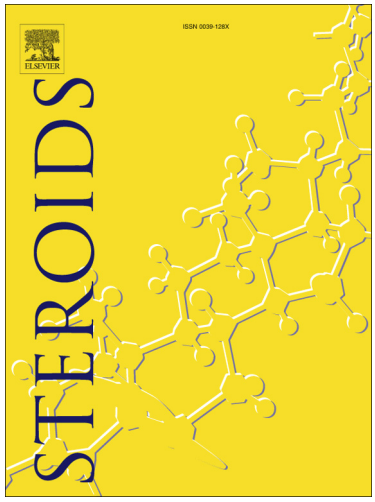


The cytotoxicity of oleanane derived aminocarboxamides depends on their aminoalkyl substituents

Michael Kahnt, Anne Loesche, Immo Serbian, Sophie Hoenke, Lucie Fischer, Ahmed Al-Harrasi, René Csuk

PII: S0039-128X(19)30112-6
DOI: https://doi.org/10.1016/j.steroids.2019.05.014
Reference: STE 8422

To appear in: *Steroids*

Received Date: 20 March 2019
Revised Date: 27 May 2019
Accepted Date: 29 May 2019

Please cite this article as: Kahnt, M., Loesche, A., Serbian, I., Hoenke, S., Fischer, L., Al-Harrasi, A., Csuk, R., The cytotoxicity of oleanane derived aminocarboxamides depends on their aminoalkyl substituents, *Steroids* (2019), doi: https://doi.org/10.1016/j.steroids.2019.05.014

The cytotoxicity of oleanane derived aminocarboxamides depends on their aminoalkyl substituents

Michael Kahnt [a], Anne Loesche [a], Immo Serbian [a], Sophie Hoenke [a], Lucie Fischer [a], Ahmed Al-Harrasi [b], René Csuk*[,a]

[a] *Martin-Luther-University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany*

[b] University of Nizwa, Natural and Medical Sciences Research Center, P.O. Box 33, Birkat Al Mauz, Nizwa 616, Sultanate of Oman.

*Corresponding author: Prof. Dr. René Csuk
Martin-Luther University Halle-Wittenberg
Organic Chemistry
Kurt-Mothes-Str. 2
D-06120 Halle (Saale)
Germany
Tel.: +49 345 5525660
Fax: +49 345 5527030
e-mail: rene.csuk@chemie.uni-halle.de

Abstract

Several oligo-methylene diamine derived carboxamides of oleanolic and maslinic acid have been prepared, and substitutions of the terminal primary amine as well as variations of the length of alkyl chain of the diamine moiety were made. Biological evaluation of their cytotoxic activity was performed using photometric sulforhodamin B assays employing a panel of different human cancer cell lines. These experiments showed most of the carboxamides to be cytotoxic with EC_{50} values below 10 µM. Prolongation of the alkyl chain length initially reduced EC_{50} values to a minimum, but a decrease in cytotoxicity was observed for longer alkyl chains. Variation of substituents at the terminal nitrogen atom, however, did not influence EC_{50} values at all. Noteworthy results were obtained particularly for compounds **4**, **6** and **23** as indicated by EC_{50} values lower than 2 µM, and in case of a maslinic derivative **23** even an increased tumor/non-tumor cell selectivity was observed. These compounds were further

investigated using fluorescence microscopy and flow cytometry analysis, which revealed **6** to show indications of apoptosis.

Keywords: Oleanolic acid; maslinic acid; cytotoxicity; triterpenoids, carboxamides

1 Introduction

Medicinal herbs have been playing an important role in traditional medicine for thousands of years. In search of rescue for their disease, people have been searching for drugs in nature ever since. The oldest written evidence of plants' medicinal usage is approximately 5000 years old.[1] Nowadays nearly 25% of all plant species in the world have some kind of medicinal use.[2] Due to their broad spectrum of biological and pharmacological properties such as anti-inflammatory, antimalarial, antitumor and antiviral activities, natural products are considered to be ideal candidates for modern drug discovery. A recent study revealed about 50% of all new approved drugs in the period between 1981 and 2014 being natural products or natural product related.[3] One of the most prevalent disease in present time remains cancer, resulting in approximately 9.6 million cancer deaths worldwide in 2018.[4] With more than 50% of all new anticancer drugs (1981-2014), natural products and derivatives thereof still play a key role in treating cancer.[3] A promising class of natural products are triterpenes including (among others) oleanolic (Fig. 1, **OA**) and maslinic acid (Fig. 1, **MA**), both of which can be found in and isolated from plants. They show some promising biological properties such as antioxidant [5-8], antiviral [5, 9-11] and antitumor activity [5, 12-18]. In this study, we focused on the improvement of the antitumor activity of both triterpenes by chemical modification. Oleanolic derivatives were previously investigated by Heller et al., showing ethylene diamine derived carboxamides to be of an increased cytotoxicity as compared to their parent compounds.[19] Previous studies, however, focused on the investigation of the influence of the substitution at the primary amine of the ethylene diamine moiety.[19, 20] Also included in this study was an ursolic carboxamide holding an extended alkyl length (derived from tetramethylene diamine), that showed promising cytotxic activity in the range of 1.3–3.2 μM.[20] Therefore, we decided to additionally vary the length of the α,ω-diamine moiety to evaluate its influence on cytotoxicity. For successful cancer treatment, cytotoxicity towards cancer cells (as indicated by low EC_{50} values) is very important, but gaining selectivity between tumor and non-tumor cells is even more essential. Recent studies revealed maslinic acid derivatives to show increased

cytotoxicity being accompanied by an improved tumor–non-tumor cell selectivity - as compared to their analogs derived from oleanolic acid.[21-23] Keeping this in mind, we prepared a small library of carboxamides of oleanolic and maslinic acid and compared both cytotoxicity as well as selectivity.

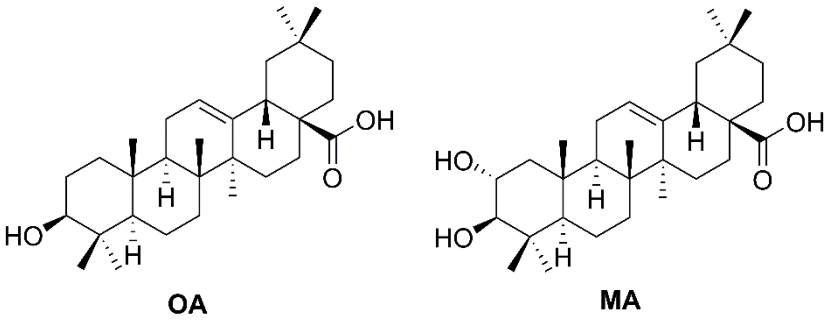
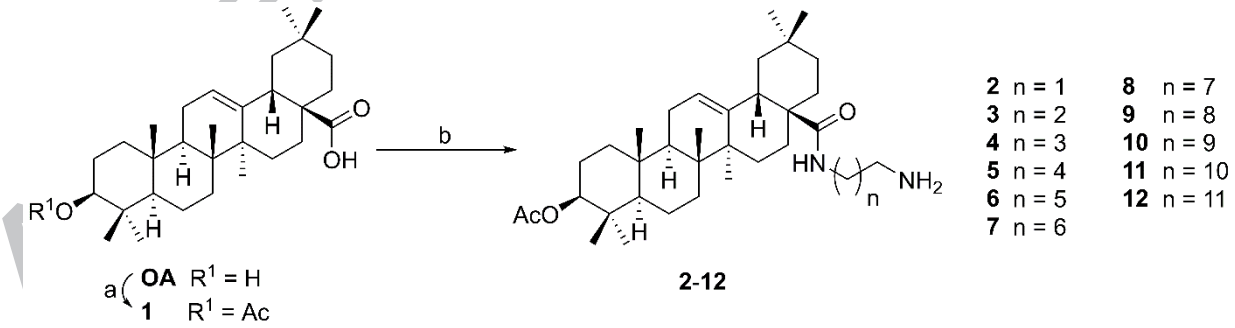


Fig. 1: Structures of oleanolic (**OA**) and maslinic acid (**MA**).

2 Results and Discussion

Oleanolic acid (**OA**) was obtained from Carbone Scientific (London, UK) in 98.6% purity and maslinic acid (**MA**) was synthesized as previously described.[24, 25] Acetylation of both triterpenoic acids afforded acetates **1** (Scheme 1) and **13** (Scheme 2) in excellent yields. Treatment of acetate **1** with oxalyl chloride and α,ω-alkyl diamines of various chain length provided compounds **2-12** (Scheme 1).



Scheme 1: Synthesis of oleanolic carboxamides **2-12**: a) Ac_2O, DCM, NEt_3, DMAP, 25 °C, 2 days, 90%; b) oxalyl chloride, DCM, DMF, 0–25 °C, 15 h, then amine, 25 °C, 2 h, 36-60%.

The cytotoxicity of oleanolic carboxamides **2-12** was determined in sulforhodamine B (SRB) assays.[26] The results of this screening are compiled in Table 1.

Table 1: Cytotoxicity of compounds **2-12** and oleanolic acid (**OA**): EC_{50} values from SRB assays after 96 h of treatment are given in μM (n.d. not detected); the values are averaged from three independent experiments each performed in triplicate; confidence interval CI = 95%.

Compound	A253	A2780	HT29	MCF-7	SW1736	NIH 3T3
OA	n.d.	>30	>30	>30	n.d.	>30
2	3.1 ± 0.1	3.1 ± 0.1	2.0 ± 0.2	1.7 ± 0.2	3.9 ± 0.6	2.1 ± 0.1
3	3.1 ± 0.1	3.1 ± 0.2	2.2 ± 0.2	1.7 ± 0.1	3.1 ± 0.3	2.4 ± 0.9
4	1.2 ± 0.9	1.2 ± 0.4	1.7 ± 0.1	2.0 ± 0.1	3.6 ± 2.1	1.0 ± 0.3
5	3.8 ± 0.4	3.1 ± 0.2	1.4 ± 0.3	1.6 ± 0.3	3.5 ± 0.5	2.0 ± 0.2
6	3.1 ± 0.2	3.1 ± 0.2	1.2 ± 0.1	3.9 ± 0.9	3.8 ± 0.2	2.7 ± 0.4
7	2.8 ± 0.5	3.0 ± 0.1	1.2 ± 0.1	3.1 ± 0.2	3.5 ± 0.1	2.2 ± 0.1
8	3.7 ± 0.8	2.5 ± 0.2	1.8 ± 0.1	3.1 ± 0.7	3.5 ± 0.4	2.3 ± 0.2
9	2.4 ± 0.3	2.0 ± 0.2	1.6 ± 0.1	2.4 ± 0.2	3.8 ± 0.6	2.3 ± 0.2
10	2.7 ± 0.2	2.7 ± 0.2	3.1 ± 0.2	3.1 ± 0.1	7.0 ± 0.5	3.0 ± 0.2
11	2.3 ± 0.4	2.9 ± 0.3	3.2 ± 0.1	3.2 ± 0.3	3.5 ± 0.1	3.2 ± 0.7
12	2.5 ± 0.6	4.6 ± 0.7	5.4 ± 0.3	5.2 ± 0.2	6.9 ± 0.1	5.7 ± 0.1

The influence of an altered chain length on the cytotoxicity of the compounds shall be demonstrated comparing the EC_{50} values for the human colon adenocarcinoma cell line HT29 (Fig. 2). Thus, EC_{50} values initially decreased with increasing chain length *n* up to a minimum value of 1.2 μM (*n* = 5 (**6**) or *n* = 6 (**7**)). For *n* greater than 6, the EC_{50} values increased slightly until they reached a maximum value at *n* = 11 (**12**, EC_{50} = 5.4 ± 0.3 μM). Comparing the EC_{50} values with those obtained for non-malignant mouse fibroblasts (NIH 3T3), some information on the selectivity [which is defined as selectivity index (SI); e.g. SI = EC_{50} (NIH 3T3)/EC_{50} (HT29)] can be obtained (indicated by red numbers in Fig. 2). As a result, the most selective substance in the series holding a SI of 2.25 was compound **6**; this compound also showed the highest cytotoxicity (EC_{50} = 1.2 ± 0.1 μM). The origin of this selectivity, however, remains unclear. Investigation of the other cancer cell lines revealed also compound **4** as highly cytotoxic. This compound showed noteworthy cytotoxic activity on human submandibular gland A253 cancer cells (1.2 ± 0.9 μM), HT29 (1.7 ± 0.1 μM) and on the human ovarian cancer

cell line A2780 (1.2 ± 0.4 µM). This was the reason to select this substance together with compound **6** for an extended biological testing (Fig. 3).

