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Lupane acetates in small molecule drug hybrids: Probing their inhibitory activity for carbonic anhydrase II

Toni-Christopher Denner , Niels V. Heise , Julian Zacharias , René Csuk [*]

Organic Chemistry, Martin-Luther University Halle-Wittenberg, Kurt-Mothes-Str. 2, D-06120, Halle, Saale, Germany

ABSTRACT

Earlier studies had shown the potential of modified pentacyclic triterpenes as possible inhibitors of carbonic anhydrase II (CA II). In an extension of our earlier studies, betulin, betulinic acid and, for comparison purposes, glycyrrhetinic acid, ursolic acid and oleanolic acid were therefore converted into the respective acetates and linked to either taurinamide or de-acetylated acetazolamide via a variable linker. In particular, the derivatives **8** and **18** derived from betulinic acid or betulin and provided with a long spacer were found to be strong competitive inhibitors of CA II, thereby holding $K_i = 1.27$ and 0.20 μM, respectively.

1. Introduction

The development of inhibitors for carbonic anhydrases is an area of increasing scientific interest. Whereas many years ago these enzymes were thought to be responsible only for the fixation of carbon dioxide to bicarbonate, and therefore only important for the regulation of intracellular and extracellular pH, today many different isoenzymes are known to be involved in an almost incalculable number of biological processes. For this reason, the development of new inhibitors seems particularly important [1–5].

In the past, CA II inhibitors have only played a role in the treatment of glaucoma [6–11]. This should not be underestimated, as it is estimated that around 76 million people worldwide suffered from this eye disease in the year 2020, and in 2040 around 111.8 million persons will be affected [12]; furthermore, glaucoma is the leading cause of global irreversible blindness [13]. CA II inhibitors have recently become particularly important because CA II has also been implicated in epilepsy [14–18] (in addition to CA VII and CA IV), neuropathic pain [19–21] (in addition to CA VII) and edema [22–24] (in addition to CA I). Edema and brain swelling have also been reported as adverse effects in patients with Alzheimer's disease treated with lecanemab, donanemab or aducanumab – three anti-amyloid monoclonal antibodies [25–28].

During our studies on pentacyclic triterpenes some years ago, we became aware of the CA II inhibitory activity of some derivatives and decided to investigate this aspect in more detail [29–33].

Based on these initial findings, the pentacyclic triterpene betulinic acid (**BA**, Scheme 1) in particular offered itself as a readily available

starting material. Classical sulfonamides have been known as inhibitors of CA II since the 1950s and 1960s. In the context of a small molecule drug conjugate (SMDC) concept [34–37], conjugates between the triterpene and a suitable sulfonamide therefore appeared to be of interest. We therefore chose structural elements derived from the known inhibitors, acetazolamide [38–40] or taurinamide [41,42].
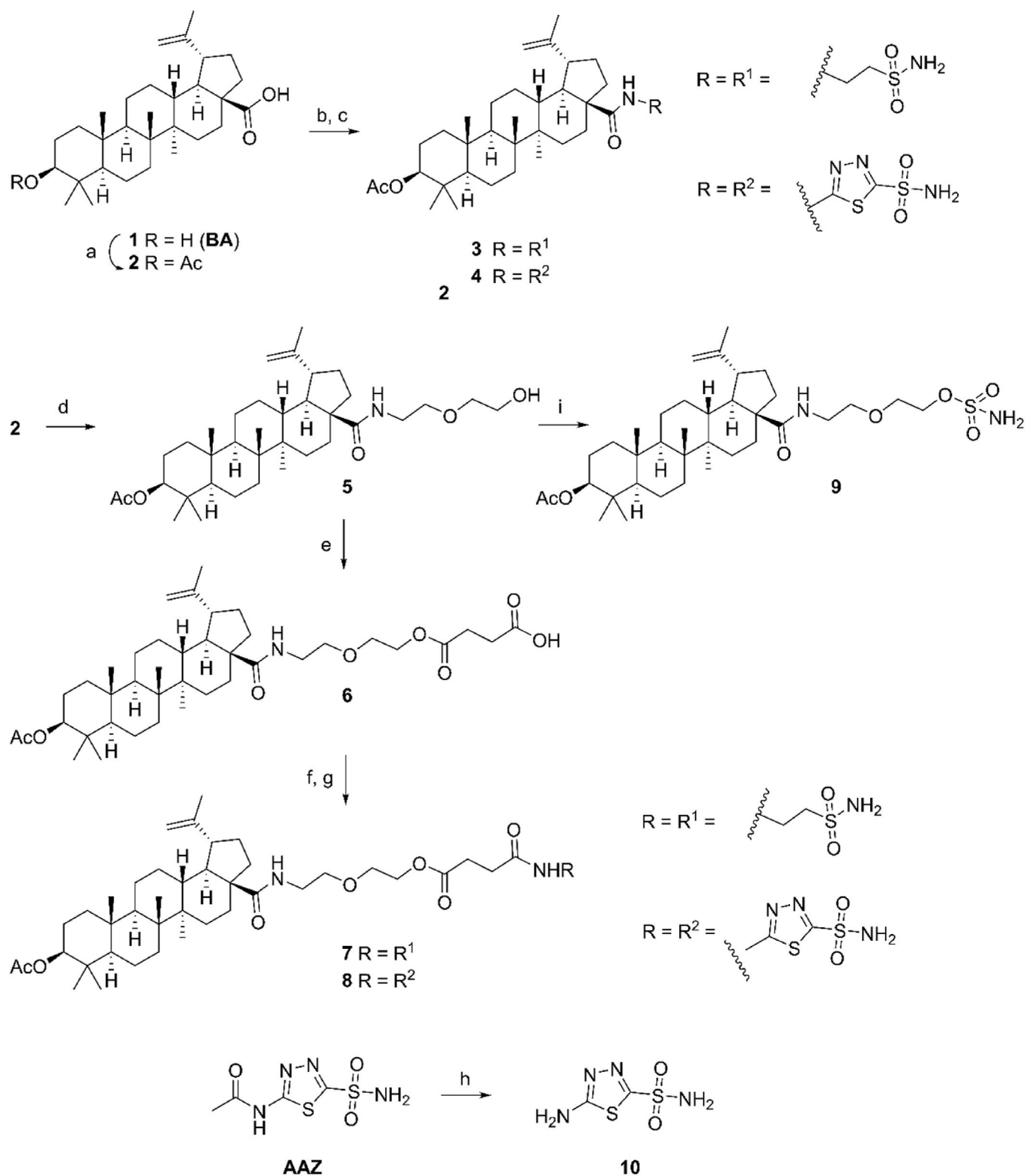
2. Results and discussion

Betulinic acid (**BA**, **1**, Scheme 1) was acetylated as previously described, and acetate **2** was obtained in 96% yield. Reaction of **2** with oxalyl chloride followed by adding either taurinamide or deacetylated acetazolamide (**10**) yielded conjugates **3** and **4** in 69% and 32% yield, respectively.

To investigate the influence of a linker onto the activity of the compounds, acetate **2** was converted *in situ* with oxalyl chloride into the corresponding acid chloride; its subsequent reaction with 2-(2-amino-ethoxy)-ethan-1-ol gave 96% of **5** whose reaction with succinic anhydride furnished **6**. The latter compound was again activated *in situ* with oxalyl chloride followed by the reaction with taurinamide or **10** to furnish conjugates **7** and **8**, respectively. In a similar way, from the reaction of **5** with sulfamoyl chloride conjugate **9** was obtained.

Acetylation of betulin (**BN**, **11**, Scheme 2) afforded diacetate **12** in 93% yield whose selective de-acetylation with catal. amounts of calcium hydride gave 84% of 3-*O*-acetyl-betulin (**13a**); 28-*O*-acetyl-betulin (**13b**) was prepared according to literature [43,44]. The former compound was reacted with sulfamoyl chloride to yield 95% of sulfamate

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Scheme 1. Reactions and conditions: a) Ac$_2$O, NEt$_3$, DMAP (cat.), 20 °C, 12 h, 96%; b) (COCl)$_2$, DMF (cat.), DCM, 20 °C, 30 min, then taurinamide, NEt$_3$, DCM, 20 °C, 12 h → 3 (69%); c) (COCl)$_2$, DMF (cat.), DCM, 20 °C, 30 min, then **10**, NEt$_3$, DCM, microwave-assisted, 120 °C, 2 h → 4 (32%); d) (COCl)$_2$, DMF (cat.), DCM, 20 °C, 30 min, then H$_2$N–(CH$_2$)$_2$–O–(CH$_2$)$_2$–OH, NEt$_3$, DCM, 12 h, 20 °C, 96%; e) pyridine, DMAP (cat.), succinic anhydride, Δ, 3.5 h, 84%; f) (COCl)$_2$, DMF (cat.), DCM, 20 °C, 30 min, then taurinamide, NEt$_3$, DCM, 20 °C, 12 h → 7 (68%); g) (COCl)$_2$, DMF (cat.), DCM, 20 °C, 30 min, then **10**, NEt$_3$, DCM, 20 °C, 12 h → 8 (80%); h) conc. aq. HCl, Δ, 3 h, 96%; i) DMA, sulfamoyl chloride, 20 °C, 24 h, 83%.

14. Interestingly, while the reaction of **13a** with sulfamoyl chloride in dry DMA exclusively furnished **14**, from the reaction of **13a** with sodium hydride/sulfamoyl chloride a mixture of 28-sulfamate **14** and 3-sulfamate **15** was obtained. The formation of **15** can be explained by in intramolecular migration of the acetyl group from position C-3 to C-28 followed by sulfamoylation. The structure of **15** was confirmed beyond doubt by MS and especially NMR studies. In addition, the MS spectra showed a quasi-molecule ion $[M - H]^-$ $m/z = 562.2$, and an independent synthesis starting from **13b** led to the same product.

Reaction of **13a** with succinic anhydride yielded 81% of spacered **16** whose reaction with taurinamide or **10** - as described above - yielded conjugates **17** and **18**, respectively.

