

An asiatic acid derived trisulfamate acts as a nanomolar inhibitor of human carbonic anhydrase VA

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ABSTRACT

This investigation delves into the inhibitory capabilities of a specific set of triterpenic acids on diverse isoforms of human carbonic anhydrase (hCA). Oleanolic acid (1), maslinic acid (2), betulinic acid (3), platanic acid (4), and asiatic acid (5) were chosen as representative triterpenoids for evaluation. The synthesis involved acetylation of parent triterpenic acids 1–5, followed by sequential reactions with oxalyl chloride and benzylamine, deacetylation of the amides, and subsequent treatment with sodium hydride and sulfamoyl chloride, leading to the formation of final compounds 21–25.

Inhibition assays against hCAs I, II, VA, and IX demonstrated noteworthy outcomes. A derivative of betulinic acid, compound 23, exhibited a K_i value of 88.1 nM for hCA VA, and a derivative of asiatic acid, compound 25, displayed an even lower K_i value of 36.2 nM for the same isoform. Notably, the latter compound displayed enhanced inhibitory activity against hCA VA when compared to the benchmark compound acetazolamide (AAZ), which had a K_i value of 63.0 nM. Thus, this compound surpasses the inhibitory potency and isoform selectivity of the standard compound acetazolamide (AAZ). In conclusion, the research offers insights into the inhibitory potential of selected triterpenic acids across diverse hCA isoforms, emphasizing the pivotal role of structural attributes in determining isoform-specific inhibitory activity. The identification of compound 25 as a robust and selective hCA VA inhibitor prompts further exploration of its therapeutic applications.

1. Introduction

The development of inhibitors of the enzyme class of carbonic anhydrases (CAs) has gained importance for many years [1–10]. Although first described in 1933 by Meldrum and Roughton [11,12] for the efficient catalysis of the reversible hydration of CO₂ in blood to bicarbonate and protons, eight genetically distinct CA families are known today, and CAs are involved in many physiological processes. These include respiration, gluconeogenesis, adipogenesis and numerous other biosynthetic reactions. Therefore, especially human CAs (hCAs) are the subject of numerous investigations as therapeutic targets for a wide variety of diseases, including the therapy of edema, glaucoma, epilepsy, obesity, inflammatory diseases, neuropathic pain, Alzheimer's disease, oxidative stress and especially of hypoxic tumors [1–10].

The connection between cancer and individual CA isoforms, especially hCA IX and hCA XII, has been known for many years [13–19]. Both hCAs are under control of the hypoxia inducible factor [20–24]. For

individual inhibitors of hCA XI and hCA XII, it has now been demonstrated that they reduce the number of cancer stem cells, inhibit the growth of primary tumors and also slow down metastasis. In addition, they also interfere with an iron-dependent cell death mechanism (ferroptosis) [25–27].

Links between lifestyle combined with inappropriate food and cancer are now considered to be well established, and it is therefore not surprising that obesity is among the top global health problems; enzyme isoforms CAs VA and VB have been suggested as targets to develop anti-obesity drugs [28–32].

Some time ago, we were able to demonstrate the quite high cytotoxicity of pentacyclic triterpenes for human tumor cell lines with simultaneously significantly lower cytotoxicity for non-malignant fibroblasts. In addition, some compounds were found to be inhibitors of CA II [33–40]. It was therefore obvious to extend the investigations of this substance class, especially regarding compounds exhibiting isoform selectivity.

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The number of publications dealing with triterpene derivatives as inhibitors of CAs is limited. Thereby, methyl triterpenoates were shown [35] to be good inhibitors of CA II, and several derivatives of betulin and betulinic acid inhibited CA IX [36,41]. Recently, we were able to show that succinyl spaced acetazolamide hybrids of acetylated triterpenoids such as betulin, oleanolic acid, ursolic acid and glycyrrhetic acid were inhibitors of CA II [34]. Thereby, it was revealed that small structural differences govern the inhibition activity of these conjugates [34]. The latter finding called for a more intensive study of sulfamated triterpenoids and their ability to inhibit isoforms of CAs.

2. Results and discussion

Oleanolic acid (**1**), maslinic acid (**2**), betulinic acid (**3**), platanic acid (**4**) and asiatic acid (**5**) (Scheme 1) were chosen as a small library of representative triterpenoic acids. Compounds **1** and **2** are representatives of triterpenes holding an oleanane skeleton, **3** and **4** are lupanes and compound **5** is a trihydroxylated triterpenoid of the ursane type.

For the synthesis of the target compounds, parent triterpenoic acids **1–5** were acetylated, and acetates **6–10** were obtained. Their treatment with oxalyl chloride followed by the reaction with benzylamine yielded benzyl amides **11–15**, which upon de-acetylation afforded **16–20**. Their treatment with sodium hydride in dry THF followed by the addition of sulfamoyl chloride yielded final products **21–25**, respectively.

