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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Platanic acid-derived methyl 20-amino-30-norlupan-28-oates are potent cytotoxic agents acting by apoptosis

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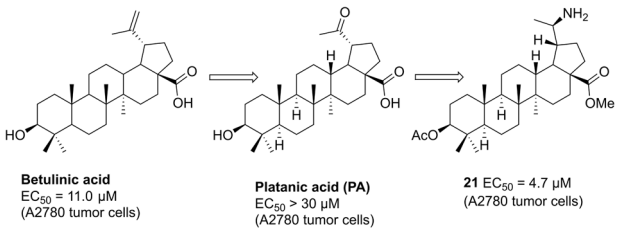
Abstract

A set of eight derivatives of betulinic acid (**2**) and 12 derivatives of platanic acid (**3**) has been prepared and screened for their cytotoxic activity using SRB assays. First synthetic approaches were focused on the preparation of augustic acid (**4**) analogs of **2** and **3** by introducing a second hydroxyl group on the A-ring of both triterpenoic acids leading to compounds **5**–**14**. Further structural modifications were performed at the C-20 keto group of the platanic acid backbone by its transformation into an oxime moiety and subsequent reduction to 20-amino-30-norlupan derivatives **17**–**24**. In the SRB assays low EC_{50} values were observed especially for *methyl (3β, 20 R)-3-acetyloxy-20-amino-30-norlupan-28-oate* (**21**), *methyl (3β, 20 S)-3-acetyloxy-20-amino-30-norlupan-28-oate* (**22**), and *(3β, 20 R)-3,28-diacetyloxy-20-amino-30-norlupane* (**24**) all holding an amino function at C-20. Compound **21** was selected for extensive biological testing that showed this compound to cause cell death by inducing apoptosis.

Graphical Abstract



Keywords Platanic acid · Betulinic acid · Cytotoxicity · Triterpenoids · Apoptosis

Introduction

Treatment of severe diseases has always been a challenge for humankind. While our early ancestors trusted in the use of extracts from leaves, fruits, and roots of plants, during the 18th and 19th century a dramatic change occurred. To put it into simple language: the use of natural products was out, and the trust in products synthesized in pharmaceutical labs was great. Nowadays we note a coexistence of these two ways to treat diseases: exploiting natural products (providing valuable synthetic backbones and scaffolds) and their derivatization (with sophisticated synthetic transformations).

There have been large efforts in surgery and irradiation of tumors. Furthermore, the development and the refinement of a broad variety of chemotherapeutics has been improved

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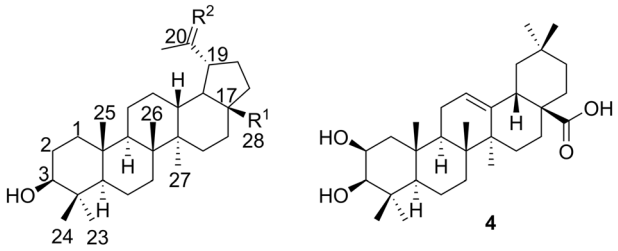
but the prognosis for many types of cancers remains poor. Our own synthetic work during recent years focused on the use of triterpenoic acids as valuable starting points for the synthesis of cytotoxic agents being intended to fight cancer. These triterpenoic acids (such as ursolic, oleanolic, tormentic, boswellic, maslinic, asiatic, or glycyrrhetinic acid) can conveniently be obtained from plants. Early in the history of humankind our ancestors made use of different parts of birch trees (*Betula platyphylla*). The bark of birches contains huge amounts of betulin (**1**, Figs. 1, 2) besides smaller amounts of betulinic acid (**2**) and several other triterpenes. While **1** is not cytotoxic at all for many human tumor cell lines, **2** shows better cytotoxic properties. As a result, many derivatives of betulinic acid have been prepared and examined in detail (Ali-Seyed et al. 2016; Csuk 2014; Gheorgheosu et al. 2014; Jonnalagadda et al. 2013; Paduch and Kandefer-Szerszen 2014; Periasamy et al. 2014; Salvador et al. 2014; Zhang et al. 2015). Recently, derivatives of platanic acid (**3**) and augustic acid (**4**) came in the focus of interest. Therefore, we decided to synthesize several analogs derived from **1** and **2**, and to investigate their potential as antitumor agents.



1 $R^1 = CH_2OH$, $R^2 = CH_2$
2 $R^1 = COOH$, $R^2 = CH_2$
3 $R^1 = COOH$, $R^2 = O$

Fig. 1 Structure of betulin (**1**), betulinic acid (**2**), platanic acid (**3**), and augustic acid (**4**) A final proof, however, for the structure of **8** was obtained after growing suitable crystals and performing a single crystal X-ray structure analysis. The results of this analysis are depicted and summarized in Fig. 2 and in the Experimental part

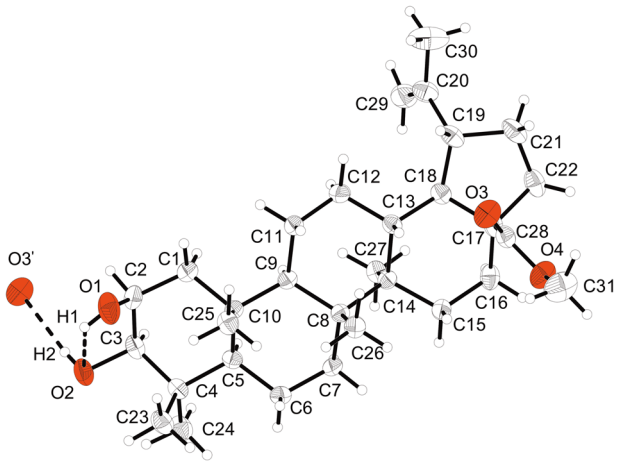


Fig. 2 Molecular structure of compound **8**. Thermal ellipsoids at the 50% probability level. Symmetry operator: i: x − 1/2, −y + 1/2, −z + 1. Intramolecular hydrogen bridge O1-H1···O2: d(O1···O2) 275.6 pm, intermolecular hydrogen bridge O2-H2···O3′: d(O2···O3) 289.6 pm. Formula $C_{31}H_{50}O_4$, $M = 486.71$, colorless crystal, $0.380 \times 0.380 \times 0.150$ mm^3, $a = 1113.90(7)$ pm, $b = 1408.39(9)$ pm, $c = 1751.92(11)$ pm, $\alpha = \beta = \gamma = 90°$, $V = 2.7484(3)$ nm^3, $\rho_{calc} = 1.176$ g cm^3, $\mu = 0.075$ mm^{-1}, $Z = 4$, orthorhombic, space group $P2_12_12_1$, $T = 220(2)$ K, 18,533 reflections, independent reflections 4827, $R_{int} = 0.0602$, Data/restraints/parameters 4827/0/352, Goof on F^2 2.204, $R_1 = 0.0442$(I > 2sigma(I)), wR2 = 0.0798(all data), CCDC: 1577240

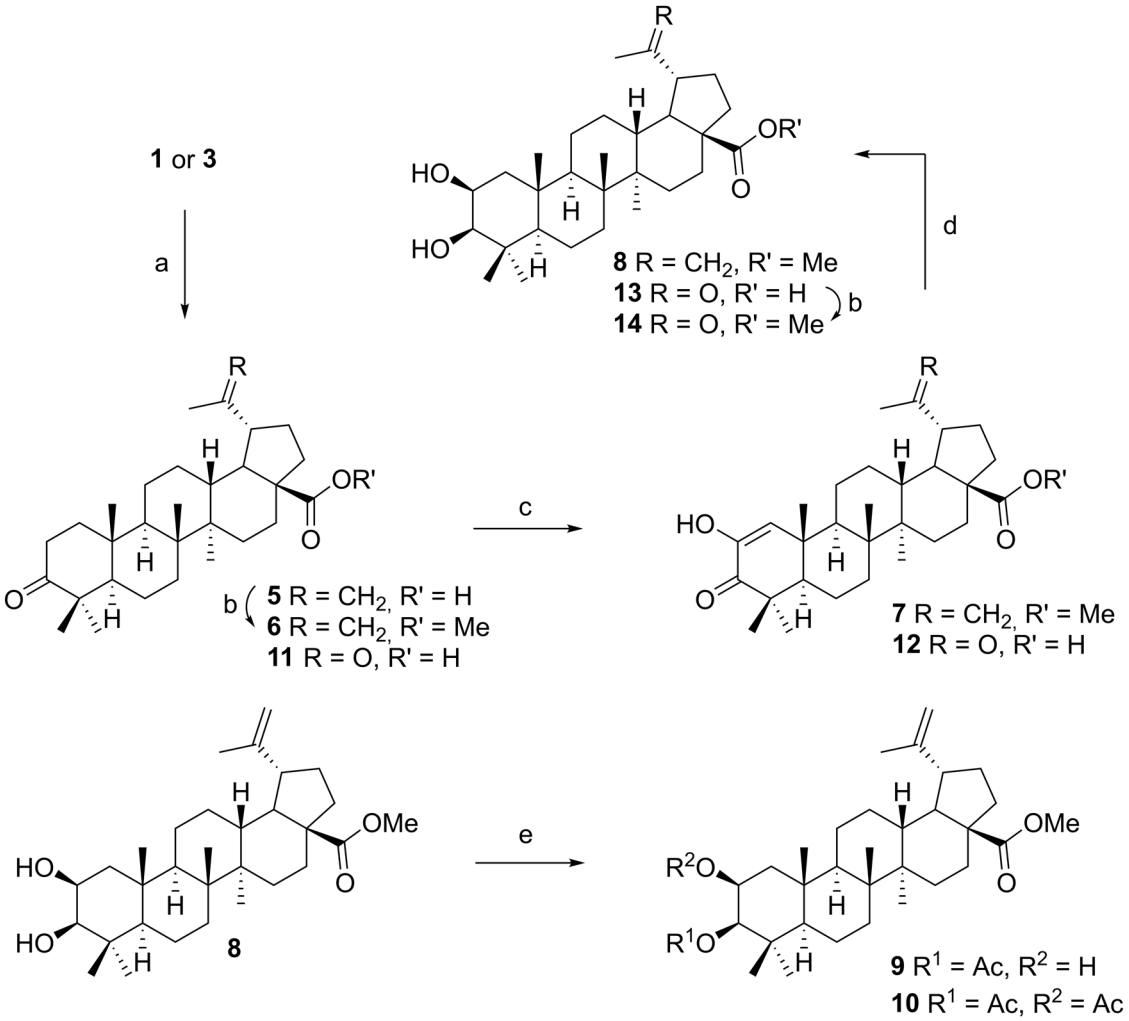
Following previous findings (Sommerwerk et al. 2016a, Sommerwerk et al. 2016b) reporting increased cytotoxicity accompanied by tumor–nontumor selectivity of augustic acid (**4**) derivatives, our objective was the introduction of a second hydroxyl moiety at C-2 of betulinic (**2**) and platanic acid (**3**). Data from literature (Ma et al. 2005, Ma et al. 2009) also revealed amino substituted triterpenoids, especially those derived from oleanolic or usrolic acid to be hightly cytotxic ($EC_{50} < 2$ µM). Therefore, we decided to synthesize several 20-amino derivatives of platanic and betulinic acid.

