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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 triterpenoic acids on diverse isoforms of human carbonic anhydrase (*h*CA). 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 triterpenoic 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 *h*CAs 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 *h*CA 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 *h*CA 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 triterpenoic acids across diverse *h*CA isoforms, emphasizing the pivotal role of structural attributes in determining isoform-specific inhibitory activity. The identification of compound **25** as a robust and selective *h*CA 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 CO_2 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 (*h*CAs) 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 *h*CA IX and *h*CA XII, has been known for many years [13–19]. Both *h*CAs are under control of the hypoxia inducible factor [20–24]. For individual inhibitors of *h*CA XI and *h*CA 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 spacered acetazolamide hybrids of acetylated triterpenoids such as betulin, oleanolic acid, ursolic acid and glycyrrhetinic 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.

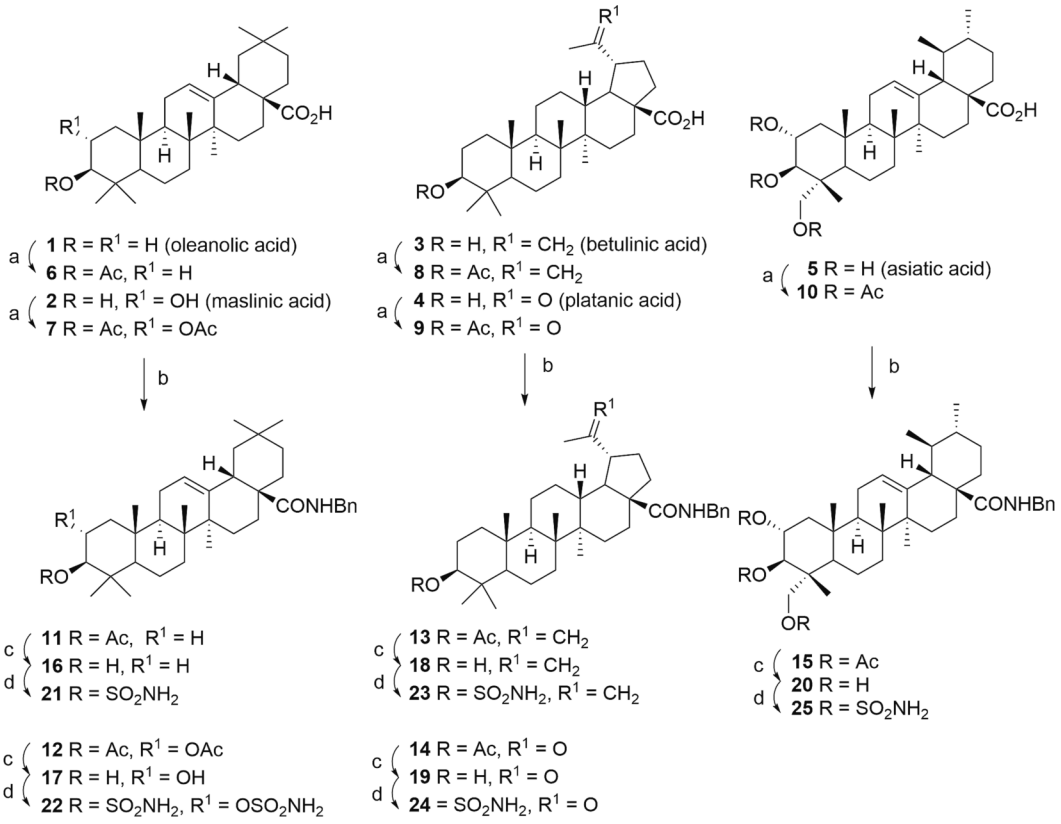
Compounds **21**–**25** were screened for their ability to inhibit *h*CAs I, II, VA, and IX, respectively. The results from these assays are summarized in Table 1 and depicted in Fig. 1.

While none of these compounds was an excellent inhibitor for *h*CA I and only a fair inhibitor of *h*CA II, especially betulinic acid derived **23** showed a K_i value of 88.1 nM for *h*CA VA, and an even lower $K_i = 36.2$ nM was measured for asiatic acid derived compound **25**. Thus, this compound was a better inhibitor for this enzyme than standard acetazolamide (**AAZ**) holding a $K_i = 63.0$ nM for *h*CA 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 *h*CA 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 *h*CA I vs *h*CA IX.

