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Ursolic and oleanolic acid derivatives with cholinesterase inhibiting potential

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A B S T R A C T

Triterpenoids are in the focus of scientific interest, and they were evaluated for many pharmacological applications among them their ability to act as inhibitors of cholinesterases. These inhibitors are still of interest as drugs that improve the life quality of patients suffering from age-related dementia illnesses especially of Alzheimer's disease. Herein, we prepared several derivatives of ursolic and oleanolic acid and screened them in Ellman's assays for their ability to inhibit acetylcholinesterase and/or butyrylcholinesterase, and for each of the active compounds the type of inhibition was determined. As a result, several compounds were shown as good inhibitors for acetylcholinesterase and butyrylcholinesterase even in a micromolar range. An ursolic acid derived hydroxyl-propinyl derivative **10** was a competitive inhibitor for butyrylcholinesterase with an inhibition constant of K_i = 4.29 μM, and therefore being twice as active as gold standard galantamine hydrobromide. The best inhibitor for acetylcholinesterase, however, was 2-methyl-3-oxo-methyl-ursoloate (**18**), acting as a mixed-type inhibitor showing K_i = 1.72 μM and K_i' = 1.28 μM, respectively.

1. Introduction

Spending their last days on earth having already lost many cognitive functions remains a major fear of elderly people. Although during the last century their lifespans increased, the so-called "old-age diseases" became more and more important. Alzheimer's disease (AD) besides cancer, strokes, heart attacks and dementia diseases became the most important diseases of the 21st century; these diseases cause the highest death rates. Nowadays, one person in 200 suffers from any kind of dementia, and this number is expected to double within the next 30 years [1,2]. Therefore, the scientific and economic interest remains unbroken, and the research for a better understanding of dementia and possible treatments especially of AD have been in progress for decades.

The large number of patients suffering from AD makes this disease of special interest within the field of dementia diseases. Many theories regarding the causes of these diseases were postulated, resulting in some therapeutic concepts. One of the most often followed ideas is the β-amyloid hypothesis basing essentially on the neurotoxic effect of β-amyloid plaques inside the human brain having been formed by the action of α-, β- and γ-secretases [3,4]. However, about 30 percent of middle-aged people have AD-equal amounts of these plaques but without suffering from AD [5,6]. In addition, several therapies basing on this theory failed to increase the cognitive abilities [7–10] by decreasing the β-amyloid plaque's concentration. Other therapeutic targets refer, for example, to inflammatory processes or mitochondrial disorder of the τ-protein [11–13].

Another concept relies on the neurotransmitter acetylcholine (ACh) since its concentration seems reduced during AD; AD typical symptoms such as amnesia or behavioral disorders [14–17] have been credited to a lowered concentration of ACh. Usually, the cleavage of this neurotransmitter is performed by the hydrolase acetylcholinesterase (AChE, E.C. 3.1.1.7) but another enzyme, butyrylcholinesterase (BChE, E.C. 3.1.1.8), seems also important for controlling the concentration of ACh in different tissues of an organism including the brain. It was assumed that BChE is able to compensate a lack of AChE activity [18,19]. Furthermore, the AChE/BChE ratio in the brain alters from 0.2 in healthy brains to 11 during advanced AD [14,20,21]. Thus, both enzymes represent interesting targets for potential AD cures or – at least - as tools for a deeper insight into AD's pathology.

Triterpenes represent a group of pharmacologically active substances having already been tested for cholinergic activities [22–24]. Triterpenes holding hopyl [25], lanostyl [26] or lupyl [27] skeletons have been targets in recent studies. Also, pentacyclic triterpenoic acids and compounds derived thereof showed an inhibitory potential for AChE in micromolar range [28]. Especially several subgroups of α- and β-amyrins were most effective inhibitors for this cholinesterase.