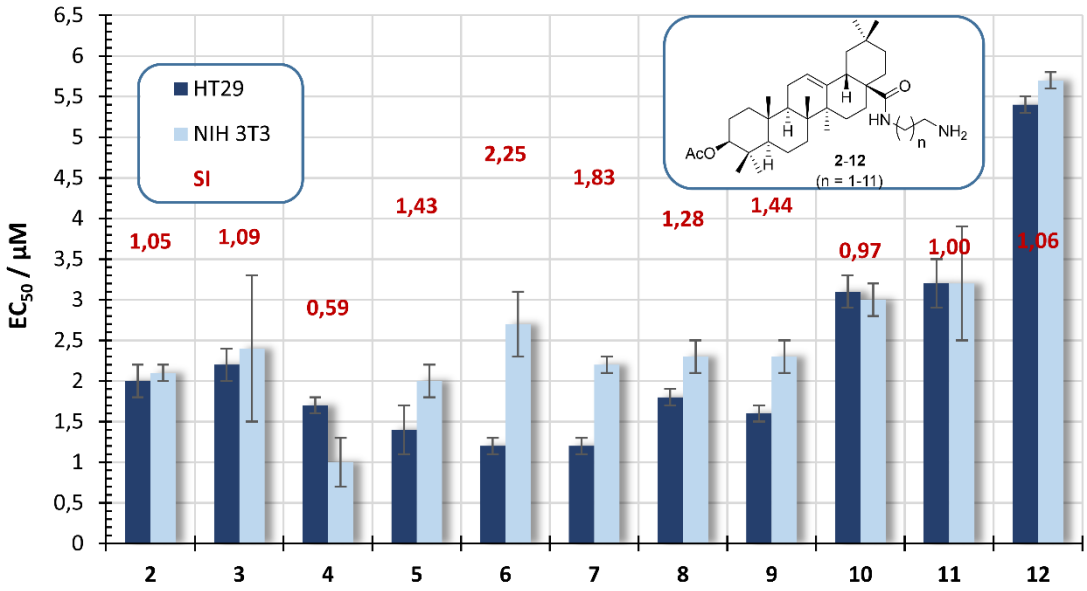


Fig. 2: Influence of chain length on EC_{50} values of compounds **2-12** for HT29 cancer cells compared to non-malignant mouse fibroblasts (NIH 3T3). The selectivity index (SI) is defined as: SI = EC_{50} (NIH 3T3)/EC_{50} (HT29).

Initial investigations were performed by using dye exclusion acridine orange (AO)/ propidium iodide (PI) assays and A2780 cancer cells (Fig. 3, A). Microscopic images of A2780 cells treated with **4** revealed – in addition to many vital cells (green staining) – the presence of some secondary necrotic/late-stage apoptotic cells, as indicated by their orange stained nuclei. Close inspection also showed protrusions of the plasma membrane in some cells (membrane blebbing, marked by white arrows in Fig. 3). These observations were also made for A2780 cells having been treated with **6** for 24 h, while significantly less vital cells were noticed. For a quantification of the apoptosis-inducing activity of **4** and **6**, flow cytometry analyses were performed using an annexin V-FITC/PI staining (Fig. 3, B). Most of the A2780 cells treated with **4** remained vital (72.2%), while 10.0% or 16.1% of the cells were apoptotic or secondary necrotic/late-stage apoptotic, respectively. Treatment of A2780 cells with **6** resulted in 56.1% annexin V-FITC-positive cells, with 43.4% of the cells still being considered vital. Close inspection of the density plots showed most of the cells having died by apoptosis (49.9%) while 6.2% of the cells were secondary necrotic/late-stage apoptotic.

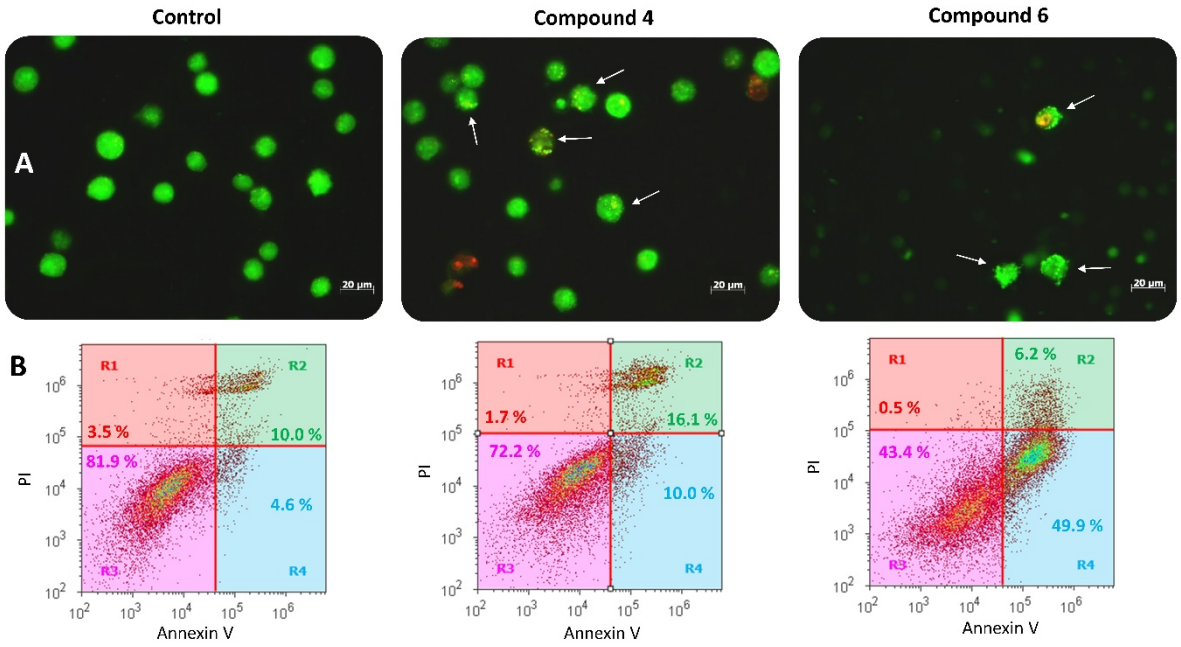
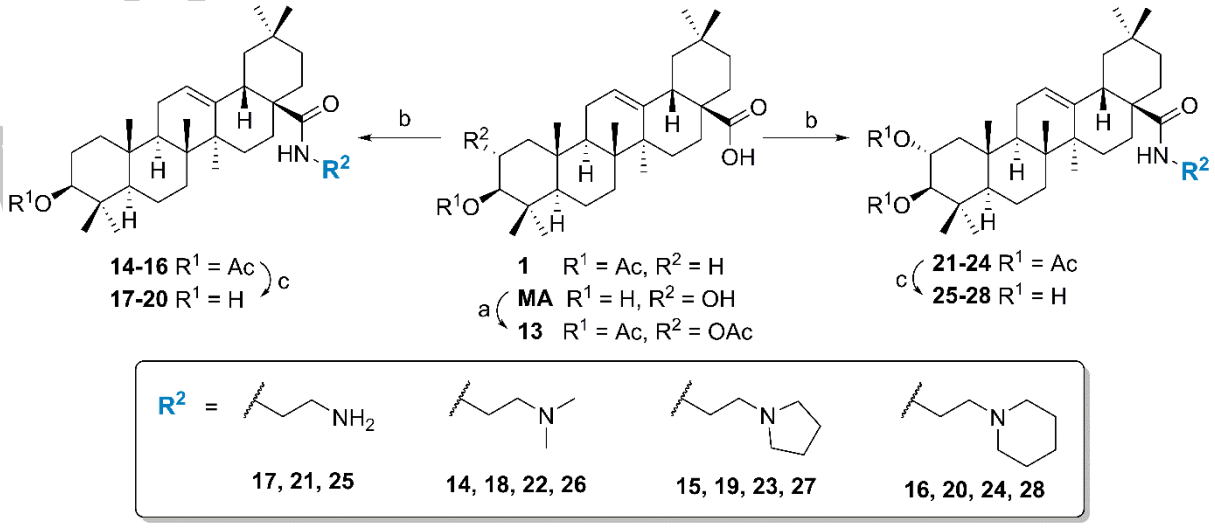


Fig. 3: (A) Fluorescence microscopic images: treatment of A2780 cells with **4** (2.4 µM) and **6** (6.2 µM) for 24 h. Scale bar = 20 µm, AO and PI were used. (B) Annexin V-FITC/PI assay: treatment of A2780 cells with **4** and **6** (2.4 µM/6.2 µM) for 24 h. Examples of density plots determined by flow cytometry (Attune® Cytometric Software v. 1.2.5). R1: necrotic, R2: secondary necrotic/late stage apoptotic, R3: vital, R4: apoptotic.

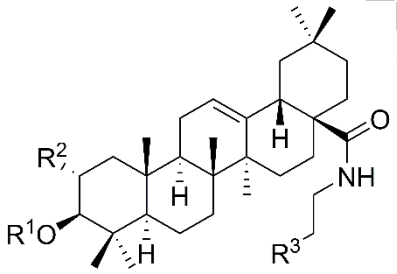
Syntheses of oleanolic and maslinic carboxamides **14-28** derived from ethylene diamine were analogous to compounds **2-12** and obtained from the acetylated triterpenoic acids by reaction with oxalyl chloride followed by adding substituted ethylenediamines (Scheme 2). Finally, the acetoxy groups were removed with an methanolic solution of potassium hydroxide.[27]



Scheme 2: Synthesis of oleanolic and maslinic carboxamides **14-28**: a) Ac_2O, DCM, NEt_3, DMAP, 25 °C, 2 days, 91%; b) oxalyl chloride, DCM, DMF, 0–25 °C, 1 h, then amine, 25 °C, 2 h, 71-96%; c) MeOH/KOH, 25 °C, 2-3 days, 60-93 %.

