oleanane from *Junellia aspera* (Verbenaceae), Cell. Mol. Biol. 53 (2007) 13–17.
[54] Y.W. Hsum, W.T. Yew, P.L. Hong, K.K. Soo, L.S. Hoon, Y.C. Chieng, L.Y. Mooi, Cancer chemopreventive activity of maslinic acid: suppression of COX-2 expression and inhibition of NF-kappaB and AP-1 activation in Raji cells, Planta Med. 77 (2011) 152–157.
[55] T. Yamagishi, D.C. Zhang, J.J. Chang, D.R. McPhail, A.T. McPhail, K.H. Lee, Antitumor agents. Part 94. The cytotoxic principles of *Hyptis capitata* and the structures of the new triterpenes hyptatic acid A and B, Phytochemistry 27 (1988) 3213–3216.
[56] M.E. Juan, J.M. Planas, V. Ruiz-Gutierrez, H. Daniel, U. Wenzel, Antiproliferative and apoptosis-inducing effects of maslinic and oleanolic acids, two pentacyclic triterpenes from olives, on HT-29 colon cancer cells, Br. J. Nutr. 100 (2008) 36–43.
[57] R. Martin, J. Carvalho-Tavares, E. Ibeas, M. Hernandez, V. Ruiz-Gutierrez, M.L. Nieto, Acidic triterpenes compromise growth and survival of astrocytoma cell lines by regulating reactive oxygen species accumulation, Cancer Res. 67 (2007) 3741–3751.
[58] V. Alexander, Chapter nine – acridine orange as a probe for cell and molecular biology, in: W.T. Mason (Ed.), Fluorescent and Luminescent Probes for Biological Activity, second ed., Academic Press, London, 1999, pp. 117–135.
[59] H.M. Shapiro, Practical Flow Cytometry, third ed., Wiley-Liss, 1995.
[60] J.A.R. Salvador, Pentacyclic Triterpenes as Promising Agents in Cancer, Nova Science Pub Inc, 2010.
[61] E. Bossy-Wetzel, D.R. Green, Detection of apoptosis by annexin V labeling, Methods Enzymol. 322 (2000) 15–18.
[62] A.J. McGahon, S.J. Martin, R.P. Bissonnette, A. Mahboubi, Y. Shi, R.J. Mogil, W.K. Nishioka, D.R. Green, The end of the (cell) line: methods for the study of apoptosis in vitro, Methods Cell. Biol. 46 (1995) 153–185.
[63] A.H. Wyllie, Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation, Nature 284 (1980) 555–556.
[64] J.M. Fang, K.C. Wang, Y.S. Cheng, The chemical constituents from the aerial part of *Rosa laevigata*, J. Chin. Chem. Soc. (Taipei) 38 (1991) 297–299.
[65] G. Ruecker, R. Mayer, J.S. Shin-Kim, Triterpene saponins from the Chinese drug "Daxueteng" (Caulis sargentodoxae), Planta Med. 57 (1991) 468–470.
[66] S.-S. Lee, S.-N. Shy, K.C.S. Liu, Triterpenes from *Paliurus hemsleyanus*, Phytochemistry 46 (1997) 549–554.
[67] C. Terreaux, M.P. Maillard, M.P. Gupta, K. Hostettmann, Triterpenes and triterpene glycosides from *Paradrymonia macrophylla*, Phytochemistry 42 (1996) 495–499.