Results and Discussion

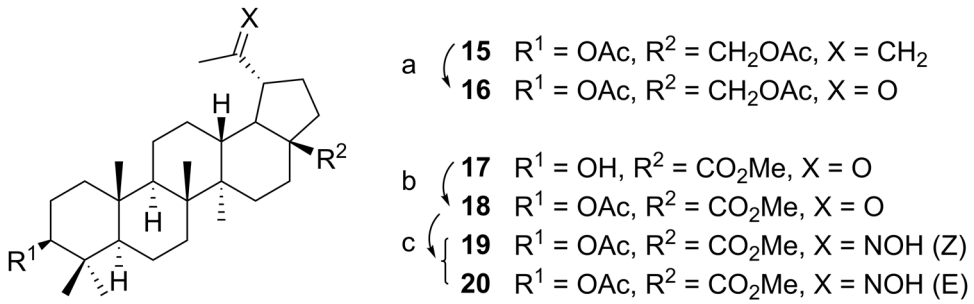
Soxhlet extraction of dry birch bark (*Betula platyphylla*) with diethyl ether delivered betulin (**1**) in excellent yields. Regioselective oxidation of **1** as previously described (Barthel et al. 2008) gave betulinic acid (**2**) while from the Jones oxidation of **1** betulonic acid (**5**) was obtained (Csuk et al. 2006; Barthel et al. 2008). Esterification of **5** gave methyl ester **6** (Xu et al. 2012). Reaction of **6** with potassium *tert*-butanolate in dry *tert*-butanol in the presence of air yielded 80% of **7**. Compound **7** was reduced with $NaBH_4$ in THF at 0 °C and 82% of **8** were isolated. Compound **8** is characterized in its ^{1}H NMR spectrum by the presence of a signal at $\delta = 4.05$ ppm (assigned to 2-H); 3-H was detected at $\delta = 3.15$ ppm. Interpretation of the NOESY spectra allowed to determine the absolute configuration of this molecule Scheme 1.

Acetylation of **8** in the presence of one equivalent of acetyl chloride in pyridine afforded a low yield of mono acetate **9**. The use of an excess of acetylating agent gave diacetate **10**, however, in excellent isolated yield.

Platanic acid derivatives **11**–**14** were prepared using the same procedure as described for the synthesis of **8**. Thus, Jones oxidation of platanic acid (**3**) gave 3-oxo-platanic acid (**11**) in almost quantitative yield. Reaction of **11** with potassium *tert*-butanolate in dry *tert*-butanol in the presence of air yielded 65% of **12**. Subsequent reduction of **12** with $NaBH_4$ in dry THF gave 2β-hydroxy-platanic acid (**13**) in



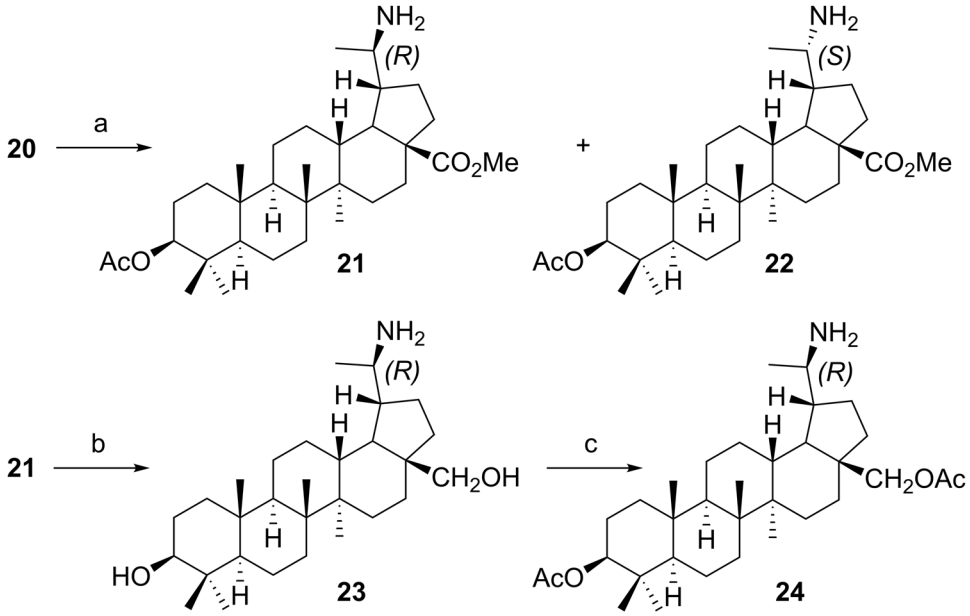
Scheme 1 Synthesis of betulin-derived compounds **5**–**10** and platanic acid-derived compounds **11**–**14**: **a** Jones oxidation, 4 h, 25 °C, 81% for **5** from **1** or 1 h, 25 °C, 98% for **11** from **3**; **b** MeI, K_2CO_3, DMF, 25 °C, 1 h, 70% (**6**) or 30 min 79% (**14**); **c** ^{t}BuOK, ^{t}BuOH, air, 40 °C, 60 min, 80% (for **7** from **6**) or ^{t}BuOK, ^{t}BuOH, THF, air, 50 °C, 4 h, 65% (for **12** from **11**); **d** $NaBH_4$, THF, EtOH, 25 °C, 60 min, 81% (for **8** from **7**) or $NaBH_4$, THF, MeOH, 25 °C, 22 h, 63% (for **13** from **12**); **e** AcCl (1 equiv.), pyridine, 25 °C, 2 days, 42% (**9**) or AcCl (3 equiv.), pyridine, 25 °C, 2 days, 52% (**10**)



Scheme 2 Synthesis of compounds **15**–**20**: **a** O_3, DCM, –60 °C, 60 min then Zn (powder) AcOH, 25 °C, 2 h, 73 %; **b** Ac_2O, pyridine, 25 °C, 4 h, 96%; **c** $HONH_2$·HCl, pyridine, 60 °C, 3 h, 13 % (for **19**) and 72 % (for **20**)

63% isolated yield. For comparison, compound **13** was transformed into its methyl ester **14** by esterification with MeI in DMF (L. Heller et al. 2017).

From the ozonolysis of betulin-3,28-diacetate (**15**) in dry DCM at −60 °C diacetate **16** was obtained (Scheme 2). Esterification of **3** gave methyl ester **17** whose acetylation yielded **18**. Reaction of **18** with hydroxylammonium chloride in dry pyridine at 60 °C for 3 h gave a mixture of oximes (20 *Z*)-**19** (13%) and (20 *E*)-**20** (72 %) that were easily separated by column chromatography. Their absolute configuration was deduced from the interpretation of NOESY spectra (L. Heller et al. 2017).



Scheme 3 Synthesis of amines **21**–**24**: **a** CH_3COONH_4, $NaBH_3CN$, MeOH, 25 °C then $TiCl_3$ (≥12% in aq. HCl (12%)), 0–25 °C, 20 h, yielding **21** (67%) and **22** (15%); **b** $LiAlH_4$, THF, 70 °C (6 h), 25 °C (24 h), 71%; **c** Boc_2O, pyridine, 25 °C, 30 min then Ac_2O, 16 h then TFA, DCM, 0–25 °C, 3 h, 74%

As previously described, reduction of the oximes **19** or **20** (L. Heller et al. 2017; Sun et al. 1998) with $NaBH_3CN$/$TiCl_3$/NH_4OAc in methanol gave the amines **21** and **22** in good yields (Scheme 3); these compounds were separated by chromatography, and their absolute configuration at C-20 was determined using ^1H NMR spectroscopy. Reaction of **21** with lithium aluminiumhydride in dry THF at 70 °C yielded **23**. Acetylation of both hydroxyl groups of **23** was performed by protecting the amino group at C-20 with di-*tert*-butyldicarbonate followed by a reaction with acetic anhydride in dry pyridine. Subsequent removal of the Boc protecting group with trifluoroacetic acid in DCM furnished **24** (Heller et al. 2017; Kahnt et al. 2018).

The cytotoxicity of all compounds was determined by sulforhodamine B (SRB) assays; the results are compiled in Table 1.

The betulinic acid derivatives **5**–**7** were found to be of moderate cytotoxicity or to be not cytotoxic at all. These results agree with results previously reported (Urban et al. 2004; Urban et al. 2005. The cytotoxicity of augustic acid analogs **8**–**10** was of the same order of magnitude as compound **5**. Thus, they show similar EC_{50} values as augustic acid (**4**). Parent platanic acid (**3**) and its analogs were not cytotoxic ($EC_{50} > 30$ µM) for the human tumor cell lines. This indicates that the presence of a carbonyl function at position C-20 has no influence onto cytotoxic properties of this compound. This finding was also deduced by comparing the results obtained for compounds **15** and **16**. For example, for betulin-3,28-diacetate (**15**) a $EC_{50} = 11.1$ µM was measured for ovarian carcinoma cells A2780, and diacetate **16** showed an EC_{50} value of 21.0 µM. The platanic acid derivatives **17** and **18** show no cytotoxic properties, too.