To test the influence of the triterpenoid backbone on the biological activity, glycyrrhetinic acid (**GA**, **19**, Scheme 3), ursolic acid (**UA**, **20**) and oleanolic acid (**OA**, **21**) were also included in the investigations.
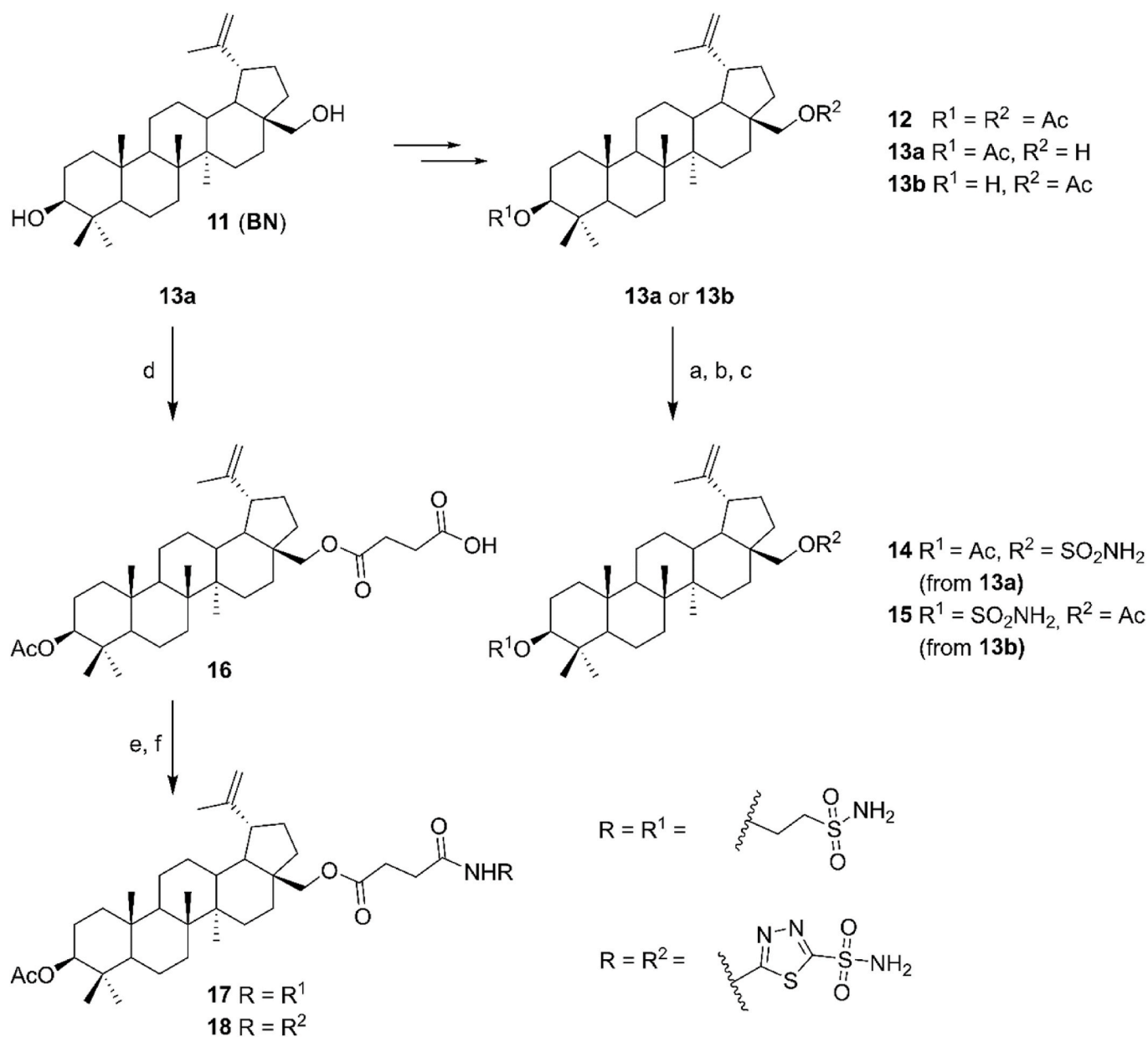
These pentacyclic triterpenoic acids were converted into acetates **22–24**; their reaction with succinic anhydride afforded **25–27** which

upon activation with oxalyl chloride and reaction with taurinamide yielded final compounds **28–30**, respectively.
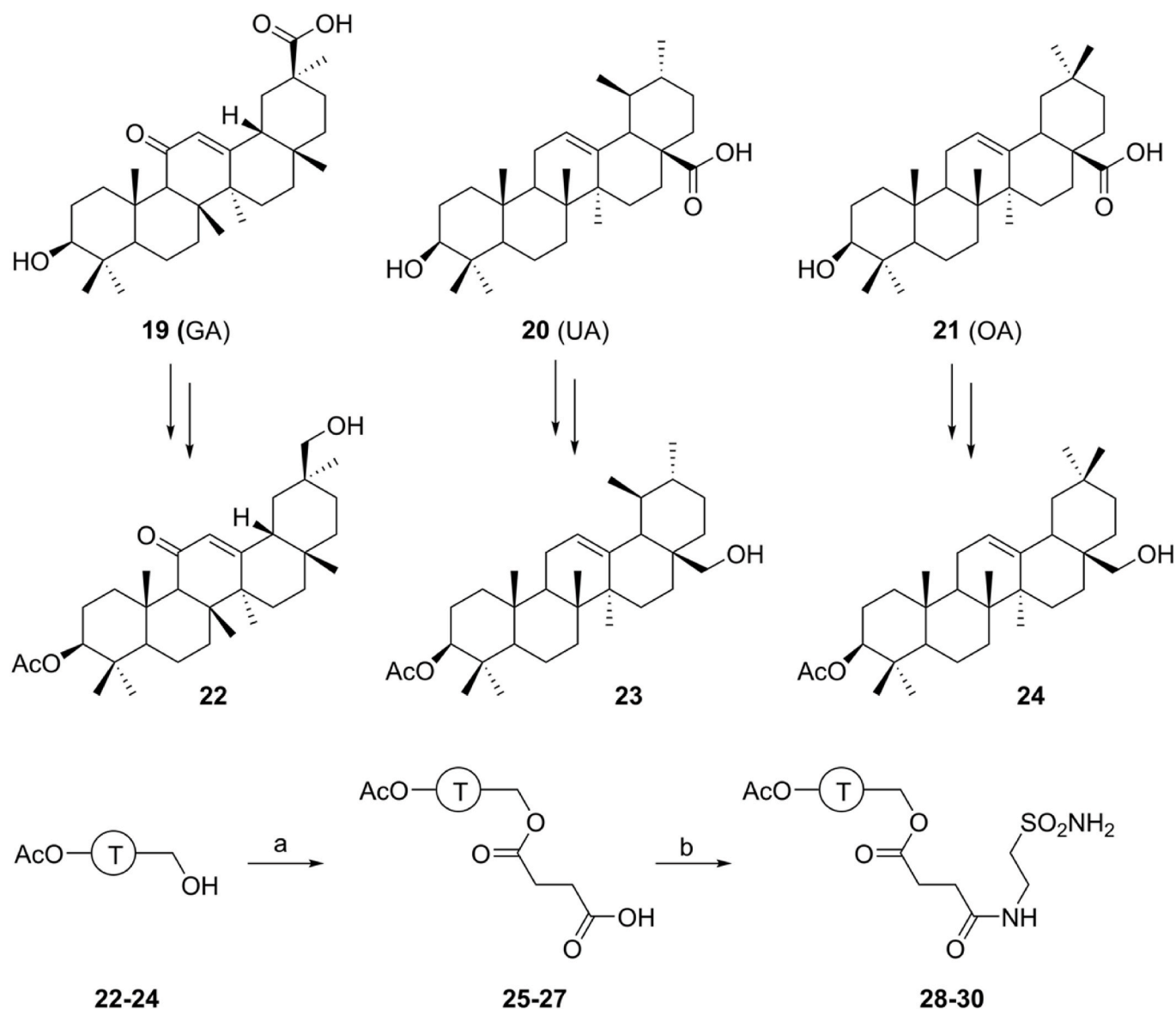
The products were subjected to enzymatic assays employing CA II; the results from these assays are compiled in Table 1.

Most compounds proved to be weak inhibitors for CA II except for **8** and **18** which were excellent inhibitors. Therefore, extra kinetic studies were performed for these compounds as well as for **9** and **14**. These compounds were shown to be competitive inhibitors, and their respective K_i values are compiled in Table 2. A Dixon plot for **8** is depicted in Fig. 1.

In this series of compounds, those with a lupane scaffold appear to be better inhibitors than those holding an ursane or an oleanane scaffold. Compounds with a longer spacer between the sulfamate group and the triterpene are better inhibitors than those with only a short-chained spacer. One reason for this could be that in compounds with a longer spacer, the triterpene does not have to penetrate the active site of the enzyme; from the structure of the active site no polar interactions with

Scheme 2. Reactions and conditions: a) DMA, sulfamoyl chloride, 24 h, 20 °C, 95% (from **13a**); b) **13a**, DCM, sulfamoyl chloride, 24 h, 20 °C, → 40% **14** and 44% **15**; c) DMA, sulfamoyl chloride, 24 h, 20 °C, 90% (from **13b**); d) according to lit. [29]; 80%; e) ethyl chloroformate, 4-methylmorpholine, THF, 20 °C 15 min, then taurinamide, Δ, 24h, → **17** (80%); f) ethyl chloroformate, 4-methylmorpholine, THF, 20 °C 15 min, then **10**, Δ, 24h, → **18** (85%).

Scheme 3. Reactions and conditions: a) according to lit. [29], b) THF, ethyl chloroformate, 4-methylmorpholine 20 °C, then taurinamide, reflux, 24 h, → **28** (68% from **25**), → **29** (81% from **26**), → **30** (70% from **27**).

Table 1

Inhibition in %) of *b*CA II by compounds **3,4, 7–9, 14, 15, 17, 18, 28–30** (at 1 μM concentration of the inhibitor); acetazolamide (**AAZ**) was used as a positive standard. All experiments were performed in triplicate with three technical replicas. Under the conditions of the assay, **AAZ** showed an inhibition of 98%.

Comp	Inhibition [%]	Comp	Inhibition [%]
3	<5	**15**	58.9 ± 3.9
4	22.5 ± 0.6	**17**	38.1 ± 0.1
7	11.1 ± 2.7	**18**	93.0 ± 0.1
8	98.4 ± 0.7	**28**	12.7 ± 0.4
9	<5	**29**	<5
14	76.4 ± 5.2	**30**	<5

Table 2

Detailed investigation of the most active inhibitors (from Table 1); K_i are given in μM.

Comp	K_i	Comp	K_i
8	1.27 ± 0.04	**15**	2.77 ± 0.03
14	1.40 ± 0.08	**18**	0.20 ± 0.03

3. Conclusion

Previous studies had shown the potential of modified pentacyclic triterpenes as possible inhibitors of carbonic anhydrase II. Betulinic, betulinic, glycyrrhetinic, ursolic and oleanolic acids appear to be of particular interest - also due to their good commercial availability. Therefore, in an extension of our previous studies, these triterpenes were converted to the corresponding acetates and coupled via a variable linker to either taurinamide or de-acetylated acetazolamide, the latter compound being a well-known and excellent inhibitor of carbonic anhydrases. As a result, in particular, derivatives **8** and **18**, derived from betulinic acid or betulin and provided with a long spacer, were found to

the triterpene can be expected anyway. Molecular modelling calculations support this assumption. A 2D representation of **8** and CA II is depicted in Fig. 2.