The cytotoxicity of oleanolic and maslinic carboxamides **14-28** was determined in sulforhodamine B (SRB) assays.[26] The results of these screenings are compiled in Table 2.

Table 2: Cytotoxicity of compounds **14-28**, oleanolic acid (**OA**) and maslinic acid (**MA**): EC_{50} values from SRB assays after 96 h of treatment are given in µM (n.d. not detected); the values are averaged from three independent experiments each performed in triplicate; confidence interval CI = 95%.



	R^1	R^2	R^3	518A2	A2780	HT29	MCF-7	A375	NIH 3T3
OA	H	H	28-COOH	>30	>30	>30	>30	n.d.	>30
MA	H	OH	28-COOH	13.7 ± 0.9	19.5 ± 0.8	28.8 ± 0.5	>30	n.d.	21.1 ± 0.2
17	H	H	NH_2	3.7 ± 0.1	n.d.	3.1 ± 0.3	1.5 ± 0.1	3.2 ± 0.2	3.8 ± 0.6
21	Ac	OAc		3.3 ± 0.2	1.8 ± 0.1	1.7 ± 0.2	1.8 ± 0.2	1.6 ± 0.1	3.4 ± 0.5
25	H	OH		8.7 ± 1.6	7.0 ± 0.8	5.0 ± 0.3	6.9 ± 1.0	6.5 ± 0.6	7.4 ± 0.8
14	Ac	H	NMe_2	3.4 ± 0.6	3.2 ± 0.1	3.9 ± 0.2	2.9 ± 0.3	3.5 ± 0.7	2.8 ± 0.3
18	H	H		3.4 ± 0.3	2.9 ± 0.4	3.7 ± 0.1	2.8 ± 0.6	2.7 ± 0.7	3.5 ± 0.6
22	Ac	OAc		n.d.	1.6 ± 0.1	2.3 ± 0.3	2.4 ± 0.2	n.d.	4.4 ± 0.4
26	H	OH		14.5 ± 0.7	4.8 ± 0.3	5.1 ± 0.6	4.7 ± 0.7	7.0 ± 0.4	7.6 ± 0.7
15	Ac	H	N	3.3 ± 0.3	3.2 ± 0.1	2.8 ± 0.2	2.7 ± 0.3	3.5 ± 0.3	2.7 ± 0.2
19	H	H		3.6 ± 0.3	2.4 ± 0.1	3.4 ± 0.8	1.3 ± 0.1	1.6 ± 0.1	2.6 ± 0.7
23	Ac	OAc		n.d.	1.5 ± 0.3	1.7 ± 0.2	2.0 ± 0.2	n.d.	4.6 ± 0.3
27	H	OH		10.7 ± 0.5	3.6 ± 0.4	4.6 ± 0.7	3.0 ± 0.5	4.6 ± 1.2	6.2 ± 2.9

16	Ac	H		4.6 ± 0.5	3.5 ± 0.8	3.5 ± 0.4	3.5 ± 0.5	4.9 ± 0.1	4.4 ± 0.6
20	H	H		1.5 ± 0.1	1.6 ± 0.1	3.4 ± 0.4	2.0 ± 0.1	1.9 ± 0.1	2.9 ± 0.1
24	Ac	OAc		1.5 ± 0.2	3.5 ± 0.1	2.0 ± 0.1	1.7 ± 0.2	0.9 ± 0.1	2.6 ± 0.3
28	H	OH		8.2 ± 0.4	7.0 ± 0.3	5.9 ± 0.2	4.9 ± 0.7	7.1 ± 0.4	2.0 ± 0.6

Evaluation of the EC_{50} values in Table 2 reveals the impact of the presence of acetyloxy groups on the cytotoxicity of the compounds. As for oleanolic carboxamides (**14-20**), the presence or absence of an acetyl moiety at position 3 had no influence on the EC_{50} values of the compounds at all. This is illustrated in Fig. 4 for HT29 cancer cells. For maslinic acid derivatives **21-28**, however, the impact of the acetoxy moieties on the cytotoxic properties of the compounds was significant. Bisacetyloxy derivatives **21-24** show low EC_{50} values in the range of 1.7 µM to 2.3 µM. Removal of these acetoxy groups (as in compounds **25-28**) more than doubled the EC_{50} values up to 5.9 µM. Contrary to the effect of the acetoxy groups, a substitution of the primary amino function did not affect cytotoxicities at all. This finding is in perfect agreement with the results from a previous investigation.[20] Furthermore, most maslinic carboxamides are significantly higher selective than those derived from oleanolic acid. That can be illustrated by comparing compounds **15** and **23** (Fig. 4). Both compounds share the same carbon skeleton but **15** is derived from oleanolic while **23** is a derivative of maslinic acid. This small structural difference led to a significant increase in selectivity from SI = 0.96 (**15**) to SI = 2.7 (**23**). This observation is consistent with previous studies [21-23], that showed maslinic acid derived derivatives to be of higher selectivity than their oleanolic acid analogs. The presence of an additional hydroxyl moiety at ring A of the triterpenoid backbone has been assumed as a possible reason for the increased selectivity. The highest selectivity of the carboxamides **14-28** was observed for maslinic acid derivative **23** and A2780 tumor cells (SI = 3.1) together with a low EC_{50} value of 1.5 ± 0.3 µM. Therefore, compound **23** was chosen for some extra biological investigations. Selectivity indices of all compounds and human tumor cell lines can be found in the supplementary material (Table 1 and Table 2).

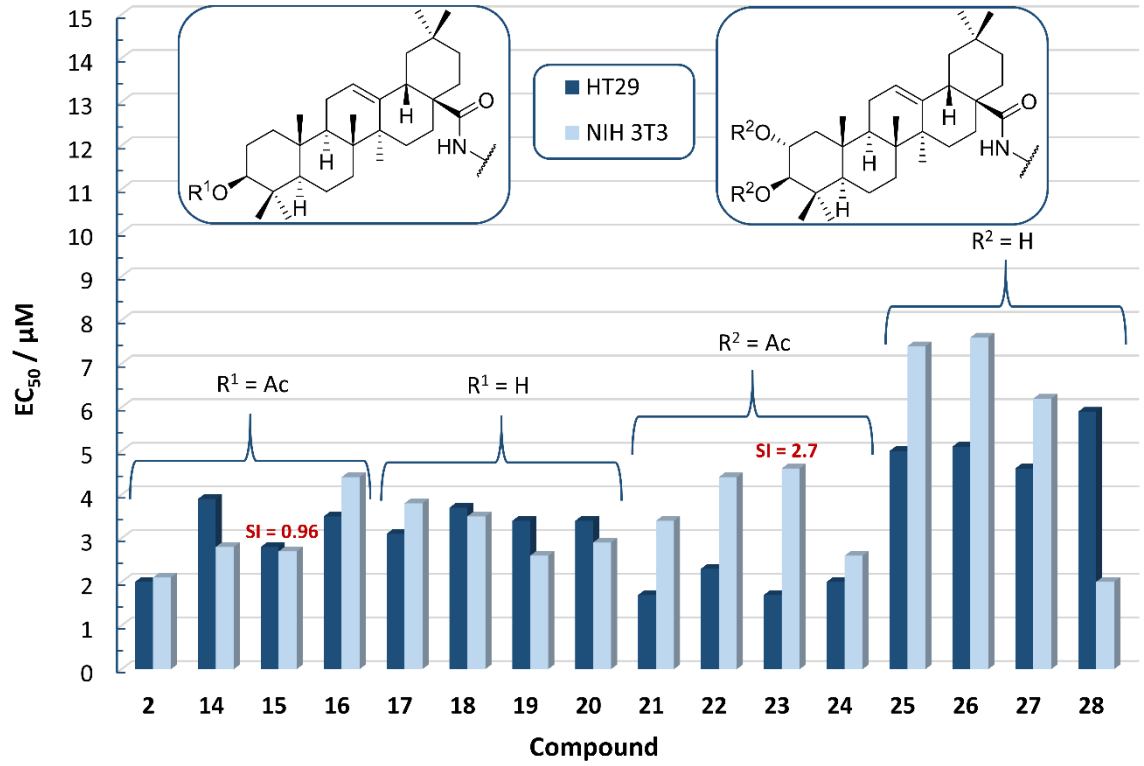


Fig. 4: Cytotoxicity of oleanolic (**2**, **14-20**) and maslinic carboxamides (**21-28**) for HT29 tumor cells vs. non-malignant mouse fibroblasts (EC_{50} from SRB assays). The selectivity index (SI) is defined as: SI = EC_{50} (NIH 3T3)/EC_{50} (HT29).

Microscopic images of A2780 cells treated with **23** for 48h showed the presence of some vital cells, and ruptures in the plasma membrane were noticed (membrane blebbing, marked by white arrows in Fig. 5). Additionally, some orange stained nuclei could be observed, thus indicating late-stage apoptotic cells. For quantification of the apoptosis-inducing activity of **23**, a flow cytometry analysis was performed using an annexin V-FITC/PI staining (Fig. 5). The density plots for the treatment of A2780 cells with **23** for 24 h and 48 h showed significant differences. After 24 h, 73.8% of the tumor cells were still vital, 12.2% were secondary necrotic/late stage apoptotic and 12.6% of the cells having died by apoptosis. After 48 h the quantity of vital cells decreased to 67.7%, while 30.5% of the cells were annexin V-FITC-positive. In addition, the number of apoptotic cells increased to 18.6 %.

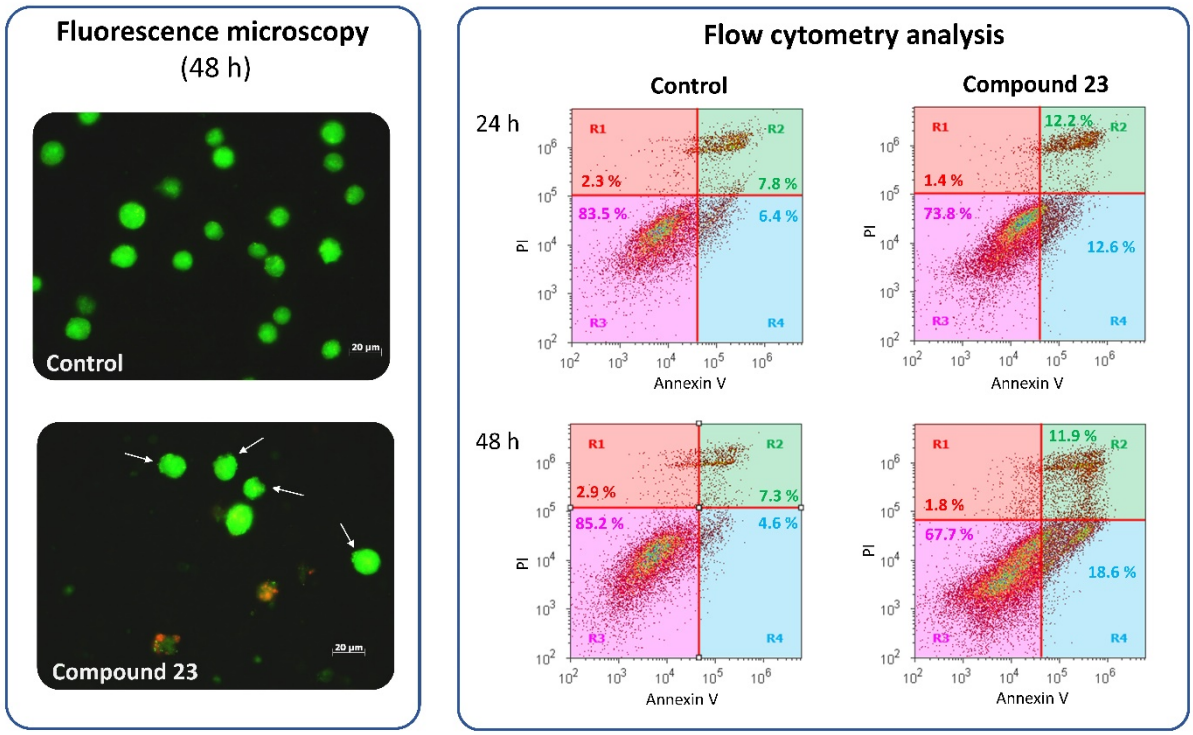


Fig. 5: Fluorescence microscopic images: treatment of A2780 cells with **23** (3.0 µM) for 48 h. Scale bar = 20 µm, AO and PI were used. Flow cytometry analysis: Annexin V-FITC/PI assay, treatment of A2780 cells with **23** (3.0 µM) for 24 h and 48 h, respectively. Examples of density plots determined by flow cytometry (Attune® Cytometric Software v. 1.2.5). R1: necrotic, R2: secondary necrotic/late stage apoptotic, R3: vital, R4: apoptotic.