Table 1 Cytotoxicity of compounds **1**–**24**

#	518A2	A549	A2780	MCF-7	NIH 3T3
1	>30	>30	>30	>30	>30
2	18.9 ± 1.7	14.6 ± 1.6	11.0 ± 1.9	14.8 ± 1.9	13.1 ± 1.1
3	>30	>30	>30	>30	>30
4	25.7 ± 0.7	>30	18.9 ± 1.8	25.1 ± 2.2	>30
5	14.2 ± 1.1	13.1 ± 0.9	4.7 ± 0.6	12.0 ± 1.5	19.4 ± 1.2
6	>30	>30	21.0 ± 1.7	>30	>30
7	>30	>30	>30	>30	>30
8	20.4 ± 2.3	17.0 ± 1.1	8.9 ± 0.6	17.8 ± 1.3	25.2 ± 2.6
9	21.4 ± 1.9	20.9 ± 0.9	11.3 ± 1.5	21.0 ± 2.4	27.3 ± 2.5
10	24.3 ± 1.7	21.3 ± 1.1	10.7 ± 3.0	29.2 ± 2.9	17.1 ± 1.5
11	>30	>30	>30	>30	>30
12	>30	>30	20.6 ± 1.7	24.8 ± 1.7	>30
13	>30	>30	>30	>30	>30
14	>30	>30	>30	>30	>30
15	10.2 ± 1.3	>30	11.1 ± 1.4	20.4 ± 2.6	>30
16	>30	>30	21.0 ± 1.7	>30	>30
17	>30	>30	>30	>30	>30
18	>30	>30	>30	>30	>30
19	9.7 ± 2.1	8.8 ± 0.7	12.9 ± 0.7	12.1 ± 0.3	12.3 ± 1.2
20	7.8 ± 2.5	8.2 ± 0.9	12.2 ± 0.5	12.4 ± 0.5	14.0 ± 2.4
21	4.1 ± 0.3	5.3 ± 0.7	4.7 ± 0.1	5.1 ± 0.1	5.6 ± 0.2
22	6.3 ± 0.2	6.6 ± 0.8	6.0 ± 0.1	4.6 ± 0.2	4.4 ± 0.6
23	23.7 ± 1.8	>30	>30	>30	16.0 ± 1.1
24	2.2 ± 0.1	2.3 ± 0.1	2.5 ± 0.1	2.1 ± 0.2	4.0 ± 0.7

EC_{50} values from SRB assays after 96 h of treatment are given in µM; the values are averaged from three independent experiments each performed in triplicate; confidence interval CI = 95%; positive control: betulinic acid (**2**)

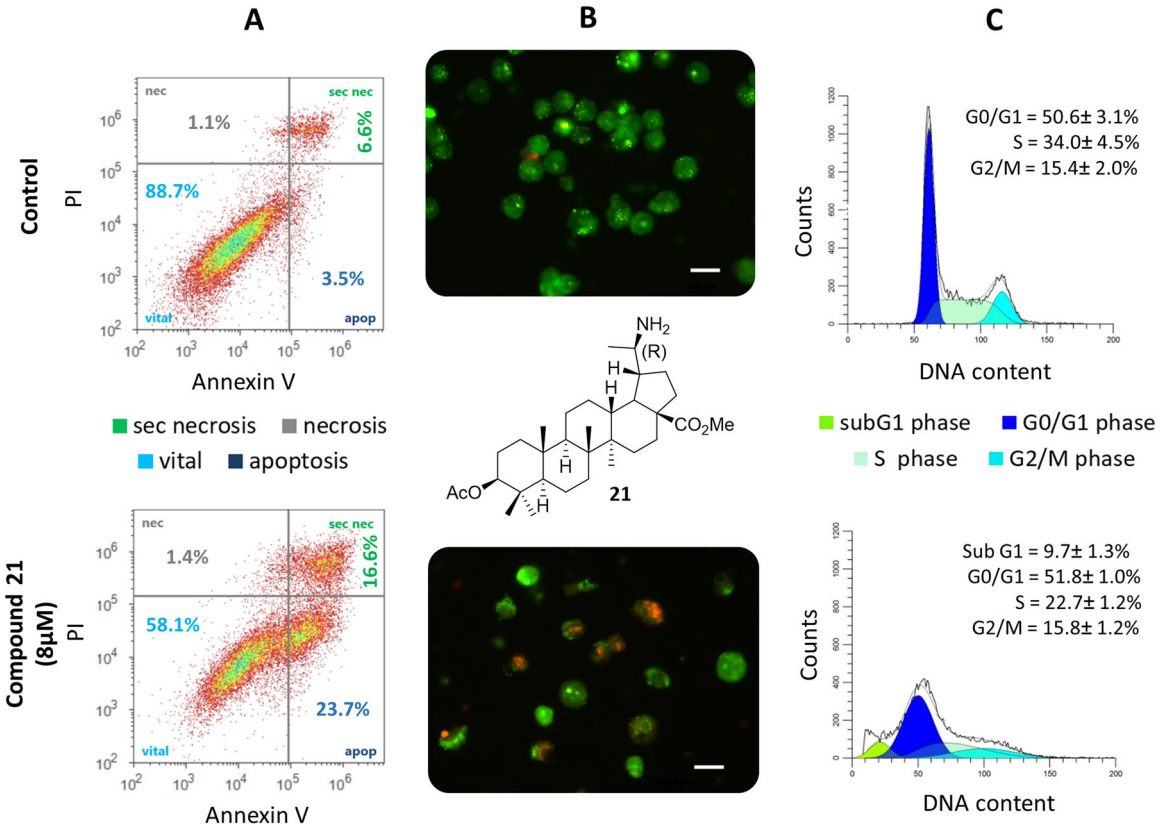


Fig. 3 **a** Annexin V-FITC/ PI assay: treatment of A2780 cells with **21** (8 µM) for 24 h. Examples of density plots determined by flow cytometry (Attune® Cytometric Software vl 1.2.5). **b** Fluorescence microscopic images (scale bar = 10 µm), AO and PI were used. **c** Representative examples for cell cycle evaluation via ModFit V 4.0.5

The idea of achieving enhanced activities by modifying C-20 was realized by synthesizing oximes **19** and **20**; in their SRB screening both compounds gave EC_{50} values in the range of 7.8–14.0 µM. The 20-amino derivatives **21** and **22**, however, were even more cytotoxic. In comparison, the 20-amino betulin derivative **23** showed low cytotoxicity. Thus, the most active compound of this series was (3*β*, 20 R)-3,28-diacetyloxy-20-amino-30-norlupane (**24**) with an EC_{50} value of 2.1 µM for MCF-7 cells.

For compound **21** extra experiments were performed by treating A2780 cells with **21** (8 µM) for one day. A dye exclusion test using acridine orange and propidium iodide (AO/PI) revealed that in some cells ruptures of the plasma membrane occurred, while the red-green color indicates a controlled cell death (Fig. 3b). To elucidate whether the growth inhibitory effects of **21** were due to cell cycle arrest, a flow cytometry analysis was performed. As shown in Fig. 3c, treatment of the cells with **21** resulted in a decrease in the S phase from 34.0 to 22.7% compared to control. However, there were no cell cycle arrests to identify. Furthermore, an accumulation (9.7% of the cell population) in sub-G1 phase, indicator of cell apoptosis, was detected after treating the cells with **21** exposure for one day. An annexin V-FITC/PI staining allowed a quantification of the apoptosis-inducing activity of compound **21**. Thus, treatment of A2780 cells with **21** resulted in about 23.7% apoptotic cells and 16.6% secondary necrotic cells, with 58.1% of the cells still being considered vital. Representative examples of the density plots determined by flow cytometry and the distributions are shown in Fig. 3a.

From these results, we assume that compound **21** is able to trigger programmed cell death, including apoptotic mechanisms.

Conclusion

Augustic acid analogs of betulinic and platanic acid have been prepared and screened for their cytotoxic activity using SRB assays. As a result, the compounds derived from betulinic acid revealed to be more potent than those from platanic acid. Therefore, further structural modifications were performed, which showed that the replacement of the keto group at position C-20 improves the cytotoxicity of the compounds. The best results were observed for the 20-amino derivatives **21**–**24**. All of these compounds showed EC_{50} values < 7 µM. The highest cytotoxic activity was

observed for *(3β, 20 R)-3,28-diacetyloxy-20-amino-30-norlupane* (**24**, $EC_{50} = 2.1 \pm 0.2$, MCF-7). Compound **21** was selected for extensive biological testing, that showed this compound mainly acts by apoptosis.

Experimental

General

NMR spectra were recorded using the Varian spectrometers Gemini 2000 or Unity 500 (δ given in ppm, J in Hz; typical experiments: H-H-COSY, HMBC, HSQC, and NOESY), MS spectra were taken on a Finnigan MAT LCQ 7000 (electrospray, voltage 4.1 kV, sheath gas nitrogen) instrument. The optical rotations were measured on a Perkin–Elmer polarimeter at 20 °C; TLC was performed on silica gel (Merck 5554, detection with cerium molybdate reagent); melting points are uncorrected (*Leica* hot stage microscope), and elemental analyses were performed on a Foss-Heraeus Vario EL (C-HNS) unit. IR spectra were recorded on a Perkin–Elmer FT-IR spectrometer Spectrum 1000. The solvents were dried according to usual procedures. The purity of the compounds was determined by HPLC and found to be >96%. Platanic acid (**3**) was obtained from Betulinines (Stříbrná Skalice, Czech Republic) in bulk quantities. Fluorescence microscopic images were recorded on an Axioskop 20 with an AxioCam MR3 (Carl Zeiss AG). Flow cytometric experiments were performed on an Attune acoustic focusing cytometer (Life Technologies GmbH). Data sets for the crystal structure were collected with a STOE-IPDS2 diffractometer. Programs used: structure solution SHELXS-97 (Sheldrick 2008); structure refinement SHELXL-97 (Sheldrick 2008), and graphics Diamond (Diamond 1997).