In a direct comparison between **17, 28, 29** and **30**, compound **17** holds unambiguously superior inhibitory properties as compared to the other compounds of this investigation.
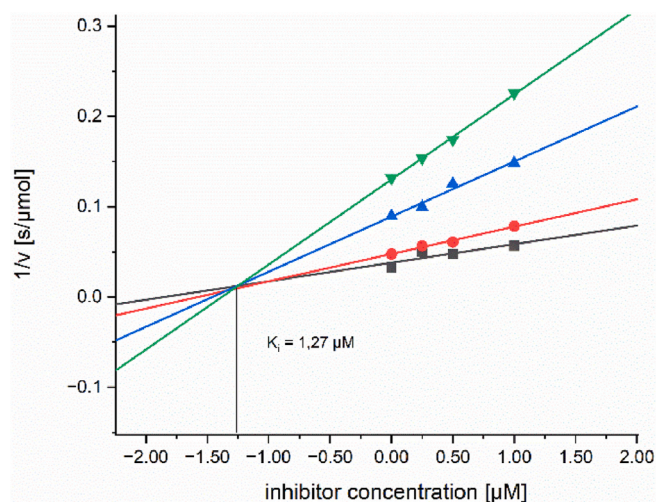
Fig. 1. Dixon plot for **8**; a K_i value of 1.27 μM was determined (concentration of the inhibitor: green: 0.75 μM, blue: 0.5 μM; red: 0.25 μM; black: 0.15 mM). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
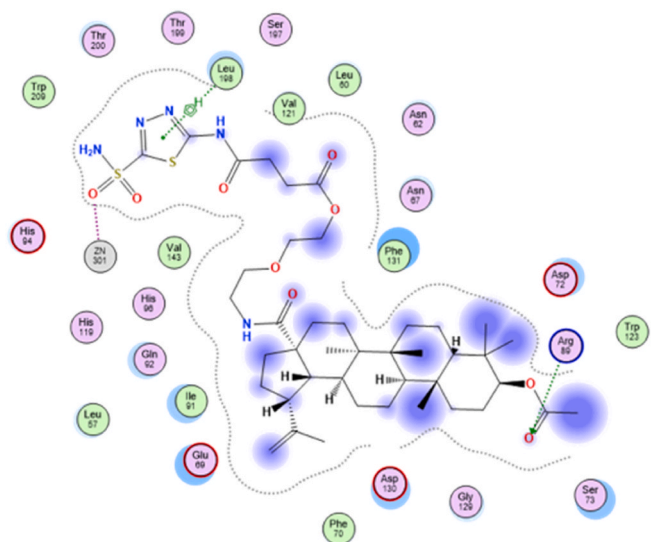

Fig. 2. Depiction of the interactions of CA II with compound **8**.

be strong competitive inhibitors of CA II holding K_i values of $K_i = 1.27 \pm 0.04$ μM (for **8**) and $K_i = 0.129 \pm 0.02$ μM (for **18**), respectively.

4. Experimental

4.1. General

Starting materials were obtained from local vendors; solvents were dried under usual conditions; equipment and assays were used as previously described [29,30,32]. Triterpenes were obtained from Betulinines (Stříbrná Skalice, CZ) and used as received.

4.2. Syntheses

4.2.1. (3β) Acetyloxy-lup-20(29)-en-28-oic acid (2)

Acetylation of betulinic acid (**1**, 10.0 g, 21.8 mmol) with acetic anhydride (250 mL, 3.25 mol) was performed [45] as previously described; recrystallization from ethanol gave **2** (8.4 g, 96%) as a colourless solid; $R_f = 0.65$ (hexanes/ethyl acetate; 3:1); m.p. 277–278 °C [lit. [45]:

277–278 °C]; $[\alpha]_D^{20} = +18.9°$ ($c = 0.013$, CHCl$_3$) [lit. [45]: $[\alpha]_D^{20} = +22.0°$ ($c = 0.049$, CHCl$_3$)]; ESI-MS (MeOH): $m/z = 497.3$ ([M − H]$^-$), 995.2 ([2M − H]$^-$), 1017.5 ([2M-2H + Na]$^-$).

4.2.2. (3β) 28-{[2-(Aminosulfonyl)ethyl]amino}-28-oxolup-20(29)-en-3-yl acetate (3)

To a solution of **2** (300 mg, 0.60 mmol) in dry DCM (10 mL), oxalyl chloride (0.2 mL, 2.4 mmol) and cat. amounts of DMF were added, and the reaction mixture was stirred for 30 min at 20 °C followed by an evaporation of the volatiles. A solution of taurinamide (150 mg, 1.2 mmol) and triethylamine (0.3 mL, 2.0 mmol) in dry DCM (20 mL) was slowly added, and the mixture was stirred overnight at 20 °C. Usual aqueous work-up followed by column chromatography (SiO$_2$, chloroform MeOH, 95:5) gave **3** (250 mg, 69%) as a s colourless solid; $R_f = 0.45$ (hexanes/ethyl acetate; 7:3); m.p. 137–140 °C; $[\alpha]_D^{20} = +1.3°$ ($c = 0.083$, MeOH); IR (ATR): $\nu = 2941$ m, 2869w, 1733 m, 1633 m, 1520 m, 1449w, 1375 m, 1327 m, 1245s, 1190w, 1136s, 1024 m, 979 m, 880w, 796w, 753 m, 569w, 489 m cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): $\delta = 6.41$ (t, $J = 6.1$ Hz, 1H, NH), 5.19 (s, 2H, NH$_2$), 4.72 (d, $J = 1.5$ Hz, 1H, 29-H$_b$), 4.59 (d, $J = 1.5$ Hz, 1H, 29-H$_a$), 4.50–4.42 (m, 1H, 3-H), 3.84–3.70 (m, 2H, 33-H), 3.37–3.21 (m, 2H, 34-H), 3.06 (dt, $J = 11.3$, 5.8 Hz, 1H, 19-H), 2.40 (dt, $J = 12.4$, 3.6 Hz, 1H, 13-H), 2.03 (s, 3H, 32-H), 1.99–1.85 (m, 2H, 7-H$_a$, 16-H$_a$), 1.82–1.73 (m, 1H, 22-H$_b$), 1.67 (s, 3H, 30-H), 1.73–1.59 (m, 2H, 1-H$_a$, 12-H$_a$), 1.61–1.52 (m, 3H, 2, 18-H), 1.52–1.29 (m, 8H, 6-H, 7-H$_b$, 11-H$_a$, 15-H$_a$, 16-H$_b$, 21-H$_a$, 22-H$_a$), 1.29–1.19 (m, 3H, 9, 11-H$_b$, 21-H$_b$), 1.14 (dt, $J = 13.7$, 3.2 Hz, 1H, 15-H$_b$), 1.02–0.96 (m, 2H, 1-H$_b$, 12-H$_b$), 0.95 (s, 3H, 27-H), 0.92 (s, 3H, 26-H), 0.83 (s, 3H, 24-H), 0.83 (s, 3H, 23-H), 0.82 (s, 3H, 25-H), 0.80–0.74 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, CDCl$_3$): $\delta = 177.3$ (C-28), 171.2 (C-31), 150.8 (C-20), 109.7 (C-29), 81.1 (C-3), 55.9 (C-17), 55.6 (C-5), 55.0 (C-34), 50.7 (C-9), 50.2 (C-18), 46.9 (C-19), 42.6 (C-14), 40.9 (C-8), 38.5 (C-1), 38.3 (C-22), 37.9 (C-10), 37.9 (C-13), 37.3 (C-4), 34.5 (C-33), 33.6 (C-7), 30.9 (C-16), 29.8 (C-21), 29.5 (C-15), 28.1 (C-23), 25.7 (C-12), 23.8 (C-2), 21.4 (C-32), 21.1 (C-11), 19.5 (C-30), 18.3 (C-6), 16.6 (C-25), 16.3 (C-26), 16.3 (C-24), 14.7 (C-27) ppm; MS (ESI, MeOH/CHCl$_3$, 4:1): $m/z = 605.2$ ([M+H]$^+$); analysis calcd for C$_{34}$H$_{56}$N$_2$SO$_5$ (604.88): C 67.51, H 9.33, N 4.63; found: C 67.39, H 9.55, N 4.42.

4.2.3. (3β) 28-{[5-(Aminosulfonyl)1,3,4-thiadiazol-2-yl]amino}-28-oxolup-20(29)-en-3-yl acetate (4)

From the reaction of **2** (500 mg, 1.0 mmol) in dry DCM (20 mL), with oxalyl chloride (0.4 mL, 4.8 mmol) and cat. amounts of DMF as described above, followed by adding **10** (230 mg, 1.3 mmol) and triethylamine (0.3 mL, 2 mmol) in dry DCM (20 mL) and subsequent stirring in a (microwave apparatus, Monowave, Anton Paar GmbH) at 120 °C for 2h, followed by usual aqueous work-up and column chromatography (SiO$_2$, CHCl$_3$/MeOH, 95:5) **4** (208 mg, 32%) was obtained as a s colourless solid; m.p. 162–165 °C; $[\alpha]_D^{20} = +13.2°$ ($c = 0.082$, MeOH); $R_F = 0.35$ (SiO$_2$, CHCl$_3$/MeOH, 9:1); UV–Vis (MeOH): λ_{max} (log ε) = 262 nm (3.62); IR (ATR): $\nu = 3263$br, 2941br, 2671w, 1733w, 1710 m, 1693 m, 1643w, 1510s, 1467 m, 1455w, 1366s, 1316w, 1291w, 1265 m, 1245s, 1174s, 1153w, 1129 m, 1070 m, 1026 m, 979 m, 944w, 915w, 889 m, 796w, 749w, 700w, 661 m, 616s, 602s, 560w, 509 m, 489w, 454w cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): $\delta = 10.63$ (s, 1H, NH), 6.69 (s, 2H, NH$_2$), 4.77–4.74 (m, 1H, 29'-H$_a$), 4.64–4.61 (m, 1H, 29-H$_b$), 4.51–4.41 (m, 1H, 3-H), 3.01 (dt, $J = 10.9$, 4.6 Hz, 1H, 19-H), 2.54–2.44 (m, 1H, 13-H), 2.27 (d, $J = 13.9$ Hz, 1H, 7-H$_a$), 2.04 (s, 3H, 32-H), 1.84–1.56 (m, 9H, 16-H$_a$, 12-H$_a$, 7-H$_b$, 30-H, 1-H$_a$, 18-H, 2-H), 1.53–1.13 (m, 12H; 6-H, 11-H$_a$, 16-H$_b$, 22-H, 9-H, 11-H$_b$, 21-H, 15-H), 1.08–1.01 (m, 2H, 1-H$_b$, 12-H$_b$), 0.98 (s, 3H, 27-H), 0.88 (s, 3H, 26-H), 0.84 (s, 3H, 24-H), 0.83 (s, 3H, 23-H), 0.82 (s, 3H, 25-H), 0.81–0.75 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, CDCl$_3$): $\delta = 175.4$ (C-28), 171.2 (C-31), 164.2 (C-34), 163.0 (C-33), 149.9 (C-20), 110.3 (C-29), 81.1 (C-3), 57.1 (C-17), 55.6 (C-5), 50.7 (C-9), 50.1 (C-18), 46.4 (C-19), 42.6 (C-14), 40.9 (C-8), 38.6 (C-1), 37.9 (C-10), 37.7 (C-13), 37.3 (C-4), 34.4 (C-22), 32.5 (C-7), 30.5 (C-

16), 29.8 (C-21), 29.7 (C-15), 28.1 (C-23), 25.6 (C-12), 23.8 (C-2), 21.5 (C-32), 21.1 (C-11), 19.5 (C-30), 18.3 (C-6), 16.6 (C-25), 16.4 (C-26), 16.3 (C-24), 14.8 (C-27) ppm; MS (ESI, MeOH/CHCl$_3$, 4:1): $m/z = 659.5$ ([M − H]$^-$); analysis calcd for C$_{34}$H$_{52}$N$_4$O$_5$S$_2$ (660.93): C 61.79, H 7.93, N 8.48; found: C 61.50, H 9.17, N 8.23.

