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Research paper

Betulinic acid derived amides are highly cytotoxic, apoptotic and selective

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ABSTRACT

Betulinic and platanic acid derived amides were prepared and screened for their cytotoxic activity. All of the compounds were shown to be cytotoxic for a panel of human tumor cell lines, and especially apoptotic betulinic acid derived compounds **6**, **8** and **19** showed low EC_{50} values. Of special interest was a 4-isoquinolinyl amide of 3-*O*-acetyl-betulinic acid (compound **19**), being the most cytotoxic compound of this series and holding EC_{50} values as low as EC_{50} = 1.48 μM (A375 melanoma cells) while being significantly less cytotoxic for non-malignant fibroblasts NIH 3T3 with a selectivity index of >91.2. This finding parallels previous results obtained for **SAA21**, a augustic acid derived compound thus making the 4-isoquinolinyl moiety to a privileged scaffold.

1. Introduction

Despite all efforts, diseases and deaths from cancer still represent the second most frequent type of death worldwide. In high-income countries, however, deaths from cancer are even more common than those resulting from a cardiovascular disease [1] (see Scheme 1)

The number of patients with cancer has risen steadily in recent years, and a forecast for the year 2040 predicts that the number of patients as well as the number of deaths by cancer will double [2]. The number of newly approved drugs increases continuously; thus in 2019 alone for Germany 25 new drugs (excluding biosimilars) have been brought to market with ten of them to treat cancer patients [3].

The development of new cytotoxic active ingredients continues to be focused on natural substances or on drugs derived from natural substances. Thus, nowadays 50% of all approved drugs can be traced back to a natural product [4]. A special role thereby is taken by terpenes and terpenoids in as much as they represent the largest family of natural compounds that have been investigated for their therapeutic effects [4,5].

More recently, triterpenes in particular have again become a focus of research, and especially triterpene carboxylic acids derived from, for example betulinic acid, maslinic acid, oleanolic acid or ursolic acid (Fig. 1).

Recent investigations have shown that the presence of an intact carboxyl function is necessary to achieve good cytotoxicity. For example, derivatives derived from betulinic acid are usually significantly more active than those derived from betulin [6–8]. The potential of those compounds containing an additional cationic residue, e.g. a quaternary ammonium salt [9,10], a phosphonium moiety [11–13], a BODIPY residue [14,15], a malachite green [16] or – of special interest - a rhodamine B residue [17–20] being connected to the basic skeleton of the triterpene with or without a suitable amine spacer, should be particularly emphasized. Interestingly, however, in the past, some triterpenoid amides also showed good and - above all - quite selective cytotoxic effects. **EM2** [21–25] - a benzylamide of diacetylated maslinic acid (Fig. 2) - is cytotoxic in the low μM range [26] (e.g. EC_{50} against A2780 ovarian cancer cells = 0.5 μM) but this compound also showed good selectivity towards cancer cells (e.g. EC_{50} against non-malignant primary human fibroblasts WW030272 = 156 μM). Furthermore, small structural differences obviously influence both cytotoxicity and selectivity. For example, **SAA9** derived from augustic acid was quite cytotoxic (EC_{50} 2.2 μM, A2780 cells), but also quite unselective (S = [EC_{50} (A2780)]/[EC_{50} (NIH 3T3)] = 0.4), whereas the analogous

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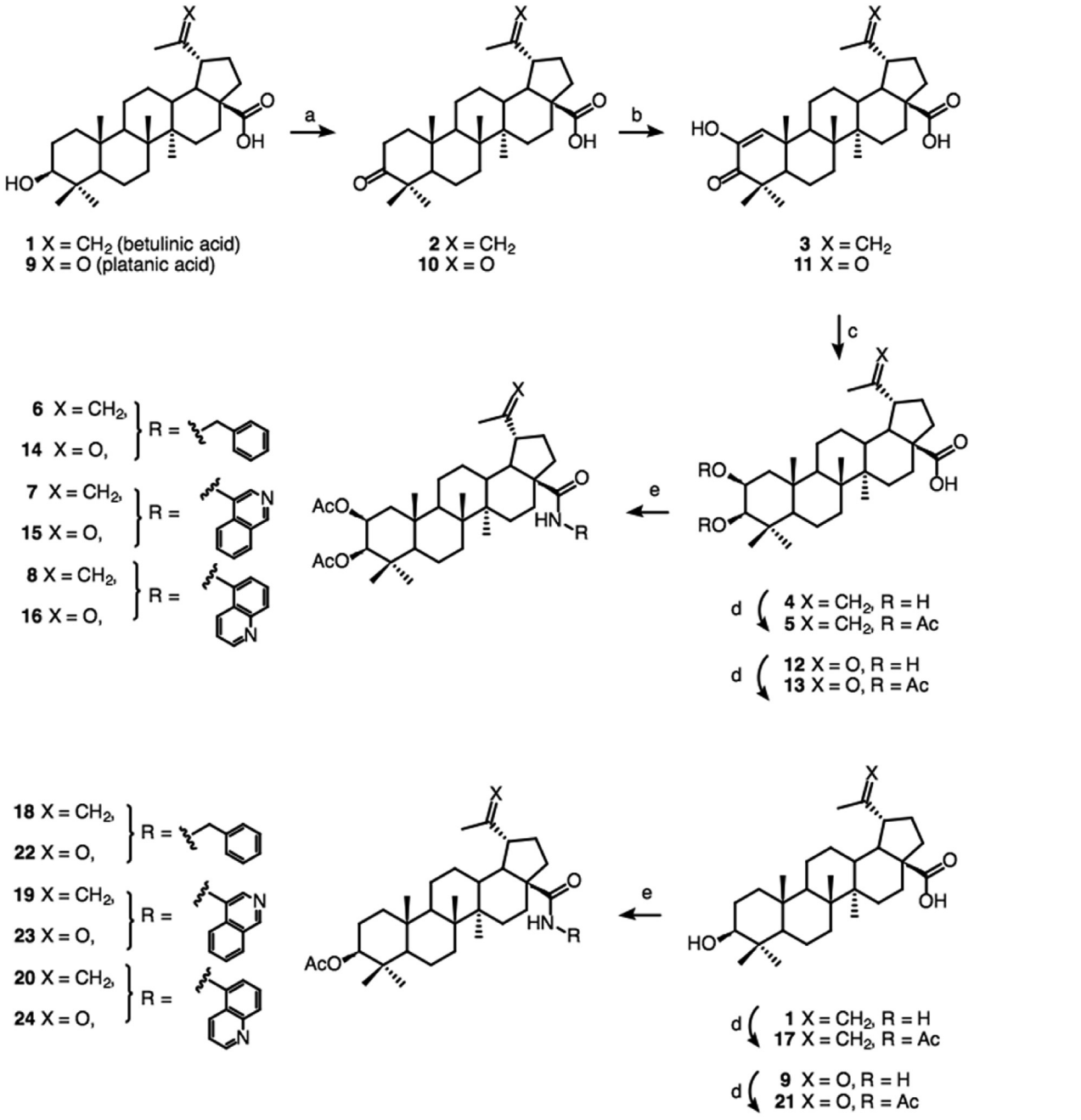


Scheme 1. Reactions and conditions: a) Jones oxidation, 76% for 2, 98% for 10; b) ^{t}BuOK, ^{t}BuOH, THF, 3 h, 50 °C, air, 97% for **3**, 85% for **11**; c) $NaBH_4$, MeOH, 23 °C, 2 h, 55% (for **4**), 74% (for **12**); d) Ac_2O, NEt_3, DMAP (cat.), DCM, 23 °C, 1 d, 70% (for **5**), 48% (for **13**); e) $(COCl)_2$, DCM, 23 °C, 1 h, then benzylamine or 4-amino-isoquinoline or 5-aminoquinoline, DCM, 23 °C, 1 h, 62% (for **6**), 55% (for **7**), 42% (for **8**), 84% (for **14**), 49% (for **15**), 82% (for **16**), 73% (for **18**), 60% (for **19**), 64% (for **20**), 76% (for **22**), 80% (for **23**), 57% (for **24**).

SAA21 derivative showed high cytotoxicity (EC_{50} = 1.2 μM, A2780) but also high selectivity (S = [EC_{50} (A2780)]/[EC_{50} (NIH 3T3)] = > 50) [27].

In the past, many derivatives obtained from betulinic acid proved to be more cytotoxic than those derived from 2,3-*O*-diacylated triterpene carboxylic acids [7,8]. To get a better insight into structure/activity relationships, we decided to synthesize derivatives holding a betulinic acid (or derived from it, platanic acid) backbone but with an extra hydroxyl group at C-2. We also wanted to investigate whether the influence of the orientation of the quinolinyl substituent, as already observed for augustic acid derivatives, was also reflected in these derivatives.

2. Results and discussion

2.1. Chemistry

The synthesis of the compounds was straightforward. Silica gel supported Jones oxidation of betulinic acid (**1**) gave betulonic acid (**2**) in 76% isolated yield. Its treatment with ^{t}BuOK/air in ^{t}BuOH/THF gave **3** whose reduction with $NaBH_4$ furnished (2β, 3β) configurated **4**. Compound **4** was acetylated to yield diacetylated **5**. Compound **5** served as a valuable starting material for the synthesis of the amides. Thereby **5** was activated with oxalyl chloride followed by the addition of either benzylamine, 4-aminoisoquinoline or 5-

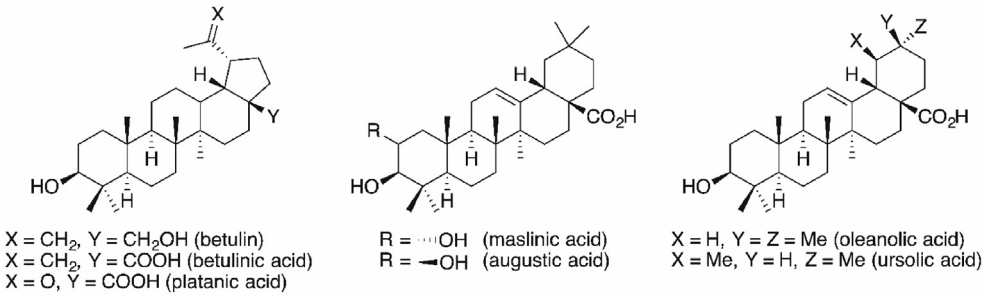


Fig. 1. Structure of betulin, betulinic acid, platanic acid, maslinic acid, augustic acid, oleanolic acid and ursolic acid.

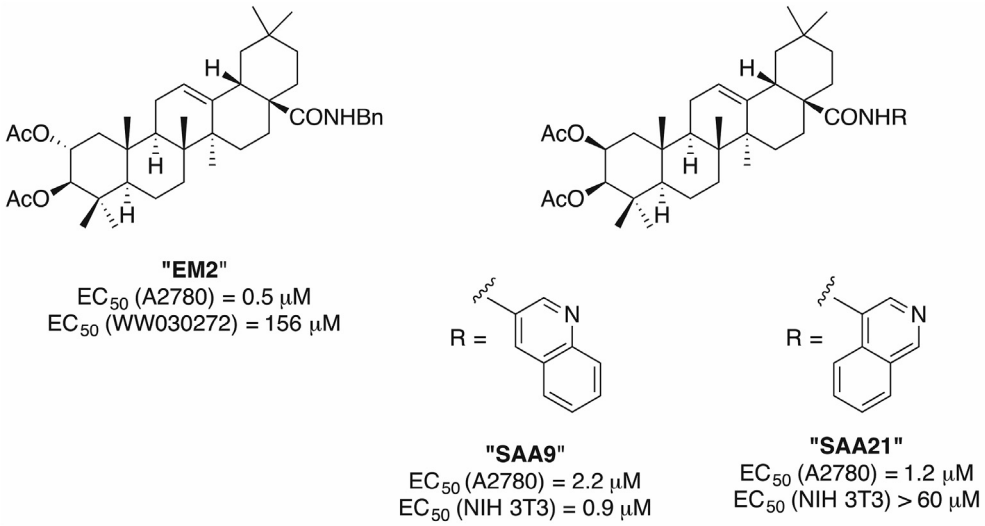


Fig. 2. Structure (and representative EC_{50} values) for maslinic acid derived **EM2** and augustic acid derived compounds **SAA9** and **SAA21**.

aminoquinoline to yield amides **6**–**8**, respectively. In a similar way, platanic acid (**9**) was oxidized, and 2-oxo compound **10** was obtained, whose oxidation (→ **11**), reduction (→**12**) followed by acetylation gave **13**. As described above, from **13** amides **14**–**16** were prepared.