Compounds **21–25** were screened for their ability to inhibit hCAs I, II, VA, and IX, respectively. The results from these assays are summarized in Table 1 and depicted in Fig. 1.

While none of these compounds was an excellent inhibitor for hCA I and only a fair inhibitor of hCA II, especially betulinic acid derived **23** showed a K_i value of 88.1 nM for hCA VA, and an even lower $K_i = 36.2$ nM was measured for asiatic acid derived compound **25**. Thus, this

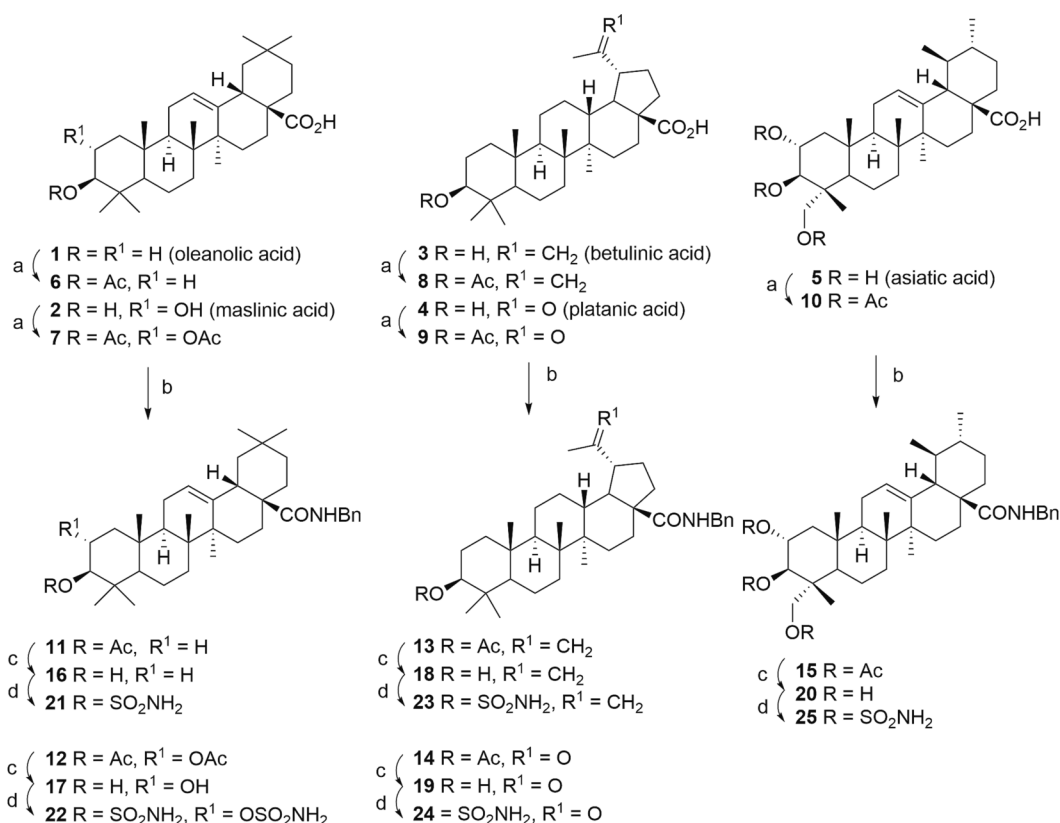
Table 1

Inhibition of carbonic anhydrase isoforms hCA I, hCA II, hCA VA and hCA IX; values were obtained as means from three different assays by a stopped flow technique as described in the Supplementary Materials File; errors were in the range of ± 5 –10 % of the reported values. Acetazolamide (AAZ) was used as a positive control. K_i values are reported in nM.

Compound	hCA I	hCA II	hCA VA	hCA IX
21	925.0	390.4	457.0	95.2
22	3781	507.7	362.3	1923
23	892.8	564.4	88.1	995.3
24	2494	428.9	206.1	1122
25	5230	297.5	36.2	1126
AAZ	250.0	12.1	63.0	25.8

compound was a better inhibitor for this enzyme than standard acetazolamide (AAZ) holding a $K_i = 63.0$ nM for hCA VA. The SI values as depicted in Fig. 2 for compounds **21–25** indicate that the former exhibits a similar selectivity towards the different isoenzymes as standard AAZ. In contrast, compound **23** held the lowest selectivity towards hCA IX. The most significant difference, however, in their respective SI values were observed for **21** and **22**, although both compounds share an oleanane skeleton, but differ in their number of hydroxyl groups. It might be assumed that the number of sulfamate groups holds a significant impact on the selectivity index for hCA I vs hCA IX.