4.2.4. (3β) 28-{[2-(2-Hydroxyethoxy)ethyl]amino}-28-oxolup-20(29)-en-3-yl acetate (5)

As described above from the reaction of **2** (1.0 g, 2 mmol) in dry DCM (20 mL) with oxalyl chloride (1.0 mL, 5.0 mmol) and cat. amounts of DMF, followed by adding 2-(2-aminoethoxy)-ethan-1-ol (0.6 mL, 5.0 mmol) and triethylamine (0.6 mL, 4 mmol) in dry DCM (20 mL), stirring for 12 h at 20 °C and usual work-up and chromatography (SiO$_2$, CHCl$_3$/MeOH, 95:5) **5** (1.12 g mg, 96%) was obtained as a colourless solid; R$_f$ = 0.38 (DCM/MeOH, 10:0.1); m.p. 205–207 °C; [α]$_D^{20}$ = +13.0° (c = 0.170, MeOH); IR (ATR): ν = 3313w, 2932 m, 2866w, 1733s, 1639 m, 1545 m, 1467w, 1444w, 1368 m, 1248vs, 1130 m, 1070w, 1026 m, 976w, 898w, 878w, 656w, 542w cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 6.02 (t, J = 5.2 Hz, 1H, NH), 4.72 (d, J = 1.4 Hz, 1H, 29-H$_b$), 4.58 (d, J = 1.4 Hz, 1H, 29-H$_a$), 4.49–4.41 (m, 1H, 3-H), 3.73 (s, 2H, 36-H), 3.60–3.52 (m, 4H, 34-H, 35-H), 3.53–3.44 (m, 1H, 33-H$_b$), 3.44–3.34 (m, 1H, 33-H$_a$), 3.10 (dt, J = 11.0, 3.9 Hz, 1H, 19-H), 2.42 (dt, J = 12.8, 3.4 Hz, 1H, 13-H), 2.21 (s, 1H, OH), 2.02 (s, 3H), 2.00–1.86 (m, 2H, 16-H$_a$, 21-H$_b$), 1.78–1.67 (m, 3H, 1-H$_b$, 12-H$_b$, 22-H$_b$), 1.67 (s, 3H, 30-H), 1.64–1.52 (m, 4H, *2-H*, 18-H, 21-H$_a$), 1.51–1.44 (m, 2H, 6-H$_a$, 15-H$_a$), 1.44–1.28 (m, 6H, 6-H$_b$, *7-H*, 11-H$_a$, 16-H$_b$, 22-H$_a$), 1.26 (s, 1H, 9-H), 1.22 (dt, J = 12.2, 4.0 Hz, 1H, 11-H$_b$), 1.16–1.09 (m, 1H, 15-H$_b$), 1.05–0.97 (m, 2H, 1-H$_a$, 12-H$_a$), 0.95 (s, 3H, 27-H), 0.92 (s, 3H, 26-H), 0.84–0.82 (m, 6H, 23, 24-H), 0.81 (s, 3H, 25-H), 0.79–0.75 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, CDCl$_3$): δ = 176.5 (C-28), 171.1 (C-31), 151.0 (C-20), 109.5 (C-29), 81.1 (C-3), 72.3 (C-35), 70.3 (C-34), 61.9 (C-36), 55.9 (C-17), 55.6 (C-5), 50.7 (C-9), 50.2 (C-18), 47.0 (C-19), 42.6 (C-14), 40.9 (C-8), 39.1 (C-33), 38.5 (C-1), 38.5 (C-22), 37.9 (C-4), 37.9 (C-13), 37.3 (C-10), 34.5 (C-7), 33.8 (C-21), 31.0 (C-16), 29.5 (C-15), 28.1 (C-23), 25.7 (C-12), 23.8 (C-2), 21.4 (C-32), 21.1 (C-11), 19.6 (C-30), 18.3 (C-6), 16.6 (C-25), 16.3 (C-24, 26), 14.7 (C-2) ppm; ESI-MS (MeOH): = 584.5 ([M − H]$^-$); 620.5 ([M+Cl]$^-$); analysis calcd for C$_{36}$H$_{59}$NO$_5$ (585.86): C 73.80, H 10.15, N 2.39; found: C 73.62, H 10.39, N 2.17.

4.2.5. 4-[(2-2-{[(3β)-(Acetyloxy)-28-oxolup-20(29)en-28-yl]amino}ethoxy)ethoxy]-4-oxo-butanoic acid (6)

Compound **5** (500 mg, 0.85 mmol) was acylated in dry pyridine [DMAP (catalytic amounts), with succinic anhydride (430 mg, 4.3 mmol)] under reflux for 3.5 h; usual aqueous work-up followed by column chromatography (SiO$_2$, CHCl$_3$/MeOH, 95:5) gave **6** (490 mg, 84%) obtained as a colourless solid; R$_f$ = 0.48 (DCM/MeOH, 10:0.5); m. p. 78–82 °C; [α]$_D^{20}$ = +16.1° (c = 0.118, MeOH); IR (ATR): ν = 2942 m, 2869w, 1733s, 1636w, 520w, 1466w, 1374 m, 1245s, 1161 m, 1130 m, 1028 m, 979 m, 881w, 545w cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 6.06 (t, J = 5.5 Hz, 1H, NH), 4.72 (d, J = 1.6 Hz, 1H, 29-H$_b$), 4.58 (d, J = 1.4 Hz, 1H, 29-H$_a$), 4.49–4.43 (m, 1H, 3-H), 4.26–4.23 (m, 2H, 36-H), 3.66–3.62 (m, 2H, 35-H), 3.55–3.50 (m, 2H, 34-H), 3.49–3.44 (m, 1H, 33-H$_a$), 3.43–3.37 (m, 1H, 33-H$_b$), 3.09 (dt, J = 11.0, 4.0 Hz, 1H, 19-H), 2.70–2.62 (m, J = 3.8 Hz, 4H, 38-H, 39-H), 2.46–2.38 (m, 1H, 13-H), 2.03 (s, 3H, 32-H), 2.00–1.86 (m, 2H, 16-H$_a$, 21-H$_b$), 1.78–1.69 (m, 2H, 1-H$_b$, 12-H$_a$), 1.67 (s, 3H, 30-H), 1.65–1.52 (m, 4H, *2-H*, 18-H, 22-H$_a$), 1.52–1.44 (m, 3H, 6-H$_a$, 15-H$_a$, 21-H$_a$), 1.44–1.29 (m, 6H, 6-H$_b$, 7-H, 11-H$_a$, 16-H$_b$, 22-H$_b$), 1.26 (d, J = 7.7 Hz, 2H, 9, 11-H$_b$), 1.16–1.10 (m, 1H, 15-H$_b$), 1.00 (s, 2H, 1-H$_a$, 12-H$_b$), 0.95 (s, 3H, 27-H), 0.92 (s, 3H, 26-H), 0.83 (s, 3H, 25-H), 0.83 (s, 3H, 23-H), 0.82 (s, 3H, 24-H), 0.79–0.76 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, CDCl$_3$): δ = 176.8 (C-28), 176.4 (C-40), 172.2 (C-37), 171.2 (C-31), 151.0 (C-20), 109.6 (C-29), 81.1 (C-3), 70.1 (C-34), 69.0 (C-35), 63.9 (C-36), 55.9 (C-17), 55.6 (C-5), 50.7 (C-9), 50.2 (C-18), 46.9 (C-19), 42.6 (C-14), 40.9 (C-8), 39.1 (C-33), 38.5 (C-1), 38.5 (C-22), 37.9 (C-4), 37.9 (C-13), 37.3 (C-

10), 34.5 (C-7), 33.8 (C-21), 31.0 (C-16), 29.5 (C-15), 29.1 (C-39), 29.0 (C-38), 28.1 (C-23), 25.7 (C-12), 23.8 (C-2), 21.4 (C-32), 21.1 (C-11), 19.6 (C-30), 18.3 (C-6), 16.6 (C-25), 16.3 (C-26), 16.3 (C-24), 14.7 (C-27) ppm; ESI-MS (MeOH): m/z = 684.6 ([M − H]$^-$); analysis calcd for C$_{40}$H$_{63}$NO$_8$ (685.93): C 70.04, H 9.26, N 2.04; found: C 69.86, H 9.33, N 1.85.

4.2.6. 2-(2-{[(3β)-3-(Acetyloxy)-28-oxolup-20(29)en-28-ylamino}ethoxy)ethyl 4-{[2-(aminosulfonyl)ethyl]amino}-4-oxobutanoate (7)

From the reaction of **6** (250 mg, 0.36 mmol) (10 mL) with oxalyl chloride (0.1 mL, 1.2 mmol) and cat. amounts of DMF in dry DCM in dry DCM for 30 min, followed by the reaction with taurinamide (75 mg, 0.6 mmol) and triethylamine (0.1 mL, 0.7 mmol) in dry DCM (20 mL) at 21C for 12 h, usual aqueous work-up and column chromatography (SiO$_2$, CHCl$_3$/MeOH, 95:5) **7** (190 mg, 68%) was obtained as a colourless, viscous solid; R$_f$ = 0.20 (CHCl$_3$/MeOH, 9:1); m.p. 44–48 °C; [α]$_D^{20}$ = +6.3° (c = 0.131, MeOH); IR (ATR): ν = 3327w, 3208w, 2944 m, 2870w, 1722w, 1688s, 1658 m, 1532 m, 1450w, 1374m, 1330 m, 1264s, 1141s, 1040s, 979w, 901w, 751s, 601w, 501 s cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 6.84 (t, J = 5.8 Hz, 1H, NH$_a$), 6.22 (t, J = 5.5 Hz, 1H, NH$_b$), 5.62–5.56 (m, 2H, NH$_2$), 4.71 (s, 1H, 29-H$_b$), 4.58 (s, 1H, 29-H$_a$), 4.48–4.42 (m, 1H, 3-H), 4.24–4.19 (m, 2H, 36-H), 3.79–3.71 (m, 2H, 41-H), 3.71–3.62 (m, 2H, 35-H), 3.57–3.51 (m, 2H, 34-H), 3.48–3.37 (m, 2H, 33-H), 3.37–3.29 (m, 2H, 42), 3.07 (dt, J = 10.7, 10.2, 3.4 Hz, 1H, 19-H), 2.67 (t, J = 6.4 Hz, 2H, 38-H), 2.51 (t, J = 6.4 Hz, 2H, 39-H), 2.47–2.36 (m, 1H, 13-H), 2.03 (s, 3H, 32), 2.01–1.84 (m, 2H, 16-H$_a$, 21-H$_b$), 1.80–1.72 (m, 1H, 1-H$_b$), 1.70–1.65 (m, 2H, 12-H$_b$, 22-H$_b$), 1.67 (s, 3H, 30-H), 1.64–1.46 (m, 5H, *2-H*, 6-H$_a$, 18, 21-H$_a$), 1.45–1.29 (m, 8H, 6-H$_b$, 7-H, 11-H, 15-H$_a$, 16-H$_b$, 22-H$_a$), 1.29–1.18 (m, 1H, 9-H), 1.18–1.09 (m, 1H, 15-H$_b$), 1.06–0.96 (m, 2H, 1-H$_a$, 12-H$_a$), 0.95 (s, 3H, 27-H), 0.92 (s, 3H, 26-H), 0.84–0.82 (m, 6H, 23, 24-H), 0.82 (s, 3H, 25-H), 0.79–0.73 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, CDCl$_3$): δ = 176.9 (C-28), 173.3 (C-37), 172.7 (C-40), 171.2 (C-31), 151.0 (C-20), 109.6 (C-29), 81.1 (C-3), 70.2 (C-34), 69.0 (C-35), 64.0 (C-36), 55.6 (C-5), 54.8 (C-42), 54.2 (C-17), 50.6 (C-9), 50.2 (C-18), 47.0 (C-19), 42.6 (C-14), 40.9 (C-8), 39.1 (C-33), 38.5 (C-1), 38.5 (C-22), 37.9 (C-4), 37.9 (C-13), 37.3 (C-10), 35.0 (C-41), 34.5 (C-7), 33.7 (C-21), 31.0 (C-39), 30.7 (C-16), 29.5 (C-38), 29.4 (C-15), 28.1 (C-23), 25.7 (C-12), 23.8 (C-2), 21.4 (C-32), 21.1 (C-11), 19.5 (C-30), 18.3 (C-6), 16.6 (C-25), 16.3 (C-26), 14.7 (C-24), 14.7 (C-27) ppm; ESI-MS (MeOH/CHCl$_3$, 4:1): m/z = 814.7 ([M+Na]$^+$); analysis calcd for C$_{42}$H$_{69}$N$_3$O$_9$S (792.08): C 63.69, H 8.78, N 5.31; found: C 63.41, H 8.93, N 5.02.