Synthesis

Isolation of betulin (1)

Soxhlet extraction of dry birch bark (*Betula platyphylla*, 180 g) with diethyl ether for two days followed by chromatography (silica gel, toluene/EtOAc/heptane/HCOOH, 80/20/10/3) gave **1** (39.6 g, 22%) as a colorless solid; m.p. 253–255 °C, lit.: 254–255 °C (Jarolim et al. 1961) $[\alpha]_D = +26.5°$ ($c = 0.9$, $CHCl_3$), lit.: $[\alpha]_D = +22.6°$ ($c = 0.1$, $CHCl_3$) (Gu et al. 2004).

Betulinic acid (2)

This compound was prepared as previously described (Barthel et al. 2008) from **1** and obtained as a colorless solid; m.p. 309–312 °C, lit.: 310–313 °C (Csuk et al. 2006) $[\alpha]_D = +8.7$ ($c = 0.4$, $CHCl_3$), lit.: $[\alpha]_D = +9.0$ ($c = 0.5$, $CHCl_3$) (Csuk et al. 2006).

Platanic acid (3)

This compound was obtained commercially from betulinines (Stříbrná Skalice, Czech Republic); as an alternative, it was obtained by ozonolysis of betulinic acid (**2**) (Vystrčil and Buděšínský 1970).

Augustic acid (4)

This compound was prepared from oleanolic acid as previously described (Sommerwerk et al. 2015a, 2015b); colorless solid; m.p. 309–311 °C, lit.: 308–310 °C (Wen et al. 2008), $[\alpha]_D = +88.1°$ ($c = 0.31$, THF), lit.:+93.5 ($c = 0.17$, pyridine) (Cheng et al. 2008).

Betulonic acid (5)

This compound was prepared from **1** by Jones oxidation as previously described ((Barthel et al. 2008) and obtained as a colorless solid; m.p. 249–252 °C, lit.: 250–254 °C (Urban et al. 2004) [15] $[\alpha]_D = +31.1°$ ($c = 0.3$, $CHCl_3$), lit.: $[\alpha]_D = +32.0$ ($c = 0.4$, $CHCl_3$) (Urban et al. 2004).

Methyl 3-oxo-lup-20(29)-en-28-oate (6)

Esterification of **5** with MeI/K_2CO_3 in dry DMF as previously described (Xu et al. 2012) gave **6** (85%) as a colorless solid; m.p. 162–165 °C, lit.: 161–165 °C (Urban et al. 2004); $[\alpha]_D = +27.2°$ ($c = 0.56$, MeOH), lit.: $[\alpha]_D = +28.0°$ ($c = 0.4$, $CHCl_3$) (Urban et al. 2004).

Methyl 2-hydroxy-3-oxolupa-1,20(29)-dien-28-oate (7)

To a suspension of potassium *tert*-butanolate (18.5 g, 164,9 mmol) in *tert*-butanol (175 ml) at 40 °C **6** (2.0 g, 4.3 mmol) was added, and stirring was continued for an additional 60 min. During this period, a stream of air was bubbled through the reaction mixture. Usual aqu. work-up followed by chromatography (silica gel, toluene/diethyl ether, 15:1) gave **7** (1.67 g, 80%) as a colorless solid; m.p. 122–126 °C, lit.: 122–124 °C (Urban et al. 2004; Urban et al. 2005); $[\alpha]_D = +26.1°$ ($c = 0.54$, MeOH), lit.: $[\alpha]_D = +3°$ ($c = 0.67$, $CHCl_3$) (Urban et al. 2005); $R_F = 0.77$ (toluene/diethyl ether, 5:1); IR (KBr): $\nu = 2948$s, 1727s, 1668m, 1646m, 1457m, 1406*m*, 1234m, 1156m, and 1133m cm^{-1}; UV/Vis (MeOH): λ_{max} (log ε) = 288 nm (4.09); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 6.41$ (s, 1H, 1-H), 5.86 (s, 1H, *OH*), 4.73 (m, 1H, 29-H_a), 4.59 (m, 1H, 29-H_b), 3.65 (s, 3H, 31-H), 2.98 (ddd, 1H, $J = 11.1$, 10.8, 4.5 Hz,

19-H), 2.29–2.19 (m, 2H, 13-H, 16-H_a), 1.93–1.85 (m, 2H, 22-H_a, 21-H_a), 1.81–1.72 (m, 1H, 12-H_a), 1.67 (s, 3H, 30-H), 1.63–1.30 (m, 15H, 22-H_b, 18-H, 16-H_b, 15-H_a, 6-H_a, 11-H_a, 6-H_b, 21-H_b, 7-H_a, 7-H_b, 9-H, 11-H_b, 5-H, 15-H_b, 12-H_b), 1.17 (s, 3H, 23-H), 1.10 (s, 3H, 25-H), 1.08 (s, 3H, 24-H), 0.96 (s, 3H, 27-H), 0.94 (s, 3H, 26-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 200.9$ (C-3), 176.4 (C-28), 150.1 (C-20), 143.8 (C-2), 128.7 (C-1), 109.7 (C-29), 56.6 (C-17), 54.1 (C-5), 51.3 (C-31), 49.5 (C-9), 47.0 (C-18), 45.9 (C-19), 44.0 (C-4), 42.8 (C-10), 41.6 (C-14), 38.7 (C-8), 38.4 (C-13), 37.0 (C-22), 34.1 (C-7), 32.2 (C-16), 30.7 (C-21), 29.7 (C-15), 27.2 (C-23), 25.5 (C-12), 21.7 (C-24), 21.3 (C-6), 20.2 (C-25), 19.5 (C-30), 18.9 (C-11), 16.6 (C-26), and 14.7 (C-27) ppm; MS (ESI, MeOH): $m/z = 1003$ (44% $[2M+K]^+$), 987 (100% $[2M+Na]^+$), 505 (36% $[M+Na]^+$), and 483 (25% $[M+H]^+$); analysis calcd for $C_{31}H_{46}O_4$ (482.71): C 77.14, H 9.61; found: C 76.89, H 9.83.

Methyl (2β, 3β) 2,3-dihydroxylup-20(29)-en-28-oate (8)

To a solution of **7** (2.28 g, 4.8 mmol) in dry THF (40 mL) and EtOH (7 mL) at 0 °C $NaBH_4$ (0.72 g, 19.2 mmol) was added. The mixture was allowed to warm to room temperature, and stirring was continued for another 60 min. Usual aq. work-up followed by chromatography (silica gel, hexanes/EtOAc, 8:2) gave **8** as a colorless solid (1.90 g, 81%); m.p. 219–221 °C, lit.: 115 °C (Krainova et al. 2016); $[\alpha]_D = +14.0°$ ($c = 0.70$, MeOH), lit.: $[\alpha]_D = +24.0°$ ($c = 0.4$, $CHCl_3$) [25]; $R_F = 0.33$ (toluene/EtOAc, 3:1); IR (KBr): $\nu = 3558$m, 3510s, 2952s, 2867m, 1709s, 1646w, 1454w, 1193m, 1173m, 1159m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.71$ (d, 1H, $J = 1.8$ Hz, 29-H_a), 4.58 (s, 1H, 29-H_b), 4.05 (m, 1H, 2-H), 3.64 (s, 3H, 31-H), 3.15 (d, 1H, 4.1 Hz, 3-H), 2.96 (ddd, 1H, $J = 11.0$, 10.6, 4.4 Hz, 19-H), 2.30–2.10 (m, 3H, 16-H_a, 13-H, 1-H_a), 1.95–1.80 (m, 2H, 21-H_a, 22-H_a), 1.70–1.65 (m, 2H, 12-H_a, 6-H_a), 1.66 (s, 3H, 30-H), 1.60–1.27 (m, 10H, 18-H, 6-H_b, 11-H, 22-H_b, 15-H_a, 16-H_b, 21-H_b, 7-H), 1.24–1.00 (m, 4H, 9-H, 15-H_b, 12-H_b, 1-H_b), 1.11 (s, 3H, 26-H), 0.96 (s, 3H, 25-H), 0.95 (s, 3H, 23-H), 0.93 (s, 3H, 27-H), 0.90 (s, 3H, 24-H), 0.73 (dd, 1H, $J = 10.8$, 2.8 Hz, 5-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 176.5$ (C-28), 150.4 (C-20), 109.5 (C-29), 78.5 (C-3), 71.2 (C-2), 56.6 (C-17), 55.4 (C-5), 51.3 (C-31), 51.1 (C-19), 49.6 (C-18), 47.0 (C-9), 44.6 (C-1), 42.6 (C-14), 40.9 (C-8), 38.4 (C-13), 38.3 (C-4), 37.0 (C-10), 37.0 (C-22), 34.4 (C-7), 32.3 (C-16), 30.7 (C-21), 29.7 (C-23), 29.7 (C-15), 25.7 (C-12), 21.2 (C-11), 19.5 (C-30), 18.2 (C-6), 17.2 (C-25), 17.2 (C-26), 16.1 (C-24), 14.8 (C-27) ppm; MS (ESI, MeOH): $m/z = 487.5$ (12.5% $[M+H]^+$), 509.6 (100% $[M+Na]^+$), 996.1 (55% $[2M+Na]^+$); analysis calcd for $C_{31}H_{50}O_4$ (486.74): C 76.50, H 10.35; found: C 74.31, H 10.37.