3 Conclusion

In this study, several carboxamides derived from oleanolic and maslinic acid have been prepared using various α,ω-diamines and *N*-substituted ethylenediamines. The cytotoxicity of the compounds was determined employing photometric sulforhodamine B assays, which showed most of the carboxamides to be cytotoxic with EC_{50} values below 10 µM. Increasing chain lengths of the α,ω-alkyldiamines seems to reduce EC_{50} values until a minimum value of 1.2 ± 0.1 µM (**6**, HT29 tumor cells). While variation of the terminal substituent of ethylene diamines did not affect the cytotoxicity at all, the presence or absence of an acetyloxy moiety at the A ring of the triterpenoic backbone had a significantly higher influence, and especially for maslinic derivatives **21**-**28** increased cytotoxicity was observed. Carboxamides derived from maslinic acid were higher selective than their oleanolic acid derived analogs. Compounds **4**, **6** and **23** were selected for further investigations regarding their mode of action using dye

exclusion acridine orange (AO)/ propidium iodide (PI) assays and flow cytometry analysis. Incubation of A2780 cells with **6** triggered apoptosis.

4 Experimental Part

Information about technical equipment, experimental procedures and full analytical data of these compounds can be found in the supplementary material. Oleanolic and maslinic acid derivatives **14**-**28** have been synthesized as previously reported.[27]

*4.1.1 General procedure A for the acetylation of triterpenoic acids (**1**, **13**)*

To a solution of the triterpenoic acid (11 mmol) in dry DCM (150 mL), triethylamine (4.6 mL, 33 mmol), acetic anhydride (3.1 mL, 33 mmol) and DMAP (cat.) were added. After stirring for two days at 25 °C a saturated solution of NH_3 in MeOH was added (3 mL), and the mixture was stirred for another 30 minutes. Dilution with DCM and subsequent aqueous work-up provided the crude acetates. Recrystallization from EtOH yielded acetates **1** (90%) and **13** (91%) as colorless solids; their spectroscopic data were in full agreement with data from the literature.

*4.1.2 General procedure B for the synthesis of oleanolic carboxamides (**2-12**)*

To an ice-cold solution of **1** (0.5 mmol) in dry DCM (10 mL), oxalyl chloride (3.2 mmol) and dry DMF (2 drops) were added. After warming to 25 °C, the mixture was stirred for 15 h. The solvent was removed under reduced pressure, re-evaporated with dry THF (4 x 15 mL), and the residue was immediately resolved in dry DCM (10 mL). This mixture was added dropwise to a solution of the amine (3.0 mmol) in dry DCM (2 mL) and stirred at 25 °C for 2 h. After usual aqueous work-up, the solvent was removed under reduced pressure, and the crude products were subjected to column chromatography (silica gel, chloroform/methanol, 9:1). Compounds **2-12** were each obtained as colorless solids.

*(3β)-3-Acetyloxy-olean-12-en-28-oic acid (**1**)*

Compound **1** was prepared according to general procedure A from oleanolic acid. Yield: 90%; m.p. 259–261 °C (Lit.: 255–257 °C[28]).

*(3β)-N-(2-Aminoethyl)-3-acetyloxy-olean-12-en-28-amide (**2**)*

Compound **2** was prepared from **1** according to general procedure B using ethylenediamine. Column chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **2** (yield: 60%); m.p. 202–205 °C (decomp.); $[\alpha]_D$ = +54.7° (*c* 0.310, $CHCl_3$); R_f = 0.20 ($CHCl_3$/MeOH, 9:1); IR (KBr): ν = 3406*br*, 2946*s*, 2878*s*, 1734*s*, 1624*m*, 1524*m*, 1466*m*, 1432*m*, 1384*vs*, 1340*m*, 1314*m*, 1248*s*, 1214*w*, 1148*w*, 1174*w*, 1148*w*, 1096*w*, 1074*w*, 1028*m*, 986*m* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.49 (*dd*, *J* = 5.6, 5.6 Hz, 1H, N*H*), 5.21 (*dd*, *J* = 3.4, 3.4 Hz, 1H, 12-H), 4.38 (*dd*, *J* = 11.7, 4.3 Hz, 1H, 3-H), 3.27 (*ddd*, *J* = 13.1, 13.1, 6.6 Hz, 1H, 31-H_a), 3.16 (*ddd*, *J* = 13.1, 13.1, 6.9 Hz, 1H, 31-H_b), 2.78 (*t*, *J* = 7.0 Hz, 2H, 32-H), 2.76 (*m*, 1H, 18-H), 1.99 (*s*, 3H, Ac), 1.93 (*ddd*, *J* = 13.3, 13.3, 4.4 Hz 1H, 16-H_a), 1.86 – 1.75 (*m*, 2H, 2-H_a, 11-H_a), 1.66 (*dd*, *J* = 13.5, 13.5 Hz, 1H, 19-H_a), 1.62 – 1.26 (*m*, 12H, 1-H_a, 2-H_b, 6-H_a, 6-H_b, 7-H_a, 9-H, 11-H_b, 15-H_a, 16-H_b, 21-H_a, 22-H_a, 22-H_b), 1.25 – 1.16 (*m*, 1H, 7-H_b), 1.10 (*s*, 3H, 27-H), 1.14 – 0.90 (*m*, 4H, 1-H_b, 15-H_b, 19-H_b, 21-H_b), 0.88 (*s*, 6H, 25-H, 30-H), 0.87 (*s*, 3H, 29-H), 0.86 – 0.81 (*m*, 1H, 5-H), 0.81 (*s*, 6H, 23-H, 24-H), 0.66 (*s*, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 177.4 (C-28), 170.1 (Ac), 143.8 (C-13), 121.5 (C-12), 79.9 (C-3), 54.5 (C-5), 46.8 (C-9), 45.9 (C-19), 45.3 (C-17), 41.2 (C-14), 40.4 (C-18), 38.9 (C-8), 38.7 (C-32), 37.5 (C-1), 37.2 (C-4), 36.9 (C-31), 36.4 (C-10), 33.5 (C-21), 32.8 (C-29), 32.6 (C-22), 32.1 (C-7), 30.4 (C-20), 27.7 (C-23), 26.9 (C-15), 25.6 (C-27), 23.6 (C-30), 23.2 (C-2), 22.9 (C-11), 22.2 (C-16), 20.9 (Ac), 17.7 (C-6), 16.7 (C-26), 16.6 (C-24), 15.0 (C-25) ppm; MS (ESI, MeOH): *m/z* = 541.3 (100%, $[M+H]^+$), 1081.3 (4%, $[2M+H]^+$); analysis calcd for $C_{34}H_{56}N_2O_3$ (540.83): C 75.51, H 10.44, N 5.18; found: C75.31, H 10.63, N 5.01.

*(3β)-N-(3-Aminopropyl)-3-acetyloxy-olean-12-en-28-amide (**3**)*

Compound **3** was prepared from **1** according to general procedure B using 1,3-diaminopropane. Column chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **3** (yield: 50%); m.p. 188–190 °C (decomp.); $[\alpha]_D$ = +30.5° (*c* 0.310, $CHCl_3$); R_f = 0.19 ($CHCl_3$/MeOH, 9:1); IR (KBr): ν = 3424*br*, 2948*m*, 2876*w*, 1734*w*, 1628*w*, 1534*w*, 1464*w*, 1432*w*, 1384*vs*, 1248*w*, 1148*vw*, 1028*w* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.45 (*dd*, *J* = 5.8, 5.8 Hz, 1H, N*H*), 5.21 (*dd*, *J* = 3.4, 3.4 Hz, 1H, 12-H), 4.38 (*dd*, *J* = 11.6, 4.3 Hz, 1H, 3-H), 3.14 – 3.00 (*m*, 2H, 33-H_a, 31-H_a, 31-H_b), 2.79 (*dd*, J = 13.3, 3.6 Hz, 1H, 18-H), 2.73 (*t*, *J* = 7.4 Hz, 2H, 33-H_a, 33-H_b), 1.99 (*s*, 3H, Ac), 1.96 – 1.88 (*m*, 1H, 16-H_a), 1.84 – 1.73 (*m*, 2H, 2-H_a, 11-H_a), 1.73 – 1.27 (*m*, 15H, 1-H_a, 2-H_b, 6-H_a, 6-H_b, 7-H_a, 9-H, 11-H_b, 15-H_a, 16-H_b, 19-H_a, 21-H_a, 22-H_a, 22-H_b, 32-H_a, 32-H_b), 1.25 – 0.89 (*m*, 5H, 1-H_a, 7-H_b, 15-H_b, 19-H_b, 21-H_b), 1.10 (*s*, 3H, 27-H), 0.88 (*s*, 3H, 25-H), 0.88 (*s*, 3H, 30-H), 0.87 (s, 3H, 29-H), 0.81 (*s*, 6H, 23-H, 24-H), 0.87 – 0.80 (*m*, 1H, 5-H), 0.66 (*s*, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 177.1 (C-28), 170.1 (Ac), 144.0 (C-

13), 121.4 (C-12), 79.9 (C-3), 54.5 (C-5), 46.9 (C-9), 46.0 (C-19), 45.4 (C-17), 41.3 (C-14), 40.4 (C-18), 38.9 (C-8), 37.6 (C-1), 37.3 (C-4), 36.9 (C-33), 36.5 (C-10), 35.8 (C-31), 33.6 (C-21), 32.9 (C-22), 32.9 (C-29), 32.2 (C-7), 30.4 (C-20), 27.8 (C-23), 27.5 (C-32), 27.0 (C-15), 25.6 (C-27), 23.6 (C-30), 23.2 (C-2), 22.9 (C-11), 22.2 (C-16), 21.0 (Ac), 17.8 (C-6), 16.9 (C-26), 16.6 (C-24), 15.1 (C-25) ppm; MS (ESI, MeOH): *m/z* = 555.4 (100%, $[M+H]^+$), 1109.2 (4%, $[2M+H]^+$) ; analysis calcd for $C_{35}H_{58}N_2O_3$ (554.86): C 75.76, H 10.54, N 5.05; found: C 75.50, H 10-74, N 4.87.