For comparison and to have an insight onto the influence of the extra hydroxyl group in these molecules (as compared to the starting materials), **1** and **9** were acetylated, and the acetates **17** and **21** were obtained. Reaction with oxalyl chloride followed by the addition of either benzylamine, 4-aminoisoquinoline or 5-aminoquinoline furnished amides **18**–**20** and **22**–**24**, respectively.

2.2. Biology

The compounds were screened for their cytotoxic activity using photometric sulforhodamine assays (SRB) [28–30], the results of which are compiled in Table 1 and Fig. 3.

The results from the SRB assays revealed for many compounds cytotoxic activity. While the parent compounds **1** and **9** were not cytotoxic within the borders of the assays (cut-off 30 μM), betulonic acid (**2**) was more cytotoxic than parent **1**. Low cytotoxicity was also observed for **3**–**5**, **9**–**13** and compound **21**. Cytotoxicity was significantly increased as soon as an amide was formed irrespective whether the terpenoid skeleton was derived from **1** or **9**. For all amides **6**–**8**, **14**,**15**, **18**–**18**, **23**, and **24** EC_{50} values in the low μM range were found with betulinic acid derived **19** (a 4-isoquinolinyl derivative) being the most cytotoxic compound of this series. These results parallel previous findings for augustic acid derived **SAA21**, and is also found for analogs **8**, **14** and **24**.

Compound **19**, the most cytotoxic compound of this study also held the highest tumor/non-tumor cell selectivity, especially for A375 melanoma cells (S = 91.2, Table 2 and Fig. 4), A2780 ovarian cancer cells (S = 61.6) and the cell line FaDu (hypopharyngeal carcinoma, S = 59.0). These results seem to indicate that the amide substituent is more important than the substitution pattern at ring E.

For compounds **6**, **8** and **19**, further investigations were carried out to get a deeper insight into their mode of being cytotoxic; thereby, A375 melanoma cell were used. After having treated the cells with the compounds for 1 day, an Annexin V assay (Fig. 5) showed that fewer cells were vital as compared to the control.

Having been treated for 2 days (Fig. 6), an even more pronounced effect was observed. The number of apoptotic cells was increased (circa 44). Approximately 20% of the cells were late-apoptotic, but only 1.5% showed a necrotic cell death. As far as **8** is concerned, about two thirds of the cells were still vital (67.4%). The number of apoptotic cells, however, was significantly increased (18.9%) as compared to necrotic (1.3%) or late apoptotic/secondary necrotic (12.4%) cells. In summary, these compounds mainly act by apoptosis. Thereby, **6** showed only a slight increase of cells being necrotic and late apoptotic and secondary necrotic range (0.7 and 7.9%, respectively), while the largest increase was observed for

Table 1
Cytotoxicity of selected compounds; SRB assay EC_{50} values [μM] after 72 h of treatment; averaged from three independent experiments performed each in triplicate; confidence interval CI = 95%. Human cancer cell lines: A375 (melanoma), HT29 (colorectal carcinoma), MCF-7 (breast adenocarcinoma), A2780 (ovarian carcinoma), FaDu (hypopharyngeal carcinoma), NIH 3T3 (non-malignant fibroblasts); n.s. not soluble under the conditions of the assay; n.d. not determined. Doxorubicin (**DR**) was used as a positive control.

	A375	HT29	MCF7	A2780	FaDu	NIH 3T3
1	>30	>30	>30	>30	>30	>30
2	14.3 ± 1.7	18.5 ± 1.9	12.0 ± 1.5	4.7 ± 0.6	17.4 ± 2.1	19.4 ± 1.2
3	13.76 ± 1.4	26.07 ± 2.8	11.96 ± 1.1	10.49 ± 1.2	14.05 ± 1.1	12.85 ± 0.9
4	11.2 ± 1.8	17.68 ± 2.2	15.8 ± 2.1	12.28 ± 2.2	16.54 ± 2.5	17.28 ± 2.3
5	15.81 ± 1.0	17.40 ± 1.2	11.25 ± 0.8	8.68 ± 1.2	13.5 ± 0.9	14.11 ± 0.9
6	3.14 ± 0.6	20.72 ± 6.1	11.3 ± 2.1	4.42 ± 1.0	5.4 ± 1.0	>70
7	3.34 ± 0.2	>15	7.33 ± 1.0	4.76 ± 0.6	4.02 ± 0.3	>30
8	2.9 ± 0.1	>30	6.26 ± 0.8	3.24 ± 0.5	3.49 ± 0.4	>120
9	>30	>30	>30	>30	>30	>30
10	>30	>30	>30	>30	>30	>30
11	>30	>30	24.8 ± 1.7	20.6 ± 1.7	>30	>30
12	>30	>30	>30	>30	>30	>30
13	22.54 ± 5.7	>30	>30	>30	>30	9.57 ± 3.5
14	2.9 ± 0.5	7.22 ± 4.3	5.2 ± 2.1	2.36 ± 1.6	5.57 ± 4.4	>30
15	4.88 ± 0.6	18.43 ± 4.9	6.96 ± 2.0	6.15 ± 0.8	6.41 ± 0.8	>30
16	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
17	19.2 ± 1.7	21.3 ± 2.0	11.0 ± 0.5	18.3 ± 0.5	7.2 ± 1.2	>30
18	4.05 ± 0.2	>30	26.8 ± 6.8	6.29 ± 0.8	9.59 ± 0.9	>30
19	1.48 ± 0.1	>30	4.65 ± 0.5	2.16 ± 0.2	2.26 ± 0.2	>135
20	3.05 ± 0.1	>30	12.12 ± 1.8	5.08 ± 0.3	5.18 ± 0.3	>30
21	>30	>30	>30	>30	>30	>30
22	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
23	5.66 ± 0.4	>30	9.34 ± 1.3	7.84 ± 0.6	7.67 ± 0.2	>30
24	4.60 ± 0.3	>30	7.06 ± 1.5	5.4 ± 0.9	6.42 ± 0.5	>30
DR	n.d.	>30	1.1 ± 0.3	0.01 ± 0.01	n.d.	1.3 ± 0.6

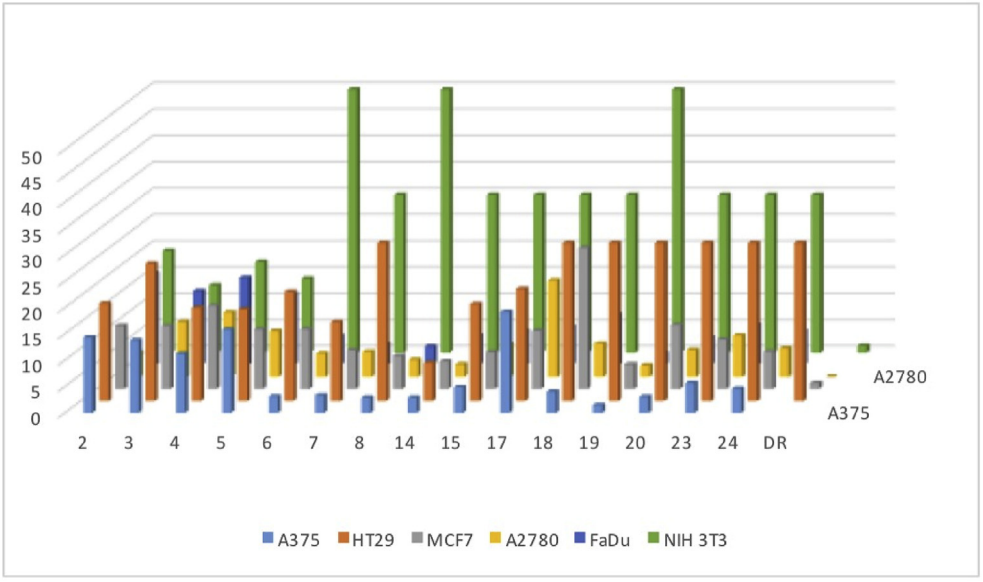


Fig. 3. Cytotoxicity of selected compounds; SRB assay EC_{50} values [μM] after 72 h of treatment.

apoptotic cells (9.5%). Compound **8** also gave an increased number of apoptotic cells, but here more were found being in the late phase. A similar behavior was observed upon treatment of the cells with **19** (8.6% apoptosis and 9.6%% late apoptosis).

Visual inspection (Fig. 7) of the cells supported the findings from the FACS investigations (vide infra). Thus, after a 24 h incubation, cell division had already been reduced/stopped. The number of apoptotic cells was slightly increased resulting in a small number of cells showing morphological differences/characteristics.

After an incubation for 48h, however, all compounds caused dramatic changes in the number and morphology of the cells due to apoptosis. For example, the cells were shrunken, strangulation occurred and also blebbing was observed.

Changes in the cell cycle (Fig. 8 and Fig. 9) were visible after 24 h of incubation. Compound **6** no longer showed a G2/M peak (0.0%), and the number of cells being in the S phase was significantly (30.3%), while more cells were in the G1 phase (63.8%). In addition, approximately 6% of the cells were in the SubG1 phase. For compound **8**, an increase of cells in the subG1 phase could be noted (3.5%). Compound **19** showed a similar behavior as **6**. Hereby, 61.1% of the cells were in G1 and 36.6% in S phase. Visual inspection of the cells supported the findings from the FACS investigations (vide

Table 2
Selectivity S = EC_{50} (NIH 3T3)/EC_{50} (tumor cell line) for the individual compounds.

	A375	HT29	MCF7	A2780	FaDu
3	0.9	0.5	1.1	1.2	0.9
4	1.5	1.0	1.1	1.4	1.0
5	0.9	0.8	1.3	1.6	1.0
6	22.3	3.4	6.2	15.8	13.0
7	9.0	2.0	4.1	6.3	7.5
8	41.4	4.0	19.2	37.0	34.4
13	0.4	0.3	0.3	0.3	0.3
14	10.3	4.2	5.8	12.7	5.4
15	6.1	1.6	4.3	4.9	4.7
17	1.6	1.4	2.7	1.6	4.2
18	7.4	1.0	1.1	4.8	3.1
19	91.2	4.5	29.0	62.5	59.7
20	9.8	1.0	2.5	5.9	5.8
23	5.3	1.0	3.2	3.8	3.9
24	6.5	1.0	4.3	5.6	4.7

infra). Thus, after a 24 h incubation, cell division had already been reduced/stopped. The number of apoptotic cells was slightly increased resulting in a small number of cells showing morphological differences/characteristics.

3. Conclusion

A small series of amides derived from betulinic acid (**1**), platanic acid (**9**) and (2β, 3β)-dihydroxy-20(29)-en-28-oic acid (**4**) as well as (2β, 3β)-20-oxo-30-norlupan-28-28-oic acid (**12**) was prepared and screened for their cytotoxic activity. All of the compounds were shown to be cytotoxic for a panel of human tumor cell lines. Especially betulinic acid derived compounds **6**, **8** and **19** held excellent cytotoxicity. They act mainly by apoptosis. Compound **19**, a 4-isoquinolinyl amide of 3-*O*-acetyl-betulinic acid, was the most cytotoxic compound holding EC_{50} values as low as EC_{50} = 1.48 μM (A375 melanoma cells) while being significantly less cytotoxic for non-malignant fibroblasts NIH 3T3 with a selectivity index of >91.2. This finding parallels previous results obtained for **SAA21**, an augustic acid derived compound.