Methyl (2β, 3β) 3-acetyloxy-2-hydroxylup-20(29)-en-28-oate (9)

Acetylation of **8** (0.08 g, 0.16 mmol) with acetyl chloride (0.013 g, 0.014 mL, 0.19 mmol) in dry pyridine (3 mL) at room temperature for two days followed by usual work-up and chromatography (silica gel, hexanes/EtOAc, 8:2) gave **9** (0.036 g, 42%) as a colorless solid; m.p. 217 °C; $[\alpha]_D = +25.3°$ ($c = 0.5$, MeOH); $R_F = 0.28$ (hexanes/EtOAc, 8:2); IR (KBr): $\nu = 3496$s, 3073m, 2947s, 2869s, 1728s, 1643m, 1451s, 1433m, 1376s, 1318m, 1247s, 1189s, 1156s, 1134s, 1107m, 1070m, 1054m, 1030s, 980s, 883m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.72$ (s, 1H, 29-H_a), 4.60 (s, 1H, 29-H_b), 4.59 (d, $J = 4.1$ Hz, 1H, 3-H), 4.09 (m, 1H, 2-H), 3.64 (s, 3H, 31-H), 2.97 (dt, 1H, $J = 10.7$, 4.4 Hz, 19-H), 2.24–2.08 (m, 3H, 1-H_a, 13-H, 16-H_a), 2.12 (s, 3H, 33-H), 1.92–1.82 (m, 2H, 21-H_a, 22-H_a), 1.73–1.69 (m, 2H, 6-H_a, 12-H_a), 1.67 (s, 3H, 30-H), 1.60–1.18 (m, 12H, 6-H_b, 7-H, 9-H, 11-H, 15-H, 16-H_b, 18-H, 21-H_b, 22-H_b), 1.16 (s, 3H, 26-H), 1.14–1.07 (m, 1H, 1-H_b), 1.05 (s, 3H, 25-H), 1.03–0.96 (m, 1H, 12-H_b), 0.95–0.79 (m, 1H, 5-H), 0.93 (s, 3H, 27-H), 0.91 (s, 3H, 24-H), 0.84 (s, 3H, 23-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 176.5$ (C-28), 170.4 (C-32), 150.5 (C-20), 109.6 (C-29), 80.9 (C-3), 69.6 (C-2), 56.7 (C-5), 55.6 (C-17), 51.3 (C-31), 51.2 (C-9), 49.6 (C-18), 47.1 (C-19), 44.0 (C-1), 42.7 (C-14), 41.0 (C-8), 38.4 (C-13), 37.7 (C-10), 37.2 (C-22), 37.1 (C-4), 34.4 (C-7), 32.4 (C-16), 30.8 (C-21), 29.7 (C-15), 29.3 (C-23), 25.7 (C-12), 21.3 (C-33), 21.3 (C-11), 19.6 (C-30), 18.2 (C-6), 18.1 (C-25), 17.3 (C-26), 16.2 (C-24), 14.8 (C-27) ppm; MS (ESI, MeOH): $m/z = 1079.1$ (100% $[2M+Na]^+$), 551.5 (78% $[M+Na]^+$); analysis calcd for $C_{33}H_{52}O_5$ (528.77): C 74.96, H 9.91; found: C 74.71, H 10.13.

Methyl (2β, 3β) 2,3-bis(acetyloxy)lup-20(29)-en-28-oate (10)

Acetylation of **8** (0.55 g, 1.1 mmol) in dry pyridine (16 mL) with acetyl chloride (0.26 g, 0.24 mL, 3.3 mmol) at 25 °C for two days, followed by usual work-up and chromatography (silica gel, toluene/EtOAc, 5:1) gave **10** (0.33 g, 52%) as an amorphous solid; $[\alpha]_D = +21.3°$ ($c = 0.4$, MeOH); $R_F = 0.73$ (toluene/EtOAc, 3:1); IR (KBr): $\nu =$ 2949s, 1745s, 1643w, 1368m, 1252s, 1189m, 1155m, 1134m, 1031m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 5.29 (m, 1H, 2-H), 4.71 (s, 1H, 29-H_a), 4.58 (d, $J = 4.1$ Hz, 1H, 3-H), 4.57 (m, 1H, 29-H_b), 3.64 (s, 3H, 31-H), 2.96 (dt, 1H, $J = 10.9$, 4.5 Hz, 19-H), 2.24–2.12 (m, 2H, 16-H_a, 13-H), 2.03–1.92 (m, 1H, 1-H_a), 2.00 (s, 3H, 35-H), 1.99 (s, 3H, 33-H), 1.91–1.80 (m, 2H, 22-H_a, 21-H_a), 1.73–1.60 (m, 2H, 12-H_a, 6-H_a), 1.66 (s, 3H, 30-H), 1.59–1.17 (m, 12H, 18-H, 6-H_b, 22-H_b, 7-H, 16-H_b, 21-H_b, 15-H_a, 11-H, 9-H, 1-H_b), 1.16–1.05 (m, 1H, 15-H_b)), 1.07 (s, 3H, 26-H),

1.04–0.96 (m, 1H, 12-H_b), 0.99 (s, 3H, 25-H), 0.95–0.83 (m, 1H, 5-H), 0.93 (s, 3H, 27-H), 0.91 (s, 3H, 24-H), 0.85 (s, 3H, 23-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 176.4$ (C-28), 170.5 (C-34), 170.0 (C-32), 150.3 (C-20), 109.6 (C-29), 78.0 (C-3), 69.6 (C-2), 56.6 (C-17), 55.3 (C-5), 51.2 (C-31), 51.1 (C-9), 49.5 (C-18), 47.1 (C-19), 42.6 (C-1), 42.3 (C-14), 40.9 (C-8), 38.3 (C-13), 37.5 (C-4), 37.1 (C-10), 37.0 (C-22), 34.3 (C-7), 32.3 (C-16), 30.7 (C-21), 29.6 (C-15), 29.1 (C-23), 25.5 (C-12), 21.3 (C-35), 21.1 (C-11), 21.0 (C-33), 19.4 (C-30), 18.1 (C-6), 17.6 (C-25), 16.8 (C-26), 16.2 (C-24), 14.7 (C-27) ppm; MS (ESI, MeOH): *m/z* = 1163.2 (100% $[2M+Na]^+$), 593.5 (20% $[M+Na]^+$); analysis calcd for $C_{35}H_{54}O_6$ (570.81): C 73.65, H 9.54; found: C 85.42, H 9.72.

3-Oxo-platanic acid (11)

A suspension of **3** (10.5 g, 21.8 mmol) and silica gel (100 mL) in acetone (500 mL) was stirred for 30 min. After cooling to 0 °C Jones reagent (freshly prepared from CrO_3 (2.5 g, 24.9 mmol), water (10 mL), and concd. H_2SO_4 (2.5 mL)) was slowly added, and the mixture was stirred for 30 min at 25 °C. Then MeOH (5 mL) was added, and stirring was continued for another 30 min. The solvents were removed under diminished pressure, and the resulting solid was extracted with diethyl ether (Soxhlet apparatus, 6 h). Evaporation of the ether furnished **11** (9.74 g, 98%) as a white solid; m.p. 231–233 °C, lit.: 230–233 °C (Baratto et al. 2013); $[\alpha]_D = +5.4°$ ($c = 0.39$, $CHCl_3$), lit.: $[\alpha]_D = +31°$ ($c = 0.1$, $CHCl_3$) (Samoshina et al. 2003); $R_F = 0.5$ (toluene/EtOAc/heptane/HCOOH, 80:26:10:5); IR (KBr): ν = 32946s, 2869s, 1706s, 1683s, 1455m, 1379m, 1354m, 1321w, 1278m, 1243m, 1203m, 1166m, 1140m, 1082w cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.24$ (ddd, $J = 11.3$, 11.3, 4.9 Hz, 1H, 19-H), 2.51–2.37 (m, 2H, 2-H_a, 2-H_b), 2.32–2.26 (m, 1H, 16-H_a), 2.18 (s, 3H, 29-H), 2.19–1.96 (m, 4H, 21-H_a, 18-H, 13-H, 22-H_a), 1.91–1.84 (m, 1H, 1-H_a), 1.64–1.21 (m, 14H, 22-H_b, 21-H_b, 15-H_a, 16-H_b, 6-H_a, 11-H_a, 7-H_a, 7-H_b, 9-H, 1-H_b, 11-H_b, 6-H_b, 5-H, 15-H_b), 1.16–1.07 (m, 2H, 12-H_a, 12-H_b), 1.06 (s, 3H, 23-H), 1.01 (s, 3H, 27-H), 1.00 (s, 3H, 24-H), 0.94 (s, 3H, 26-H), 0.90 (s, 3H, 25-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 218.6$ (C-3), 212.4 (C-20), 181.9 (C-28), 56.4 (C-17), 54.9 (C-5), 51.3 (C-19), 49.8 (C-9), 49.2 (C-18), 47.4 (C-4), 42.4 (C-14), 40.7 (C-8), 39.7 (C-1), 37.8 (C-13), 37.0 (C-10), 36.8 (C-22), 34.2 (C-2), 33.6 (C-7), 31.5 (C-16), 30.2 (C-29), 29.8 (C-15), 28.4 (C-21), 27.3 (C-12), 26.9 (C-23), 21.5 (C-11), 21.1 (C-24), 19.8 (C-6), 16.1 (C-25), 15.8 (C-26), 14.8 (C-27) ppm; MS (ESI, MeOH): *m/z* = 455 (100% $[M-H]^-$), 911 (77% $[2M-H]^-$); analysis calcd for $C_{29}H_{44}O_4$ (456.67): C 76.27, H 9.71; found: C 76.07, H 9.88.