Only oleanolic acid derived compound **21** was a moderate inhibitor of hCA IX with a $K_i = 95.2$ nM. Due to its high selectivity towards hCA I (Fig. 2), **21** might be an interesting starting point for the development of drugs to deal with brain edema. This seems of special interest inasmuch as a potential association between neuronal cell swelling induced by plaques and the symptomatic manifestations of Alzheimer's disease have been discovered. Concerning Alzheimer's disease ramifications



Scheme 1. Synthesis of sulfamated triterpenoic acid derivatives **21–25**; reactions and conditions: a) Ac₂O, Et₃N, DMAP (cat.), DCM, 24 h, 20 °C: **6** (90 %), **7** (91 %), **8** (90 %), **9** (86 %), **10** (87 %); b) (COCl)₂, DCM, DMF (cat.), 0 °C → room temperature 3 h, then Bn-NH₂, room temperature, 1 h: **11** (93 %), **12** (89 %), **13** (93 %), **14** (85 %), **15** (93 %); c) KOH in MeOH, 20 °C: **16** (92 %), **17** (90 %), **18** (92 %), **19** (87 %), **20** (84 %); d) NaH, THF then ClSO₂NH₂, room temperature, 1 week: **21** (86 %), **22** (84 %), **23** (87 %), **24** (86 %), **25** (89 %).

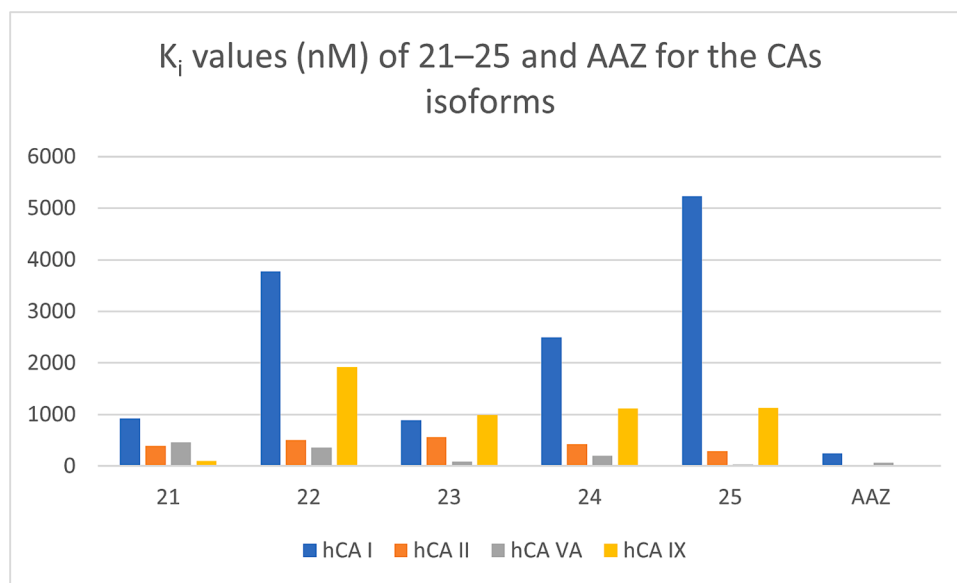


Fig. 1. Inhibition of hCAs I, II, VA and IX by triterpenoid sulfamates 21–25 and acetazolamide (AAZ) for comparison; K_i values are reported in nM.

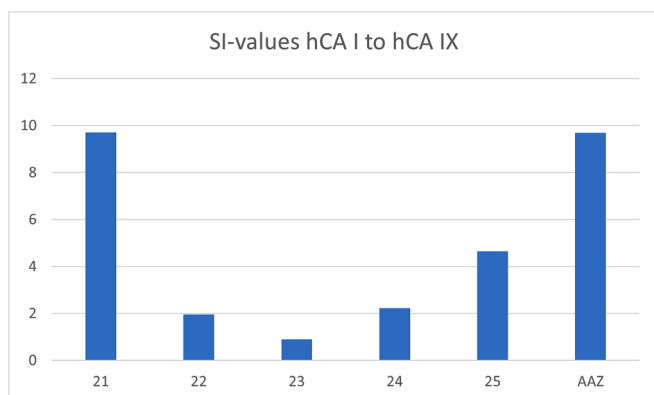


Fig. 2. Selectivity index (SI) (ratio K_i (hCA I) vs K_i (hCA IX)) of 21–25 and of standard AAZ showing 21 of holding a selectivity for hCA IX similarly to that of AAZ.