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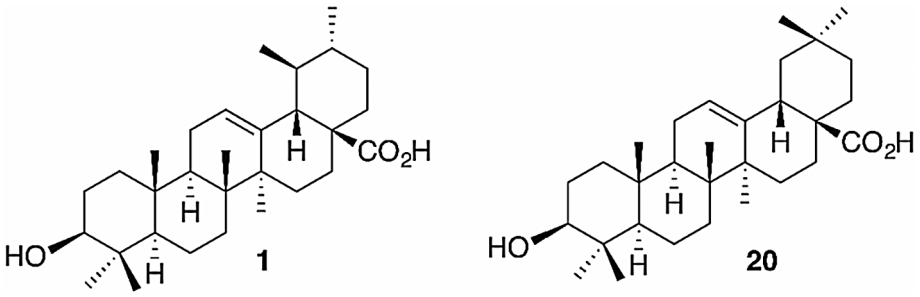


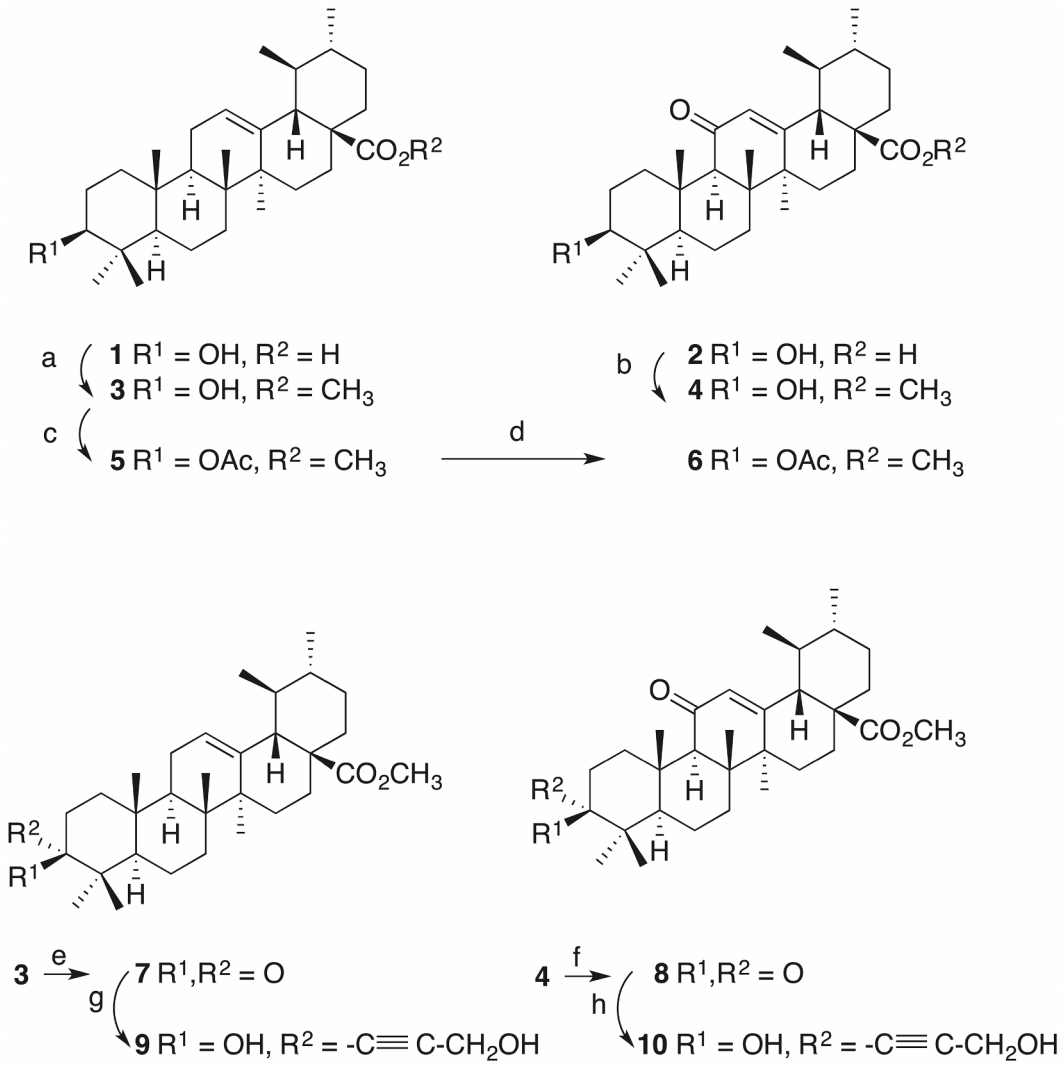
Fig. 1. Structure of ursolic acid (1) and oleanolic acid (20).

Furthermore, AChE inhibition has been found for derivatives of oleanolic acid [29–32], ursolic acid [33] as well as of glycyrrhetinic acid [34] or platanic acid derived compounds [35]. Therefore, we decided to synthesize and to test several ursolic and oleanolic acid (Fig. 1) derived compounds in Ellman's assays for their ability to act as inhibitors of AChE as well as of BChE and to obtain inhibitory constants (K_i and K_i').