2-Hydroxy-3,20-dioxo-1-en-30-norlupan-28-oic acid (12)

To a suspension of potassium *tert*-butanolate (1.5 g, 13.37 mmol) in *tert*-butanol (26 ml) and tetrahydrofuran (3.5 mL) **11** (0.5 g, 1.09 mmol) was added, and stirring was continued for 4 h at 50 °C. During this period, a stream of air was bubbled through the reaction mixture. Usual aq. work-up followed by chromatography (silica gel, hexanes/EtOAc, 3:2) gave **12** (334 mg, 65%) as a colorless solid; m. p. 224–228 °C; $[\alpha]_D = +9.0°$ ($c = 0.66$, MeOH), $R_F = 0.46$ (toluene/EtOAc/heptane/HCOOH, 80:26:10:5); IR (KBr): ν = 3433s, 2947s, 2871m, 1709s, 1668s, 1455m, 1406m, 1382m, 1359m, 1236m, 1166m, 1132m, 1055m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 6.41$ (s, 1H, 1-H), 3.25 (ddd, $J = 11.2$, 11.2, 4.8 Hz, 1H, 19-H), 2.34–2.27 (m, 1H, 16-H_a), 2.19 (s, 3H, 29-H), 2.19–1.97 (m, 4H, 18-H, 21-H_a, 13-H, 22-H_a), 1.68–1.37 (m, 12H, 11-H_a, 9-H, 22-H_b, 5-H, 21-H_b, 15-H_a, 6-H_a, 6-H_b, 16-H_b, 7-H_a, 7-H_b, 11-H_b), 1.28–1.12 (m, 3H, 15-H_b, 12-H_a, 12-H_b), 1.20 (s, 3H, 23-H), 1.12 (s, 3H, 25-H), 1.09 (s, 3H, 24-H), 1.01 (s, 3H, 27-H), 0.98 (s, 3H, 26-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 212.1$ (C-20), 201.3 (C-3), 181.8 (C-28), 144.1 (C-2), 128.9 (C-1), 56.4 (C-17), 54.1 (C-5), 51.3 (C-19), 49.1 (C-18), 45.7 (C-9), 44.1 (C-4), 42.7 (C-14), 41.6 (C-8), 38.8 (C-10), 37.8 (C-13), 36.8 (C-22), 34.0 (C-7), 31.5 (C-16), 30.2 (C-29), 29.7 (C-15), 28.4 (C-21), 27.3 (C-23), 27.1 (C-12), 21.7 (C-24), 21.2 (C-11), 20.4 (C-25), 18.9 (C-6), 16.5 (C-26), 14.8 (C-27) ppm; MS (ESI, MeOH): *m/z* = 471 (100%, $[M+H]^+$), 493 (14%, $[M+Na]^+$); analysis calcd for $C_{29}H_{42}O_5$ (470.65): C 74.01, H 9.00; found: C 73.72, H 9.19.

(2β, 3β) 2,3-Dihydroxy-20-oxo-30-norlupan-28-oic acid (13)

To a solution of **12** (217 mg, 0.46 mmol) in dry THF (10 mL) and MeOH (2 mL) at 0 °C $NaBH_4$ (32 mg, 0.85 mmol) was added. The mixture was allowed to warm to room temperature, and stirring was continued for another 22 h. Usual aq. work-up followed by chromatography (silica gel, chloroform/acetone, 4:1) gave **13** as a colorless solid (138 mg, 63%); m.p. 266–270 °C; $[\alpha]_D = -6.2°$ ($c = 0.215$, $CHCl_3$); $R_F = 0.43$ (toluene/EtOAc/heptane/HCOOH, 80:26:10:5); IR (KBr): $\nu = 3436$br s, 2944s, 2870m, 1698s, 1450m, 1378m, 1358m, 1322w, 1278w, 1242m, 1190m, 1170m, 1134m, 1044m cm^{-1}; ^{1}H NMR (400 MHz, THF-d_8): $\delta = 3.91$ (ddd, $J = 4.0$, 4.0, 2.9 Hz, 1H, 2-H), 3.22 (ddd, $J = 11.2$, 11.2, 4.3 Hz, 1H, 19-H), 3.02 (d, $J = 4.0$ Hz, 1H, 3-H), 2.24 (ddd, $J = 12.6$, 3.1, 3.1 Hz, 1H, 16-H_a), 2.19–1.96 (m, 4H, 13-H, 1-H_a, 18-H, 21-H_a), 2.08 (s, 3H, 29-H), 1.92–1.86 (m, 1H, 22-H_a), 1.59–1.23 (m, 11H, 15-H_a, 6-H_a, 6-H_b, 11-H_a, 22-H_b, 21-H_b, 7-H_a, 7-H_b, 16-H_b, 11-H_b, 9-H), 1.16 (s, 3H, 25-H), 1.18–1.00 (m, 4H, 15-H_b, 12-H_a, 12-H_b, 1-H_b), 0.99 (s, 3H, 27-H), 0.95 (s,

6H, 23-H, 24-H), 0.94 (s, 3H, 26-H), 0.82–0.77 (dd, J = 10.3, 3.0 Hz, 1H, 5-H) ppm; ^{13}C NMR (100 MHz, THF-d_8): δ = 210.4 (C-20), 177.6 (C-28), 78.7 (C-3), 72.0 (C-2), 56.7 (C-17), 56.6 (C-5), 52.1 (C-19), 52.0 (C-9), 49.8 (C-18), 45.6 (C-1), 43.2 (C-14), 41.7 (C-8), 39.2 (C-4), 38.3 (C-13), 38.0 (C-10), 37.6 (C-22), 35.3 (C-7), 32.6 (C-16), 30.7 (C-15), 30.1 (C-23), 29.6 (C-29), 29.0 (C-21), 28.2 (C-12), 22.1 (C-11), 19.1 (C-6), 17.8 (C-24), 17.6 (C-25), 16.5 (C-26), and 15.1 (C-27) ppm; MS (ESI, MeOH): m/z = 475 (12%, $[M+H]^+$), 492 (100%, $[M+NH_4]^+$), 971 (86%, $[2M+Na]^+$); analysis calcd for $C_{29}H_{46}O_5$ (474.68): C 73.38, H 9.77; found: C 73.05, H 9.90.

Methyl (2β, 3β)-2,3-dihydroxy-20-oxo-30-norlupan-28-oate (14)

Esterification of **13** (98 mg, 0.21 mmol) in dry DMF (2 mL) with K_2CO_3 (120 mg, 0.87 mmol) and MeI (0.03 mL, 0.48 mmol) as previously described (Xu et al. 2012; Heller et al. 2017) gave **14** as a colorless solid (80 mg, 79%); m.p. 227–229 °C; $[\alpha]_D$ = –10.7° (c = 0.38, $CHCl_3$); R_F = 0.31 (toluene/EtOAc/heptane/HCOOH, 80:26:10:5); MS (ESI, MeOH): m/z = 506 (100%, $[M+NH_4]^+$), 999 (20%, $[2M+Na]^+$).

(3β)-3,28-Diacetyloxylup-20(29)-ene (15)

This compound was prepared according to (Gauthier et al. 2006) from **1**; colorless solid; m.p. 219–221 °C, lit.: 223–224 °C (Gauthier et al. 2006); $[\alpha]_D$ = +22.1° (c = 0.8, $CHCl_3$), lit.: 19.7° (c = 1.7, $CHCl_3$) (Gauthier et al. 2006).

(3β) 3,28-Diacetyloxy-30-norlup-20-one (16)

Ozone was bubbled through a solution of **15** (0.9 g, 1.71 mmol) in dry DCM (50 mL) at −60 °C for 60 min. After warming to room temperature powdered Zn (1.0 g, 0.015 mol) and glacial acetic acid (10 mL) were added within 1 h. The suspension was stirred for another hour, followed by usual aq. work-up and chromatography (silica gel, hexanes/EtOAc, 9:1) to afford **16** (0.66 g, 73%) as a colorless solid; m.p. 185–187 °C, lit.: 188–190 °C (Flekhter et al. 2005); $[\alpha]_D$ = –10.9° (c = 0.50, $CHCl_3$), lit.: $[\alpha]_D$ = –9.8° (c = 0.75, $CHCl_3$) (Vystrčil and Buděšínský 1970); R_F = 0.42 (hexanes/EtOAc, 8:2); IR (KBr): ν = 2948s, 2871s, 1737s, 1457m, 1392s, 1368s, 1316w, 1247s, 1169m, 1106w, 1032s, 979s cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.44 (dd, 1H, J = 10.9, 5.2 Hz, 3-H), 4.18 (d, 1H, J = 11.1 Hz, 28-H_a), 3.75 (d, 1H, J = 11.1 Hz, 28-H_b), 2.63 (dt, 1H, J = 11.3, 5.8 Hz, 19-H), 2.13 (s, 3H, 29-H), 2.11–1.95 (m, 2H, 15-H_a, 18-H), 2.05 (s, 3H, 31-H), 2.00 (s, 3H, 33-H), 1.88–1.75 (m, 2H, 16-H_a, 22-H_a), 1.70–1.13 (m, 15H, 1-H_a, 2-H, 6-H, 7-H, 9-H, 11-H, 12-H_a, 13-H, 15-H_b), 16-H_b, 22-H_b), 1.12–0.89 (m, 4H, 1-H_b, 2-H, 21-H), 0.99 (s, 3H, 26-H), 0.97 (s, 3H, 27-H), 0.82 (s, 6H, 24-H, 25-H), 0.81 (s, 3H, 23-H), 0.76 (d, 1H, J = 9.9 Hz, 5-H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 211.6 (C-20), 171.5 (C-32), 170.9 (C-30), 80.8 (C-3), 62.5 (C-28), 55.3 (C-5), 51.7 (C-19), 50.1 (C-9), 49.4 (C-18), 46.3 (C-17), 42.5 (C-14), 40.8 (C-8), 38.3 (C-1), 37.8 (C-4), 37.0 (C-10), 36.4 (C-13), 34.4 (C-22), 34.0 (C-7), 29.6 (C-29), 29.3 (C-16), 27.9 (C-23), 27.5 (C-21), 27.2 (C-12), 27.0 (C-15), 23.6 (C-2), 21.3 (C-33), 21.0 (C-31), 20.8 (C-11), 18.1 (C-6), 16.5 (C-24), 16.1 (C-25), 16.0 (C-26), 14.6 (C-27) ppm; MS (ESI, MeOH): m/z = 546.5 (16% $[M+NH_4]^+$), 551.5 (26% $[M+Na]^+$), 815.9 (63% $[3M+2Na]^{2+}$), and 1079.2 (100% $[2M+Na]^+$); analysis calcd for $C_{33}H_{52}O_5$ (528.77): C 74.96, H 9.91; found: C 74.65, H 10.16.