4. Experimental

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, NOESY), MS spectra were taken on a Finnigan MAT LCQ 7000 (electrospray, voltage 4.1 kV, sheath

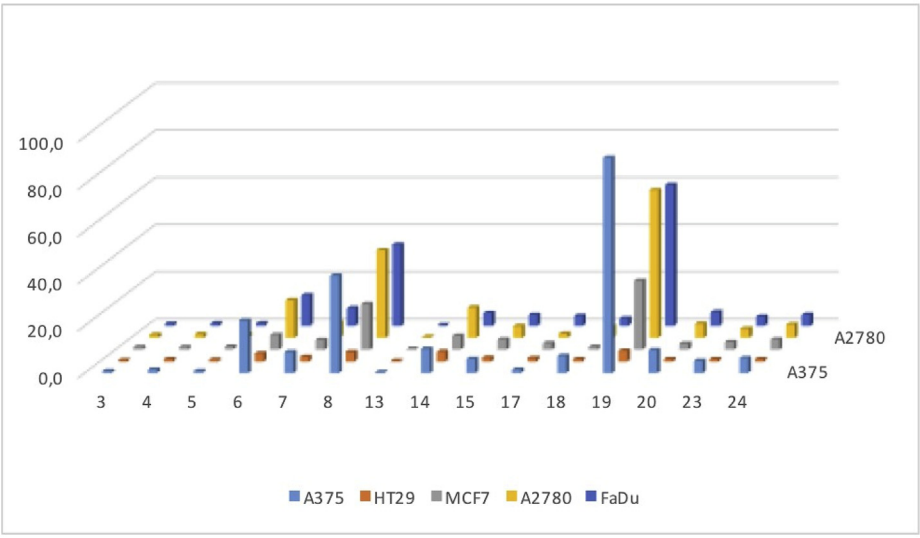
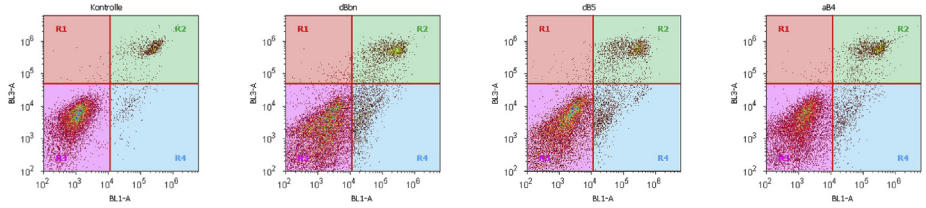
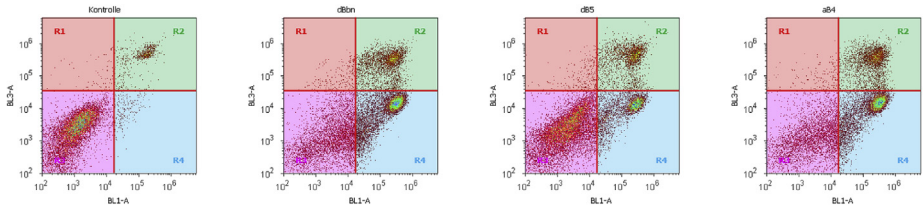


Fig. 4. Selectivity S = EC_{50} (NIH 3T3)/EC_{50} (tumor cell line) of selected compounds.



	control	compound **6**	compound **8**	Compound **19**
R1	0.331	0.667	0.749	0.351
R2	7.352	7.930	10.295	9.649
R3	90.123	81.857	80.405	83.505
R4	2.194	9.546	6.495	8.551

Fig. 5. Annexin V-FITC/PI assay: treatment of A375 cells with **6**, **8** and **19** (6.2 μM/4.8 μM/3.0 μM) for 24 h. Examples of density plots determined by flow cytometry (Attune® Cytometric Software v. 1.2.5). R1: necrotic, R2: secondary necrotic/late stage apoptotic, R3: vital, R4: apoptotic.



	control	compound **6**	compound **8**	Compound **19**
R1	0.547	1.449	1.342	1.115
R2	5.108	20.078	12.388	21.382
R3	92.723	34.287	67.409	33.194
R4	1.622	44.187	18.861	44.309

Fig. 6. Annexin V-FITC/PI assay: treatment of A375 cells with **6**, **8** and **19** (6.2 μM/4.8 μM/3.0 μM) for 48 h. Examples of density plots determined by flow cytometry (Attune® Cytometric Software v. 1.2.5). R1: necrotic, R2: secondary necrotic/late stage apoptotic.

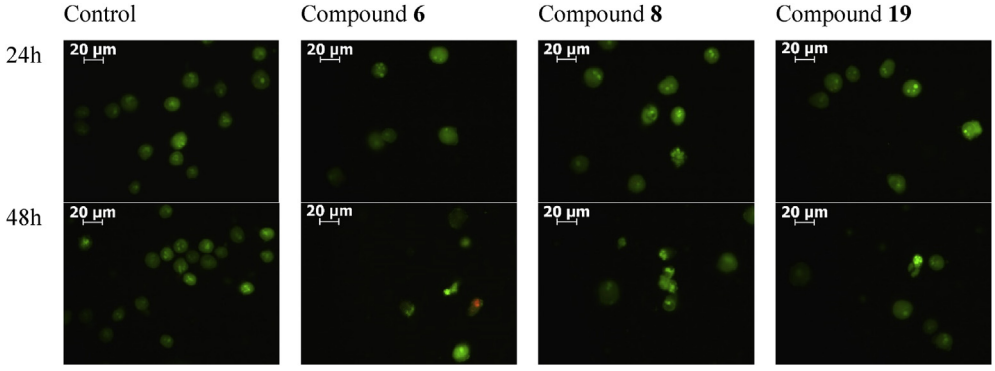
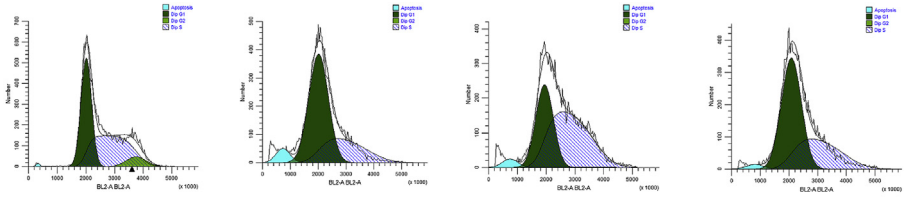


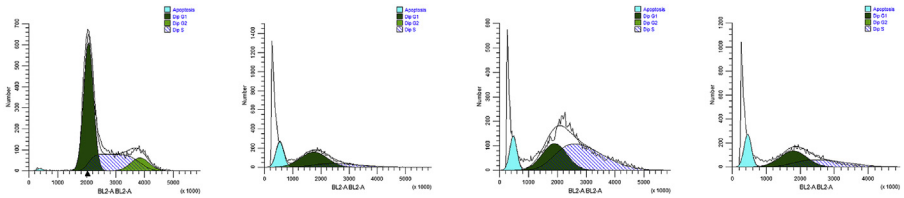
Fig. 7. Microscopic evaluation of the A375 cells treated with compounds **6**, **8**, **19**.



	control	compound **6**	compound **8**	Compound **19**
subG1	0.46%	5.98%	3.53%	2.38%
G1	44.59%	63.76%	37.51%	61.06%
S	47.46%	30.27%	58.53%	36.56%
G2/M	7.48%	0.00%	0.43%	0.00%

Fig. 8. Cell Cycle investigation. Examples for histogram determined by flow cytometry after 24 h, green G1 G2/M, striped S phase, blue subG1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

gas nitrogen) instrument. The optical rotations were measured either on a JASCO P-2000 or 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 (CHNS) unit. IR spectra were recorded on a Perkin Elmer FT-IR spectrometer Spectrum 1000 or a Perkin-Elmer Spectrum Two (UATR Two Unit). UV-VIS spectra were taken on a Perkin-Elmer Lambda 14 spectrometer or on a Perkin-Elmer Lambda 750 S (UV/VIS/NIR) spectrometer. The solvents were dried according to usual procedures. The purity of the compounds was determined by HPLC and found to be >96%. Column chromatography was performed on a Büchi Reveleris Prep purification system using Chromabond Flash cartridges (SiOH, 40.63 μm) from Macherey-Nagel or Reveleris high resolution cartridges from Büchi. Betulinic acid and platanic acid were obtained from Betulinines (Stříbrná Skalice, Czech Republic) in bulk quantities and used as received. A description of the biological screening, additional



	control	compound **6**	compound **8**	Compound **19**
subG1	0.57%	25.69%	11.49%	23.32%
G1	57.30%	52.38%	31.39%	41.85%
S	31.30%	21.92%	57.06%	34.84%
G2/M	10.83%	0.00%	0.00%	0.00%

Fig. 9. Cell Cycle investigation. Examples for histogram determined by flow cytometry after 48 h, green G1 G2/M, striped S phase, blue subG1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

spectroscopic data and depictions of the ^{1}H and ^{13}C NMR spectra are provided in the supplementary materials file.

4.1. General procedure for acetylation (GP A)

One equivalent of each triterpene carboxylic acid (compounds **1**, **4**, **9**, **12**) was dissolved in dry dichloromethane (DCM, 100 mL), and acetic anhydride (3 eq. per hydroxyl group), triethylamine (3 eq.) and catalytic amounts of DMAP were added. The reaction mixture was stirred at 23 °C for 1 day. A methanolic solution of ammonia (satd., 5 mL) was added, and the reaction mixture was stirred for another 30 min at 23 °C. For workup, aq. hydrochloric acid (10%, 20 mL) was added, the aqueous phase was extracted with DCM (3 x 10 mL), the combined organic phases were washed with brine (20 mL) and dried (Na_2SO_4). The volatiles were removed under diminished pressure, and the crude product (**5**, **13**, **17**, **2**1) was purified by column chromatography.

4.2. General procedure for the synthesis of benzyl amides (GP B)

The corresponding acetylated compound (**5**, **13**, **17** and **21**) was dissolved in dry DCM (10 mL) and oxalyl chloride (4 eq.) and catalytic quantities of DMF were added. The reaction mixture was stirred at 23 °C for 1 h. The volatiles were removed under diminished pressure, the residue was dissolved in dry DCM (10 mL), and the corresponding amine (3 eq.) was added. The reaction mixture was stirred at 23 °C for 1 h. Usual aqueous workup followed column chromatography gave compounds **6**, **14**, **18** and **22**, respectively.

4.3. General procedure for the synthesis of quinolinyl amides (GP C)

The corresponding acetylated compound (**5**, **13**, **17** and **21**) was dissolved in dry DCM (10 mL), and activated with oxalyl chloride (4 eq.) and catalytic quantities of DMF as described above. The volatiles were removed under reduced pressure, the residue was dissolved in dry DCM (4 mL), and dry pyridine (8 mL), and the respective amine (3 eq.) and catalytic quantities of DMAP and triethylamine were added. The reaction mixture was stirred with the help of microwave irradiation (Anton Parr, Graz/Austria, Monowave) at 120 °C for 2 h. Usual aqueous workup followed by column chromatographic purification gave compounds **7**, **8**, **15**, **16**, **19**, **20**, **23** and **24**, respectively.

4.4. Syntheses

4.4.1. 3-Oxolup-20(29)-en-28-oic acid (betulonic acid, 2)

Oxidation of betulinic acid (**1**, 10.0 g, 21.9 mmol) with silica gel supported Jones reagent [31] [from acetone (500 mL), CrO_3 (2.51 g, 25.1 mmol), H_2SO_4 (2.5 mL), H_2O (10 mL), silica gel (100 mL)] at 0 °C for 1 h followed by quenching the reaction by the addition of MeOH (5 mL), removal of the solvents and Soxhlet-extraction of the residue with diethyl ether (600 mL, 3.5 h) and column chromatography (SiO_2, hexanes/EtOAc, EtOAc: 3% → 15%) gave **2** (6.23 g, 76%) as a colorless solid; m.p. 246–248 °C (lit.: [32] 245–247 °C); $[\alpha]_D$ = +37.4° (*c* 0.283, $CHCl_3$) [lit.: [33] $[\alpha]_D$ = +33.8° (*c* 0.33 $CHCl_3$)]; R_F = 0.42 (SiO_2, hexanes/EtOAc, 7:1).

4.4.2. 2-Hydroxy-3-oxolupa-1,20(29)-dien-28-oic acid (3) [173106-22-4]

To a solution of **2** (8.55 g, 18.8 mmol) in ^{t}BuOH (500 mL) and THF (70 mL), ^{t}BuOK (30.0 g, 0.267 mol) [34] was added, and the mixture was stirred for 3 h at 50 °C while air was bubbled through. The solvents were removed under diminished pressure, and the residue was dissolved in EtOAc (100 mL). Usual aqueous workup followed by column chromatography (SiO_2, hexanes/EtOAc, EtOAc: 5% → 20%) gave **3** (7.41 g, 97%) as a colorless solid; m.p. 204–205 °C (lit.: [35] 204–205 °C); $[\alpha]_D$ = +40.5° (*c* 0.265, $CHCl_3$) [lit.: [35] $[\alpha]_D$ = +13° (*c* 0.75, $CHCl_3$)]; R_F = 0.68 (SiO_2, hexanes/EtOAc, 3:1).