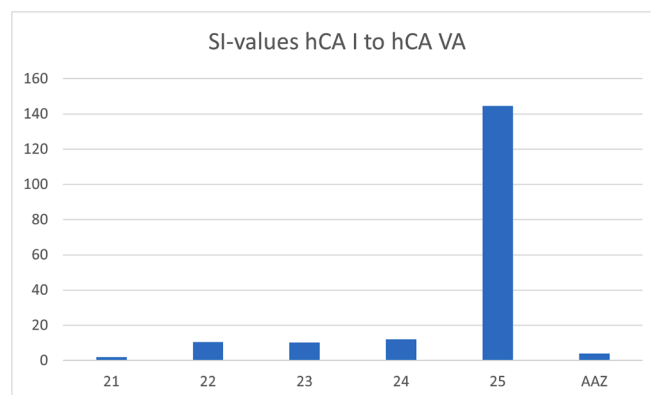


Fig. 3. Selectivity index (SI) (ratio K_i (hCA I) vs K_i (hCA VA)) of 21–25 and of standard AAZ showing 25 of holding a high selectivity of 144.5 especially for hCA VA.

subsequently to lecanemab or donanemab treatment [42–44], namely brain edema and hemorrhage, it was observed that these complications affected a substantial proportion of participants - up to approximately one-third - in the clinical trial cohort [42]. Furthermore, an activation of microglial cells within regions of cerebral edema is evident in brain swelling patients. This observation propounds the proposition that these microglia might play a contributory role in Alzheimer's disease pathogenesis subsequently to therapeutic interventions. It should be noted that microglial activation is also discernible in Alzheimer's disease models and is currently acknowledged as one of the primary pathological hallmarks of this disease [45]. Unspecific CA inhibitors (pan-CA inhibitors) are presently applied to reduce brain swelling and brain edema [46,47], and the development of more specific inhibitors is called for. Compound 21 might be a good candidate for further development.

The results from the CA screening also show that there is a strong dependence of isoform-specific inhibitory activity and the skeleton of the triterpenoid scaffold. Some more calculated selectivity values (calculated from the ratio of the K_i-values) are depicted in Figs. 3 and 4. As a result, asiatic acid derived compound 25 was the best inhibitor for hCA VA holding a K_i = 36.2 nM and a high selectivity towards this enzyme (as compared to hCA I) of 144.5.

Hence, compound 25 is a better inhibitor for this enzyme holding

also a higher selectivity for this isoform than standard compound acetazolamide (AAZ). Comparison of isoform selectivity concerning hCA II vs hCA VA shows (Fig. 4) compounds 23 and 25 to be more selective towards hCA VA than standard AAZ.

Fig. 4 shows a comparison for the isoform selectivity towards hCA II and hCA VA. It is evident that compounds 23 and 25 exhibit stronger selectivity towards hCA VA than hCA II. This selectivity is even greater than that of AAZ. In this context, compounds 21 and 22 (both holding an oleanane skeleton) showed less selectivity towards hCA VA. Compounds 23 and 24 (both lupanes) demonstrated more pronounced differences in their selectivity. Compound 23, derived from betulinic acid, displayed a threefold higher selectivity towards hCA VA as compared to the platonic acid-derived compound 24. Trihydroxylated compound 25 (ursane type) exhibited the highest selectivity among all compounds of this study.

Parent triterpenes are well known for their poor solubility in water. While parent parent triterpenoic acids are almost insoluble (solubility between 0.01 and 0.02 µg/mL) in water, compound 25 showed an about 20-fold improved solubility. The excellent properties of this compound call for additional biological testing. Due to legal restrictions on the implementation of animal experiments to determine in vivo toxicity, toxicity was estimated using the ProTox II (<https://tox-new.charite.de/>)

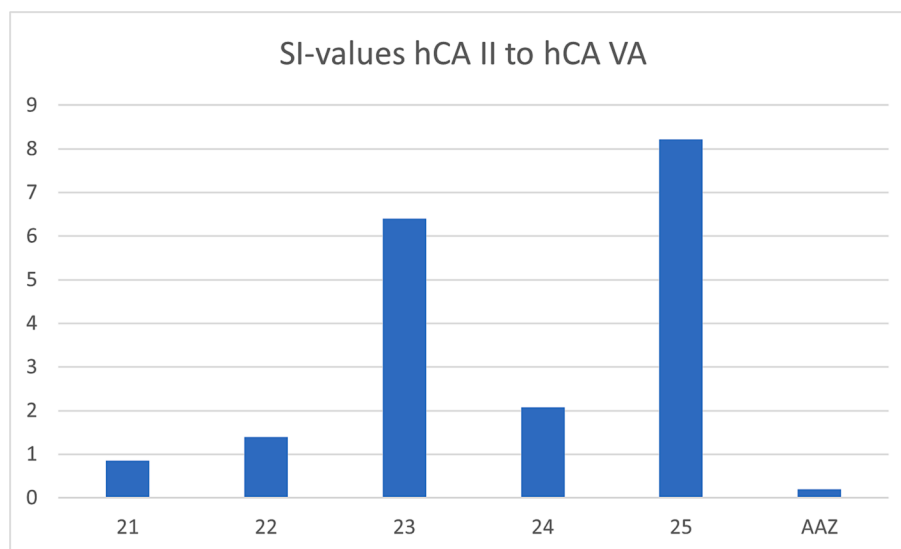


Fig. 4. Selectivity index (SI) (ratio K_i (hCA II) vs K_i (hCA VA)) of **21–25** and of standard **AAZ** showing **23** and **25** to be significantly more selective for hCA VA than to hCA II and performing better than **AAZ**.