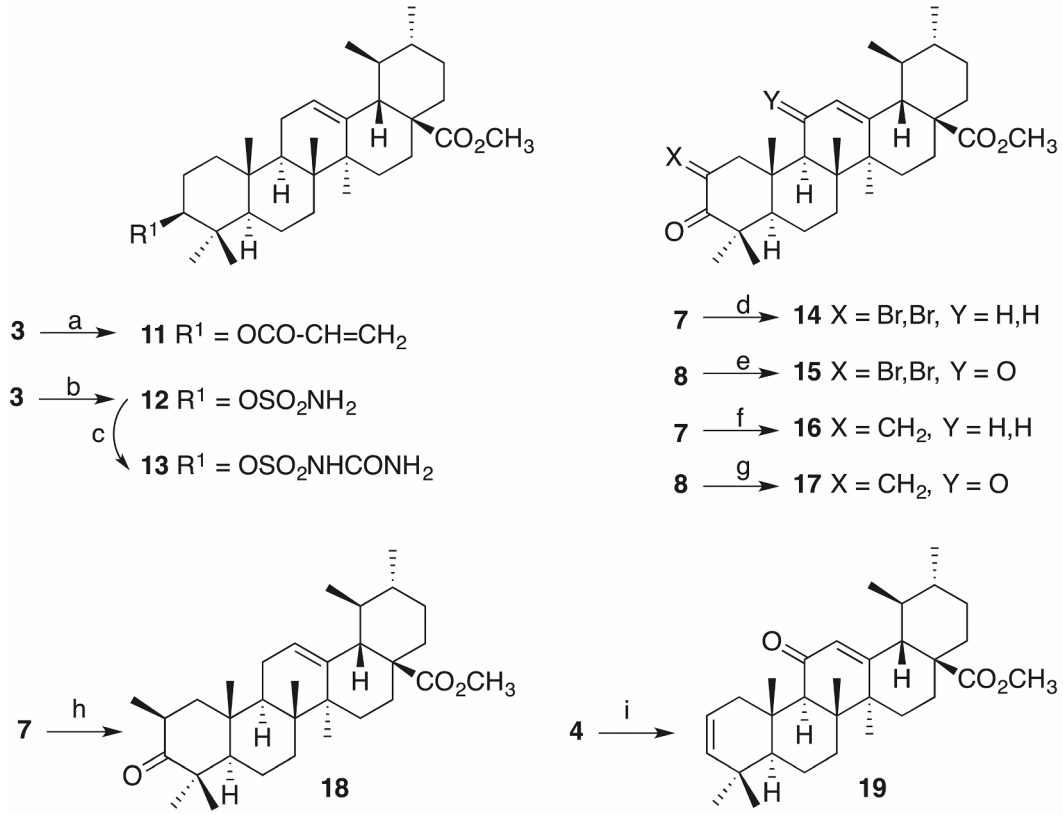
2. Results and discussion

2.1. Chemistry

Reaction of ursolic acid (**1**, Scheme 1) or of its 11-oxo analogue **2** [36] with methyl iodide in the presence of potassium carbonate gave esters **3** and **4** in good yields. These esters served as valuable starting



Scheme 1. Synthesis of ursolic acid (1) derived compounds 2–10: (a) K_2CO_3, MeI, DMF, 12 h, 25 °C, 78%; (b) K_2CO_3, MeI, DMF, 12 h, 25 °C, 82%; (c) Ac_2O, TEA, DCM, 12 h, 25 °C, 87%; (d) $Na_2Cr_2O_7$ AcOH, NHS, acetone, 2 d, 40 °C, 65%; (e) CrO_3, H_2SO_4, acetone, 1 h, 25 °C, 88%; (f) CrO_3, H_2SO_4, acetone, 1 h, 25 °C, 88%; (g) LDA, THF, −78 °C → 25 °C, 53%; h) LDA, THF, −78 °C → 25 °C, 63%.