Only oleanolic acid derived compound **21** was a moderate inhibitor of *h*CA IX with a $K_i = 95.2$ nM. Due to its high selectivity towards *h*CA 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

Table 1
Inhibition of carbonic anhydrase isoforms *h*CA I, *h*CA II, *h*CA 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	*h*CA I	*h*CA II	*h*CA VA	*h*CA 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



Scheme 1. Synthesis of sulfamated triterpenoic acid derivatives **21**–**25**; reactions and conditions: a) Ac_2O, Et_3N, 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-NH_2, 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 $ClSO_2NH_2$, room temperature, 1 week: **21** (86 %), **22** (84 %), **23** (87 %), **24** (86 %), **25** (89 %).

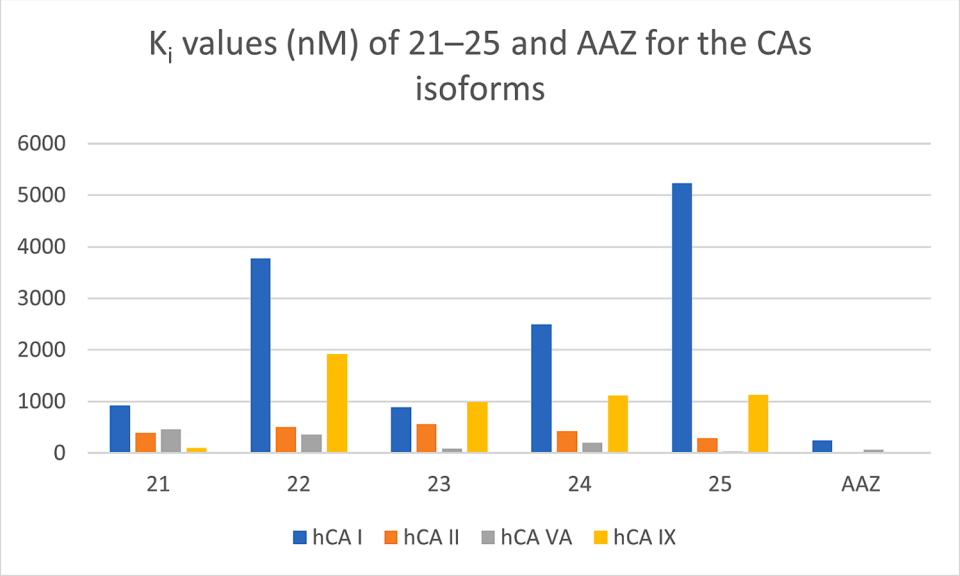


Fig. 1. Inhibition of *h*CAs I, II, VA and IX by triterpenoid sulfamates **21**–**25** and acetalzolamide (**AAZ**) for comparison; K_i values are reported in nM.

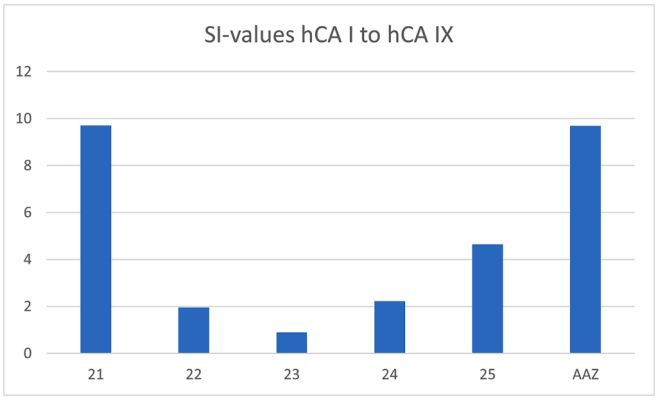


Fig. 2. Selectivity index (SI) (ratio K_i (*h*CA I) vs K_i (*h*CA IX)) of **21**–**25** and of standard **AAZ** showing **21** of holding a selectivity for *h*CA IX similarly to that of **AAZ**.