[protox_II](#)) as an alternative. This resulted in an estimated calculated toxicity of $LD_{50} > 3$ g/kg. Future studies will also include investigations concerning the cytotoxicity of these compounds.

3. Conclusion

The present study investigates the inhibitory potential of a selected group of triterpenoic acids on various human carbonic anhydrase (hCA) isoforms. Oleanolic acid (**1**), maslinic acid (**2**), betulinic acid (**3**), platanic acid (**4**), and asiatic acid (**5**) were chosen as representative triterpenoids for analysis. To synthesize the target compounds, parent compounds underwent acetylation, followed by reaction with oxalyl chloride and benzylamine, de-acetylation of the amides followed by their reaction with sodium hydride and sulfamoyl chloride to yield final sulfamoylated benzyl amides **21–25**, respectively.

The inhibitory potential of these compounds was assessed against hCAs I, II, VA, and IX. A betulinic acid-derived compound **23** displayed a K_i value of 88.1 nM for hCA VA, while asiatic acid-derived compound **25** demonstrated an even lower K_i value of 36.2 nM for the same isoform. Notably, compound **25** exhibited superior inhibitory activity against hCA VA compared to the standard compound acetazolamide (**AAZ**), which displayed a K_i value of 63.0 nM. The study's findings reveal a strong correlation between isoform-specific inhibitory activity and the triterpenoid scaffold's structural framework. Selectivity values calculated from the K_i ratios illustrate this relationship, with asiatic acid-derived compound **25** emerging as the most potent hCA VA inhibitor, displaying both a low K_i value of 36.2 nM but also a selectivity of 144.5 compared to hCA I. In conclusion, this study sheds light on the inhibitory potential of a selected group of triterpenoic acids against various hCA isoforms. The results underscore the importance of structural characteristics in determining isoform-specific inhibitory activity. The identification of asiatic acid-derived compound **25** as a potent and selective inhibitor for hCA VA warrants further exploration for its potential therapeutic applications especially for the treatment of obesity.

4. Experimental

4.1. General

Starting materials were obtained from local vendors [oleanolic acid, betulinic acid and platanic acid were obtained from betulinines (Stříbrná Skalice, Czech Republic), asiatic acid and maslinic acid were

bought from Merck (Darmstadt, Germany)]; solvents were dried under usual conditions; equipment was used as previously described. Details of the biological testing, analytical data for **21–24** and representative NMR spectra (^1H and ^{13}C) are provided in the [Supplementary File](#). The physical data (m.p., $[\alpha]_D^{20}$, IR, UV/Vis, ^1H NMR, ^{13}C NMR, MS) of known compounds **6–24** described in this manuscript was in full agreement with data described in the literature.

4.2. Acetylation of the triterpenoic acids (General procedure A, GPA)

Acetylation of **1–5** (1 equiv.) was performed in dry DCM with acetic anhydride (3 equiv.), Et_3N (3 equiv.) and DMAP (catal. amount) for 24 h at ambient temperatures. Usual aqueous work-up followed by recrystallization from EtOH yielded products **6–10**.

4.3. Synthesis of the benzylamides **11–15** (General procedure B, GPB)

The acetylated triterpenoic acid **6–10** (1 equiv.) was dissolved in dry DCM, oxalyl chloride (4.0 equiv.) and dry DMF (cat. amount) were added at 0 °C, stirring at room temperature was continued for 3 h, the volatiles were removed, and benzyl amine (2 equiv.) was added. Stirring at room temperature was continued for another hour followed by usual workup and chromatography to afford **11–15**.

4.4. Deacetylation of **11–15** (General procedure C, GPC)

To a solution of methanolic KOH (1.2 equiv.) a solution of **11–15** was slowly added, and stirring at 20 °C was continued until TLC showed completeness of the reaction. The product was precipitated with cold aq. HCl (3.5 %), collected, dried, and purified by chromatography to afford **16–20**.

4.5. Synthesis of the sulfamates **21–25** (General procedure D, GPD)

To a solution of **16–20** (0.5 mmol) in dry THF (25 mL), NaH (freshly washed with dry *n*-hexane, 0.80 mmol) was added at 5 °C, and the mixture was stirred until the evolution of gases had ceased. A solution of sulfamoyl chloride (1.0 mmol) in dry THF (2 mL) was added, and stirring at room temperature was continued for 1 week. The volatiles were removed under diminished pressure, the residue was parted between water (100 mL) and diethyl ether (100 mL); usual workup of the organic layer followed by chromatography furnished **21–25** each as a white

solid.