Scheme 2. Synthesis of compounds 11–19: (a) TEA, acryloyl chloride, DMAP, $CHCl_3$, 30 min, 25 °C, 45%; (b) NaH, THF, 0 °C → 25 °C, 30 min then sulfamoyl chloride, THF, 5 d, 25 °C, 69%; (c) NaH, THF, 0 °C → 25 °C, 30 min then CDI, NH_3, THF, 3 h, 25 °C,95%; (d) Br_2, AcOH, 15 min, 25 °C, 80%; (e) Br_2, AcOH, 15 min, 25 °C, 93%; (f) K_2CO_3, paraformaldehyde, DMF, 1 h, 90 °C, 45%; (g) K_2CO_3, paraformaldehyde, DMF, 1 h, 90 °C, 44%; (h) LDA, MeI, THF, −78 °C → 25 °C, 71%; (i) 3,3-dimethylglutarimide, PPh_3, DEAD, THF, 12 h, 0 °C, 93%.

materials for the syntheses to follow. Acetylation of **3** or **4** with acetic anhydride and triethylamine in dry DCM furnished acetates **5** and **6**, respectively. Jones oxidation of **3** or **4** gave 3-oxo compounds **7** and **8**. Their reaction with lithium diisopropylamide in THF followed by adding 2-propyn-1-ol yielded 3-hydroxyprop-1-ynyl derivatives **9** and **10**, respectively. Compound **9** is characterized in its ^{13}C NMR spectrum by the presence of the alkynyl carbons detected at $\delta = 88.9$ and 83.7 ppm. The ^1H NMR chemical shift of 33-H was determined at $\delta = 4.26$ ppm.

The 3-*O*-acryloyl derivative **11** (Scheme 2) was obtained from the reaction of **3** with acryloyl chloride in the presence of triethylamine and catalytic amounts of DMAP. Reaction of **3** with sodium hydride and sulfamoyl chloride in THF gave **12** which was converted into the corresponding carbamoylsulfamate **13** using sodium hydride, 1,1′-carbonyldiimidazole and a saturated solution of ammonia. Products dibrominated at position C-2 were obtained from the 3-oxo esters **7** and **8**. Thus, **7** or **8** was allowed to react with bromine in glacial acetic acid, and compounds **14** and **15** were obtained.

Reaction of **7** and **8** with paraformaldehyde in DMF under basic conditions gave the 2-methylenated derivatives **16** and **17**, respectively. Compound **16** is characterized in its ^1H NMR spectrum by the presence of a methylene group resulting in signals at $\delta = 5.93$ ppm and 5.07 ppm (for **17**: $\delta_H = 5.95$ and 5.18 ppm, respectively). In the ^{13}C NMR spectrum signals at $\delta = 141.9$ and 123.6 ppm (for **17**: $\delta_C = 141.8$ and 124.1 ppm) were assigned to this group. Treatment of **7** with LDA and iodomethane in dry THF yielded compound **18**. Compound **19** holding a double bond in ring A was synthesized as previously reported [37].

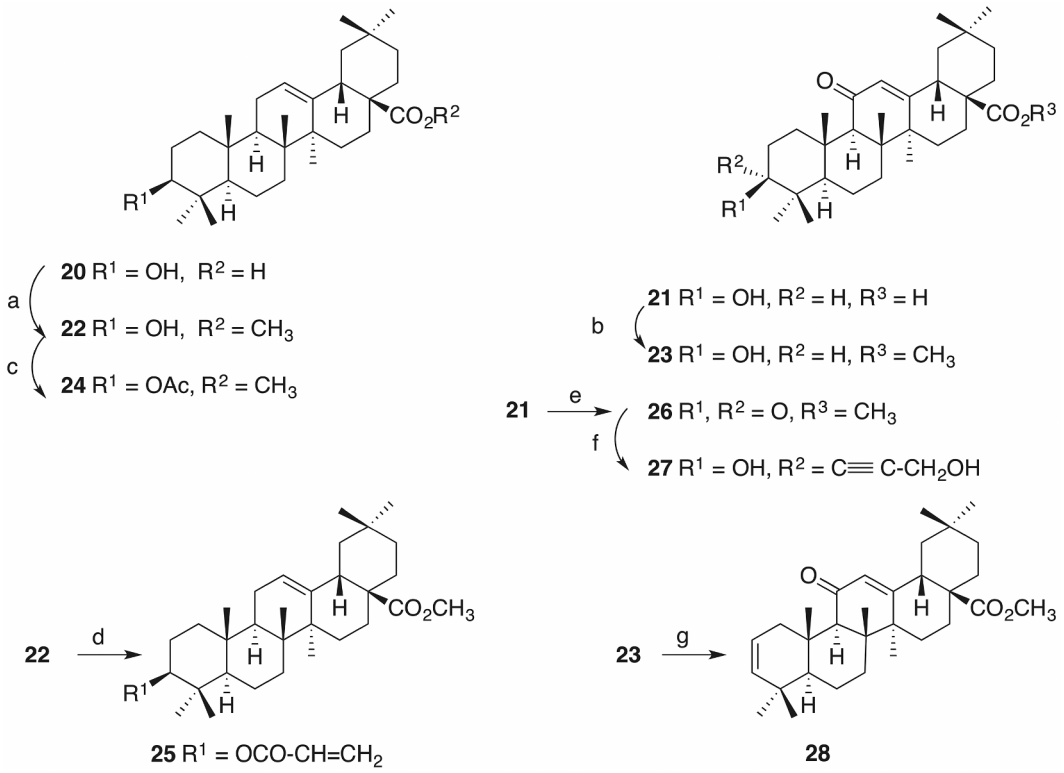
Following the procedures given for the synthesis of the derivatives of ursolic acid, starting from oleanolic acid (**20**) and 11-oxo-oleanolic acid **21** [38] the corresponding methyl esters **22** and **23** were prepared (Scheme 3). Acetylation of **22** afforded **24**; from the reaction of **22** with acryloyl chloride/trimethylamine/DMAP acryloylated **25** was obtained in 63% isolated yield. The olefinic protons of this compound were detected in the ^1H NMR spectrum at $\delta = 6.30$, 6.04 and 5.73 ppm, respectively.