4.4.3. 2β,3β-Dihydroxylup-20(29)-en-28-oic acid (4)

To a solution of **3** (8.525 g, 18.2 mmol) in THF (200 mL) and MeOH (40 mL), $NaBH_4$ (1.0 g, 26.4 mmol) [31] was added, and the mixture was stirred for 2 days at 23 °C. Usual aqueous workup followed by column chromatography (SiO_2, hexanes/EtOAc, EtOAc: 10% → 30%) gave **4** (4.03 g, 55%) as a colorless solid; m.p. 270–273 °C (decomp.) (lit.: [36] 273–276 °C); $[\alpha]_D$ = +32.4° (c 0.21, $CHCl_3$) [lit.: [36] $[\alpha]_D$ = +31.1 (*c* 0.262, pyridine)]; R_F = 0.54 (SiO_2, hexanes/EtOAc, 1:1).

4.4.4. 2β,3β-Diacetyloxy-lup-20(29)-en-28-oic acid (5)

Acetylation of **4** (2.38 g, 5.0 mmol) according to GPA followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 3% → 15%) gave **5** (1.97 g, 70%) as a colorless solid; m.p. 260–265 °C (decomp.) (lit.: [36] 246–248 °C); $[\alpha]_D$ = +34.6° (*c* 0.199, $CHCl_3$); R_F = 0.63 (SiO_2, hexanes/EtOAc, 7:1); IR (ATR): ν = 2944*m*, 1742*s*, 1366*m*, 1230*s*, 1189*m*, 1031 m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.32 (*m*, 1H, 2-H), 4.74 (*s*, 1H, 29-H_a), 4.66–4.56 (*m*, 2H, 3-H, 29-H_b), 3.00 (*m*, 1H, 19-H), 2.32–2.09 (*m*, 2H, 16-H_a, 13-H), 2.03 (*s*, 3H, 34-H), 2.02 (*s*, 3H, 32-H), 2.07–1.92 (*m*, 1H, 1-H_a), 1.96 (*m*, 2H, 21-H_a, 22-H_a), 1.72 (*d*,

J = 1.6 Hz, 2H, 12-H_a, 6-H_a), 1.69 (*s*, 3H, 30-H), 1.65−1.14 (*m*, 13H, 18-H, 6-H_b, 22-H_b, 7-H_b, 16-H_b, 21-H_b, 15-H_a, 11-H, 9-H, 1-H_b, 15-H_b), 1.11 (*s*, 3H, 26-H), 1.02 (*s*, 3H, 25-H), 0.99 (*m*, 1H, 12-H_b), 0.97 (*s*, 3H, 27-H), 0.96 (*s*, 3H, 24-H), 0.92 (*m*, 1H, 5-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 181.38 (C-28), 170.90 (C-33), 170.41 (C-31), 150.39 (C-20), 109.97 (C-29), 78.11 (C-3), 69.79 (C-2), 56.49 (C-17), 55.39 (C-5), 51.09 (C-9), 49.41 (C-18), 47.08 (C-19), 42.76 (C-1), 42.35 (C-14), 40.97 (C-8), 38.53 (C-13), 37.56 (C-4), 37.20 (C-10), 37.16 (C-22), 34.31 (C-7), 32.32 (C-16), 30.72 (C-21), 29.71 (C-15), 29.14 (C-23), 25.54 (C-12), 21.41 (C-34), 21.14 (C-11), 21.02 (C-32), 19.51 (C-30), 18.15 (C-6), 17.64 (C-25), 16.89 (C-26), 16.35 (C-24), 14.76 (C-27) ppm; MS (ESI, MeOH): *m/z* 437 (8%, $[M + H\text{-}2HOAc]^+$), 497 (10%, $[M + H\text{-}HOAc]^+$), 1135 (100%, $[2M + Na]^+$); analysis calcd for $C_{34}H_{52}O_6$ (556.77): C 73.34, H 9.41; found: C 73.11, H 9.63.

4.4.5. Benzyl 2β,3β-Diacetyloxy-lup-20(29)-en-28-amide (6)

Reaction of **5** (250 mg, 0.45 mmol) with benzyl amine (0.3 mL, 2.8 mmol) according to GPB followed by chromatography (SiO_2, EtOAc/hexanes, EtOAc 8% → 20%) gave **6** (180 mg, 62%) as a colorless solid; m.p. 138−141 °C; $[\alpha]_D$ = +39.0° (*c* 0.222, $CHCl_3$); R_F = 0.64 (SiO_2, toluene/EtOAc/heptane/HCOOH, 80:26:10:5); IR (ATR): ν = 2943*w*, 1741*s*, 1620*w*, 1365*m*, 1231*s*, 1188*m*, 1029*m*, 698 m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38−7.24 (*m*, 5H, 37-H, 38-H, 39-H, 40-H, 41-H), 5.88 (*t*, J = 5.7 Hz, 1H, NH) 5.36−5.26 (*m*, 1H, 2-H_a), 4.74 (*d*, J = 2.0 Hz, 1H, 29-H_a), 4.60 (*m*, 2H, 29-H_b, 3-H), 4.48 (*dd*, J = 15.1, 5.8 Hz, 1H, 35-H_a), 4.37 (*dd*, J = 14.6, 5.6 Hz, 1H, 35-H_b), 3.16 (*td*, J = 11.0, 4.1 Hz, 1H, 19-H), 2.55−2.44 (*td*, 1H, 13-H), 2.03 (*s*, 3H, 32-H), 2.01 (*s*, 3H, 34-H), 2.06−1.87 (*m*, 3H, 1-H, 16-H_a, 21-H_a), 1.68 (*s*, 3H, 30-H), 1.81−1.21 (*m*, 16H, 22-H_a, 12-H, 16-H_b, 18-H, 6-H, 22-H_b, 21-H_b, 7-H, 11-H, 1-H_b, 9-H), 1.12 (*s*, 1H, 15-H), 1.10 (*s*, 3H, 26-H), 1.02 (*s*, 3H, 25-H), 0.95 (*s*, 3H, 27-H), 0.93 (*s*, 3H, 24-H), 0.90 (*m*, 1H, 5-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 176.01 (C-28), 170.88 (C-33), 170.40 (C-31), 150.97 (C-20), 139.32 (C-36), 128.80 (C-38, C-40), 127.93 (C-37, C-41), 127.47 (C-39), 109.61 (C-29), 78.13 (C-3), 69.76 (C-2), 55.78 (C-17), 55.42 (C-5), 51.27 (C-9), 50.32 (C-18), 46.83 (C-19), 43.94 (C-8), 43.44 (C-35), 42.82 (C-4), 42.38 (C-1), 41.05 (C-10), 38.56 (C-22), 37.77 (C-13), 34.43 (C-7), 33.94 (C-16), 31.02 (C-21), 29.46 (C-15), 29.11 (C-23), 25.72 (C-12), 21.41 (C-32), 21.25 (C-11), 21.02 (C-34), 19.63 (C-30), 18.18 (C-6), 17.67 (C-25), 16.92 (C-26), 16.42 (C-24), 14.69 (C-27) ppm; MS (ESI, MeOH): *m/z* 646 (100%, $[M+H]^+$), 668 (76%, $[M+Na]^+$), 1291 (52%, $[2M + H]^+$), 1313 (84%, $[2M + Na]^+$); analysis calcd for $C_{41}H_{59}NO_4$ (645.93): C 76.24, H 9.21, N 2.17; found: C 76.02, H 9.37, N 2.30.

4.4.6. Isoquinolin-4-yl 2β,3β-diacetyloxy-lup-20(29)-en-28-amide (7)

According to GPC from **5** (150 mg, 0.27 mmol) and 4-aminoisoquinoline (150 mg, 1.0 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 20% → 55%) **7** (76 mg, 42%) was obtained as an off-white solid; m.p. 186−190 °C (decomp.); $[\alpha]_D$ = −3.2° (*c* 0.216, $CHCl_3$); R_F = 0.56 (SiO_2, $CHCl_3$/MeOH, 95:5); UV−Vis ($CHCl_3$): λ_{max} (log ε) = 282 nm (4.20), 323 nm (4.24); IR (ATR): ν = 2942*m*, 1741*s*, 1367*m*, 1251*s*, 1187*m*, 1030*m*, 577*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.12 (*s*, 1H, 38-H), 8.75 (*s*, 1H, 36-H), 8.01 (*d*, J = 8.2 Hz, 1H, 40-H), 7.83−7.70 (*m*, 2H, 42-H, 41-H), 7.68−7.59 (*m*, 1H, 43-H), 5.32 (*td*, 1H, 2-H), 4.75 (*s*, 1H, 29-H_a), 4.66−4.56 (*m*, 2H, 29-H_b, 3-H), 3.19 (*s*, 1H, 19-H), 2.59 (*td*, 1H, 13-H), 2.18−1.92 (*m*, 3H, 1-H_a, 12-H_a, 21-H_a), 2.02 (*s*, 3H, 32-H), 2.02 (*s*, 3H, 34-H), 1.72 (*s*, 3H, 30-H), 1.91−1.19 (*m*, 17H, 12-H_b, 18-H, 1-H_b, 21-H_b, 22-H, 16-H, 7-H, 11-H, 15-H, 9-H, 17-H), 1.10 (*s*, 3H, 26-H), 1.03 (*s*, 3H, 27-H), 1.02 (*m*, 6H, 24-H, 25-H), 0.98−0.91 (*m*, 1H, 5-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 175.20 (C-28), 170.68 (C-33), 170.19 (C-31), 150.33 (C-20), 149.89 (C-38), 138.61 (C-36), 131.07 (C-35), 130.56 (C-41), 128.75 (C-39), 128.11 (C-40), 127.31 (C-43), 124.76 (C-44), 120.64 (C-42), 109.65 (C-39), 77.88 (C-3), 69.49 (C-2), 56.68 (C-17), 55.57 (C-5), 51.05 (C-9), 50.19 (C-18), 46.43 (C-19), 42.71 (C-8), 42.17 (C-4), 40.90 (C-10), 38.44 (C-1), 37.58 (C-13), 37.32 (C-22), 36.94 (C-14), 34.22 (C-7), 34.08 (C-16), 30.82 (C-21), 29.61 (C-15), 28.88 (C-23), 25.49 (C-12), 21.17 (C-32), 21.02 (C-11), 20.79 (C-34), 19.45 (C-30), 17.92 (C-6), 17.43 (C-25), 16.69 (C-26), 16.28 (C-24), 14.54 (C-27) ppm; MS (ESI, MeOH): *m/z* 683 (92%, $[M+H]^+$), 705 (44%, $[M+Na]^+$), 1365 (100%, $[2M + H]^+$), 1388 (36%, $[2M + Na]^+$); analysis calcd for $C_{43}H_{58}N_2O_5$ (682.95): C 75.62, H 8.56, N 4.10; found: C 75.48, H 8.76, N 4.24.