4.2.7. 2-(2-{[(3β)-3-(Acetyloxy)-28-oxolup-20(29)en-28-yl]amino}ethoxy)ethyl 4-{[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]amino}-4-oxo-butanoate (8)

From the reaction of **6** (250 mg, 0.36 mmol) with oxalyl chloride (0.1 mL, 1.2 mmol) and cat. amounts of DMF as described above, followed by a reaction with **10** (100.0 mg, 0.5 mmol) and triethylamine (0.1 mL, 0.7 mmol) for 12 h at 20 °C and usual work-up and column chromatography (SiO$_2$, CHCl$_3$/MeOH, 95:5) **8** (246 mg, 80%) was obtained as a s colourless solid; R$_f$ = 0.10 (CHCl$_3$/MeOH, 95:5); m.p. 92–94 °C; [α]$_D^{20}$ = +9.5° (c = 0.082, MeOH); UV–Vis (MeOH): λ_{max} (log ε) = 264 nm (3.77); IR (ATR): ν = 2943 m, 2870w, 1731 m, 1713 m, 1638w, 1525 m, 1449w, 1365 m, 1248s, 1174s, 1132 m, 1028w, 979w, 913w, 754 m, 732w, 654w, 603 m, 509 m cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$): δ = 12.19 (s, 1H, NH$_b$), 6.75 (s, 2H, NH$_2$), 6.19 (t, J = 5.5 Hz, 1H, NH$_a$), 4.71 (s, 1H, 29-H$_b$), 4.58 (s, 1H, 29-H$_a$), 4.46 (dd, J = 10.3, 5.8 Hz, 1H, 3-H), 4.25 (dd, J = 3.3 Hz, 2H, 36-H), 3.67 (t, J = 4.3 Hz, 2H, 35-H), 3.55 (t, J = 5.1 Hz, 2H, 34-H), 3.42 (qq, J = 13.8, 5.2 Hz, 2H, 33-H), 3.06 (dt, J = 10.9, 4.3 Hz, 1H, 19-H), 2.97 (t, J = 6.0 Hz, 2H, 38-H), 2.81 (t, J = 6.4 Hz, 2H, 39-H), 2.40 (dt, J = 12.6, 3.2 Hz, 1H, 13-H), 2.03 (s, 3H, 32), 1.99 (dt, J = 12.3, 2.5 Hz, 1H, 21-H$_b$), 1.94–1.88 (m, 1H, 16-H$_a$), 1.80–1.73 (m, 1H, 1-H$_b$), 1.71–1.62 (m, 2H, 12-H$_b$, 22-H$_b$), 1.67 (s, 3H, 30-H), 1.63–1.52 (m, 4H, *2-H*, 18, 21-H$_a$), 1.52–1.28 (m, 8H, *6-H, 7-H,*

11-H$_a$, 15-H$_b$, 16-H$_b$, 22-H$_a$), 1.28–1.23 (m, 1H, 9-H), 1.21 (*dt*, *J* = 12.4, 3.9 Hz, 1H, 11-H$_b$), 1.13 (d, *J* = 13.2 Hz, 1H, 15-H$_a$), 1.04–0.96 (m, 2H, 1-H$_a$, 12-H$_a$), 0.95 (s, 3H, 27-H), 0.91 (s, 3H, 26-H), 0.84–0.81 (m, 6H, 23, 24-H), 0.81 (s, 3H, 25-H), 0.77 (d, *J* = 9.7 Hz, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, CDCl$_3$): δ = 177.1 (C-28), 172.7 (C-31), 171.2, 170.9 (C-40), 164.6 (C-42), 162.7 (C-41), 150.9 (C-20), 109.7 (C-29), 81.1 (C-3), 70.2 (C-34), 68.9 (C-35), 64.3 (C-36), 55.9 (C-17), 55.6 (C-5), 50.6 (C-9), 50.2 (C-18), 47.0 (C-19), 42.6 (C-8), 40.9 (C-14), 39.2 (C-33), 38.5 (C-1, C-22), 38.0 (C-13), 37.9 (C-4), 37.3 (C-10), 34.5 (C-7), 33.8 (C-21), 31.0 (C-38), 30.9 (C-16), 29.5 (C-15), 28.9 (C-39), 28.1 (C-23), 25.7 (C-12), 23.8 (C-2), 21.5 (C-32), 21.1 (C-11), 19.5 (C-30), 18.3 (C-6), 16.6 (C-25), 16.3 (C-26), 16.3 (C-24), 14.8 (C-27)) ppm; ESI-MS (MeOH/CHCl$_3$, 4:1): *m/z* = 846.9 ([M − H]⁻); analysis calcd for C$_{42}$H$_{65}$N$_5$O$_9$S$_2$ (848.12): C 59.48, H 7.73, N 8.26; found: C 59.26, H 8.01, N 7.96.

4.2.8. (3β)-28-[(2-{2-[(Aminosulfonyl)oxy]ethoxy}ethyl)amino]-28-oxolup-20(29)-en-3-yl acetate (9)

From the reaction of **5** (120 mg, 0.2 mmol) with sulfamoyl chloride (2 equ.) in dry DMA (2 mL) at 21 °C for 1 day followed by usual aqueous work-up and column chromatography (SiO$_2$, CHCl$_3$/MeOH, 95:5) **9** (113 mg, 83%) was obtained as a colourless solid; R$_f$ = 0.23 (SiO$_2$, CHCl$_3$/MeOH, 95:5); m.p. 114–117 °C; IR (ATR): ν = 3330*br*, 3259*br*, 3072*w*, 2942 *m*, 2869*w*, 1733*w*, 1713 *m*, 1638 *m*, 1518 *m*, 1465*w*, 1451 *m*, 1368*s*, 1316*w*, 1246*s*, 1181*s*, 1130 *m*, 1025*s*, 979 *m*, 923*s*, 885*w*, 775 *m*, 656*w*, 598*w*, 551*s*, 513*w*, 488*w*, 466*w* cm⁻¹; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.58 (s, 1H, NH), 7.45 (s, 2H, NH$_2$), 4.68–4.64 (m, 1H, 29-H$_a$), 4.55–4.51 (m, 1H, 29-H$_b$), 4.39–4.33 (m, 1H, 3-H), 4.10 (s, 2H, 34-H), 3.62 (s, 2H, 35-H), 3.41 (s, 2H, 36-H), 3.29–3.09 (m, 2H, 33-H), 3.04–2.98 (m, 1H, 19-H), 2.58–2.52 (m, 1H, 13-H), 2.12 (d, *J* = 11.5 Hz, 1H, 16-H$_a$), 1.99 (s, 3H, 32-H), 1.79–1.65 (m, 2H, 21-H$_a$, 22-H$_a$), 1.65–1.55 (m, 6H, 2-H$_a$, 12-H$_a$, 1-H$_a$, 30-H), 1.53–1.20 (m, 13H, 2-H$_b$, 18-H, 6-H, 16-H$_b$, 11-H$_a$, 22-H$_b$, 15-H$_a$, 7-H, 9-H, 21-H$_b$, 11-H$_b$), 1.19–0.99 (m, 3H; 15-H$_b$, 1-H$_b$, 12-H$_b$), 0.92 (s, 3H, 27-H), 0.85 (s, 3H, 24-H), 0.80 (s, 3H, 26-H), 0.79 (s, 7H, 5-H, 23-H, 25-H) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 175.7 (C-28), 170.1 (C-31), 150.9 (C-20), 109.2 (C-29), 79.9 (C-3), 69.1 (C-36), 68.0 (C-34), 68.0 (C-35), 54.9 (C-17), 54.7 (C-5), 49.8 (C-9), 49.6 (C-18), 46.2 (C-19), 41.9 (C-14), 40.3 (C-8), 38.1 (C-33), 37.8 (C-1), 37.6 (C-22), 37.3 (C-4), 36.7 (C-13), 36.6 (C-10), 33.8 (C-7), 32.3 (C-16), 30.3 (C-21), 28.8 (C-15), 27.7 (C-23), 25.2 (C-12), 23.4 (C-2), 20.9 (C-32), 20.6 (C-11), 19.0 (C-30), 17.7 (C-6), 16.4 (C-25), 15.9 (C-26), 15.8 (C-24), 14.3 (C-27) ppm; ESI-MS (MeOH): *m/z* = 665.3 ([M+H]⁺), 687.9 ([M+Na]⁺; analysis calcd for C$_{36}$H$_{60}$N$_2$O$_7$S (664.94) C 65.03, H 9.10, N 4.21; found: C 64.79, H 4.39, N 3.97.

4.2.9. 5-Amino-1,3,4-thiadiazole-2-sulfonamide (10)

A solution of acetazolamide (10.0 g, 45.2 mmol) was heated under reflux in conc. HCl (60 mL) for 3 h [46]. After neutralization with aq. NaOH, saturation with NaCl and extraction with THF removal of the organic solvent gave **10** as a white solid (7.8 g, 96%); m.p. 197 °C decomp. (lit. [46]: 195 °C); R$_f$ = 0.32 (SiO$_2$, CHCl$_3$/MeOH, 9:1); UV–Vis (MeOH): λ$_{max}$ (log ε) = 278 nm (3.80) IR (ATR): ν = 3428*w*, 3321 *m*, 2639*w*, 1601*s*, 1490*s*, 1448 *m*, 1338*s*, 1172 *m*, 1139 *m*, 939 *m*, 647*s*, 581 s cm⁻¹; 1H NMR (500 MHz, DMSO-d_6): δ = 8.04 (s, 2H, NH$_2$), 7.84 (s, 2H, NH$_2$) ppm; ^{13}C NMR (126 MHz, DMSO-d_6): δ = 171.7, 157.9 ppm; ESI-MS (MeOH): *m/z* = 179.0 ([M − H]⁻).