Methyl (3β) 3-hydroxy-20-oxo-30-norlupan-28-oate (17)

This compound was prepared according to (Sommerwerk et al. 2015a, 2015b) from **3**; colorless solid; m.p. 242–246 °C, lit.: 240–244 °C (Mayer 1996); R_F = 0.38 (toluene/ethyl acetate/n-heptane/HCOOH, 80:26:10:5); $[\alpha]_D$ = −28.2° (c = 0.52, $CHCl_3$), lit.: $[\alpha]_D$ = –30.0° (c = 0.40, $CHCl_3$) (Mayer 1996); MS (ESI, MeOH): m/z = 473 (60%, $[M+H]^+$), 490 (98%, $[M+NH_4]^+$), 495 (74%, $[M+Na]^+$, 505 (20%, $[M+H+MeOH]^+$), 731 (70%, $[M+2Na]^{2+}$), and 967 (98%, $[2M+Na]^+$).

Methyl (3β)-3-acetyloxy-20-oxo-30-norlupan-28-oate (18)

This compound was prepared according to (Vystrčil and Buděšínský 1970) from **17**; colorless solid m.p. 201–204 °C, lit.: 205–207 °C (Vystrčil and Buděšínský 1970); R_F = 0.66 (toluene/ethyl acetate/n-heptane/HCOOH, 80:25:30:4); $[\alpha]_D$ = −8.2° (c = 0.35, $CHCl_3$), lit.: $[\alpha]_D$ = –14.6° (c = 0.80, $CHCl_3$) [21]; MS (ESI, MeOH): m/z = 455 (70%, $[M+H\text{-}HOAc]^+$), 537 (26%, $[M+Na]^+$), 749 (100%, $[3M+2Na]^{2+}$), and 1051 (78%, $[2M+Na]^+$).

Methyl (3β, 20Z) 3-acetyloxy-20-hydroxyimino-30-norlupan-28-oate (19) and methyl (3β, 20E) 3-acetyloxy-20-hydroxyimino-30-norlupan-28-oate (20)

A solution of **18** (1.2 g, 2.34 mmol) and hydroxylammonium chloride (1.0 g, 14.95 mmol) in pyridine (12 mL) was stirred at 60 °C for 3 h. The solvent was removed under reduced pressure by re-evaporating with toluene (3 × 20 mL). The residue was dissolved in dichloromethane (20 mL) and washed successively with diluted HCl (0.1 M, 1 × 50 mL) and water (3 × 30 mL). The aqueous layer was extracted with dichloromethane (3 × 20 mL), and the combined organic layers were dried with Na_2SO_4, and the solvent was evaporated. The residue was fractionated by

column chromatography (silica gel, hexanes/ethyl acetate, 4:1) to afford compound **19** (164 mg, 13%) and compound **20** (892 mg, 72%).

Data for **19**: white solid; m.p. 244–247 °C, lit.: 245–248 °C (Vystrčil et al. 1986); $R_F = 0.48$ (toluene/ethyl acetate/n-heptane/HCOOH, 80:26:10:5); $[\alpha]_D = +45.0°$ ($c = 0.32$, $CHCl_3$), lit.: $[\alpha]_D = +30°$ ($c = 0.4$, $CHCl_3$) (Vystrčil et al. 1986); MS (ESI, MeOH): $m/z = 530$ (94%, $[M+H]^+$), 795 (34%, $[3M+2H]^{2+}$), 806 (10%, $[3M+H+Na]^{2+}$), and 1059 (100%, $[2M+H]^+$.

Data for **20**: white solid; m.p. 245–247 °C, lit.: 244–246 °C (Vystrčil et al. 1986); $R_F = 0.56$ (toluene/ethyl acetate/n-heptane/HCOOH, 80:26:10:5); $[\alpha]_D = +6.1°$ ($c = 0.36$, $CHCl_3$), lit.: $[\alpha]_D = +8.0°$ ($c = 0.4$, $CHCl_3$) (Vystrčil et al. 1986); MS (ESI, MeOH): $m/z = 530$ (100%, $[M+H]^+$), 1059 (44%, $[2M+H]^+$).

Methyl (3β, 20 R)-3-acetyloxy-20-amino-30-norlupan-28-oate (21) and methyl (3β, 20 S)-3-acetyloxy-20-amino-30-norlupan-28-oate (22)

To a solution of **20** (1.15 g, 2.17 mmol) and ammonium acetate (1.0 g, 13.0 mmol) in MeOH (120 mL), sodium cyanoborohydride (0.4 g, 6.4 mmol) was added under N_2 atmosphere. The reaction was cooled to 0 °C, and titanium trichloride (≥12% in HCl (12%), 4 mL, 3.9 mmol) was added dropwise during 30 min. The mixture was stirred at room temperature for 20 h and then treated with 2 N sodium hydroxid until pH = 10. The aqueous solution was extracted with DCM (3 × 200 mL), and the organic layer was dried over anhydrous Na_2SO_4; the solvent was concentrated in vacuo. The residue was fractionated by column chromatography (silica gel, chloroform/methanol/NH_4OH, 90:10:0.1) to afford **21** (751 mg, 67%) and **22** (168 mg, 15%).

Data for **21**: white solid; m.p. 208–211 °C; $R_F = 0.37$ ($CHCl_3$/MeOH/NH_4OH, 90:10:0.1); $[\alpha]_D = -18.4°$ ($c = 0.46$, $CHCl_3$); IR (KBr): $\nu = 3440$sbr, 2948s, 2872m, 1729s, 1636w, 1457m, 1384s, 1246s, 1166m, 1135m, 1028w, 980w cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 4.46$ (dd, $J = 10.6$, 5.7 Hz, 1H, 3-H), 3.66 (s, 3H, 30-H), 3.31 (dd, $J = 12.6$, 6.0 Hz, 1H, 20-H), 2.41 (dd, $J = 16.2$, 8.6 Hz, 1H, 19-H), 2.30–2.16 (m, 2H, 16-H_a, 13-H), 2.03 (s, 3H, 32-H), 1.84–1.74 (m, 1H, 22-H_a), 1.73–1.56 (m, 8H, 1-H_a, 21-H_a, 2-H, 12-H_a), 1.55–1.23 (m, 9H, H-18, 11-H_a, 6-H_a, 22-H_b, 16-H_a, 15-H_a, 6-Hb, 7-H, 11-H_b, 9-H, 12-H_b), 1.22 (d, $J = 6.6$ Hz, 3H, 29-H), 1.18–1.05 (m, 1H, 15-H_b), 1.04–0.94 (m, 1H, 1-H_b), 0.97 (s, 3H, 27-H), 0.90 (s, 3H, 26-H), 0.85 (s, 3H, 25-H), 0.83 (s, 6H, 23-H, 24-H), 0.80–0.72 (m, 1H, 5-H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 176.7$ (C-28), 171.0 (C-31), 81.0 (C-3), 57.1 (C-17), 55.6 (C-5), 51.5 (C-30), 50.2 (C-9), 49.9 (C-20), 48.5 (C-18), 44.4 (C-19), 42.6 (C-14), 40.8 (C-8), 38.5 (C-1), 38.2 (C-13), 37.9 (C-4), 37.2 (C-10), 36.6 (C-22), 34.4 (C-7), 31.6 (C-16), 29.8 (C-15), 28.1 (C-23), 27.1 (C-12), 23.8 (C-2), 22.3 (C-21), 21.7 (C-29), 21.4 (C-32), 21.0 (C-11), 18.3 (C-6), 16.6 (C-24), 16.2 (C-25), 16.1 (C-26), 14.5 (C-27) ppm; MS (ESI, MeOH): $m/z = 516$ (100%, $[M+H]^+$); analysis calcd for $C_{32}H_{53}NO_4$ (515.78): C 74.52, H 10.36, N 2.72; found: C 74.39, H 10.50, N 2.51.

Data for **22**: white solid, m.p. 218–220 °C; $R_F = 0.31$ ($CHCl_3$/MeOH/NH_4OH, 90:10:0.1); $[\alpha]_D = -10.7°$ ($c = 0.285$, $CHCl_3$); IR (KBr): $\nu = 2948$s, 2871m, 1732s, 1456w, 1368m, 1246s, 1156m, 1135m, 1029w, 980m cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 4.46$ (dd, $J = 10.4$, 5.9 Hz, 1H, 3-H), 3.65 (s, 3H, 30-H), 3.09 (dd, $J = 12.4$, 5.9 Hz, 1H, 20-H), 2.37–2.16 (m, 3H, 19-H, 16-H_a, 13-H), 2.03 (s 3H, 32-H), 1.85–1.77 (m, 1H, 22-H_a), 1.73–1.45 (m, 8H, 1-H_a, 12-H_a, 2-H, 21-H, 6-H_a, 11-H_a), 1.43–1.25 (m, 9H, H-18, 6-H_b, H-16$_b$, 15-H_a, 7-H, 11-H_b, 9-H, 22-H_b), 1.26–1.05 (m, 2H, 12-H_b, 15-H_b), 1.06 (d, $J = 6.6$ Hz, 3H, 29-H), 1.03–0.94 (m, 1H, 1-H_b), 0.95 (s, 3H, 27-H), 0.90 (s, 3H, 26-H), 0.85 (s, 3H, 25-H), 0.83 (s, 3H, 23-H), 0.83 (s, 3H, 24-H), 0.80–0.74 (m, 1H, 5-H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 176.9$ (C-28), 171.1 (C-31), 81.1 (C-3), 57.1 (C-17), 55.6 (C-5), 51.4 (C-30), 50.3 (C-9), 48.6 (C-18), 48.5 (C-20), 45.2 (C-19), 42.6 (C-14), 40.8 (C-8), 38.5 (C-1), 38.2 (C-13), 37.9 (C-4), 37.2 (C-10), 37.2 (C-22), 34.4 (C-7), 32.0 (C-16), 29.8 (C-15), 28.1 (C-23), 27.1 (C-12), 23.8 (C-2), 23.5 (C-29), 22.3 (C-21), 21.5 (C-32), 21.0 (C-11), 18.3 (C-6), 16.6 (C-24), 16.3 (C-25), 16.1 (C-26), 14.7 (C-27) ppm; MS (ESI, MeOH): $m/z = 516$ (100%, $[M+H]^+$); analysis calcd for $C_{32}H_{53}NO_4$ (515.78): C 74.52, H 10.36, N 2.72; found: C 74.34, H 10.47, N 2.43.