*(3β)-N-(4-Aminobutyl)-3-acetyloxy-olean-12-en-28-amide (**4**)*

Compound **4** was prepared from **1** according to general procedure B using 1,4-diaminobutane. Column chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **4** (yield: 46%); m.p. 149–152 °C; $[\alpha]_D$ = +31.6° (*c* 0.190, $CHCl_3$); R_f = 0.16 ($CHCl_3$/MeOH, 9:1); IR (KBr): ν = 3428*br*, 2946*m*, 2876*w*, 1736*w*, 1630*m*, 1534*w*, 1452*m*, 1384*m*, 1246*m*, 1092*w*, 1028*m* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.32 (*dd*, *J* = 5.6, 5.6 Hz, 1H, N*H*), 5.21 (*dd*, *J* = 3.2, 3.2 Hz, 1H, 12-H), 4.38 (*dd*, *J* = 11.7, 4.3 Hz, 1H, 3-H), 3.04 (*ddd*, *J* =12.7, 12.7, 6.5 Hz, 1H, 31-H_a), 2.96 (*ddd*, *J* =12.8, 12.8, 6.6 Hz, 1H, 31-H_b), 2.79 (*dd*, J = 13.3, 3.8 Hz, 1H, 18-H), 2.72 (*t*, *J* = 7.4 Hz, 2H, 34-H_a, 34-H_b), 1.99 (*s*, 3H, Ac), 1.95 – 1.85 (*m*, 1H, 16-H_a), 1.84 – 1.77 (*m*, 2H, 2-H_a, 11-H_a), 1.66 (*dd*, *J* = 13.5, 13.5 Hz, 1H, 19-H_a), 1.66 – 1.16 (*m*, 17H, 1-H_a, 2-H_b, 6-H_a, 6-H_b, 7-H_a, 7-H_b, 9-H, 11-H_b, 15-H_a, 16-H_b, 21-H_a, 22-H_a, 22-H_b, 32-H_a, 32-H_b, 33-H_a, 33-H_b), 1.14 – 0.87 (*m*, 4H, 1-H_a, 15-H_b, 19-H_b, 21-H_b), 1.10 (*s*, 3H, 27-H), 0.89 (*s*, 3H, 25-H), 0.88 (*s*, 3H, 30-H), 0.87 (*s*, 3H, 29-H), 0.85 – 0.82 (*m*, 1H, 5-H), 0.81 (*s*, 6H, 23-H, 24-H), 0.67 (*s*, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 176.2 (C-28), 170.1 (Ac), 144.1 (C-13), 121.2 (C-12), 79.9 (C-3), 54.5 (C-5), 46.9 (C-9), 46.0 (C-19), 45.2 (C-17), 41.3 (C-14), 40.4 (C-18), 38.9 (C-8), 38.7 (C-34), 38.1 (C-31), 37.5 (C-1), 37.2 (C-4), 36.5 (C-10), 33.6 (C-21), 32.9 (C-29), 32.8 (C-22), 32.2 (C-7), 30.4 (C-20), 27.8 (C-23), 27.0 (C-15), 26.2 (C-33), 25.6 (C-27), 25.0 (C-32), 23.6 (C-30), 23.2 (C-2), 22.9 (C-11), 22.2 (C-16), 21.0 (Ac), 17.8 (C-6), 16.8 (C-26), 16.6 (C-24), 15.0 (C-25) ppm; MS (ESI, MeOH): *m/z* = 569.4 (100%, $[M+H]^+$), 1137.5 (10%, $[2M+H]^+$), 1159,6 (4%, $[2M+Na]^+$); analysis calcd for $C_{36}H_{60}N_2O_3$ (568.89): C 76.01, H 10.63, N 4.92; found: C 75.88, H 10.87, N 4.67.

*(3β)-N-(5-Aminopentyl)-3-acetyloxy-olean-12-en-28-amide (**5**)*

Compound **5** was prepared from **1** according to general procedure B using 1,5-diaminopentane. Column chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **5** (yield: 49%); m.p. 161–164 °C; $[\alpha]_D$ = +39.3° (*c* 0.330, $CHCl_3$); R_f = 0.20 ($CHCl_3$/MeOH, 9:1); IR (KBr): ν = 3424*br*, 2946*s*,

2876*m*, 1734*m*, 1628*m*, 1532*w*, 1466*w*, 1432*w*, 1384*vs*, 1318*w*, 1246*m*, 1214*w*, 1188*w*, 1176*vw*, 1148*w*, 1096*w*, 1074*w*, 1028*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.23 (*dd*, *J* = 5.5, 5.5 Hz, 1H, N*H*), 5.20 (*dd*, *J* = 3.4, 3.4 Hz, 1H, 12-H), 4.39 (*dd*, *J* = 11.6, 4.3 Hz, 1H, 3-H), 3.03 (*ddd*, *J* =13.0, 13.0, 7.0 Hz, 1H, 31-H_a), 2.94 (*ddd*, *J* =13.0, 13.0, 6.9 Hz, 1H, 31-H_b), 2.81 – 2.76 (*m*, 1H, 18-H), 2.75 (*t*, *J* = 7.7 Hz, 2H, 35-H_a, 35-H_b), 1.99 (*s*, 3H, Ac), 1.91 (*ddd*, *J* = 14.3, 14.3, 3.2 Hz, 1H, 16-H_a), 1.84 – 1.77 (*m*, 2H, 2-H_a, 11-H_a), 1.66 (*dd*, *J* = 13.4, 13.4 Hz, 1H, 19-H_a), 1.62 – 1.16 (*m*, 19H, 1-H_a, 2-H_b, 6-H_a, 6-H_b, 7-H_a, 7-H_b, 9-H, 11-H_b, 15-H_a, 16-H_b, 21-H_a, 22-H_a, 22-H_b, 32-H_a, 32-H_b, 33-H_a, 33-H_b, 34-H_a, 34-H_b), 1.10 (*s*, 3H, 27-H), 1.11 – 0.89 (*m*, 4H, 1-H_a, 15-H_b, 19-H_b, 21-H_b), 0.89 (*s*, 3H, 25-H), 0.88 (*s*, 3H, 30-H), 0.87 (s, 3H, 29-H), 0.86 – 0.83 (*m*, 1H, 5-H), 0.81 (*s*, 6H, 23-H, 24-H), 0.67 (*s*, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 176.1 (C-28), 170.1 (Ac), 144.1 (C-13), 121.2 (C-12), 79.9 (C-3), 54.5 (C-5), 46.8 (C-9), 46.0 (C-19), 45.2 (C-17), 41.3 (C-14), 40.4 (C-18), 38.9 (C-8), 38.8 (C-35), 38.5 (C-31), 37.5 (C-1), 37.2 (C-4), 36.5 (C-10), 33.6 (C-21), 32.9 (C-29), 32.8 (C-22), 32.3 (C-7), 30.4 (C-20), 28.6 (C-32), 27.8 (C-23), 27.0 (C-15), 26.7 (C-34), 25.6 (C-27), 23.6 (C-30), 23.3 (C-33), 23.2 (C-2), 22.9 (C-11), 22.3 (C-16), 20.9 (Ac), 17.8 (C-6), 16.8 (C-26), 16.6 (C-24), 15.0 (C-25) ppm; MS (ESI, MeOH): *m/z* = m/z = 583.4 (100%, $[M+H]^+$), 1165.4 (15%, $[2M+H]^+$); analysis calcd for $C_{37}H_{62}N_2O_3$ (582.91): C 76.24, H 10.72, N 4.81; found: C 76.02, H 10.96, N 4.63.

*(3β)-N-(6-Aminohexyl)-3-acetyloxy-olean-12-en-28-amide (**6**)*

Compound **6** was prepared from **1** according to general procedure B using 1,6-diaminohexane. Column chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **3** (yield: 50%); m.p. 125–129 °C; $[\alpha]_D$ = +34.4° (*c* 0.335, $CHCl_3$); R_f = 0.21 ($CHCl_3$/MeOH, 9:1); IR (KBr): ν = 3444*br*, 2942*m*, 2864*vw*, 1734*vw*, 1634*m*, 1538*vw*, 1462*vw*, 1432*vw*, 1384*vs*, 1248*w*, 1089*w*, 1028*w* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.22 (*dd*, *J* = 5.6, 5.6 Hz, 1H, N*H*), 5.20 (*dd*, *J* = 3.4, 3.4 Hz, 1H, 12-H), 4.38 (*dd*, *J* = 11.5, 4.4 Hz, 1H, 3-H), 3.02 (*ddd*, *J* = 12.8, 12.8, 6.8 Hz, 1H, 31-H_a), 2.95 (*ddd*, *J* = 12.8, 12.8, 6.9 Hz, 1H, 31-H_b), 2.81 – 2.75 (*m, 1H*, 18-H), 2.76 (*t*, *J* = 7.6 Hz, 2H, 36-H_a, 36-H_b), 1.99 (*s*, 3H, Ac), 1.89 (*ddd*, *J* = 14.9, 14.9, 4.0 Hz 1H, 16-H_a), 1.83 – 1.76 (*m*, 2H, 2-H_a, 11-H_a), 1.65 (*dd*, *J* = 13.4, 13.4 Hz, 1H, 19-H_a), 1.60 – 1.17 (*m*, 21H, 1-H_a, 2-H_b, 6-H_a, 6-H_b, 7-H_a, 7-H_b, 9-H, 11-H_b, 15-H_a, 16-H_b, 21-H_a, 22-H_a, 22-H_b, 32-H_a, 32-H_b, 33-H_a, 33-H_b, 34-H_a, 34-H_b, 35-H_a, 35-H_b), 1.09 (*s*, 3H, 27-H), 1.11 – 0.88 (*m*, 4H, 1-H_b, 15-H_b, 19-H_b, 21-H_b), 0.88 (*s*, 3H, 25-H), 0.87 (s, 3H, 30-H), 0.86 (*s*, 3H, 29-H), 0.86 – 0.80 (*m*, 1H, 5-H), 0.81 (*s*, 6H, 23-H, 24-H), 0.67 (*s*, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ =

176.1 (C-28), 170.1 (Ac), 144.2 (C-13), 121.2 (C-12), 79.9 (C-3), 54.6 (C-5), 46.9 (C-9), 46.0 (C-19), 45.2 (C-17), 41.3 (C-14), 40.5 (C-18), 38.9 (C-8), 38.9 (C-36), 38.7 (C-31), 37.6 (C-1), 37.3 (C-4), 36.5 (C-10), 33.7 (C-21), 32.9 (C-29), 32.8 (C-22), 32.3 (C-7), 30.4 (C-20), 29.0 (C-33), 27.8 (C-23), 27.1 (C-34), 27.0 (C-15), 26.1 (C-32), 25.6 (C-27), 25.6 (C-35), 23.6 (C-30), 23.2 (C-2), 22.9 (C-11), 22.3 (C-16), 21.0 (Ac), 17.8 (C-6), 16.8 (C-26), 16.7 (C-24), 15.0 (C-25) ppm; MS (ESI, MeOH): *m/z* = 597.5 (100%, $[M+H]^+$), 1193.5 (5%, $[2M+H]^+$); analysis calcd for $C_{38}H_{64}N_2O_3$ (596.94): C 76.46, H 10.81, N 4.69; found: C 76.20, H 11.04, N 4.42.