4.6. Syntheses

4.6.1. 3-O-Acetyl-oleanolic acid (6)

From **1** by GPA, compound **6** was obtained in 90 % yield; white solid; m.p. 260–262 °C (lit.: [48] m.p. 267–268 °C); $[\alpha]_D^{20} = +69.4^\circ$ (c 0.30, CHCl₃) [lit.: [49] $[\alpha]_D^{20} = +74.0^\circ$ (c 1.0, CHCl₃)]; MS (ESI, MeOH): $m/z = 499.2$ (55 %, [M + H]⁺).

4.6.2. 2,3-Di-O-acetyl-maslinic acid (7)

From **2** by GPA, compound **7** was obtained in 91 % yield; white solid; m.p. 224–226 °C (lit.: [50] 170–173 °C); $[\alpha]_D^{20} = +30.0^\circ$ (c 0.45, CHCl₃) [lit.: [50] $[\alpha]_D^{20} = +30.0^\circ$ (c 0.83, CHCl₃)]; MS (ESI, MeOH): $m/z = 557.5$ (52 %, [M + H]⁺).

4.6.3. 3-O-Acetyl-betulinic acid (8)

From **3** by GPA, compound **8** was obtained in 90 % yield; white solid; m.p. 280–283 °C (lit.: [51] 277–278 °C); $[\alpha]_D^{20} = +20.0^\circ$ (c 0.25, CHCl₃) [lit.: [51] $[\alpha]_D^{20} = +22.0^\circ$ (c 0.49, CHCl₃)]; MS (ESI, MeOH): $m/z = 497.5$ (26 %, [M + H]⁺).

4.6.4. 3-O-Acetyl-platanic acid (9)

From **4** by GPA, compound **9** was obtained in 86 % yield; white solid; m.p. 266–268 °C (lit.: [52] 256–259 °C); $[\alpha]_D^{20} = -9.4^\circ$ (c 0.4, CHCl₃) [lit.: [52] $[\alpha]_D^{20} = -9.1^\circ$ (c 0.34, CHCl₃)]; MS (ESI, MeOH): $m/z = 999.5$ (100 %, [2 M + H]⁺).

4.6.5. 2,3,23-Tri-O-acetyl-asiatic acid (10)

From **5** by GPA, compound **10** was obtained in 87 % yield; white solid; m.p. 162–164 °C (lit.: [53] 159–161 °C); $[\alpha]_D^{20} = +37.2^\circ$ (c 0.4, CHCl₃) [lit.: [53] $[\alpha]_D^{20} = +35.96^\circ$ (c 0.34, CHCl₃)]; MS (ESI, MeOH): $m/z = 615.3$ (17 %, [M + H]⁺).

4.6.6. 3β-Acetyloxy-N-benzyl-olean-12-en-28 amide (11)

From **6** by GPB, compound **11** was obtained in 93 % yield; white solid; m.p. 257–260 °C (lit.: [50] 254–259 °C); $[\alpha]_D^{20} = +30.1^\circ$ (c 0.5, CHCl₃) [lit.: [50] $[\alpha]_D^{20} = +29^\circ$ (c 0.56, CHCl₃)]; MS (ESI, MeOH): $m/z = 588.7$ (60 %, [M + H]⁺).

4.6.7. (2α,3β)-Bis(acetyloxy)-N-benzyl-olean-12-en-28-amide (12)

From **7** by GPB, compound **12** was obtained in 89 % yield; white solid; m.p. 144–146 °C (lit.: [50] 143–145 °C); $[\alpha]_D^{20} = -6.9^\circ$ (c 0.4, CHCl₃) [lit.: [50] $[\alpha]_D^{20} = -7.0^\circ$ (c 0.32, CHCl₃)]; MS (ESI, MeOH): $m/z = 646.5$ (100 %, [M + H]⁺).

4.6.8. 3β-Acetyloxy-N-benzyl-lup-20(29)en-28-amide (13)

From **8** by GPB, compound **13** was obtained in 93 % yield; white solid; m.p. 124–126 °C (lit.: [54] 124–127 °C); $[\alpha]_D^{20} = +24.1^\circ$ (c 0.45, CHCl₃) [lit.: [54] $[\alpha]_D^{20} = +23.2^\circ$ (c 0.35, CHCl₃)]; MS (ESI, MeOH): $m/z = 588.4$ (52 %, [M + H]⁺).

4.6.9. 3β-Acetyloxy-N-benzyl-30-oxo-30-norlupan-28-amide (14)

From **9** by GPB, compound **14** was obtained in 85 % yield; white solid; m.p. 288–290 °C (lit.: [55] 290 °C (decomp.); $[\alpha]_D^{20} = +1.7^\circ$ (c 0.25, CHCl₃) [lit.: [55] $[\alpha]_D^{20} = +0.5^\circ$ (c 0.159, CHCl₃)]; MS (ESI, MeOH): $m/z = 590.1$ (100 %, [M + H]⁺).