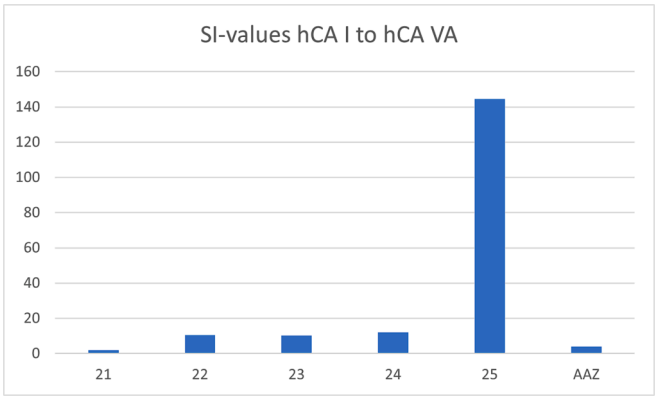


Fig. 3. Selectivity index (SI) (ratio K_i (*h*CA I) vs K_i (*h*CA VA)) of **21**–**25** and of standard **AAZ** showing **25** of holding a high selectivity of 144.5 especially for *h*CA VA.

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 *h*CA VA holding a K_i = 36.2 nM and a high selectivity towards this enzyme (as compared to *h*CA 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 *h*CA II vs *h*CA VA shows (Fig. 4) compounds **23** and **25** to be more selective towards *h*CA VA than standard **AAZ**.

Fig. 4 shows a comparison for the isoform selectivity towards *h*CA II and *h*CA VA. It is evident that compounds **23** and **25** exhibit stronger selectivity towards *h*CA VA than *h*CA 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 *h*CA 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 *h*CA VA as compared to the platanic 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/

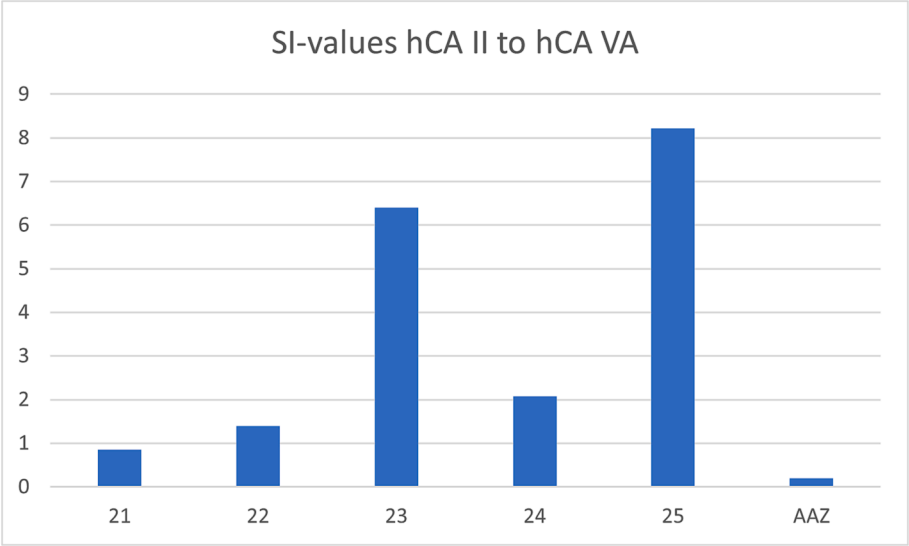


Fig. 4. Selectivity index (SI) (ratio K_i (*h*CA II) vs K_i (*h*CA VA)) of **21**–**25** and of standard **AAZ** showing **23** and **25** to be significantly more selective for *h*CA VA than to *h*CA II and performing better than **AAZ**.