*(3β)-N-(7-Aminoheptyl)-3-acetyloxy-olean-12-en-28-amide (**7**)*

Compound **7** was prepared from **1** according to general procedure B using 1,7-diaminoheptane. Column chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **7** (yield: 52%); m.p. 120–124 °C; $[\alpha]_D$ = +35.4° (*c* 0.075, $CHCl_3$); R_f = 0.30 ($CHCl_3$/MeOH, 9:1); IR (KBr): ν = 3440*br*, 2940*s*, 2860*m*, 1734*m*, 1628*m*, 1524*m*, 1438*m*, 1384*s*, 1246*m*, 1080*m*, 1028*m* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.24 (*dd*, *J* = 5.5, 5.5 Hz, 1H, N*H*), 5.21 (*dd*, *J* = 3.3, 3.3 Hz, 1H, 12-H), 4.38 (*dd*, *J* = 11.5, 4.4 Hz, 1H, 3-H), 3.02 (*ddd*, *J* = 13.3, 13.3, 7.2 Hz, 1H, 31-H_a), 2.95 (*ddd*, *J* = 12.8, 12.8, 7.5 Hz, 1H, 31-H_b), 2.78 (*dd, J = 13.4, 3.6 Hz, 1H*, 18-H), 2.72 (*t*, *J* = 7.6 Hz, 2H, 37-H_a, 37-H_b), 1.99 (*s*, 3H, Ac), 1.94 – 1.84 (*m*, 1H, 16-H_a), 1.83 – 1.76 (*m*, 2H, 2-H_a, 11-H_a), 1.65 (*dd*, *J* = 13.5, 13.5 Hz, 1H, 19-H_a), 1.61 – 1.15 (*m*, 23H, 1-H_a, 2-H_b, 6-H_a, 6-H_b, 7-H_a, 7-H_b, 9-H, 11-H_b, 15-H_a, 16-H_b, 21-H_a, 22-H_a, 22-H_b, 32-H_a, 32-H_b, 33-H_a, 33-H_b, 34-H_a, 34-H_b, 35-H_a, 35-H_b, 36-H_a, 36-H_b), 1.09 (*s*, 3H, 27-H), 1.11 – 0.88 (*m*, 4H, 1-H_b, 15-H_b, 19-H_b, 21-H_b), 0.88 (*s*, 3H, 25-H), 0.87 (*s*, 3H, 30-H), 0.86 (*s*, 3H, 29-H), 0.85 – 0.80 (*m*, 1H, 5-H), 0.81 (*s*, 6H, 23-H, 24-H), 0.67 (*s*, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 176.0 (C-28), 170.1 (Ac), 144.2 (C-13), 121.2 (C-12), 79.9 (C-3), 54.6 (C-5), 46.9 (C-9), 46.0 (C-19), 45.2 (C-17), 41.3 (C-14), 40.4 (C-18), 38.9 (C-8), 38.8 (C-37), 38.7 (C-31), 37.6 (C-1), 37.2 (C-4), 36.5 (C-10), 33.7 (C-21), 32.9 (C-29), 32.7 (C-22), 32.3 (C-7), 30.4 (C-20), 29.0 (C-33), 28.3 (C-34), 27.8 (C-23), 27.1 (C-35), 26.9 (C-15), 26.4 (C-32), 25.9 (C-36), 25.6 (C-27), 23.6 (C-30), 23.2 (C-2), 22.9 (C-11), 22.3 (C-16), 21.0 (Ac), 17.8 (C-6), 16.8 (C-26), 16.6 (C-24), 15.0 (C-25) ppm; MS (ESI, MeOH): *m/z* = 611.1 (100%, $[M+H]^+$), 1222.5 (6%, $[2M+H]^+$); analysis calcd for $C_{39}H_{66}N_2O_3$ (610.97): C 76.67, H 10.89, N 4.59; found: C 76.31, H 11.00, N 4.29.

*(3β)-N-(8-Aminooctyl)-3-acetyloxy-olean-12-en-28-amide (**8**)*

Compound **8** was prepared from **1** according to general procedure B using 1,8-diaminooctane. Column chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **8** (yield: 38%); m.p. 143–146 °C;

$[\alpha]_D$ = +28.4° (*c* 0.320, $CHCl_3$); R_f = 0.22 ($CHCl_3$/MeOH, 9:1); IR (KBr): ν = 3426*br*, 2940*w*, 2860*w*, 1734*w*, 1636*w*, 1532*w*, 1466*w*, 1432*w*, 1384*vs*, 1246*w*, 1214*vw*, 1148*vw*, 1028*w* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.20 (*dd*, *J* = 5.5, 5.5 Hz, 1H, N*H*), 5.21 (*dd*, *J* = 3.2, 3.2 Hz, 1H, 12-H), 4.38 (*dd*, *J* = 11.6, 4.3 Hz, 1H, 3-H), 3.02 (*ddd*, *J* = 13.1, 13.1, 7.0 Hz, 1H, 31-H_a), 2.95 (*ddd*, *J* = 12.9, 12.9, 7.0 Hz, 1H, 31-H_b), 2.82 – 2.73 (*m*,3H, 18-H, 38-H_a, 38-H_b), 1.99 (*s*, 3H, Ac), 1.89 (*ddd*, *J* = 14.6, 14.6, 3.6Hz, 1H 16-H_a), 1.83 – 1.77 (*m*, 2H, 2-H_a, 11-H_a), 1.66 (*dd*, *J* = 13.4, 13.4 Hz, 1H, 19-H_a), 1.64 – 1.16 (*m*, 25H, 1-H_a, 2-H_a, 2-H_b, 6-H_a, 6-H_b, 7-H_a, 7-H_b, 9-H, 11-H_b, 15-H_a, 16-H_b, 21-H_a, 22-H_a, 22-H_b, 32-H_a, 32-H_b, 33-H_a, 33-H_b, 34-H_a, 34-H_b, 35-H_a, 35-H_b, 36-H_a, 36-H_b, 37-H_a, 37-H_b), 1.10 (*s*, 3H, 27-H), 1.15 – 0.87 (*m*, 4H, 1-H_b, 15-H_b, 19-H_b, 21-H_b), 0.88 (*s*, 3H, 25-H), 0.87 (s, 3H, 30-H), 0.86 (*s*, 3H, 29-H), 0.85 – 0.82 (*m*, 1H, 5-H), 0.81 (*s*, 6H, 23-H, 24-H), 0.67 (*s*, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 176.0 (C-28), 170.1 (Ac), 144.2 (C-13), 121.2 (C-12), 79.9 (C-3), 54.5 (C-5), 46.9 (C-9), 46.0 (C-19), 45.2 (C-17), 41.3 (C-14), 40.5 (C-18), 38.9 (C-8), 38.9 (C-38), 38.8 (C-31), 37.6 (C-1), 37.2 (C-4), 36.5 (C-10), 33.7 (C-21), 32.9 (C-29), 32.8 (C-22), 32.3 (C-7), 30.4 (C-20), 29.1 (C-32), 28.7 (CH_2), 28.6 (CH_2), 27.8 (C-23), 27.0 (C-36), 27.0 (C-15), 26.5 (C-33), 25.8 (C-37), 25.6 (C-27), 23.6 (C-30), 23.2 (C-2), 22.9 (C-11), 22.3 (C-16), 21.0 (Ac), 17.8 (C-6), 16.8 (C-26), 16.6 (C-24), 15.0 (C-25) ppm; MS (ESI, MeOH): *m/z* = 625.5 (100%, $[M+H]^+$), 1249.5 (8%, $[2M+H]^+$); analysis calcd for $C_{40}H_{68}N_2O_3$ (625.00): C 76.87, H 10.97, N 4.48; found: C 76.58, H 11.18, N 4.17.

*(3β)-N-(9-Aminononyl)-3-acetyloxy-olean-12-en-28-amide (**9**)*

Compound **9** was prepared from **1** according to general procedure B using 1,9-diaminononane. Column chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **9** (yield: 50%); m.p. 143–146 °C; $[\alpha]_D$ = +27.5° (*c* 0.370, $CHCl_3$); R_f = 0.21 ($CHCl_3$/MeOH, 9:1); IR (KBr): ν = 3426*br*, 2930*m*, 2858*m*, 1734*w*, 1630*m*, 1530*w*, 1464*w*, 1432*w*, 1384*vs*, 1246*m*, 1214*w*, 1186*w*, 1148*w*, 1096*w*, 1074*w*, 1028*w* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.20 (*dd*, *J* = 5.4, 5.4 Hz, 1H, N*H*), 5.20 (*dd*, *J* = 3.4, 3.4 Hz, 1H, 12-H), 4.38 (*dd*, *J* = 11.6, 4.4 Hz, 1H, 3-H), 3.02 (*ddd*, *J* = 12.8, 12.8, 6.9 Hz, 1H, 31-H_a), 2.94 (*ddd*, *J* = 12.7, 12.7, 6.7 Hz, 1H, 31-H_b), 2.81 – 1.77 (*m*, 1H, 18-H), 2.76 (*t*, *J* = 7.6 Hz, 2H, 39-H_a, 39-H_b), 1.99 (*s*, 3H, Ac), 1.89 (*ddd*, *J* = 14.3, 14.3, 3.2 Hz, 1H, 16-H_a), 1.83 – 1.77 (*m*, 2H, 2-H_a, 11-H_a), 1.66 (*dd*, *J* = 13.5, 13.5 Hz, 1H, 19-H_a), 1.61 – 1.14 (*m*, 27H, 1-H_a, 2-H_b, 6-H_a, 6-H_b, 7-H_a, 7-H_b, 9-H, 11-H_b, 15-H_a, 16-H_b, 21-H_a, 22-H_a, 22-H_b, 32-H_a, 32-H_b, 33-H_a, 33-H_b, 34-H_a, 34-H_b, 35-H_a, 35-H_b, 36-H_a, 36-H_b, 37-H_a, 37-H_b, 38-H_a, 38-H_b), 1.10 (*s*, 3H, 27-H), 1.10 – 0.87 (*m*, 4H, 1-H_b, 15-H_b, 19-H_b, 21-H_b), 0.88 (*s*, 3H, 25-H), 0.87 (*s*, 3H, 30-H), 0.86 (*s*, 3H, 29-H), 0.86 – 0.82 (*m*, 1H, 5-H), 0.81 (*s*, 6H, 23-H, 24-H),

0.67 (*s*, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 176.0 (C-28), 170.1 (Ac), 144.2 (C-13), 121.2 (C-12), 79.9 (C-3), 54.5 (C-5), 46.9 (C-9), 46.0 (C-19), 45.2 (C-17), 41.3 (C-14), 40.5 (C-18), 38.9 (C-8, C-39) 38.8 (C-31), 37.5 (C-1), 37.2 (C-4), 36.5 (C-10), 33.7 (C-21), 32.9 (C-29), 32.8 (C-22), 32.3 (C-7), 30.4 (C-20), 29.1 (C-33), 28.9 (CH_2), 28.7 (CH_2), 28.5 (CH_2), 27.8 (C-23), 27.0 (C-37), 26.9 (C-15), 26.6 (C-32), 25.8 (C-38), 25.6 (C-27), 23.6 (C-30), 23.2 (C-2), 22.9 (C-11), 22.3 (C-16), 21.0 (Ac), 17.8 (C-6), 16.8 (C-26), 16.6 (C-24), 15.0 (C-25) ppm; MS (ESI, MeOH): *m/z* = 639.5 (100%, $[M+H]^+$), 1277.4 (9%, $[2M+H]^+$); analysis calcd for $C_{41}H_{70}N_2O_3$ (639.02): C 77.06, H 11.04, N 4.38; found: C 76.86, H 11.17, N 4.07.