4.4.7. Quinolin-5-yl 2β,3β-diacetyloxy-lup-20(29)-en-28-amide (8)

According to GPC from **5** (250 mg, 0.45 mmol) and 5-aminoquinoline (250 mg, 1.7 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 20% → 55%) **8** (130 mg, 42%) was obtained as an off-white solid; m.p. 249−252 °C (decomp.); $[\alpha]_D$ = +2.9° (*c* 0.162, $CHCl_3$); R_F = 0.53 (SiO_2, $CHCl_3$/MeOH, 95:5); UV−Vis ($CHCl_3$): λ_{max} (log ε) = 305 nm (4.47), 317 nm (4.45); IR (ATR): ν = 2942*m*, 1741*s*, 1474*m*, 1366*m*, 1232*s*, 1187*m*, 1030*m*, 798*m*, 730 m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.84 (*d*, J = 3.1 Hz, 1H, 41-H), 8.29 (*d*, J = 8.4 Hz, 1H, 36-H), 8.04−7.94 (*m*, 1H, 37-H), 7.77−7.66 (*m*, 2H, 38-H, 43-H), 7.46 (*dd*, J = 8.6, 4.4 Hz, 1H, 42-H), 5.31 (*m*, 1H, 2-H), 4.74 (*s*, 1H, 29-H_a), 4.65−4.58 (*m*, 2H, 29-H_b, 3-H), 3.16 (*td*, 1H, 19-H), 2.58 (*td*, 1H, 13-H), 2.32 (*m*, 1H, 16-H_a), 2.02 (*s*, 3H, 32-H), 2.02 (*s*, 3H, 34-H), 2.20−1.95 (*m*, 3H, 1-H_a, 21-Ha, 22-H_a), 1.72 (*s*, 3H, 30-H), 1.88−1.21 (*m*, 16H, 16-H_b, 12-H, 15-H_a, 22-H_b, 18-H, 6-H, 21-H_b, 7-H, 11-H, 15-H_b, 9-H, 1-H_b), 1.10 (*s*, 3H, 26-H), 1.03 (*s*, 3H, 27-H), 1.01 (*m*, 6H, 24-H, 25-H), 0.93 (*m*, 1H, 5-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 175.66 (C-28), 170.91 (C-33), 170.41 (C-31), 150.54 (C-20), 148.81 (C-41), 148.39 (C-39), 133.54 (C-44), 132.84 (C-36), 130.38 (C-43), 125.85 (C-37), 124.09 (C-35), 123.17 (C-38), 120.89 (C-42), 109.92 (C-29), 78.10 (C-3), 69.71 (C-2), 56.92 (C-17), 55.44 (C-5), 51.27 (C-9), 50.43 (C-18), 46.68 (C-19), 42.93 (C-8), 42.40 (C-1), 41.14 (C-10), 38.62 (C-22), 37.83 (C-13), 37.55 (C-14), 37.17 (C-4), 34.46 (C-7), 34.15 (C-16), 31.05 (C-21), 29.86 (C-15), 29.10 (C-23), 25.71 (C-12), 21.40 (C-32), 21.27 (C-11), 21.02 (C-34), 19.66 (C-30), 18.15 (C-6), 17.66 (C-25), 16.92 (C-26), 16.54 (C-24), 14.78 (C-27) ppm; MS (ESI, MeOH): *m/z* 683 (100%, $[M+H]^+$), 705 (22%, $[M+Na]^+$), 1365 (44%, $[2M + H]^+$), 1387 (58%, $[2M + Na]^+$); analysis calcd for $C_{43}H_{58}N_2O_5$ (682.95): C 75.62, H 8.56, N 4.10; found: C 75.41, H 8.74, N 4.29.

4.4.8. 3,20-Dioxo-30-norlupan-28-oic acid (10)

Silica gel assisted Jones oxidation [31] of platanic acid (**9**, 5.0 g, 10.9 mmol) followed by column chromatography (SiO_2, hexanes/EtOAc, EtOAc: 10% → 30%) gave **10** (4.90 g, 98%) as a colorless solid; m.p. 230 °C (decomp.) (lit.: [37] 232−233 °C); $[\alpha]_D$ = +4.2° (*c* 0.308, $CHCl_3$) (lit.: [37] $[\alpha]_D$ = −11° (*c* 1.0, C_6H_6)]; R_F = 0.64 (SiO_2, hexanes/EtOAc, 1:1).

4.4.9. 2-Hydroxy-3,20-dioxo-30-norlupan-1-en −28-oic acid (11)

Following the procedure [34] given for the synthesis of **3**, from **10** (4.846 g, 10.6 mmol) **11** (4.30 g, 85%) was obtained and directly used in the next step; an analytical sample showed m.p. 225−228 °C (lit.: [29] 224−228 °C); $[\alpha]_D$ = +9.2° (*c* 0.71, MeOH) [lit.: [29] $[\alpha]_D$ = +9.0° (*c* 0.66, MeOH); MS (ESI, MeOH): *m/z* 472 (14%, $[M+H]^+$), 493 (100%, $[M+Na]^+$), 509 (32%, $[M+K]^+$).

4.4.10. 2β,3β-dihydroxy-20-oxo-30-norlupan-28-oic acid (12)

To a solution of **11** (4.9 g, 10.4 mmol) in THF (100 mL) and MeOH (20 mL), $NaBH_4$ (0.5 g, 13.2 mmol) [31] was added in several portions, and the mixture was stirred for 1 d at 23 °C. Usual aqueous work-up followed by chromatography (SiO_2, $CHCl_3$/acetone, acetone: 5% → 20%) gave **12** (3.7 g, 74%) as a colorless solid; m.p. 265−269 °C (lit.: [29] 266−270 °C) $[\alpha]_D$ = −11.2° (*c* 0.130, $CHCl_3$)

(lit.: $[\alpha]_D = -6.2°$ (*c* 0.215, $CHCl_3$)]; $R_F = 0.31$ (SiO_2, hexanes/EtOAc, 1:1).

4.4.11. 2β,3β-Diacetyloxy-20-oxo-30-norlupan-28-oic acid (13)

According to GPA from **12** (1.75 g, 7.4 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 5% → 50%), **13** (993 mg, 48%) was obtained as a colorless solid; m.p. 150 °C; $[\alpha]_D = +6.9°$ (*c* 0.146, $CHCl_3$); $R_F = 0.33$ (SiO_2, hexanes/EtOAc, 3:1); IR (ATR): ν = 2944*w*, 1740*m*, 1365*m*, 1231*s*, 1189*w*, 1134*w*, 1031*m*, 946*w*, 752*m*, 667*w*, 605*w*, 510*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.31 (*td*, 2H, 2-H), 4.60 (*d*, *J* = 3.9 Hz, 1H, 3-H), 3.24 (*td*, *J* = 11.3, 4.7 Hz, 2H, 19-H), 2.29 (*m*, 1H, 16-H_a), 2.18 (*s*, 3H, 29-H), 2.15−2.05 (*m*, 1H, 18-H), 2.02 (*s*, 3H, 33-H), 2.01 (*s*, 3H, 31-H), 2.06−1.92 (*m*, 3H, 1-H_a, 13-H, 22-H_a), 1.67−1.15 (*m*, 13H, 22-H_b, 6-H, 15-H_a, 21-H, 16-H_b, 7-H, 11-H, 1-H_b, 15-H_b), 1.10 (*s*, 3H, 25-H), 1.08 (*s*, 2H, 12-H), 1.01 (*s*, 3H, 24-H), 1.00 (*s*, 3H, 27-H), 0.95 (*m*, 1H, 5-H), 0.93 (*s*, 3H, 26-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 212.12 (C-20), 181.60 (C-28), 170.84 (C-32), 170.37 (C-30), 78.02 (C-3), 69.79 (C-2), 56.34 (C-17), 55.32 (C-5), 51.35 (C-19), 50.90 (C-9), 49.26 (C-18), 42.55 (C-14), 42.27 (C-1), 40.85 (C-8), 37.62 (C-13), 37.55 (C-4), 37.15 (C-10), 36.84 (C-22), 34.18 (C-7), 31.59 (C-16), 30.21 (C-29), 29.71 (C-15), 29.12 (C-23), 28.41 (C-21), 27.19 (C-12), 21.39 (C-33), 21.13 (C-11), 21.00 (C-31), 18.10 (C-6), 17.61 (C-24), 16.87 (C-25), 16.29 (C-26), 14.79 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 439 (28%, $[M + H\text{-}HOAc]^+$), 499 (32%, $[M + H\text{-}2HOAc]^+$), 576 (100%, $[M + NH_4]^+$), 581 (48%, $[M+Na]^+$); analysis calcd for $C_{33}H_{50}O_7$ (558.76): C 70.94, H 9.02; found: C 70.73, H 9.24.

4.4.12. Benzyl 2β,3β-diacetyloxy-20-oxo-30-norlupan-28-amide (14)

According to GPB from **13** (12.41 g, 2.22 mmol) and benzylamine (0.49 mL, 4.5 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, 3:2), **14** (1.21 g, 84%) was obtained as a colorless solid; m.p. 150−154 °C; $[\alpha]_D = +21.5°$ (*c* 0.355, $CHCl_3$); $R_F = 0.49$ (SiO_2, hexanes/EtOAc, 3:1); UV−Vis ($CHCl_3$): λ_{max} (log ε) = 259 nm (3.22); IR (ATR): ν = 2943*m*, 1741*s*, 1660*m*, 1516*m*, 1231*s*, 1188*m*, 1030*m*, 699*m*, 603*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.36−7.23 (*m*, 5H, 36-H, 37-H, 38-H, 39-H, 40-H), 5.97 (*t*, *J* = 5.7 Hz, 1H, NH) 5.30 (*td*, *J* = 3.6 Hz, 1H, 2-H), 4.59 (*d*, *J* = 3.9 Hz, 1H, 3-H), 4.51−4.44 (*m*, 1H, 34-H_a), 4.38−4.30 (*m*, 1H, 34-H_b), 3.46 (*td*, *J* = 11.2, 4.1 Hz, 1H, 19-H), 2.27 (*td*, *J* = 12.1, 4.1 Hz, 1H, 13-H), 2.16 (*s*, 3H, 29-H), 2.12−2.03 (*m*, 2H, 18-H, 21-H_a), 2.02 (*s*, 3H, 33-H), 2.01 (*s*, 3H, 31-H), 1.98−1.93 (*m*, 1H, 1-H_b), 1.93−1.85 (*m*, 1H, 16-H_a), 1.81−1.73 (*m*, 1H, 22-H_a), 1.71−1.19 (*m*, 12H, 16-H_b, 22-H_b, 6-H, 21-H_b, 15-H_a, 7-H, 11-H, 1-H_b, 9-H), 1.14 (*m*, 1H, 15-H_b), 1.09 (s, 3H, 25-H), 1.13−1.05 (*m*, 2H, 12-H), 1.01 (*s*, 3H, 24-H), 0.97 (*s*, 3H, 27-H), 0.95−0.92 (*m*, 1H, 5-H), 0.89 (*s*, 3H, 26-H), 0.87 (*s*, 3H, 23-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 212.76 (C-20), 175.70 (C-28), 170.65 (C-32), 170.19 (C-30), 138.96 (C-35), 128.68 (C-38, C-37), 127.73 (C-40, C-36), 127.39 (C-38), 77.89 (C-3), 69.60 (C-2), 55.48 (C-17), 55.22 (C-5), 50.97 (C-19), 50.93 (C-9), 49.92 (C-18), 43.30 (C-34), 42.42 (C-14), 42.17 (C-1), 40.79 (C-8), 37.95 (C-22), 37.39 (C-4), 36.99 (C-10), 36.68 (C-13), 34.15 (C-7), 32.98 (C-16), 30.30 (C-29), 29.33 (C-15), 28.94 (C-23), 28.54 (C-21), 27.13 (C-12), 21.24 (C-33), 21.06 (C-11), 20.84 (C-31), 17.97 (C-6), 17.50 (C-24), 16.73 (C-25), 16.19 (C-26), 14.60 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 648 (72%, $[M+H]^+$), 994 (52%, $[3M+2Na]^{2+}$), 1295 (34%, $[2M + H]^+$), 1317 (72%, $[2M + Na]^+$); analysis calcd for $C_{40}H_{57}NO_6$ (647.90): C 74.15, H 8.87, N 2.16; found: C 73.97, H 9.03, N 2.31.