4.6.10. (2α,3β,4α) 2,3,23-Tris(acetyloxy)-N-benzyl-urs-12-en-28-amide (15)

From **10** by GPB, compound **15** was obtained in 93 % yield; white solid; m.p. 137–139 °C (lit.: [53] 136–138 °C); $[\alpha]_D^{20} = +0.75^\circ$ (c 0.40,

CHCl₃) [lit.: [53] $[\alpha]_D^{20} = +0.68^\circ$ (c 0.345, CHCl₃)]; ESI (MS, MeOH): $m/z = 704.5$ (100 %, [M + H]⁺).

4.6.11. 3β-Hydroxy-N-benzyl-olean-12-en-28-amide (16)

From **11** by GPC, compound **16** was obtained in 92 % yield; white solid; m.p. 247–249 °C (lit.: [50] 247–249 °C); $[\alpha]_D^{20} = +31.3^\circ$ (c 0.70, CHCl₃) [lit.: [50] $[\alpha]_D^{20} = +30.4^\circ$ (c 0.56, CHCl₃)]; MS (ESI, MeOH): $m/z = 546.7$ (40 %, [M + H]⁺).

4.6.12. (2α, 3β)-Dihydroxy-N-benzyl-olean-12-en-28-amide (17)

From **12** by GPC, compound **17** was obtained in 90 % yield; white solid; m.p. 149–151 °C (lit.: [50] 148–151 °C); $[\alpha]_D^{20} = +32.1^\circ$ (c 0.50, CHCl₃) [lit.: [50] $[\alpha]_D^{20} = +29^\circ$ (c 0.29, CHCl₃)]; MS (ESI, MeOH): $m/z = 563.3$ (17 %, [M + H]⁺).

4.6.13. 3β-Hydroxy-N-benzyl-lup-20(29)en-28-amide (18)

From **13** by GPC, compound **18** was obtained in 92 % yield; white solid; m.p. 247–248 °C (lit.: [54] 246–248 °C); $[\alpha]_D^{20} = +15.1^\circ$ (c 0.45, CHCl₃) [lit.: [54] $[\alpha]_D^{20} = +14.3^\circ$ (c 0.31, CHCl₃)]; MS (ESI, MeOH): $m/z = 546.4$ (100 %, [M + H]⁺).

4.6.14. 3β-Hydroxy-20-oxo-N-benzyl-30-norlupan-28-amide (19)

From **14** by GPC, compound **19** was obtained in 87 % yield; white solid; m.p. 267–268 °C (lit.: [41] 266–268 °C); $[\alpha]_D^{20} = -6.2^\circ$ (c 0.25, CHCl₃) [lit.: [41] $[\alpha]_D^{20} = -6.1^\circ$ (c 0.3, CHCl₃)]; MS (ESI, MeOH): $m/z = 548.2$ (100 %, [M + H]⁺).

4.6.15. (2α, 3β, 4α)-2,3,23-Trihydroxy-N-benzyl-urs-12-en-28-amide (20)

From **15** by GPC, compound **20** was obtained in 84 % yield; white solid; m.p. 160–163 °C (lit.: [56] 155–159 °C); $[\alpha]_D^{20} = +29.5^\circ$ (c 0.45, CHCl₃) [lit.: [56] $[\alpha]_D^{20} = +27.26^\circ$ (c 0.31, CHCl₃)]; MS (ESI, MeOH): $m/z = 578.2$ (100 %, [M + H]⁺).

4.6.16. (3β)-[(Aminosulfonyl)oxy]-N-benzyl-olean-12-en-28-amide (21)

From **16** by GPD, compound **21** was obtained in 86 % yield; white, amorphous solid; $[\alpha]_D^{20} = +27.9^\circ$ (c 0.25, CHCl₃) [lit.: [41] $[\alpha]_D^{20} = +28.1^\circ$ (c 0.385, CHCl₃)]; MS (ESI, MeOH): $m/z = 625.2$ (100 %, [M + H]⁺).

4.6.17. (2α, 3β)-Bis[(aminosulfonyl)oxy]-N-benzyl-olean-12-en-amide (22)

From **17** by GPD, compound **22** was obtained in 84 % yield; white, amorphous solid; $[\alpha]_D^{20} = +7.5^\circ$ (c 0.25, CHCl₃) [lit.: [41] $[\alpha]_D^{20} = +7.3^\circ$ (c 0.3, CHCl₃)]; MS (ESI, MeOH): $m/z = 720.2$ (100 %, [M + H]⁺).

4.6.18. (3β)-[(Aminosulfonyl)oxy]-N-benzyl-lup-20(29)-en-28-amide (23)

From **18** by GPD, compound **23** was obtained in 87 % yield; white, amorphous solid; $[\alpha]_D^{20} = +18.4^\circ$ (c 0.5, CHCl₃) [lit.: [41] $[\alpha]_D^{20} = +17.2^\circ$ (c 0.345, CHCl₃)]; MS (ESI, MeOH): $m/z = 625.4$ (100 %, [M + H]⁺).