*(3β)-N-(10-Aminodecyl)-3-acetyloxy-olean-12-en-28-amide (**10**)*

Compound **10** was prepared from **1** according to general procedure B using 1,10-diaminodecane. Column chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **10** (yield: 46%); m.p. 108–110 °C; $[\alpha]_D$ = +32.5° (*c* 0.320, $CHCl_3$); R_f = 0.32 ($CHCl_3$/MeOH, 9:1); IR (KBr): ν = 3442*br*, 2928*s*, 2854*m*, 1734*m*, 1636*m*, 1526*m*, 1464*m*, 1370*m*, 1246*s*, 1028*m* cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.21 (*dd*, *J* = 5.5, 5.5 Hz, 1H, N*H*), 5.21 (*dd*, *J* = 3.2, 3.2 Hz, 1H, 12-H), 4.39 (*dd*, *J* = 11.6, 4.4 Hz, 1H, 3-H), 3.03 (*ddd*, *J* = 12.6, 12.6, 6.5 Hz, 1H, 31-H_a), 2.94 (*ddd*, *J* = 12.7, 12.7, 6.7 Hz, 1H, 31-H_b), 2.78 (*dd*, *J* = 13.1, 3.4 Hz, 1H, 18-H), 2.69 (*t*, *J* = 7.5 Hz, 2H, 40-H_a, 40-H_b), 1.99 (*s*, 3H, Ac), 1.89 (*ddd*, *J* = 14.2, 14.2, 3.1 Hz, 1H, 16-H_a), 1.83 – 1.77 (*m*, 2H, 2-H_a, 11-H_a), 1.66 (*dd*, *J* = 13.5, 13.5 Hz, 1H, 19-H_a), 1.74 – 1.17 (*m*, 29H, 1-H_a, 2-H_b, 6-H_a, 6-H_b, 7-H_a, 7-H_b, 9-H, 11-H_b, 15-H_a, 16-H_b, 21-H_a, 22-H_a, 22-H_b, 32-H_a, 32-H_b, 33-H_a, 33-H_b, 34-H_a, 34-H_b, 35-H_a, 35-H_b, 36-H_a, 36-H_b, 37-H_a, 37-H_b, 38-H_a, 38-H_b, 39-H_a, 39-H_b), 1.10 (*s*, 3H, 27-H), 1.12 – 0.88 (*m*, 4H, 1-H_b, 15-H_b, 19-H_b, 21-H_b), 0.88 (*s*, 3H, 25-H), 0.88 (*s*, 3H, 30-H), 0.86 (*s*, 3H, 29-H), 0.86 – 0.81 (*m*, 1H, 5-H), 0.81 (*s*, 6H, 23-H, 24-H), 0.67 (*s*, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 176.0 (C-28), 170.1 (Ac), 144.2 (C-13), 121.2 (C-12), 79.9 (C-3), 54.5 (C-5), 46.9 (C-9), 46.0 (C-19), 45.2 (C-17), 41.3 (C-14), 40.5 (C-18), 39.4 (C-40), 38.9 (C-8), 38.8 (C-31), 37.5 (C-1), 37.2 (C-4), 36.5 (C-10), 33.7 (C-21), 32.9 (C-29), 32.7 (C-22), 32.3 (C-7), 30.4 (C-20), 29.1 (C-33), 29.0 (CH_2), 28.9 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 28.3 (C-38), 27.8 (C-23), 26.9 (C-15), 26.6 (C-32), 26.0 (C-39), 25.6 (C-27), 23.6 (C-30), 23.2 (C-2), 22.9 (C-11), 22.3 (C-16), 21.0 (Ac), 17.8 (C-6), 16.8 (C-26), 16.6 (C-24), 15.0 (C-25) ppm; MS (ESI, MeOH): *m/z* = 653.5 (100%, $[M+H]^+$), 1305.5 (5%, $[2M+H]^+$); analysis calcd for $C_{42}H_{72}N_2O_3$ (653.05): C 77.25, H 11.11, N 4.29; found: C 77.04, H 11.39, N 4.09.

*(3β)-N-(11-Aminoundecyl)-3-acetyloxy-olean-12-en-28-amide (**11**)*

Compound **11** was prepared from **1** according to general procedure B using 1,11-diaminoundecane. Column chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **11** (yield: 36%); m.p. 162–165 °C; $[\alpha]_D$ = +24.3° (*c* 0.315, $CHCl_3$); R_f = 0.18 ($CHCl_3$/MeOH, 9:1); IR (KBr): ν = 3426*br*, 2928*m*, 2854*m*, 1734*w*, 1718*w*, 1636*m*, 1526*w*, 1466*w*, 1432*w*, 1384*vs*, 1246*m*, 1148 *vw*, 1028*m* cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.20 (*dd*, *J* = 5.5, 5.5 Hz, 1H, N*H*), 5.20 (*dd*, *J* = 3.3, 3.3 Hz, 1H, 12-H), 4.38 (*dd*, *J* = 11.6, 4.3 Hz, 1H, 3-H), 3.03 (*ddd*, *J* = 12.9, 12.9, 6.8 Hz, 1H, 31-H_a), 2.93 (*ddd*, *J* = 12.6, 12.6, 6.7 Hz, 1H, 31-H_b), 2.81 – 2.73 (*m*, 3H, 18-H, 41-H_a, 41-H_b), 1.99 (*s*, 3H, Ac), 1.89 (*ddd*, *J* = 14.1, 14.1, 2.8 Hz, 1H, 16-H_a), 1.82 – 1.77 (*m*, 2H, 2-H_a, 11-H_a), 1.65 (*dd*, *J* = 13.5, 13.5 Hz, 1H, 19-H_a), 1.61 – 1.16 (*m*, 31H, 1-H_a, 2-H_b, 6-H_a, 6-H_b, 7-H_a, 7-H_b, 9-H, 11-H_b, 15-H_a, 16-H_b, 21-H_a, 22-H_a, 22-H_b, 32-H_a, 32-H_b, 33-H_a, 33-H_b, 34-H_a, 34-H_b, 35-H_a, 35-H_b, 36-H_a, 36-H_b, 37-H_a, 37-H_b, 38-H_a, 38-H_b, 39-H_a, 39-H_b, 40-H_a, 40-H_b), 1.45 – 0.86 (*m*, 4H, 1-H_b, 15-H_b, 19-H_b, 21-H_b), 1.09 (*s*, 3H, 27-H, 0.87 (*s*, 3H, 25-H), 0.87 (*s*, 3H, 30-H), 0.86 (*s*, 3H, 29-H), 0.85 – 0.81 (*m*, 1H, 5-H), 0.80 (*s*, 3H, 23-H), 0.80 (*s*, 3H, 24-H), 0.67 (*s*, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 176.1 (C-28), 170.1 (Ac), 144.3 (C-13), 121.2 (C-12), 79.9 (C-3), 54.6 (C-5), 46.9 (C-9), 46.1 (C-19), 45.2 (C-17), 41.3 (C-14), 40.5 (C-18), 38.9 (C-41), 38.9 (C-8), 38.8 (C-31), 37.6 (C-1), 37.3 (C-4), 36.5 (C-10), 33.7 (C-21), 33.0 (C-29), 32.8 (C-22), 32.3 (C-7), 30.4 (C-20), 29.1 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 29.0 (CH_2), 28.6 (CH_2), 27.8 (C-23), 27.0 (CH_2), 27.0 (C-15), 26.7 (C_K^*), 25.9 (C_K^*), 25.6 (C-27), 23.6 (C-30), 23.2 (C-2), 22.9 (C-11), 22.3 (C-16), 21.0 (Ac), 17.8 (C-6), 16.9 (C-26), 16.6 (C-24), 15.0 (C-25) ppm; MS (ESI, MeOH): *m/z* = 667.5 (100%, $[M+H]^+$), 1333.6 (5%, $[2M+H]^+$); analysis calcd for $C_{43}H_{74}N_2O_3$ (667.08): C 77.42, H 11.18, N 4.20; found: C 77.21, H 11.42, N 4.12.

*(3β)-N-(12-Aminododecyl)-3-acetyloxy-olean-12-en-28-amide (**12**)*

Compound **12** was prepared from **1** according to general procedure B using 1,12-diaminododecane. Column chromatography (SiO_2, $CHCl_3$/MeOH, 9:1) gave **12** (yield: 42%); m.p. 151–155 °C; $[\alpha]_D$ = +35.9° (*c* 0.330, $CHCl_3$); R_f = 0.38 ($CHCl_3$/MeOH, 9:1); IR (KBr): ν = 3422*br*, 2928*s*, 2854*s*, 1736*m*, 1632*s*, 1528*m*, 1464*s*, 1434*s*, 1384*s*, 1318*s*, 1246*s*, 1214*w*, 1188*w*, 1148*w*, 1096*w*, 1074*w*, 1028*m*, 1008*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 6.05 (*dd*, *J* = 4.8, 4.8 Hz, 1H, N*H*), 5.37 (*dd*, *J* = 3.4, 3.4 Hz, 1H, 12-H), 4.48 (*dd*, *J* = 9.9, 6.0 Hz, 1H, 3-H), 3.34 (*ddd*, *J* = 13.2, 13.2, 6.9 Hz, 1H, 31-H_a), 3.09 – 3.01 (*m*, 2H, 42-H_a, 42-H_b), 2.97 (*ddd*, *J* = 12.0, 12.0, 6.6 Hz, 1H, 31-H_b), 2.49 (*dd*, *J* = 12.7, 2.9 Hz, 1H, 18-H), 2.04 (*s*, 3H, Ac), 2.00 – 1.85 (*m*, 3H, 2-H_a, 11-H_a, 16-H_a), 1.80 – 1.14 (*m*, 36H, 1-H_a, 2-H_b, 6-H_a, 6-H_b, 7-H_a, 7-H_b, 9-H, 11-H_b, 15-H_a, 16-H_b, 19-H_a, 19-H_b, 21-H_a, 21-H_b, 22-H_a, 22-H_b, 32-H_a, 32-H_b,

33-H_a, 33-H_b, 34-H_a, 34-H_b, 35-H_a, 35-H_b, 36-H_a, 36-H_b, 37-H_a, 37-H_b, 38-H_a, 38-H_b, 39-H_a, 39-H_b, 40-H_a, 40-H_b, 41-H_a, 41-H_b), 1.15 (*s*, 3H, 27-H), 1.10 – 0.98 (*m*, 2H, 1-H_b, 15-H_b), 0.93 (*s*, 3H, 25-H), 0.90 (*s*, 3H, 30-H), 0.89 (*s*, 3H, 29-H), 0.86 (*s*, 3H, 23-H), 0.85 (*s*, 3H, 24-H), 0.86 – 0.81 (*m*, 1H, 5-H), 0.76 (*s*, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 178.5 (C-28), 171.2 (Ac), 145.2 (C-13), 122.9 (C-12), 81.0 (C-3), 55.4 (C-5), 47.6 (C-9), 46.9 (C-19), 46.4 (C-17), 42.5 (C-18), 42.2 (C-14), 40.7 (C-42), 39.7 (C-31), 39.5 (C-8), 38.3 (C-1), 37.8 (C-4), 37.0 (C-10), 34.3 (C-21), 33.1 (C-29), 32.5 (C-22), 32.5 (C-7), 30.9 (C-20), 29.6 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.4 (C-35), 29.2 (CH_2), 28.2 (C-23), 27.6 (CH_2), 27.4 (C-15), 27.2 (C-34), 26.6 (CH_2), 25.8 (C-27), 23.9 (C-16), 23.8 (C-30), 23.7 (C-2), 23.7 (C-11), 21.4 (Ac), 18.3 (C-6), 17.0 (C-26), 16.8 (C-24), 15.6 (C-25) ppm; MS (ESI, MeOH): *m/z* = 681.5 (100%, $[M+H]^+$), 1361.6 (5%, $[2M+H]^+$); analysis calcd for $C_{44}H_{76}N_2O_3$ (681.10): C 77.59, H 11.25, N 4.11; found: C 77.28, H 11.49, N 3.97.