(3β, 20 R) 3-Hydroxy-20-amino-30-norlupan-28-ol (23)

Compound **23** (245 mg, 71%) was obtained from **21** (400 mg, 0.78 mmol) by reduction within $LiAlH_4$ in THF (1 M, 6 mL) as previously described (Heller et al. 2017); white solid, m.p. 240–246 °C; $R_F = 0.37$ ($CHCl_3$/MeOH/NH_4OH, 90:10:0.1); $[\alpha]_D = -47.8°$ ($c = 0.29$, pyridine); MS (ESI, MeOH): $m/z = 446$ (100%, $[M+H]^+$).

(3β, 20 R) 3,28-Diacetyloxy-20-amino-30-norlupane (24)

Di-*tert*-butyldicarbonate (70 mg, 0.32 mmol) was added to a solution of **23** (150 mg, 0.33 mmol) in pyridine (2 mL) (Kahnt et al. 2018), and stirring at 25 °C was continued for 30 min. Without additional work-up, acetic anhydride (0.1 mL, 1.06 mmol) was added, and the mixture was stirred at room temperature for 16 h. The reaction mixture was then diluted with toluene (3 × 20 mL), and all organic solvents were removed under reduced pressure to yield a crude solid (170 mg) which was used without further purification. After

dissolving the residue in dichloromethane (50 mL), the mixture was cooled to 0 °C, and trifluoroacetic acid (0.08 mL, 1 mmol) was added. Cooling was removed, and the reaction mixture was allowed to warm up to room temperature. Stirring was continued for additional 3 h, then the reaction mixture was concentrated to dryness under reduced pressure, re-dissolved in dichloromethane and re-evaporated. The residue was purified by column chromatography (silica gel, $CHCl_3$/MeOH/NH_4OH, 90:10:0.1) to afford **24** (130 mg, 74%) as a white solid; m.p. 204–206 °C; $R_F = 0.49$ ($CHCl_3$/MeOH/NH_4OH, 90:10:0.1); $[\alpha]_D = -24.4°$ ($c = 0.33$, $CHCl_3$), IR (KBr): $\nu = 3442$br s, 2946m, 1736m, 1636m, 1524w, 1458w, 1384vs, 1245m, 1031m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.44$ (dd, $J = 10.4$, 5.9 Hz, 1H, 3-H), 4.21 (d, $J = 11.1$ Hz, 1H, 28-H_a), 3.82 (d, $J = 11.1$ Hz, 1H, 28-H_b), 3.60 (q, $J = 6.1$ Hz, 1H, 20-H), 2.44 (ddd, $J = 11.1$, 11.1, 5.3 Hz, 1H, 19-H), 2.05 (s, 3H, 33-H), 2.03 (s, 3H, 31-H), 1.98 (td, $J = 11.4$, 3.7 Hz, 1H, 22-H_a), 1.92–1.83 (m, 1H, 16-H_a), 1.82–1.54 (m, 9H, H-18, 21-H, 13-H, 1-H_a, 2-H, 12-H_a, 15-H_a), 1.50–1.44 (m, 1H, 6-H_a), 1.43–1.31 (m, 5H, 6-Hb, 11-H, 7-H), 1.37 (d, $J =$ 6.6 Hz, 3H, 29-H), 1.30–1.20 (m, 2H, 9-H, H-16$_b$), 1.09–1.01 (m, 3H, 22-H_b, 15-H_b, 12-H_b), 1.04 (s, 3H, 27-H), 1.00–0.93 (m, 1H, 1-H_b), 0.99 (s, 3H, 25-H), 0.85 (s, 3H, 23-H), 0.83 (s, 3H, 24-H), 0.82 (s, 3H, 26-H), 0.79–0.70 (m, 1H, 5-H) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta = 171.6$ (C-32), 171.0 (C-30), 80.9 (C-3), 62.3 (C-28), 55.6 (C-5), 51.0 (C-20), 49.8 (C-9), 47.8 (C-18), 46.7 (C-17), 43.6 (C-19), 43.0 (C-14), 41.0 (C-8), 38.4 (C-1), 37.9 (C-4), 37.2 (C-13), 37.1 (C-10), 34.2 (C-7), 33.5 (C-22), 29.3 (C-16), 28.1 (C-23), 27.2 (C-15), 27.0 (C-12), 23.8 (C-2), 21.6 (C-21), 21.4 (C-31), 21.1 (C-33), 20.8 (C-11), 19.6 (C-29), 18.3 (C-6), 16.6 (C-24), 16.2 (C-25), 16.2 (C-26), 14.3 (C-27) ppm; MS (ESI, MeOH): $m/z = 530$ (100%, $[M+H]^+$); analysis calcd for $C_{33}H_{55}NO_4$ (529.81): C 74.81, H 10.46, N 2.64; found: C 74.77, H 10.63, N 2.37.

Biological assays

Cell lines and culture conditions

The cell lines used are human cancer cell lines: 518A2 (melanoma), A549 (alveolar basal epithelial adenocarcinoma), A2780 (ovarian carcinoma), MCF-7 (breast adenocarcinoma), and non-malignant mouse fibroblasts NIH 3T3. Cultures were maintained as monolayers in RPMI 1640 medium with L-glutamine (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) supplemented with 10% heat inactivated fetal bovineserum (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and penicillin/streptomycin (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) at 37 °C in a humidified atmosphere with 5% CO_2.

Cytotoxic assay

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (Kiton-Red S, ABCR) micro culture colorimetric assay. Cells were seeded into 96-well plates on day 0 at appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with six different concentrations (1, 3, 7, 12, 20, and 30 μM) minimum. The final concentration of DMSO/DMF never exceeded 0.5 %, which was non-toxic to the cells. After a 96 h treatment, the supernatant medium from the 96-well plates was discarded, the cells were fixed with 10% trichloroacetic acid (TCA) and allowed to rest at 4 °C. After 24 h fixation, the cells were washed in a strip washer and dyed with SRB solution (100 μL, 0.4 % in 1 % acetic acid) for about 20 min. After dying, the plates were washed four times with 1% acetic acid to remove the excess of the dye and allowed to air-dry overnight. Tris base solution (200 μL, 10 mM) was added to each well and absorbance was measured at $\lambda = 570$ nm using a 96-well plate reader (Tecan Spectra, Crailsheim, Germany). The EC_{50} values were averaged from three independent experiments performed each in triplicate calculated from semi logarithmic dose response curves applying a non-linear 4 P Hills-slope equation (GraphPad Prism5; variables top and bottom were set to 100 and 0, respectively).

AO/PI dye exclusion test

Morphological characteristics of cell death were analyzed employing an AO/PI assay using human cancer cell line A2780. Approximately $8{\cdot}10^5$ cells were seeded in cell culture flasks (25 cm^2), and the cells were allowed to grow up for 24 h. After removing of the used medium, the substance loaded fresh medium was reloaded (or a blank new medium as a control). After 24 h, the content of the flask was collected and centrifuged (1200 rpm, 4 °C), the pellet was gently suspended in phosphate-buffered saline (PBS (w/w), 1 mL) and centrifuged again. The PBS was removed, and the pellet gently suspended in PBS (50 μL) again. 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 (1 μg/mL, 10 μL).

Cell cycle investigations

Approximately $8{\cdot}10^5$ cells (A2780) were seeded in cell culture flasks (25 cm^2), and the cells were allowed to grow up for 24 h. After removing of the used medium, the substance loaded fresh medium was reloaded (or a blank fresh medium as a control). After 24 h, only the adherent cells

were harvested, centrifuged (1200 rpm, 4 °C), and washed twice with PBS (w/w), 1 mL). The cells were counted and approximately $1{\cdot}10^6$ cells were fixed with ethanol (70%, 4 °C, 24 h). After centrifugation (1200 rpm, 4 °C) the cells were washed with staining buffer (PBS (w/w), containing 2% FCS and 0.01% NaN_3 (2% in H_2O), 1 mL) and centrifuged. The pellet was gently suspended in RNAase A (100 µL, 100 mg/mL) and incubated for 30 min at 37 °C. After resuspending cells in 1 mL PI buffer (20 µL PI solution (1 mg/mL) in staining buffer) and incubating for 30 min at room temperature in the dark, the cells were analyzed using the Attune® FACS machine; collecting data from the BL-2A channel. Doublet cells were excluded from the measurements by plotting BL-2A against BL-2H. For each cell cycle distribution 20,000 events were collected in technical triplicates, each sample was measured in duplicates. Cell cycle distribution was calculated using ModFitLT™ (Verity Software House, Topsham,US).

Annexin V-FITC/ PI assay

Approximately $8{\cdot}10^5$ cells (A2780) were seeded in cell culture flasks (25 cm^2), and the cells were allowed to grow up for 24 h. After removing of the used medium, the substance loaded fresh medium was reloaded (or a blank fresh medium as a control). After 24 h, the cells were harvested, centrifuged (1200 rpm, 4 °C), and washed twice with PBS (w/w), 1 mL). The cells were counted and approximately $1{\cdot}10^6$ cells were washed with Annexin V binding buffer (BioLegend®, San Diego,US) and treated with propidium iodide solution (3 µL, 1 mg/mL) and Annexin V-FITC (5 µL, BioLegend®, San Diego, US) for 15 min in the dark at room temperature. After adding Annexin V binding buffer (400 µL) the suspension was analyzed using Attune® FACS machine. After gating for living cells, the data from detectors BL-1A and BL-3A were collected (20,000 events) in technical triplicates. The assay was performed in duplicates; cell distribution was calculated using Attune® Software.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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