4.6.19. (3β)-[(Aminosulfonyl)oxy]-N-benzyl-20-oxo-30-norlupan-28-amide (24)

From **19** by GPD, compound **24** was obtained in 86 % yield; white, amorphous solid; $[\alpha]_D^{20} = -3.1^\circ$ (c 0.25, CHCl₃) [lit.: [41] $[\alpha]_D^{20} = -2.9^\circ$ (c 0.34, CHCl₃)]; MS (ESI, MeOH): $m/z = 627.5$ (35 %, [M + H]⁺).

4.6.20. (2α, 3β, 4α)-2,3,23-Tris[(aminosulfonyl)oxy]-N-benzyl-urs-12-en-28-amide (25)

From **20** by GPD, compound **25** was obtained in 89 % yield; white solid; m.p. 198–199 °C $[\alpha]_D^{20} = +8.59$ (c 0.136, MeOH); $R_f = 0.26$ (SiO₂, CHCl₃/MeOH, 9:1); UV–Vis (CHCl₃): $\lambda_{\max}$ (log ϵ) = 259 nm (3.25); IR (ATR): $\nu = 3361w, 3278w, 3062w, 2923w, 2870w, 1627w, 1557w,$

1525w, 1497w, 1455w, 1360 s, 1176vs, 1012w, 971 m, 960 s, 924 s, 877w, 828 s, 754w, 743w, 723w, 699w, 666w, 651w, 641w, 601w, 580 m, 555 m, 537 m, 497w, 482w, 460w cm⁻¹; ¹H NMR (400 MHz, DMSO-*d*₆): δ = 7.73 (t, *J* = 6.0 Hz, 1H, NH), 7.48–7.10 (m, 11H, NH₂, 33-H, 34-H, 35-H, 36-H, 37-H), 5.21 (t, *J* = 3.6 Hz, 1H, 12-H), 4.67 (ddd, *J* = 11.8, 9.8, 4.7 Hz, 1H, 2-H), 4.37 (d, *J* = 9.9 Hz, 1H, 3-H), 4.20 (qd, *J* = 15.0, 5.8 Hz, 2H, 31-H), 3.90–3.80 (m, 2H, 24-H), 2.32 (dd, *J* = 12.5, 4.5 Hz, 1H, 1-H_a), 2.23 (d, *J* = 10.7 Hz, 1H, 18-H), 2.00–1.62 (m, 4H, 16-H_a, 11-H_a, 15-H_a, 16-H_b), 1.61–1.20 (m, 11H, 22-H, 9-H, 7-H_a, 6-H, 21-H_a, 19-H, 21-H_b, 5-H, 11-H_b), 1.20–1.06 (m, 2H, 7-H_b, 1-H_b), 1.05 (s, 3H, 27), 0.97 (s, 4H, 26, 20-H), 0.92 (s, 4H, 30, 15-H_b), 0.84 (d, *J* = 6.4 Hz, 3H, 29), 0.81 (s, 3H, 23), 0.52 (s, 3H, 25) ppm; ¹³C NMR (101 MHz, DMSO-*d*₆): δ = 176.1 (C-28), 140.0 (C-32), 138.6 (C-13), 128.0 (C-34, C-36), 127.3 (C-33, C-37), 126.4 (C-35), 124.0 (C-12), 82.6 (C-3), 76.2 (C-2), 69.2 (C-24), 51.9 (C-18), 46.6 (C-5, C-9), 46.5 (C-17), 44.1 (C-1), 42.8 (C-4), 42.2 (C-31), 41.6 (C-14), 39.0 (C-8, C-10), 38.7 (C-19), 38.4 (C-20), 37.0 (C-22), 31.8 (C-7), 30.4 (C-21), 27.2 (C-15), 23.5 (C-16), 23.2 (C-27), 23.0 (C-11), 21.1 (C-30), 17.2 (C-6), 17.1 (C-29), 16.7 (C-25), 16.2 (C-26), 13.5 (C-23) ppm; MS (ESI, MeOH): *m/z* = 812.9 (100 %, [M–H]⁺); anal. calcd. for C₃₇H₅₈N₄S₃O₁₀ (815.07): C 54.52, H 7.17, N 6.87; found: C 54.27, H 7.39, N 6.55.

CRedit authorship contribution statement

Toni C. Denner: Writing – review & editing, Writing – original draft, Investigation. **Niels V. Heise:** Writing – review & editing, Writing – original draft, Investigation. **Immo Serbian:** Writing – review & editing, Writing – original draft, Investigation. **Andrea Angeli:** Writing – review & editing, Writing – original draft, Investigation. **Claudiu T. Supuran:** Writing – review & editing, Writing – original draft, Validation, Supervision. **René Csuk:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Investigation, Conceptualization.

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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