4.4.13. Isoquinolin-4-yl 2β,3β -diacetyloxy-20-oxo-30-norlupan-28-amide (15)

According to GPC from **13** (137 mg, 0.24 mmol) and 4-aminoisoquinoline (150 mg, 1.0 mmol) **15** (83 mg, 49%) was obtained as an off-white solid; m.p. 205−208 °C (decomp.); $[\alpha]_D = -14.2°$ (*c* 0.258, $CHCl_3$); $R_F = 0.20$ (SiO_2, $CHCl_3$/MeOH, 95:5); UV−Vis ($CHCl_3$): λ_{max} (log ε) = 228 nm (3.78); IR (ATR): ν = 2943*w*, 1741*m*, 1666*w*, 1588*w*, 1449*w*, 1396*m*, 1368*m*, 1253*s*, 1188*m*, 1032*m*, 947*w*, 873*w*, 780*w*, 750*w*, 653*w*, 622*w*, 576*w*, 497*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.13 (*s*, 1H, 37-H), 8.71 (*s*, 1H, 35-H), 8.02 (*d*, *J* = 8.2 Hz, 1H, 39-H), 7.82−7.72 (*m*, 2H, 42-H, 40-H), 7.65 (*m*, 1H, 41-H), 5.31 (*m*, 1H, 2-H), 4.60 (*d*, *J* = 3.9 Hz, 1H, 3-H), 3.45 (*td*, *J* = 11.3, 5.7 Hz, 1H, 19-H), 2.41−2.32 (*m*, 1H, 13-H), 2.29−2.20 (*m*, 2H, 16-H_a, 18-H), 2.18 (*m*, 1H, 21-H_a), 2.17 (*s*, 3H, 29-H), 2.09 (*m*, 1H, 22-H_a), 2.02 (*s*, 3H, 33-H), 2.01 (*s*, 3H, 31-H), 1.97 (*m*, 1H, 1-H_b), 1.88 (*m*, 1H, 16-H_b), 1.74 (*m*, 2H, 15-H, 22-H_b), 1.62−1.21 (*m*, 10H, 21-H_b, 6-H, 7-H, 11-H, 15-H_b, 9-H, 1-H_b), 1.09 (*s*, 3H, 25-H), 1.07 (*m*, 2H, 12-H), 1.06 (*s*, 3H, 27-H), 1.02 (*s*, 3H, 24-H), 1.00 (*s*, 3H, 25-H), 0.97−0.91 (*m*, 1H, 5-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 212.60 (C-20), 175.39 (C-28), 170.86 (C-32), 170.39 (C-30), 150.25 (C-37), 138.78 (C-35), 131.38 (C-34), 130.94 (C-40), 128.97 (C-38), 128.44 (C-39), 128.28 (C-43), 127.66 (C-41), 120.82 (C-42), 78.04 (C-3), 69.73 (C-2), 56.77 (C-17), 55.40 (C-5), 51.11 (C-19), 50.86 (C-9), 50.11 (C-18), 42.72 (C-14), 42.34 (C-1), 41.03 (C-8), 38.18 (C-22), 37.55 (C-4), 37.16 (C-10), 36.89 (C-13), 34.34 (C-7), 33.53 (C-16), 30.51 (C-29), 29.91 (C-15), 29.10 (C-23), 28.66 (C-21), 27.29 (C-12), 21.39 (C-33), 21.22 (C-11), 21.01 (C-31), 18.12 (C-6), 17.65 (C-24), 16.89 (C-25), 16.44 (C-26), 14.84 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 685 (100%, $[M+H]^+$); analysis calcd for $C_{42}H_{56}N_2O_6$ (684.92): C 73.65, H 8.24, N 4.09; found: C 73.40, H 8.42, N 4.17.

4.4.14. Quinolin-5-yl 2β,3β -diacetyloxy-20-oxo-30-norlupan-28-amide (16)

According to GPC fron **13** (244 mg, 0.44 mmol) and 5-aminoquinoline (25 mg, 1.8 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 10% → 60%) **16** (244 mg, 82%) was isolated as an off-white solid; m.p. 290−292 °C (decomp.); $[\alpha]_D = -28.6°$ (*c* 0.213, $CHCl_3$); $R_F = 0.26$ (SiO_2, $CHCl_3$:MeOH, 95:5); UV−Vis ($CHCl_3$): λ_{max} (log ε) = 304 nm (4.34), 316 nm (4.31); IR (ATR): ν = 2939*w*, 1741*m*, 1364*m*, 1249*s*, 1027*w*, 809 s cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.93 (*d*, *J* = 2.8 Hz, 1H, 40-H), 8.17 (*d*, *J* = 8.5 Hz, 1H, 42-H), 8.02 (*dd*, *J* = 6.9, 2.5 Hz, 1H, 41-H), 7.78−7.68 (*m*, 2H, 37-H, 35-H), 7.45 (*dd*, *J* = 8.6, 4.2 Hz, 1H, 36-H), 5.34−5.28 (*m*, 1H, 2-H), 4.60 (*d*, *J* = 3.9 Hz, 1H, 3-H), 3.51−3.41 (*m*, 1H, 19-H), 2.41−2.31 (*m*, 1H, 13–H), 2.23 (*m*, 3H, 16-H_a, 18-H, 21-H_a), 2.17 (*s*, 3H, 29-H), 2.14−2.04 (*m*, 1H, 22-H_a), 2.02 (*s*, 3H, 33-H), 2.01 (*s*, 3H, 31-H), 1.97 (*s*, 1H, 1-H_a), 1.89 (*m*, 1H, 16-H_b), 1.80−1.67 (*m*, 1H, 15-H_a, 22-H_b), 1.64−1.22 (*m*, 10H, 21-H_b, 6-H, 7-H, 11-H, 15-H_b, 9-H, 1-H_b), 1.10 (*s*, 3H, 25-H), 1.13−1.03 (*m*, 2H, 12-H), 1.06 (*s*, 3H, 27-H), 1.02 (*s*, 3H, 24-H), 1.00 (*s*, 3H, 26-H), 0.97−0.91 (*m*, 1H, 5-H), 0.88 (*s*, 3H, 23-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 212.51 (C-20), 175.35 (C-28), 170.84 (C-32), 170.36 (C-30), 150.19 (C-40), 147.96 (C-38), 132.88 (C-34), 130.79 (C-42), 129.57 (C-35), 127.46 (C-41), 123.84 (C-43), 122.61 (C-37), 121.14 (C-36), 78.02 (C-3), 69.72 (C-2), 56.75 (C-17), 55.41 (C-5), 51.11 (C-19), 50.91 (C-9), 50.13 (C-18), 42.73 (C-14), 42.35 (C-1), 41.05 (C-8), 38.18 (C-22), 37.56 (C-4), 37.16 (C-10), 36.94 (C-13), 34.35 (C-7), 33.53 (C-16), 30.49 (C-29), 29.92 (C-15), 29.10 (C-23), 28.67 (C-21), 27.28 (C-12), 21.40 (C-33), 21.24 (C-11), 21.01 (C-31), 18.12 (C-6), 17.66 (C-24), 16.89 (C-25), 16.47 (C-26), 14.85 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 685 (100%, $[M+H]^+$), 707 (8%, $[M+Na]^+$), 1370 (18%, $[2M + H]^+$), 1391 (10%, $[2M + Na]^+$); analysis calcd for $C_{42}H_{56}N_2O_6$ (684.92): C 73.65, H 8.24, N 4.09; found: C 73.47, H 8.39, N 4.20.

4.4.15. 3β-Acetyloxy-lup-20(29)-en-28-oic acid (17) [10376-50-8]

According to GPA from **1** (0.5 g, 1.10 mmol) followed by crystallization from EtOH, **17** (489 mg, 89%) was obtained as a colorless solid; m.p. 275−278 °C (lit.: [35] 277−278 °C); $[\alpha]_D = +20.6°$ (*c* 0.350, $CHCl_3$) [lit.: [35] $[\alpha]_D = +22°$ (*c* 0.49, $CHCl_3$)]; $R_F = 0.58$ (SiO_2, hexanes/EtOAc, 4:1).

4.4.16. Benzyl 3β-acetyloxy-lup-20(29)-en-28-amide (18)

According to GPB from **17** (407 mg, 0.82 mmol) and benzylamine (0.3 mL, 2.8 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, 9:1), **18** (350 mg, 73%) was obtained as a colorless solid; m.p. 135 °C, 210 °C (decomp.) (lit.: [30,38] 124–127 °C); $[\alpha]_D = 20.9°$ (*c* 0.348, $CHCl_3$) [lit.: [30,38] $[\alpha]_D = +23.2°$ (*c* 0.35, $CHCl_3$)]; $R_F = 0.65$ (SiO_2, hexanes/EtOAc, 7:1).

4.4.17. Isoquinolin-4-yl 3β-acetyloxy-lup-20(29)-en-28-amide (19)

According to GPC from **17** (300 mg, 0.60 mmol) and 4-aminoisoquinoline (300 mg, 2.1 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 15% → 60%), **19** (214 mg, 56%) was obtained as an off-white solid; m.p. 235 °C (decomp.); $[\alpha]_D = -10.7°$ (*c* 0.124, $CHCl_3$); $R_F = 0.5$ (SiO_2, $CHCl_3$/MeOH, 95:5); UV–Vis ($CHCl_3$): λ_{max} (log ε) = 229 nm (3.87); IR (ATR): ν = 2943*m*, 2870*w*, 1731*m*, 1656*w*, 1585*w*, 1516*w*, 1468*m*, 1411*w*, 1391*m*, 1373*m*, 1244*s*, 1182*w*, 1135*w*, 1106*w*, 1026*m*, 979*m*, 884*m*, 797*w*, 778*w*, 749*s*, 559*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.08 (*s*, 1H, 36-H), 8.69 (*s*, 1H, 34-H), 7.99 (*d*, *J* = 8.2 Hz, 1H, 38-H), 7.82–7.70 (*m*, 2H, 40-H, 39-H), 7.63 (*t*, *J* = 7.1 Hz, 1H, 41-H), 4.74 (*s*, 1H, 29-H_a), 4.62 (*s*, 1H, 19-H_b), 4.50–4.42 (*m*, 1H, 3-H), 3.23–3.14 (*m*, 1H, 19-H), 2.58 (*m*, 1H, 13-H), 2.33–2.24 (*m*, 1H, 16-H_a), 2.12–2.05 (*m*, 2H, 22-H_a, 21-H_a), 2.03 (*s*, 3H, 32-H), 1.71 (*s*, 3H, 30-H), 1.86–1.17 (*m*, 16H, 16-H_b, 12-H_a, 15-H_a, 1-H_a, 18-H, 22-H_b, 2-H, 21-H_b, 11-H_a, 7-H, 6-H_b, 15-H_b, 9-H, 11-H_b), 1.03 (*s*, 3H, 27-H), 1.00 (*s*, 3H, 25-H), 0.97 (*m*, 2H, 12-H_b, 1-H_b), 0.84 (*m*, 6H, 23-H, 25-H), 0.83 (*s*, 3H, 24-H), 0.79 (*m*, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 175.58 (C-28), 171.18 (C-31), 150.68 (C-20), 149.77 (C-36), 138.49 (C-34), 131.49 (C-33), 130.90 (C-39), 128.88 (C-37), 128.68 (C-42), 128.35 (C-38), 127.62 (C-41), 121.15 (C-40), 109.80 (C-29), 81.08 (C-3), 56.93 (C-17), 55.65 (C-5), 50.76 (C-9), 50.45 (C-18), 46.66 (C-19), 42.77 (C-14), 41.03 (C-8), 38.64 (C-1), 38.59 (C-4), 37.95 (C-10), 37.89 (C-13), 37.29 (C-22), 34.56 (C-7), 34.21 (C-16), 31.06 (C-21), 30.00 (C-15), 28.08 (C-23), 25.79 (C-12), 23.84 (C-2), 21.45 (C-32), 21.12 (C-11), 19.68 (C-30), 18.34 (C-6), 16.62 (C-24), 16.38 (C-25, C-26), 14.83 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 626 (76%, $[M+H]^+$), 1249 (100%, $[2M + H]^+$); analysis calcd for $C_{41}H_{56}N_2O_3$ (624.91): C 78.80, H 9.03, N 4.48; found: C 78.61, H 9.18, N 4.61.