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 (*h*CA) 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 *h*CAs I, II, VA, and IX. A betulinic acid-derived compound **23** displayed a K_i value of 88.1 nM for *h*CA 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 *h*CA 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 *h*CA VA inhibitor, displaying both a low K_i value of 36.2 nM but also a selectivity of 144.5 compared to *h*CA I. In conclusion, this study sheds light on the inhibitory potential of a selected group of triterpenoic acids against various *h*CA 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 *h*CA 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 (^{1}H and ^{13}C) are provided in the Supplementary File. The physical data (m.p., $[\alpha]_D^{20}$, IR, UV/Vis, ^{1}H 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° (*c* 0.30, $CHCl_3$) [lit.: [49] $[\alpha]_D^{20}$ = +74.0° (*c* 1.0, $CHCl_3$)]; 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° (*c* 0.45, $CHCl_3$) [lit.: [50] $[\alpha]_D^{20}$ = +30.0° (*c* 0.83, $CHCl_3$)]; 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° (*c* 0.25, $CHCl_3$) [lit.: [51] $[\alpha]_D^{20}$ = +22.0° (*c* 0.49, $CHCl_3$)]; 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° (*c* 0.4, $CHCl_3$) [lit.: [52] $[\alpha]_D^{20}$ = –9.1° (*c* 0.34, $CHCl_3$)]; 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° (*c* 0.4, $CHCl_3$) [lit.: [53] $[\alpha]_D^{20}$ = +35.96° (*c* 0.34, $CHCl_3$)]; 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° (*c* 0.5, $CHCl_3$) [lit.: [50] $[\alpha]_D^{20}$ = +29° (*c* 0.56, $CHCl_3$)]; 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° (*c* 0.4, $CHCl_3$) [lit.: [50] $[\alpha]_D^{20}$ = –7.0° (*c* 0.32, $CHCl_3$); 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° (*c* 0.45, $CHCl_3$) [lit.: [54] $[\alpha]_D^{20}$ = +23.2° (*c* 0.35, $CHCl_3$); 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° (*c* 0.25, $CHCl_3$) (lit.: [55] $[\alpha]_D^{20}$ = + 0.5° (*c* 0.159, $CHCl_3$); 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° (*c* 0.40, $CHCl_3$) [lit.: [53] $[\alpha]_D^{20}$ = +0.68° (*c* 0.345, $CHCl_3$); 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° (*c* 0.70, CHCl3) [lit.: [50] $[\alpha]_D^{20}$ = +30.4° (*c* 0.56, $CHCl_3$); 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° (*c* 0.50, $CHCl_3$) [lit.: [50] $[\alpha]_D^{20}$ = +29° (*c* 0.29, $CHCl_3$); 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° (*c* 0.45, $CHCl_3$) [lit.: [54] $[\alpha]_D^{20}$ = +14.3° (*c* 0.31, $CHCl_3$); 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° (*c* 0.25, $CHCl_3$) [lit.: [41] $[\alpha]_D^{20}$ = –6.1° (*c* 0.3, $CHCl_3$); 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° (*c* 0.45, $CHCl_3$) [lit.: [56] $[\alpha]_D^{20}$ = +27.26° (*c* 0.31, $CHCl_3$); 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° (*c* 0.25, $CHCl_3$) [lit.: [41] $[\alpha]_D^{20}$ = +28.1° (*c* 0.385, $CHCl_3$); 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° (*c* 0.25, $CHCl_3$) [lit.: [41] $[\alpha]_D^{20}$ = +7.3° (*c* 0.3, $CHCl_3$); 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° (*c* 0.5, $CHCl_3$) [lit.: [41] $[\alpha]_D^{20}$ = +17.2° (*c* 0.345, $CHCl_3$); 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° (*c* 0.25, $CHCl_3$) [lit.: [41] $[\alpha]_D^{20}$ = –2.9° (*c* 0.34, $CHCl_3$); 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 (SiO_2, $CHCl_3$/MeOH, 9:1); UV–Vis ($CHCl_3$): λ_{max} (log ε) = 259 nm (3.25); IR (ATR): ν = 3361*w*, 3278*w*, 3062*w*, 2923*w*, 2870*w*, 1627*w*, 1557*w*,

1525*w*, 1497*w*, 1455*w*, 1360 *s*, 1176*vs*, 1012*w*, 971 *m*, 960 *s*, 924 *s*, 877*w*, 828 *s*, 754*w*, 743*w*, 723*w*, 699*w*, 666*w*, 651*w*, 641*w*, 601*w*, 580 *m*, 555 *m*, 537 *m*, 497*w*, 482*w*, 460*w* cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.73$ (t, $J = 6.0$ Hz, 1H, NH), 7.48 – 7.10 (*m*, 11*H*, NH_2, 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*, 11*H*, 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; ^{13}C NMR (101 MHz, DMSO-d_6): $\delta = 176.1$ (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 $C_{37}H_{58}N_4S_3O_{10}$ (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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