4.2.10. 3,28-Di-O-acetyl-betulin (12)

Compound **12** was prepared from betulin (**11**,10.0 g, 32 mmol) by acetylation with acetic anhydride as previously [47] described, and recrystallization from ethanol gave **12** (11.8 g, 93%) as a white solid; R$_f$ = 0.70 (SiO$_2$, hexanes/ethyl acetate, 8:2); m.p. 220–222 °C (lit. [47]: 216–218 °C); [α]$_D^{20}$ = +17.5° (*c* = 0.032, CHCl$_3$), [lit. [47]: [α]$_D^{20}$ = +19.7° (CHCl$_3$)]; ESI-MS (MeOH): *m/z* = 467.5 ([M + H-HOAc]⁺).

4.2.11. 3-O-acetyl-betulin (13a)

Compound **13a** was prepared from **12** by selective deacetylation with cat. amounts of CaH$_2$ as previously [47] described; R$_f$ = 0.40 (SiO$_2$, hexanes/ethyl acetate, 8:2); m.p. 258 °C (lit. [47]: 258–260 °C); [α]$_D^{20}$ = +26.2° (*c* = 0.013, CHCl$_3$), [lit. [47]: [α]$_D^{20}$ = +25.7° (CHCl$_3$)]; ESI-MS (MeOH): *m/z* = 992.0 ([2 M + Na]⁺).

4.2.12. 28-O-acetyl-betulin (13b)

Compound **13b** was prepared from **11** as previously [43,44] reported; R$_f$ = 0.25 (SiO$_2$, hexanes/ethyl acetate, 9:1); m.p. 206–208 °C (lit. [43]: 205–208 °C); [α]$_D^{20}$ = +8.8° (*c* = 1.1, CHCl$_3$), [lit.: [44][α]$_D^{20}$ = +8.5° (*c* = 1.58, CHCl$_3$)]; ESI-MS (MeOH): *m/z* = 992.3 ([2 M]⁺).

4.2.13. (3β)-28-[(Aminosulfonyl)oxy]lup-20(29)-en-3-yl acetate (14)

Method A: To a solution of **13a** (200 mg, 0.41 mmol) in dry DMA (2.0 mL) at 0 °C, sulfamoyl chloride (2 eq.) was added, and the reaction mixture was stirred at 20 °C for 1 day. Usual aqueous work up followed by column chromatography (SiO$_2$, CHCl$_3$/MeOH, 95:5) gave **14** (220 mg, 95%) as a colourless solid; m.p. 130–134 °C; [α]$_D^{20}$ = +10.4° (*c* = 0.163, MeOH); R$_f$ = 0.35 (SiO$_2$, hexanes/ethyl acetate, 8:2); IR (ATR): ν = 2944 *m*, 2872*w*, 1711 *m*, 1456*w*, 1367*s*, 1265*s*, 1255*s*, 1181*s*, 1028*w*, 972*s*, 917 *m*, 902 *m*, 574*s*, 606*w*, 549 *m*, 500*w* cm⁻¹; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 4.83 (s, 2H, NH$_2$), 4.71–4.66 (m, 1H, 29-H$_a$), 4.63–4.57 (m, 1H, 29-H$_b$), 4.46 (*dd*, *J* = 10.4, 5.6 Hz, 1H, 3-H), 4.39 (d, *J* = 8.2 Hz, 1H, 28-H$_a$), 3.94 (d, *J* = 9.4 Hz, 1H, 28-H$_b$), 2.39 (*dt*, *J* = 10.7, 5.9 Hz, 1H, 19-H), 2.04 (s, 3H, 32), 2.01–1.85 (m, 3H, 16-H$_a$, 21-H$_a$, 22-H$_a$), 1.74 (*dt*, *J* = 13.7, 4.2 Hz, 1H, 15-H$_b$), 1.68 (s, 3H, 30-H), 1.67–1.58 (m, 5H, 1-H$_b$, 2-H, 12-H$_b$, 13-H, 18-H), 1.55–1.35 (m, 6H, 6-H$_a$, 7-H, 11-H$_a$, 21-H$_b$), 1.33–1.24 (m, 3H, 9-H, 16-H$_b$), 1.20 (*dd*, *J* = 12.6, 4.3 Hz, 1H, 11-H$_b$), 1.06 (s, 4H, 1-H$_a$, 12-H$_a$, 15-H$_a$, 22-H$_b$), 1.03 (s, 3H, 24-H), 0.97 (s, 3H, 27-H), 0.85–0.84 (m, 6H, 23, 25-H), 0.83 (s, 3H, 26-H), 0.79 (d, *J* = 9.5 Hz, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, CDCl$_3$): δ = 171.3 (C-31), 149.8 (C-20), 110.3 (C-29), 81.1 (C-3), 70.5 (C-28), 55.5 (C-5), 50.4 (C-9), 48.9 (C-18), 47.9 (C-19), 46.8 (C-17), 42.8 (C-14), 41.0 (C-8), 38.5 (C-1), 37.9 (C-13), 37.2 (C-4, C-10), 34.3, 34.2 (C-7, C-22), 29.5 (C-16), 29.3 (C-21), 28.1 (C-23), 27.0 (C-15), 25.2 (C-12), 23.8 (C-2), 21.5 (C-32), 20.9 (C-11), 19.2 (C-30), 18.3 (C-6), 16.6 (C-26), 16.3 (C-25), 16.1 (C-24), 14.9 (C-27) ppm; ESI-MS (MeOH/CHCl$_3$, 4:1): *m/z* = 562.3 ([M − H]⁻); analysis calcd for C$_{32}$H$_{53}$NO$_5$S (563.83): C 68.17, H 9.48, N 2.48; found: C 67.83, H 9.63, 2.03.

Method B: To a solution of **13a** (200 mg, 0.41 mmol) in dry DCM (20 mL), sodium hydride (4 eq.) and sulfamoyl chloride (2 eq.) were added at 0 °C, and the reaction mixture was stirred at 20 °C for 1 day. Usual aqueous work up followed by column chromatography (SiO$_2$, CHCl$_3$/MeOH, 95:5) gave **14** (90 mg, 40%) as a colourless solid; data as above.

4.2.14. (3β)-3-[(Aminosulfonyl)oxy]lup-20(29)-en-28-yl acetate (15)

Compound **13b** (200 mg, 0.41 mmol) was dissolved in dry DCM (20 mL), and at 0 °C, sodium hydride (4 eq.) and sulfamoyl chloride (2 eq.) were added; the reaction mixture was stirred at 20 °C for 1 day. Usual aqueous work up followed by column chromatography (SiO$_2$, CHCl$_3$/MeOH, 95:5) gave **15** (100 mg, 44%) as a colourless solid; m.p. 110–112 °C; [α]$_D^{20}$ = +10.5° (*c* = 0.111, MeOH); R$_f$ = 0.3 (SiO$_2$, hexanes/ethyl acetate, 8:2); IR (ATR): ν = 2944 *m*, 2871*w*, 1738 *m*, 1717 *m*, 1455*w*, 1364*s*, 1236*s*, 1180*s*, 1033 *m*, 933*s*, 909*vs*, 883*s*, 838 *m*, 753 *m*, 586*w*, 548 *m*, 513*w* cm⁻¹; ^{1}H NMR (500 MHz, CDCl$_3$): δ = 4.81 (s, 2H, NH$_2$), 4.68 (s, 1H, 29-H$_a$), 4.59 (s, 1H, 29-H$_b$), 4.24 (d, *J* = 11.2 Hz, 1H, 28-H$_a$), 4.21 (*dd*, *J* = 12.1, 4.7 Hz, 1H, 3-H), 3.84 (d, *J* = 11.0 Hz, 1H, 28-H$_b$), 2.44 (*dt*, *J* = 11.1, 5.7 Hz, 1H, 19-H), 2.06 (s, 3H, 32), 2.03 (*dd*, *J* = 13.8, 3.9 Hz, 1H, 2-H$_b$), 1.99–1.93 (m, 1H, 21-H$_b$), 1.88–1.81 (m, 2H, 2-H$_a$, 16-H$_b$), 1.80–1.76 (m, 1H, 22-H$_a$), 1.75–1.72 (m, 1H, 1-H$_a$), 1.73–1.62 (m, 3H, 12-H$_b$, 13, 15-H$_b$), 1.68 (s, 4H, 30-H), 1.60 (d, *J* = 11.7 Hz, 1H, 18-H), 1.55–1.49 (m, 1H, 6-H$_a$), 1.46–1.35 (m, 5H, 6-H$_b$, 7-H, 11-H$_a$, 21-H$_a$), 1.29–1.17 (m, 3H, 9-H, 11-H$_b$, 16-H$_a$), 1.11–1.04 (m,

2H, 12-H$_a$, 15-H$_a$, 22-H$_b$), 1.03 (s, 3H, 26-H), 1.01 (s, 3H, 23-H), 1.00–0.93 (m, 1H, 1-H$_b$), 0.96 (s, 3H, 27-H), 0.85 (s, 3H, 24-H), 0.84 (s, 3H, 25-H), 0.77 (d, $J = 9.0$ Hz, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, CDCl$_3$): $\delta = 171.8$ (C-31), 150.2 (C-20), 110.0 (C-29), 92.0 (C-3), 62.9 (C-28), 55.8 (C-5), 50.4 (C-9), 48.9 (C-18), 47.8 (C-19), 46.4 (C-17), 42.8 (C-8), 41.0 (C-14), 38.9 (C-1), 38.7 (C-4), 37.7 (C-13), 37.1 (C-10), 34.7 (C-7), 34.2 (C-22), 29.9 (C-16), 29.7 (C-21), 28.2 (C-23), 27.2 (C-12), 25.3 (C-15), 24.6 (C-2), 21.2 (C-32), 21.0 (C-11), 19.3 (C-30), 18.4 (C-6), 16.3 (C-25), 16.2 (C-26), 16.2 (C-24), 14.9 (C-27) ppm; ESI-MS (MeOH/CHCl$_3$, 4:1): $m/z = 562.2$ ([M − H]$^-$); analysis calcd for C$_{32}$H$_{53}$NO$_5$S (563.83): C 68.17, H 9.48, N 2.48; found: C 67.93, H 9.61, N 2.25.

4.2.15. *4-{[(3β)-3-(Acetyloxy)lup-20(29)-en-28-yl]oxy}-4-oxobutanoic acid (16)*

Compound **16** was prepared from **13** and succinic anhydride as previously [29] described; m.p. 123–124 °C [lit. [29]: 122–125 °C]; [α]$_D^{20}$ = +8.4° (*c* = 0.023, MeOH) [lit. [29]: [α]$_D^{20}$ = +12.1° (*c* = 0.198, MeOH)]; ESI-MS (MeOH): $m/z = 583.6$ ([M − H]$^-$).