Oxidation of **21** gave a 3,11-dioxo-oleanoate **26** whose reaction with LDA and 2-propyn-1-ol afforded **27** in 52% yield. Reaction of **23** with triphenylphosphane, 3,3-dimethylglutarmide and diethyl azodicarboxylate in THF gave 87% of a 2,12-diene **28**. Sulfamoylated **29** was prepared from **22** (Scheme 4) according to our previously published procedure [39]; this compound was further transformed into carbamoylsulfamated **30**. The diene **28** served as a starting material for the synthesis of the 1,9-endoperoxide **31** and epoxide **32**, respectively [34]. Bromination of **32** gave **33** whose Jones-oxidation furnished **34**.

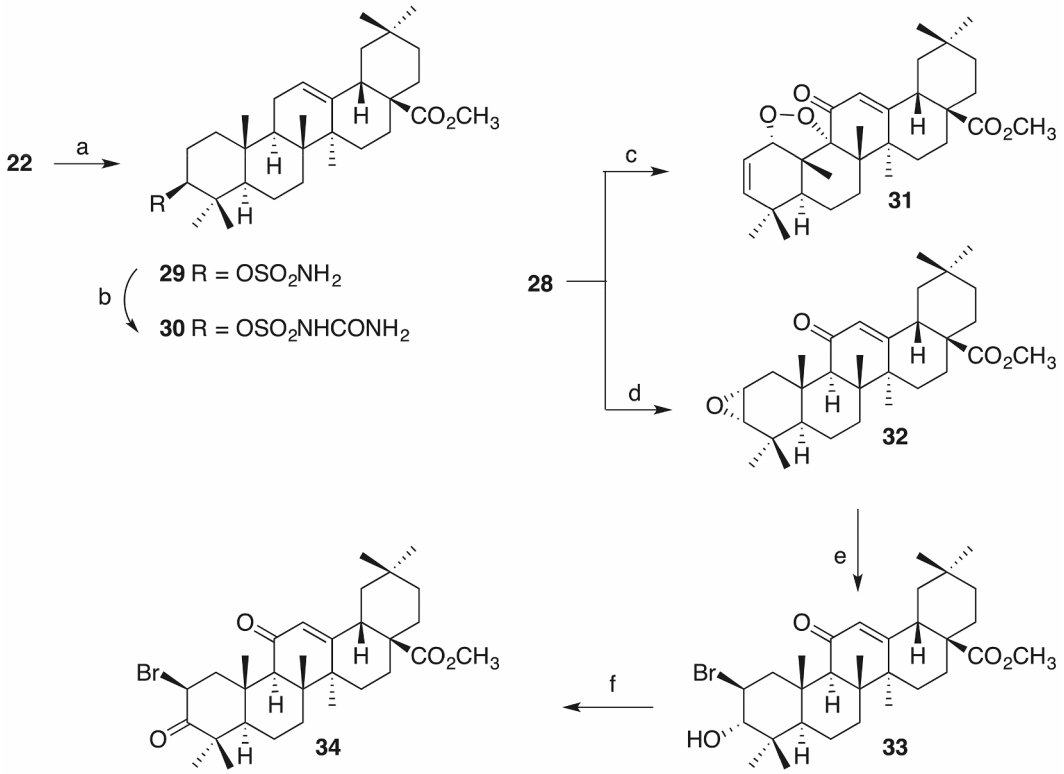
2.2. Biology

Compounds **1**–**34** and galantamine hydrobromide (**GH**, as a standard) were subjected to Ellman's assays to measure their ability to inhibit the enzymes acetylcholinesterase (from *Electrophorus electricus*, electric eel) and butyrylcholinesterase (from equine serum). Cholinesterases from different species are very similar. Due to these large and functional conservations, AChE from the electric eel, and BChE from equine serum can be used as suitable models for the corresponding enzymes from humans. As a result, while most of the compounds inhibited AChE, only two compounds displayed a significant activity for BChE; several compounds were insoluble under the conditions of the assay. The results from these assays are compiled in Table 1.

For the parent triterpenoic acids inhibition constants for AChE in low micromolar magnitude were measured. Thus, ursolic acid (**1**) gave a



Scheme 3. Synthesis of 21–28: (a) K_2CO_3, MeBr, DMF, 18 h, 25 °C, 88%; (b) K_2CO_3, MeBr, DMF, 18 h, 25 °C, 90%; (c) Ac_2O, TEA, DCM, 12 h, 25 °C, 95%; (d) TEA, acryloyl chloride, DMAP, $CHCl_3$, 30 min, 25 °C, 63%; (e) CrO_3, H_2SO_4, acetone, 1 h, 25 °C, 75%; (f) LDA, THF, −78 °C → 25 °C, 52%; (g) 3,3-dimethylglutarimide, PPh_3, DEAD, THF, 12 h, 0 °C, 96%.