*(2α,3β)-2,3-Diacetyloxy-olean-12-en-28-oic acid (**13**)*

Compound **13** was prepared according to general procedure A from maslinic acid. Yield: 91%; m.p. 172–175 °C (Lit.: 170–173 °C[23]).

Acknowledgments

We'd like to thank Dr. R. Kluge for measuring the ESI-MS spectra and Dr. D. Ströhl and his team for the NMR spectra, but also Mrs. J. Wiese, MSc and Mrs. V. Simon for measuring the IR spectra and optical rotations. The cell lines were kindly provided by Dr. Th. Müller (Dept. of Haematology/Oncology, Martin-Luther Universität Halle-Wittenberg). Financial support by the ''WissenschaftsCampus Halle WCH" (W13004216 to R.C.) and the Oman Research Council (ORG/HSS/14/004 to A. A.-H.) is gratefully recognized.

Appendix A. Supplementary material

Supplementary data related to this article can be found, in the online version, at …

References

[1] B.B. Petrovska, Historical review of medicinal plants' usage, Pharmacogn. Rev. 6 (2012) 1-5.

[2] R. Verpoorte, Medicinal Plants: A Renewable Resource for Novel Leads and Drugs, in: K.G. Ramawat (Ed.), Herbal Drugs: Ethnomedicine to Modern Medicine, Springer, Berlin, Heidelberg, 2009, pp. 1-5.

[3] D.J. Newman, G.M. Cragg, Natural Products as Sources of New Drugs from 1981 to 2014, J. Nat. Prod. 79 (2016) 629-661.

[4] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, Ca-Cancer J. Clin. 68 (2018) 394-424.

[5] P.-J. Anna, R. Aleksandra, R. Maria, Molecular Mechanisms of Biological Activity of Oleanolic Acid - A Source of Inspiration for A New Drugs Design, Mini-Rev. Org. Chem. 11 (2014) 330-342.

[6] M.P. Montilla, A. Agil, M.C. Navarro, M.I. Jiménez, A. García-Granados, A. Parra, M.M. Cabo, Antioxidant activity of maslinic acid, a triterpene derivative obtained from Olea europaea, Planta Med. 69 (2003) 472-474.

[7] Y. Allouche, G. Beltrán, J.J. Gaforio, M. Uceda, M.D. Mesa, Antioxidant and antiatherogenic activities of pentacyclic triterpenic diols and acids, Food Chem. Toxicol. 48 (2010) 2885-2890.

[8] L. Huang, T. Guan, Y. Qian, M. Huang, X. Tang, Y. Li, H. Sun, Anti-inflammatory effects of maslinic acid, a natural triterpene, in cultured cortical astrocytes via suppression of nuclear factor-kappa B, Eur. J. Pharmacol. 672 (2011) 169-174.

[9] S. Taniguchi, Y. Imayoshi, E. Kobayashi, Y. Takamatsu, H. Ito, T. Hatano, H. Sakagami, H. Tokuda, H. Nishino, D. Sugita, Production of bioactive triterpenes by Eriobotrya japonica calli, Phytochemistry 59 (2002) 315-323.

[10] A. Parra, F. Rivas, P.E. Lopez, A. Garcia-Granados, A. Martinez, F. Albericio, N. Marquez, E. Muñoz, Solution-and solid-phase synthesis and anti-HIV activity of maslinic acid derivatives containing amino acids and peptides, Bioorg. Med. Chem. 17 (2009) 1139-1145.

[11] L.Y. Mooi, N.A. Wahab, N.H. Lajis, A.M. Ali, Chemopreventive properties of phytosterols and maslinic acid extracted from Coleus tuberosus in inhibiting the expression of EBV early-antigen in Raji cells, Chem. Biodiversity 7 (2010) 1267-1275.

[12] D.-W. Mu, H.-Q. Guo, G.-B. Zhou, J.-Y. Li, B. Su, Oleanolic acid suppresses the proliferation of human bladder cancer by Akt/mTOR/S6K and ERK1/2 signaling, Int. J. Clin. Exp. Pathol. 8 (2015) 13864-13870.

[13] Y.Y. Zhu, H.Y. Huang, Y.L. Wu, Anticancer and apoptotic activities of oleanolic acid are mediated through cell cycle arrest and disruption of mitochondrial membrane potential in HepG2 human hepatocellular carcinoma cells, Mol. Med. Rep. 12 (2015) 5012-5018.

[14] F.J. Reyes, J.J. Centelles, J.A. Lupiáñez, M. Cascante, (2α,3β)-2,3-Dihydroxyolean-12-en-28-oic acid, a new natural triterpene from Olea europea, induces caspase dependent apoptosis selectively in colon adenocarcinoma cells, FEBS Lett. 580(27) (2006) 6302-6310.

[15] F.J. Reyes-Zurita, G. Pachón-Peña, D. Lizárraga, E.E. Rufino-Palomares, M. Cascante, J.A. Lupiáñez, The natural triterpene maslinic acid induces apoptosis in HT29 colon cancer cells by a JNK-p53-dependent mechanism, BMC cancer 11 (2011) 154.

[16] A. Parra, F. Rivas, S. Martin-Fonseca, A. Garcia-Granados, A. Martinez, Maslinic acid derivatives induce significant apoptosis in b16f10 murine melanoma cells, Eur. J. Med. Chem. 46 (2011) 5991-6001.

[17] C. Li, Z. Yang, C. Zhai, W. Qiu, D. Li, Z. Yi, L. Wang, J. Tang, M. Qian, J. Luo, Maslinic acid potentiates the anti-tumor activity of tumor necrosis factor α by inhibiting NF-κB signaling pathway, Mol. Cancer 9 (2010) 73.

[18] D.-M. Wu, D. Zhao, D.-Z. Li, D.-Y. Xu, W.-F. Chu, X.-F. Wang, Maslinic acid induces apoptosis in salivary gland adenoid cystic carcinoma cells by Ca 2+-evoked p38 signaling pathway, Naunyn-Schmiedeberg's Arch. Pharmacol. 383 (2011) 321-330.

[19] L. Heller, A. Knorrscheidt, F. Flemming, J. Wiemann, S. Sommerwerk, I.Z. Pavel, A. Al-Harrasi, R. Csuk, Synthesis and proapoptotic activity of oleanolic acid derived amides, Bioorg. Chem. 68 (2016) 137-151.

[20] M. Kahnt, L. Fischer , A. Al-Harrasi, R. Csuk, Ethylenediamine Derived Carboxamides of Betulinic and Ursolic Acid as Potential Cytotoxic Agents, Molecules 23 (2018) 2558.

[21] S. Sommerwerk, L. Heller, J. Kuhfs, R. Csuk, Urea derivates of ursolic, oleanolic and maslinic acid induce apoptosis and are selective cytotoxic for several human tumor cell lines, Eur. J. Med. Chem. 119 (2016) 1-16.

[22] 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.

[23] B. Siewert, E. Pianowski, A. Obernauer, R. Csuk, Towards cytotoxic and selective derivatives of maslinic acid, Bioorg. Med. Chem. 22 (2014) 594-615.

[24] S. Sommerwerk, R. Csuk, Convenient and chromatography-free partial syntheses of maslinic acid and augustic acid, Tetrahedron Lett. 55 (2014) 5156-5158.

[25] S. Sommerwerk, L. Heller, I. Serbian, R. Csuk, Straightforward partial synthesis of four diastereomeric 2,3-dihydroxy-olean-12-en-28-oic acids from oleanolic acid, Tetrahedron 71 (2015) 8528-8534.

[26] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drug screening, JNCI, J. Natl. Cancer Inst. 82 (1990) 1107-1112.

[27] A. Loesche, M. Kahnt, I. Serbian, W. Brandt, R. Csuk, Triterpene-Based Carboxamides Act as Good Inhibitors of Butyrylcholinesterase, Molecules 24 (2019) 948.

[28] M.H.L. Wong, H.K. Bryan, I.M. Copple, R.E. Jenkins, P.H. Chiu, J. Bibby, N.G. Berry, N.R. Kitteringham, C.E. Goldring, P.M. O'Neill, B.K. Park, Design and Synthesis of Irreversible

Analogues of Bardoxolone Methyl for the Identification of Pharmacologically Relevant Targets and Interaction Sites, J. Med. Chem. 59 (2016) 2396-2409.

Highlights

* Oleanolic acid and maslinic acid occur in many plants

* These triterpenoids show promising biological activities

* Their substituted ethylene diamine derived carboxamides are highly cytotoxic

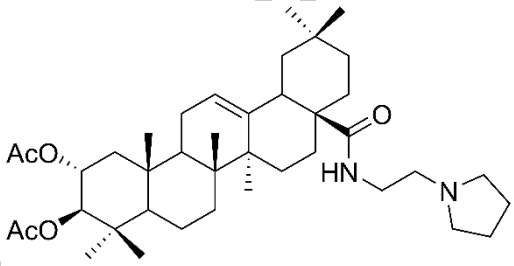
* The alkyl chain length of the diamine units influences the cytotoxicity

* Low EC_{50} values were observed for these compounds and human tumor cells

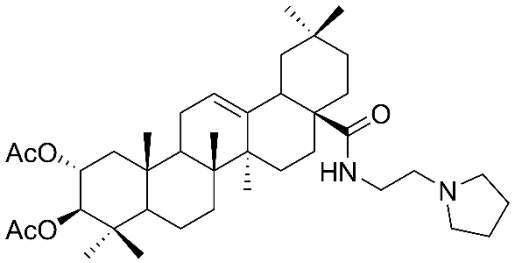
* Maslinic acid derivatives are higher selective than oleanolic analogs

* Cell death in the human tumor cells is triggered by apoptosis

Graphical abstract



EC_{50} = 1.5 μM (A2780)
EC_{50} = 4.6 μM (NIH 3T3)



EC_{50} = 1.5 μM (A2780)
EC_{50} = 4.6 μM (NIH 3T3)