4.4.18. Quinolin-5-yl 3β-acetoxy-lup-20(29)-en-28-amide (20)

According to GPC from **17** (200 mg, 0.4 mmol) and 5-aminoquinoline (200 mg, 1.4 mmol) followed by chromatography (SiO_2: hexanes/EtOAc, EtOAc: 25% → 50%), **20** (160 mg, 64%) was obtained as an off-white solid; m.p. 277–280 °C (decomp.); $[\alpha]_D = +21.2°$ (*c* 0.261, $CHCl_3$); $R_F = 0.54$ (SiO_2, $CHCl_3$/MeOH, 95:5); UV–Vis ($CHCl_3$): λ_{max} (log ε) = 236 nm (3.93); IR (ATR): ν = 2943*m*, 1730*m*, 1649*w*, 1594*w*, 1477*m*, 1392*w*, 1367*m*, 1244*s*, 1181*w*, 1028*m*, 979*m*, 883*m*, 797*s*, 755*m*, 651*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.91–8.86 (*m*, 1H, 39-H), 8.15 (*d*, *J* = 8.5 Hz, 1H, 41-H), 7.97 (*dd*, *J* = 6.8, 2.6 Hz, 1H, 34-H), 7.71–7.63 (*m*, 2H, 36-H, 35-H), 7.40 (*dd*, *J* = 8.5, 4.2 Hz, 1H, 40-H), 4.74 (*s*, 1H, 29-H_a), 4.62 (*s*, 1H, 29-H_b), 4.50–4.41 (*m*, 1H, 3-H), 3.19 (*td*, *J* = 10.9, 4.7 Hz, 1H, 19-H), 2.57 (*td*, *J* = 12.6, 3.4 Hz, 1H, 13-H), 2.30–2.20 (*m*, 1H, 16-H_a), 2.11–1.99 (*m*, 2H, 21-H_a, 22-H_a), 2.03 (*s*, 3H, 32-H), 1.71 (*s*, 3H, 30-H), 1.89–1.15 (*m*, 17H, 16-H_b, 12-H_a, 15-H_a, 9-H, 1-H_a, 22-H_b, 2-H, 6-H_a, 21-H_b, 11-H_a, 7-H, 6-H_b, 15-H_b, 18-H, 11-H_b), 1.03 (*s*, 3H, 27-H), 1.11–0.91 (*m*, 2H, 12-H_b, 1-H_b), 0.99 (*s*, 3H, 24-H), 0.84 (*s*, 3H. 23-H), 0.83 (*s*, 3H, 25-H), 0.82 (*s*, 3H, 26-H), 0.79 (*m*, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 175.51 (C-28), 171.17 (C-31), 150.66 (C-20), 150.13 (C-39), 148.40 (C-37), 133.14 (C-42), 130.88 (C-41), 129.47 (C-35), 127.29 (C-34), 123.89 (C-33), 122.56 (C-36), 121.00 (C-40), 109.81 (C-29), 81.06 (C-3), 56.88 (C-17), 55.64 (C-5), 50.75 (C-18), 50.46 (C-9), 46.71 (C-19), 42.76 (C-14), 41.03 (C-8), 38.64 (C-4), 38.64 (C-1), 37.94 (C-22), 37.91 (C-13), 37.28 (C-10), 34.56 (C-7), 34.19 (C-16), 31.06 (C-21), 29.99 (C-15), 28.07 (C-23), 25.77 (C-12), 23.84 (C-2), 21.45 (C-32), 21.12 (C-11), 19.66 (C-30), 18.32 (C-6), 16.62 (C-24), 16.38 (C-25, C-26), 14.83 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 625 (100%, $[M+H]^+$), 1249 (44%, $[2M + H]^+$); $C_{41}H_{56}N_2O_3$ (624.91): C 78.80, H 9.03, N 4.48; found: C 78.53, H 9.23, N 4.57.

4.4.19. 3β-Acetyloxy-20-oxo-30-norlupan-28-oic acid (21)

According to GPA from **9** (15.02 g, 32.8 mmol) followed by crystallization from EtOH, **21** (13.8 g, 84%) was obtained as a colorless solid; m.p. 268–270 °C (decomp.) (lit.: [39] 252–255 °C); $[\alpha]_D = -9.1°$ (*c* 0.34, $CHCl_3$) [lit.: [39] $[\alpha]_D = -9.5°$ (*c* 0.80, $CHCl_3$)]; $R_F = 0.50$ (SiO_2, toluene/EtOAc/heptane/HCOOH, 80:26:10:5).

4.4.20. Benzyl 3β-acetyloxy-20-oxo-30-norlupan-28-amide (22)

According to GPB from **21** (250 mg, 0.5 mmol) and benzylamine (0.2 mL, 1.9 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 5% → 30%) **22** (244 mg, 76%) [40] was obtained as a colorless solid; m.p. 290 °C (decomp.); $[\alpha]_D = +0.5°$ (*c* 0.159, $CHCl_3$); $R_F = 0.35$ (SiO_2, hexanes/EtOAc, 3:1); IR (ATR): ν = 2943*m*, 1733*m*, 1654*m*, 1519*m*, 1453*w*, 1367*m*, 1244*s*, 1027*m*, 979*m*, 753*m*, 698*m*, 609*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.37–7.27 (*m*, 5H, 35-H, 37-H, 38-H, 34-H, 36-H), 5.95 (*t*, *J* = 5.7 Hz, 1H, NH), 4.48 (*m*, 2H, 3-H, 32-H_a), 4.37–4.27 (*m*, 1H, 32-H_b), 3.48 (*m*, 1H, 19-H), 2.26 (*m*, 1H, 13-H), 2.17 (*s*, 3H, 29-H), 2.13–2.04 (*m*, 1H, 18-H), 2.03 (*s*, 3H, 31-H), 1.88 (*m*, 1H, 16-H_a), 1.77 (*m*, 1H, 21-H_a), 1.68–1.20 (*m*, 17H, 1-H_a, 2-H, 6-H, 7-H, 9-H, 11-H, 12-H, 15-H, 16-H_b, 21-H_b, 22-H_b), 1.19–1.02 (*m*, 1H, 1-H_b), 0.98 (*s*, 3H, 27-H), 0.88 (*s*, 3H, 26-H), 0.84–0.80 (*m*, 9H, 23-H, 26-H, 27-H), 0.78 (*m*, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 213.01 (C-20), 175.88 (C-28), 171.09 (C-30), 139.12 (C-33), 128.84 (C-35, C-37), 127.89 (C-34, C-38), 127.55 (C-36), 81.01 (C-3), 55.66 (C-17), 55.55 (C-5), 51.17 (C-19), 50.57 (C-9), 50.19 (C-18), 43.46 (C-32), 42.42 (C-14), 40.84 (C-8), 38.52 (C-1), 38.11 (C-4), 37.94 (C-22), 37.27 (C-10), 36.94 (C-13), 34.41 (C-7), 33.13 (C-16), 30.47 (C-29), 29.62 (C-15), 28.72 (C-21), 28.08 (C-23), 27.39 (C-12), 23.81 (C-2), 21.44 (C-31), 21.09 (C-11), 18.32 (C-6), 16.63 (C-24), 16.36 (C-25), 16.22 (C-26), 14.82 (C-27) ppm; MS (ESI, MeOH): *m*/*z* 590 (100%, $[M+H]^+$), 1179 (66%, $[2M + H]^+$), 1201 (54%, $[2M + Na]^+$); analysis calcd for $C_{38}H_{55}NO_4$ (589.86): C 77.38, H 9.40, N 2.37; found: C 77.19, H 9.65, N 2.50.

4.4.21. Isoquinolin-4-yl 3β-acetyloxy-20-oxo-30-norlupan-28-amide (23)

According to GPC from **21** (250 mg, 0.5 mmol) and 4-aminoisoquinoline (250 mg, 1.7 m mol) followed by chromatography SiO_2, hexanes/EtOAc, EtOAc: 20% → 80%) **23** (228 mg, 72%) was obtained as an off-white solid; m.p. 235 °C (decomp.); $[\alpha]_D = -20.3°$ (*c* 0.211, $CHCl_3$); $R_F = 0.26$ (SiO_2, $CHCl_3$/MeOH, 95:5); UV–Vis ($CHCl_3$): λ_{max} (log ε) = 228 nm (3.74); IR (ATR): ν = 2943*m*, 1710*m*, 1585*w*, 1516*w*, 1467*m*, 1368*m*, 1245*s*, 1183*w*, 1024*m*, 979*m*, 893*w*, 749*s*, 574*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 9.12 (*s*, 1H, 35-H), 8.72 (*s*, 1H, 33-H), 8.02 (*d*, *J* = 8.1 Hz, 1H, 37-H), 7.78 (*m*, 2H, 38-H, 39-H), 7.65 (*t*, *J* = 7.3 Hz, 1H, 40-H), 4.47 (*dd*, *J* = 10.9, 5.3 Hz, 1H, 3-H), 3.50–3.42 (*m*, 1H, 19-H), 2.36 (*m*, 1H, 13-H), 2.30–2.20 (*m*, 2H, 16-H_a, 18-H), 2.17 (*s*, 3H, 29-H), 2.15–2.06 (*m*, 1H, 22-H_a), 2.03 (*s*, 3H, 31-H), 1.92–1.82 (*m*, 1H, 16-H_b), 1.78–1.14 (*m*, 15H, 22-H_b, 15-H_a, 1-H_a, 2-H, 21-H, 6-H_a, 11-H_a, 7-H, 15-H_b, 6-H_b, 9-H, 11-H_b), 1.08 (*s*, 2H, 12-H), 1.06 (*s*, 3H, 27-H), 0.99 (*s*, 3H, 25-H), 0.96 (*s*, 1H, 1-H_b), 0.83 (*d*, *J* = 7.1 Hz, 9H, 23-H, 24-H, 26-H), 0.80 (*m*, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 212.90 (C-20), 175.69 (C-28), 171.38 (C-30), 150.31 (C-35), 138.85 (C-33), 131.70 (C-32), 131.26 (C-38), 128.70 (C-37), 127.98 (C-40), 121.22 (C-39), 81.25 (C-3), 57.06 (C-17), 55.83 (C-5), 51.14 (C-19), 50.86 (C-9), 50.47 (C-18), 42.82 (C-14), 41.19 (C-8), 38.80 (C-1), 38.43 (C-4), 38.21 (C-22), 37.54 (C-10), 37.24 (C-13), 34.71 (C-7), 33.76 (C-16), 30.77 (C-29), 30.32 (C-15), 28.93 (C-21), 28.33 (C-23), 27.65 (C-12), 24.07 (C-2), 21.70 (C-31), 21.36 (C-11), 18.57 (C-6), 16.88 (C-24), 16.62 (C-25), 16.57 (C-26), 15.15 (C-

27) ppm; MS (ESI, MeOH): *m/z* 627 (100%, $[M+H]^+$), 1253 (58%, $[2M+H]^+$); analysis calcd for $C_{40}H_{54}N_2O_4$ (626.88): C 76.64, H 8.68, N 4.47; found: C 76.51, H 8.96, N 4.62.

4.4.22. Quinolin-5-yl 3β -acetyloxy-20-oxo-30-norlupan-28-amide (24)

According to GPC from **21** (250 mg, 0.5 mmol) and 5-aminoquinoline (250 mg, 1.7 mmol) followed by chromatography (SiO_2, hexanes/EtOAc, EtOAc: 25% → 80%) **24** (177 mg, 57%) was obtained as an off-white solid; m.p. 277–280 °C (decomp.); $[\alpha]_D = -35.9°$ (*c* 0.106, $CHCl_3$); $R_F = 0.36$ (SiO_2, $CHCl_3$/MeOH, 95:5); UV–Vis ($CHCl_3$): λ_{max} (log ε) = 235 nm (3.95); IR (ATR): ν = 2944*m*, 1732*m*, 1711*m*, 1595*w*, 1515*w*, 1476*m*, 1367*m*, 1244*s*, 1182*w*, 1133*w*, 1024*m*, 979*m*, 798*s*, 752*s*, 665*w*, 610*w*, 496*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.90 (*d*, *J* = 3.0 Hz, 1H, 38-H), 8.21 (*d*, *J* = 8.4 Hz, 1H, 40-H), 8.03 (*d*, *J* = 7.7 Hz, 1H, 33-H), 7.71 (*m*, 2H, 35-H, 34-H), 7.45 (*dd*, *J* = 8.5, 4.3 Hz, 1H, 39-H), 4.51–4.42 (*m*, 1H, 3-H), 3.47 (*m*, 1H, 19-H), 2.36 (*s*, 1H, 13-H), 2.25 (*m*, 2H, 16-H_a, 18-H), 2.17 (*s*, 3H, 29-H), 2.14–2.05 (*m*, 2H, 21-H_a, 22-H_a), 2.03 (*s*, 3H, 31-H), 1.88 (*m*, 1H, 16-H_b), 1.80–1.16 (*m*, 14H, 22-H_b, 15-H_a, 1-H_a, 2-H, 6-H_a, 11-H_a, 7-H, 15-H_b, 6-H_b, 9-H, 11-H_b, 21-H_b), 1.09 (*s*, 2H, 12-H), 1.07 (*s*, 3H, 27-H), 0.99 (*s*, 3H 25-H), 0.97 (*s*, 1H, 1-H_b), 0.83 (*m*, 9H, 23-H, 24-H, 26-H), 0.79 (*s*, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 212.59 (C-20), 175.45 (C-28), 171.12 (C-30), 149.77 (C-38), 133.06 (C-36), 131.51 (C-40), 129.82 (C-34), 127.05 (C-33), 123.97 (C-32), 122.86 (C-35), 121.06 (C-39), 107.47 (C-41), 80.97 (C-3), 56.79 (C-17), 55.57 (C-5), 50.94 (C-19), 50.59 (C-9), 50.24 (C-18), 42.57 (C-14), 40.95 (C-8), 38.55 (C-1), 38.17 (C-4), 37.95 (C-22), 37.29 (C-10), 37.04 (C-13), 34.46 (C-7), 33.48 (C-16), 30.49 (C-29), 30.07 (C-15), 28.69 (C-21), 28.08 (C-23), 27.38 (C-12), 23.81 (C-2), 21.45 (C-31), 21.12 (C-11), 18.31 (C-6), 16.63 (C-24), 16.37 (C-25), 16.35 (C-26), 14.91 (C-27) ppm; MS (ESI, MeOH): *m/z* 628 (100%, $[M+H]^+$), 1253 (24%, $[2M+H]^+$); analysis calcd for $C_{40}H_{54}N_2O_4$ (626.88): C 76.64, H 8.68, N 4.47; found: C 76.46, H 8.87, N 4.65.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2020.112815.