4.2.16. *(3β)-3-(Acetyloxy)lup-20(29)-en-28-yl-4-2-(aminosulfonyl) ethylamino-4-oxo butanoate (17)*

To a solution of **16** (1.00 g, 1.7 mmol) in dry THF (50 mL), 4-methylmorpholine (2 eq.) and ethyl chloroformate (2 eq.) were added. The reaction mixture was stirred at 20 °C for 15 min. Taurinamide (1.2 eq.) was added, and the mixture was heated under reflux for 24 h. The solvent was removed, the residue dissolved in CHCl$_3$, washed with aq. NaOH (2 M), water and brine and dried (MgSO$_4$). Chromatography (hexanes/ethyl acetate, 3:7) gave **17** (950 mg, 80%) as a white solid; m. p. 123–126 °C; [α]$_D^{20}$ = +6.7° (*c* = 0.096, MeOH); R$_f$ = 0.28 (SiO$_2$, hexanes/ethyl acetate, 3:7); IR (ATR): ν = 3355*w*, 2943 *m*, 2872*w*, 1731*s*, 1660 *m*, 1544*w*, 1452*w*, 1391*w*, 1367 *m*, 1333 *m*, 1244*s*, 1191*w*, 1146*s*, 1107*w*, 1027 *m*, 979 *m*, 900*w*, 883 *m*, 609*w*, 547*w*, 498 m cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$): δ = 6.69 (s, 1H, NH), 5.79–4.90 (m, 2H, NH$_2$), 4.68 (s, 1H, 29-H$_a$), 4.58 (s, 1H, 29-H$_b$), 4.46 (*dd*, $J = 10.4$, 5.8 Hz, 1H, 3-H), 4.26 (d, $J = 10.8$ Hz, 1H, 28-H$_a$), 3.86 (d, $J = 10.7$ Hz, 1H, 28-H$_b$), 3.83–3.73 (m, 2H, 37-H), 3.42–3.24 (m, 2H, 38-H), 2.79–2.64 (m, 2H, 35-H), 2.59–2.46 (m, 2H, 34-H), 2.46–2.34 (m, 1H, 19-H), 2.03 (s, 3H, 32-H), 1.98–1.88 (m, 1H, 16-Ha), 1.85–1.78 (m, 1H, 16-Hb), 1.77–1.72 (m, 1H, 22-H$_a$), 1.67 (s, 3H, 30-H), 1.70–1.54 (m, 7H, 1-H$_a$, 2-H, 12-H$_a$, 13-H, 15-H$_a$, 18-H), 1.53–1.45 (m, 1H, 6-H$_a$), 1.44–1.34 (m, 5H, 6-H$_b$, 7-H, 11-H$_a$, 21-H$_a$), 1.32–1.13 (m, 3H, 9-H, 11-H$_b$, 21-H$_b$), 1.10–0.98 (m, 4H, 1-H$_b$, 12-H$_b$, 15-H$_b$, 22-H$_b$), 1.02 (s, 3H, 26-H), 0.96 (s, 3H, 27-H), 0.85–0.83 (m, 6H, 23-H, 24-H), 0.83 (s, 3H, 25-H), 0.78 (d, $J = 9.3$ Hz, 1H, 5-H) ppm; ^{13}C NMR (126 MHz, CDCl$_3$): δ = 173.8 (C-33), 172.8 (C-31), 171.2 (C-36), 150.1 (C-20), 110.1 (C-29), 81.1 (C-3), 63.5 (C-28), 55.5 (C-5), 54.5 (C-38), 50.4 (C-9), 48.9 (C-18), 47.8 (C-19), 46.6 (C-17), 42.8 (C-14), 41.0 (C-8), 38.5 (C-1), 37.9 (C-4), 37.7 (C-13), 37.2 (C-10), 35.1 (C-22), 34.7 (C-7), 34.3 (C-37), 31.1 (C-34), 29.9 (C-35), 29.7 (C-16), 29.6 (C-21), 28.1 (C-24), 27.2 (C-15), 25.3 (C-12), 23.8 (C-2), 21.4 (C-32), 20.9 (C-11), 19.2 (C-30), 18.3 (C-6), 16.6 (C-25), 16.3 (C-26), 16.2 (C-23), 14.9 (C-27) ppm; ESI-MS (MeOH): $m/z = 714.0$ ([M+Na]$^+$); analysis calcd for C$_{38}$H$_{62}$N$_2$O$_7$S (690.97): C 66.05, H 9.04, N 4.05; found: C 65.87, H 9.32, N 3.86.

4.2.17. *(3β)-3-(Acetyloxy)lup-20(29)-en-28-yl 4-{[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]amino}-4-oxobutanoate (18)*

Compound **18** was prepared as described for **17** starting from **10**; yield: 620 mg (85%); m.p. 162–164 °C [lit. [29]: 161–164 °C]; R$_f$ = 0.55 (SiO$_2$, hexanes/ethyl acetate, 7:3); ESI-MS (MeOH): $m/z = 745.7$ ([M − H]$^-$).

4.2.18. *(3β, 20β) 3-Acetyloxy-29-hydroxyolean-12-en-11-one (22)*

Compound **22** (2.26 g, 85%) was prepared as previously [29] reported from **19** (2.6 g, 5.52 mmol) and obtained as a colourless solid; m.

p. 265–267 °C (lit. [29]: 264–266 °C); R$_f$ = 0.45 (silica gel, CHCl$_3$/Et$_2$O/hexanes/HCOOH, 25:25:43:7); [α]$_D^{20}$ = +87.9° (*c* = 0.06, CHCl$_3$), [lit. [29]: [α]$_D^{20}$ = +91.6° (*c* = 0.129, CHCl$_3$)]; ESI-MS (MeOH/CHCl$_3$, 4:1): $m/z = 497.9$ ([M − H]$^-$).

4.2.19. *(3β)-28-Hydroxyursan-12-en-3-yl acetate (23)*

Compound **23** (3.58 g, 65%) was prepared as previously [48] reported from **20** (6.0 g, 11.4 mmol); m.p. 260–263 °C (lit. [48]: 258–261 °C); R$_f$ = 0.50 (silica gel, hexanes/ethyl acetate, 8:2); [α]$_D^{20}$ = +67.8° (*c* = 0.11, CHCl$_3$) [lit. [49]: [α]$_D^{20}$ = +70.5° (*c* = 0.145, CHCl$_3$)]; ESI-MS (MeOH): $m/z = 485.4$ ([M + H]$^+$).

4.2.20. *(3β)-28-Hydroxyolean-12-en-3-yl acetate (24)*

Compound **24** (3.75 g, 68%) was prepared as previously [50,51] reported from **21** (6.0 g, 11.4 mmol); m.p. 236–238 °C (lit. [50]: 233–234 °C); R$_f$ = 0.55 (silica gel, hexanes/ethyl acetate, 8:2); [α]$_D^{20}$ = +65.1° (*c* = 0.12, CHCl$_3$) [lit. [51]: [α]$_D^{20}$ = +71° (*c* = 0.70, CHCl$_3$)]; ESI-MS (MeOH): $m/z = 485.3$ ([M + H]$^+$).

4.2.21. *4-{[(3β,20β)3-(Acetyloxy)-11-oxoolean-12-en-30-yl]oxy}-4-oxobutanoic acid (25)*

Compound **25** (630 mg g, 96%) was prepared as previously [29] reported from **22** (450 mg, 0.55 mmol); m.p. 110–113 °C (lit. [29]: m.p. 109–111 °C); R$_f$ = 0.45 (silica gel, hexanes/ethyl acetate, 1:1); [α]$_D^{20}$ = +109.2° (*c* = 0.07, CHCl$_3$) (lit. [29]: [α]$_D^{20}$ = +110.5° (*c* = 0.118, CHCl$_3$)); MS (ESI, MeOH/CHCl$_3$, 4:1) $m/z = 600.0$ ([M + H]$^+$).

4.2.22. *4-{[(3β)-3-(Acetyloxy)urs-12-en-28-yl]oxy}-4-oxobutanoic acid (26)*

Compound **26** (0.84 g g, 78%) was prepared as previously [29] reported from **23** (0.9 g, 1.8 mmol); m.p. 110–113 °C (lit. [29]: m.p. 112–114 °C); R$_f$ = 0.50 (silica gel, hexanes/ethyl acetate, 7:3); [α]$_D^{20}$ = +45.7° (*c* = 0.11, CHCl$_3$) [lit. [29]: [α]$_D^{20}$ = +42.7° (*c* = 0.131, CHCl$_3$)]; MS (ESI, MeOH/CHCl$_3$, 4:1): $m/z = 583.9$ ([M − H]$^-$).

4.2.23. *4-{[(3β)-3-(Acetyloxy)-olean-12-en-28-yl]oxy}-4-oxobutanoic acid (27)*

Compound **27** (0.9 g, 84%) was prepared as previously [29] reported from **24** (0.9 g, 1.8 mmol); m.p. 124–127 °C (lit. [29]: 124–126 °C); R$_f$ = 0.45 (silica gel, hexanes/ethyl acetate, 7:3); [α]$_D^{20}$ = +45.2° (*c* = 0.04, CHCl$_3$) [lit. [29]: [α]$_D^{20}$ = +49.6° (*c* = 0.126, CHCl$_3$)]; MS (ESI, MeOH/CHCl$_3$, 4:1): $m/z = 583.9$ ([M − H]$^-$).

4.2.24. *(3β) 3-(Acetyloxy)-11-oxoolean-12-en-28-yl-4-(2-sulfamoylethyl)-4-oxobutanoate (28)*

To a solution of **25** (30 mg, 0.05 mmol) in dry THF (5 mL), 4-methylmorpholine (2 eq.) and ethyl chloroformate (2 eq.) were added. The reaction mixture was stirred at 20 °C for 15 min. Taurinamide (1,2 eq.) was added, and the mixture was heated under reflux for 24 h. The solvent was removed, the residue dissolved in CHCl$_3$, washed with aq. NaOH (2 M), water and brine and dried (MgSO$_4$). Chromatography (hexanes/ethyl acetate, 3:7) gave **28** (24 mg, 68%) as a white solid; m.p. 108–110 °C; [α]$_D^{20}$ = +47.1° (c 0.199, MeOH); R$_f$ = 0.29 (SiO$_2$, hexanes/ethyl acetate, 3:7); IR (ATR): ν = 3346*w*, 2929 *m*, 2874*w*, 1728*s*, 1657*s*, 1544*w*, 1456*w*, 1388*w*, 1366*w*, 1332 *m*, 1244*s*, 1207*w*, 1191*w*, 1144*s*, 1092*w*, 1028 *m*, 985 *m*, 902*w*, 752*w*, 562*w*, 496*w* cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 6.92–6.57 (m, 1H, NH), 5.60 (s, 1H, 12-H), 5.88–4.98 (m, 2H, NH$_2$), 4.50 (dt, $J = 11.4$, 5.5 Hz, 1H, 3-H), 4.34–4.21 (m, 1H, 30-H$_a$), 3.91–3.70 (*m*, 3 zH, 30-H$_b$, 37-H), 3.43–3.21 (m, 2H, 38-H), 2.81 (d, $J = 16.5$ Hz, 1H, 19-H$_a$), 2.71 (s, 2H, 35-H), 2.63–2.48 (m, 2H, 34-H), 2.48–2.40 (m, 1H, 1-H$_a$), 2.40–2.34 (m, 1H, 9-H), 2.24–2.12 (m, 1H, 18-H), 2.04 (s, 3H, 32-H), 1.92–1.00 (m, 16H, 15-H$_a$, 16-H$_a$, 15-H$_b$, 2-H, 6-H$_a$, 7-H$_a$, 6-H$_b$, 22-H$_a$, 7-H$_b$, 22-H$_b$, 16-H$_b$, 21-H, 1-H$_b$, 19-H$_b$), 1.36 (s,