References

[1] G.R. Dagenais, D.P. Leong, S. Rangarajan, F. Lanas, P. Lopez-Jaramillo, R. Gupta, R. Diaz, A. Avezum, G.B.F. Oliveira, A. Wielgosz, S.R. Parambath, P. Mony, K.F. Alhabib, A. Temizhan, N. Ismail, J. Chifamba, K. Yeates, R. Khatib, O. Rahman, K. Zatonska, K. Kazmi, L. Wei, J. Zhu, A. Rosengren, K. Vijayakumar, M. Kaur, V. Mohan, A. Yusufali, R. Kelishadi, K.K. Teo, P. Joseph, S. Yusuf, Variations in common diseases, hospital admissions, and deaths in middle-aged adults in 21 countries from five continents (PURE): a prospective cohort study, Lancet 395 (2020) 785–794.
[2] J. Ferlay, M. Colombet, I. Soerjomataram, C. Mathers, D.M. Parkin, M. Pineros, A. Znaor, F. Bray, Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods, Int. J. Canc. 144 (2019) 1941–1953.
[3] NN, accessed 2020-04-17, https://www.vfa.de/de/presse/pressemitteilungen/pm-036-2019-bilanz-2019-neue-medikamente-bringen-fortschritt-fuer-patienten.html last.
[4] D.J. Newman, G.M. Cragg, Natural products as sources of new drugs from 1981 to 2014, J. Nat. Prod. 79 (2016) 629–661.
[5] J. Gershenzon, N. Dudareva, The function of terpene natural products in the natural world, Nat. Chem. Biol. 3 (2007) 408–414.
[6] J.A.R. Salvador, Pentacyclic Triterpenes as Promising Agents in Cancer, Nova Science Publishers, Inc., New York, 2010.
[7] R. Csuk, in: G.G.E. Chen, P.B.S.E. Lai (Eds.), Targeting Cancer by Betulin and Betulinic Acid in "Novel Apoptotic Regulators in Carcinogenesis", Springer, Dordrecht, 2012, pp. 267–287.
[8] R. Csuk, Betulinic acid and its derivatives: a patent review (2008-2013), Expert Opin. Ther. Pat. 24 (2014) 913–923.
[9] D. Biedermann, B. Eigenrova, M. Hajduch, J. Sarek, Synthesis and Evaluation of Biological Activity of the Quaternary Ammonium Salts of Lupane-, Oleanane-, and Ursane-type Acids, Synthesis, 2010, pp. 3839–3848.
[10] V.E. Kataev, I.Y. Strobykina, L.Y. Zakharova, Quaternary ammonium derivatives of natural terpenoids. Synthesis and properties, Russ. Chem. Bull. 63 (2014) 1884–1900.
[11] A.Y. Spivak, D.A. Nedopekina, R.R. Khalitova, R.R. Gubaidullin, V.N. Odinokov, Y.P. Bel'skii, N.V. Bel'skaya, V.A. Khazanov, Triphenylphosphonium cations of betulinic acid derivatives: synthesis and antitumor activity, Med. Chem. Res. 26 (2017) 518–531.
[12] A.Y. Spivak, D.A. Nedopekina, E.R. Shakurova, R.R. Khalitova, R.R. Gubaidullin, V.N. Odinokov, U.M. Dzhemilev, Y.P. Bel'skii, N.V. Bel'skaya, S.A. Stankevich, E.V. Korotkaya, V.A. Khazanov, Synthesis of lupane triterpenoids with triphenylphosphonium substituents and studies of their antitumor activity, Russ. Chem. Bull. 62 (2013) 188–198.
[13] J. Zielonka, J. Joseph, A. Sikora, M. Hardy, O. Ouari, J. Vasquez-Vivar, G. Cheng, M. Lopez, B. Kalyanaraman, Mitochondria-targeted triphenylphosphonium-based compounds: syntheses, mechanisms of action, and therapeutic and diagnostic applications, Chem. Rev. 117 (2017) 10043–10120.
[14] B. Brandes, S. Hoenke, L. Fischer, R. Csuk, Design, synthesis and cytotoxicity of BODIPY FL labelled triterpenoids, Eur. J. Med. Chem. 185 (2020) 111858.
[15] S. Krajčovičová, J. Stankova, P. Dzubak, M. Hajduch, M. Soural, M. Urban, Synthetic approach for rapid preparation of BODIPY conjugates and their use in imaging of cellular drug uptake and distribution, Chem. A Eur J. 24 (2018).
[16] S. Friedrich, I. Serbian, S. Hoenke, R.K. Wolfram, R. Csuk, Synthesis and cytotoxic evaluation of malachite green derived oleanolic and ursolic acid piperazineamides, Med. Chem. Res. 29 (2020) 926–933.
[17] M. Kahnt, J. Wiemann, L. Fischer, S. Sommerwerk, R. Csuk, Transformation of asiatic acid into a mitocanic, bimodal-acting rhodamine B conjugate of nanomolar cytotoxicity, Eur. J. Med. Chem. 159 (2018) 143–148.
[18] S. Sommerwerk, L. Heller, C. Kerzig, A.E. Kramell, R. Csuk, Rhodamine B conjugates of triterpenoic acids are cytotoxic mitocans even at nanomolar concentrations, Eur. J. Med. Chem. 127 (2017) 1–9.
[19] R.K. Wolfram, L. Fischer, R. Kluge, D. Ströhl, A. Al-Harrasi, R. Csuk, Homopiperazine-rhodamine B adducts of triterpenoic acids are strong mitocans, Eur. J. Med. Chem. 155 (2018) 869–879.
[20] R.K. Wolfram, L. Heller, R. Csuk, Targeting mitochondria: esters of rhodamine B with triterpenoids are mitocanic triggers of apoptosis, Eur. J. Med. Chem. 152 (2018) 21–30.
[21] I.Z. Pavel, R. Csuk, C. Danciu, S. Avram, F. Baderca, A. Cioca, E.A. Moaca, C.V. Mihali, I. Pinzaru, D.M. Muntean, C.A. Dehelean, Assessment of the anti-angiogenic and anti-inflammatory properties of a maslinic acid derivative and its potentiation using zinc chloride, Int. J. Mol. Sci. 20 (2019).
[22] I.Z. Pavel, C. Danciu, C. Oprean, C.A. Dehelean, D. Muntean, R. Csuk, D.M. Muntean, In vitro evaluation of the antimicrobial ability and cytotoxicity on two melanoma cell lines of a benzylamide derivative of maslinic acid, Anal. Cell Pathol. (2016), 2787623.
[23] I.Z. Pavel, C.A. Dehelean, L. Farczadi, D.M. Muntean, L. Vlase, C. Danciu, R. Csuk, F. Birsasteanu, D.M. Muntean, Assessment of a maslinic acid derivative and its metabolite in rat blood by liquid chromatography coupled with mass spectrometry, Rev. Chim.-Bucharest 68 (2017) 1089–1094.
[24] I.Z. Pavel, O.M. Duicu, I. Scurtu, D. Coricovac, C. Dehelean, R. Csuk, D.M. Muntean, Assessment of the effects of a maslinic acid derivative in isolated mouse liver mitochondria, Eur. J. Clin. Invest. 45 (2015), 29-29.
[25] I.Z. Pavel, A.E. Parvu, C.A. Dehelean, L. Vlase, R. Csuk, D.M. Muntean, Assessment of the antioxidant effect of a maslinic acid derivative in an experimental model of acute inflammation, FARMACIA 65 (2017) 390–395.
[26] B. Siewert, E. Pianowski, A. Obernauer, R. Csuk, Towards cytotoxic and selective derivatives of maslinic acid, Bioorg. Med. Chem. 22 (2014) 594–615.
[27] S. Sommerwerk, L. Heller, J. Kuhfs, R. Csuk, Selective killing of cancer cells with triterpenoic acid amides - the substantial role of an aromatic moiety alignment, Eur. J. Med. Chem. 122 (2016) 452–464.
[28] M. Kahnt, L. Heller, P. Grabandt, A. Al-Harrasi, R. Csuk, Platanic acid: a new scaffold for the synthesis of cytotoxic agents, Eur. J. Med. Chem. 143 (2018) 259–265.
[29] M. Kahnt, L. Heller, A. Al-Harrasi, R. Schäfer, R. Kluge, C. Wagner, C. Otgonbayar, R. Csuk, Platanic acid-derived methyl 20-amino-30-norlupan-

28-oates are potent cytotoxic agents acting by apoptosis, Med. Chem. Res. 27 (2018) 1757–1769.
[30] J. Wiemann, L. Heller, V. Perl, R. Kluge, D. Ströhl, R. Csuk, Betulinic acid derived hydroxamates and betulin derived carbamates are interesting scaffolds for the synthesis of novel cytotoxic compounds, Eur. J. Med. Chem. 106 (2015) 194–210.
[31] S. Sommerwerk, R. Csuk, Convenient and chromatography-free partial syntheses of maslinic acid and augustic acid, Tetrahedron Lett. 55 (2014) 5156–5158.
[32] A. Barthel, S. Stark, R. Csuk, Oxidative transformations of betulinol, Tetrahedron 64 (2008) 9225–9229.
[33] Y. Fukuda, K. Sakai, S. Matsunaga, H. Tokuda, R. Tanaka, Cancer chemopreventive effect of orally administrated lupane-type triterpenoid on ultraviolet light B induced photocarcinogenesis of hairless mouse, Canc. Lett. 240 (2006) 94–101.
[34] Y. Wei, C.-M. Ma, M. Hattori, Synthesis and evaluation of A-seco type triterpenoids for anti-HIV-1protease activity, Eur. J. Med. Chem. 44 (2009) 4112–4120.
[35] M. Urban, J. Sarek, J. Klinot, G. Korinkova, M. Hajduch, Synthesis of A-seco derivatives of betulinic acid with cytotoxic activity, J. Nat. Prod. 67 (2004) 1100–1105.
[36] J. Hao, X. Zhang, P. Zhang, J. Liu, L. Zhang, H. Sun, Efficient access to isomeric 2,3-dihydroxy lupanes: first synthesis of alphitolic acid, Tetrahedron 65 (2009) 7975–7984.
[37] N.G. Komissarova, N.G. Belenkova, O.V. Shitikova, L.V. Spirikhin, M.S. Yunusov, Oxidative decarboxylation of triterpene C-28 acids of lupane series, Nat. Prod. Commun. 3 (2008) 1569–1574.
[38] R.K. Wolfram, L. Heller, R. Csuk, Targeting mitochondria: esters of rhodamine B with triterpenoids are mitocanic triggers of apoptosis, Eur. J. Med. Chem. 152 (2018) 21–30.
[39] A. Vystrcil, M. Budesinsky, Triterpenes. XVI. Unusual epimerization of the C-19 acetyl group in 20-oxo-30-norlupane derivatives, Collect. Czech Chem. Commun. 35 (1970) 295–311.
[40] T. Durst, Z. Merali, J.T. Arnason, E.P. Sanchez-Vindas, L.J. Poveda Alvarez, Anxiolytic Marcgraviaceae Compositions Containing Betulinic Acid, Betulinic Acid Derivatives, and Methods of Preparation and Use, 2002, p. CA2446748C.