3H, 27-H), 1.17 (s, 3H, 25-H), 1.14 (s, 3H, 26-H), 1.12 (s, 3H, 24-H), 1.02 (s, 3H, 29-H), 0.87 (m, 6H, 23-H, 28-H), 0.82–0.79 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, CDCl$_3$): δ = 209.86 (C-11), 173.30 (C-33), 173.22 (C-36), 172.51 (C-31), 170.99 (C-13), 128.03 (C-12), 80.53 (C-3), 74.47 (C-30), 63.46 (C-9), 54.99 (C-5), 54.36 (C-38), 46.74 (C-18), 44.27 (C-8), 43.41 (C-14), 38.84 (C-19), 38.21 (C-1), 37.84 (C-4), 36.99 (C-10), 36.74 (C-22), 35.91 (C-20), 34.44 (C-37), 32.66 (C-7), 32.22 (C-17), 31.02 (C-34), 30.72 (C-35), 29.40 (C-21), 28.53 (C-28), 28.02 (C-23), 26.40 (C-15), 25.77 (C-16), 24.01 (C-29), 23.54 (C-2), 23.31 (C-27), 21.29 (C-32), 19.47 (C-6), 18.78 (C-26), 18.74 (C-24), 16.66 (C-25) ppm; ESI-MS (MeOH): m/z = 727.3 ([M+Na]$^+$), 705.2 ([M+H]$^+$); analysis calcd for C$_{38}$H$_{60}$N$_2$O$_8$S (704.96): C 64.74, H 8.58, N 3.97; found: C 64.45, H 8.76, N 3.55.

4.2.25. *(3β) 3-(Acetyloxy)urs-12-en-28-yl-4-(2-sulfamoylethyl)-4-oxobutanoate* **(29)**

Reaction of **26** (100 mg, 0,17 mmol) in dry THF (10 mL) with 4-methylmorpholine (2 eq.), ethyl chloroformate (2 eq.) and taurinamide (1.2 eq.) under reflux for 24 h followed by chromatography (hexanes/ethyl acetate, 3:7) gave **29** (95 mg, 81%) as a white solid; m.p. 117–120 °C; $[\alpha]_D^{20}$ = +27.6° (c = 0.094, MeOH); R$_f$ = 0.34 (SiO$_2$, hexanes/ethyl acetate, 3:7); IR (ATR): ν = 3347w, 2947 m, 2925 m, 2670w, 1732 m, 1658 m, 1544w, 1456w, 1389w, 1369w, 1332 m, 1244s, 1176w, 1145s, 1095w, 1025 m, 1005 m, 985w, 902w, 753w, 664w, 607w, 496w cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$): δ = 6.73–6.50 (m, 1H, NH), 5.56–4.84 (m, 2H, NH$_2$), 5.17–5.11 (m, 1H, 12-H), 4.56–4.44 (m, 1H, 3-H), 4.07 (d, J = 10.8 Hz, 1H, 28-H$_a$), 3.82–3.69 (m, 2H, 37-H), 3.62 (d, J = 10.8 Hz, 1H, 28-H$_b$), 3.39–3.26 (m, 2H, 38-H), 2.76–2.60 (m, 2H, 35-H), 2.57–2.39 (m, 2H, 34-H), 2.04 (s, 3H, 32-H), 1.99–1.85 (m, 3H, 11-H, 16-H$_a$), 1.77–1.69 (m, 1H, 15-H$_a$), 1.69–1.59 (m, 3H, 1-H$_a$, 2-H), 1.59–1.50 (m, 4H, 6-H$_a$, 7-H$_a$, 9-H, 22-H$_a$), 1.49–1.37 (m, 4H, 6-H$_b$, 18-H, 19-H, 21-Ha), 1.37–1.27 (m, 3H, 7-H$_b$, 22-H$_b$), 1.25–1.16 (m, 2H, 16-H$_b$, 21-H$_b$), 1.09 (s, 3H, 27-H), 1.12–1.04 (m, 2H, 1-H$_b$, 15-H$_b$), 0.97 (s, 6H, 2-H 5, 30-H), 0.93 (s, 3H, 29-H), 0.87 (s, 3H, 23-H), 0.86 (s, 3H, 24-H), 0.85–0.83 (m, 1H, 5-H), 0.81 (s, 3H, 26-H) ppm; ^{13}C NMR (126 MHz, CDCl$_3$): δ = 173.6 (C-33), 172.8 (C-36), 171.2 (C-31), 138.3 (C-13), 125.7 (C-12), 81.1 (C-3), 71.9 (C-28), 55.4 (C-5), 54.5 (C-38), 54.4 (C-18), 47.7 (C-9), 42.1 (C-14), 40.1 (C-8), 39.5 (C-20), 39.3 (C-19), 38.6 (C-1), 37.8 (C-4), 37.1 (C-10), 36.9 (C-17), 35.8 (C-22), 35.1 (C-37), 32.8 (C-7), 31.2 (C-34), 30.6 (C-21), 29.5 (C-35), 28.2 (C-23), 26.2 (C-15), 23.7 (C-2), 23.5 (C-16), 23.5 (C-11), 23.5 (C-27), 21.4 (C-29), 21.4 (C-32), 18.3 (C-6), 17.4 (C-26), 16.9 (C-24, C-30), 15.9 (C-25) ppm; ESI-MS (MeOH): m/z = 714.2 (100%, [M+Na]$^+$); analysis calcd for C$_{38}$H$_{62}$N$_2$O$_7$S (690.98): C 66.05, H 9.04, N 4.05; found: C 65.83, H 9.29, N 3.76.

4.2.26. *(3β) 3-(Acetyloxy)olean-12-en-28-yl-4-(2-sulfamoylethyl)-4-oxobutanoate* **(30)**

Reaction of **27** (35 mg, 0,06 mmol) in dry THF (5 mL) with 4-methylmorpholine (2 eq.), ethyl chloroformate (2 eq.) and taurinamide (1.2 eq.) under reflux for 24 h as described above followed by chromatography (hexanes/ethyl acetate, 3:7) gave **30** (29 mg, 70%) as a white solid; m.p. 116–118 °C; $[\alpha]_D^{20}$ = +48.4° (c 0.124, MeOH); R$_f$ = 0.35 (SiO$_2$, hexanes/ethyl acetate, 3:7); IR (ATR): ν = 3355w, 2946 m, 2926 m, 2664w, 1732 m, 1660w, 1544w, 1463w, 1386w, 1364w, 1332 m, 1245s, 1199w, 1145s, 1095w, 1027 m, 1004 m, 986 m, 967w, 904w, 754w, 731w, 660w, 495w cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 6.58 (s, 1H, NH), 5.23–5.17 (m, 1H, 12-H), 5.71–4.74 (m, 2H, NH$_2$), 4.53–4.43 (m, 1H, 3-H), 4.04 (d, J = 10.8 Hz, 1H, 28-H$_a$), 3.88–3.74 (m, 2H, 37-H), 3.69 (d, J = 10.8 Hz, 1H, 28-H$_b$), 3.43–3.22 (m, 2H, 38-H), 2.80–2.63 (m, 2H, 35-H), 2.57–2.38 (m, 2H, 34-H), 2.04 (s, 3H, 32-H), 2.02–1.98 (m, 1H, 18-H), 1.97–1.81 (m, 3H, 11-H, 16-H$_a$), 1.78–1.67 (m, 1H, 19-H$_a$), 1.66–1.58 (m, 4H, 1-H$_a$, 2-H, 15-H$_a$), 1.57–1.40 (m, 4H, 6-H$_a$, 7-H$_a$, 9-H, 22-H$_a$), 1.39–1.22 (m, 4H, 6-H$_b$, 7-H$_b$, 22-H$_b$, 21-H$_a$), 1.20–1.11 (m, 2H, 16-H$_b$, 21-H$_b$), 1.15 (s, 3H, 27-H), 1.11–0.98 (m, 3H, 1-H$_b$, 15-H$_b$,

19-H$_b$), 0.95 (s, 3H, 25-H), 0.93 (s, 3H, 26-H), 0.89 (s, 3H, 29-H), 0.87 (s, 6H, 23-H, 30-H), 0.86 (s, 3H, 24-H), 0.84–0.79 (m, 1H, 5-H) ppm; ^{13}C NMR (101 MHz, CDCl$_3$): δ = 173.6 (C-33), 172.8 (C-31), 171.2 (C-36), 143.7 (C-13), 123.0 (C-12), 81.1 (C-3), 71.4 (C-28), 55.4 (C-5), 54.5 (C-38), 47.6 (C-9), 46.3 (C-19), 42.7 (C-18), 41.8 (C-14), 39.9 (C-8), 38.4 (C-1), 37.9 (C-4), 37.0 (C-10), 36.0 (C-17), 35.1 (C-37), 34.1 (C-21), 33.3 (C-29), 32.6 (C-7), 31.6 (C-22), 31.2 (C-20), 31.0 (C-34), 29.5 (C-35), 28.2 (C-23), 26.1 (C-27), 25.7 (C-15), 23.7 (C-30), 23.7 (C-2, C-11), 22.3 (C-16), 21.4 (C-32), 18.4 (C-6), 16.9 (C-26), 16.8 (C-24), 15.7 (C-25) ppm; ESI-MS (ESI, MeOH): m/z = 714.2 ([M+Na]$^+$); analysis calcd for C$_{38}$H$_{62}$N$_2$O$_7$S (690.98): C 66.05, H 9.04, N 4.05; found: C 65.71, H 9.33, N 3.72.

4.3. CA II assay

Carbonic anhydrase II (bCAII, ≥3000 W-A units/mg from bovine erythrocytes) as well as 4-nitrophenyl acetate (4-NA) were purchased from Sigma. BMG Labtech Spectrostar Omega working in the slow kinetics mode and measuring the absorbance at λ = 415 nm applying center scanning for the enzymatic studies. In short: A mixture of 4-NA solution (125 μL, 6 mM in 50 mM Tris-HCl buffer, pH 8), enzyme solution (25 μL, 0.3 mg/mL) and compounds solutions (25 μL, 3 different concentrations and water as a blank) was incubated at 37 °C for 20 min. The substrate (25 μL, [4-NA] = 0.75 mM, 0.50 mM, 0.25 mM, 0.15 mM) was added to start the enzymatic reaction. The absorbance data was recorded under a controlled temperature of 37 °C for 30 min at 1 min intervals at λ = 415 nm. The relative inhibition was determined as the quotient of the slopes (compound divided by blank) of the linear ranges.

CRediT authorship contribution statement

Toni-Christopher Denner: Writing – review & editing, Writing – original draft, Investigation. **Niels V. Heise:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Julian Zacharias:** Writing – review & editing, Writing – original draft, Investigation. **René Csuk:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Data curation, 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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmcr.2024.100139.

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