Scheme 4. Synthesis of 29–34: (a) NaH, THF, 0 °C → 25 °C, 30 min then sulfamoyl chloride, THF, 5 d, 25 °C, 70%; (b) NaH, THF, 0 °C → 25 °C, 30 min then CDI, NH_3, THF, 3 h, 25 °C, 96%; (c) $Na_2Cr_2O_7$, AcOH, NHS, acetone, 2 d, 40 °C, 46%; (d) *m*-CPBA, MeCl, 2 d, 25 °C, 55%; (e) HBr, THF, 10 min, 10 °C, 77%; (f) CrO_3, H_2SO_4, acetone, 30 min, 25 °C, 53%.

Table 1
Inhibitory constants for galantamine hydrobromide (K_i = AChE: 0.54 ± 0.01 μM; BChE: K_i = 9.37 ± 0.67 μM) as a standard and compounds 1–34 (except insoluble and inactive ones) using Ellman's assay employing AChE (electric eel) and BChE (equine serum); four different substrate concentrations and four different inhibitor concentrations were used; K_i and K_i' are reported in μM; mean ± SE; inactive means less than 30% inhibition at 50 μM concentration.

Compound	**AChE** K_i (μM) [K_i'(μM)] (Type of inhibition)	**BChE** K_i (μM) [K_i'(μM)] (Type of inhibition)	Compound	**AChE** K_i (μM) [K_i'(μM)] (Type of inhibition)	**BChE** K_i (μM) [K_i'(μM)] (Type of inhibition)
1 (UA)	8.54 ± 1.33 [38.16 ± 3.28] (Mixed type)	inactive	**20** (OA)	11.62 ± 2.82 (competitive)	inactive
2	7.48 ± 0.53 [17.40 ± 0.63] (Mixed type)	inactive	**21**	4.22 ± 0.68[12.24 ± 0.85] (Mixed type)	inactive
3	9.48 ± 0.98 [45.90 ± 0.81] (Mixed type)	inactive	**22**	3.46 ± 0.56 [49.11 ± 7.28] (Mixed type)	inactive
10	inactive	4.29 ± 0.29 (competitive)	**27**	inactive	24.35 ± 9.07 (competitive)
12	7.39 ± 1.51 [11.1 ± 1.37] (Mixed type)	inactive	**28**	6.26 ± 2.71 [15.06 ± 2.00] (Mixed type)	inactive
14	11.50 ± 1.75 [35.83 ± 1.21] (Mixed type)	inactive	**29**	6.37 ± 0.26 [26.69 ± 0.18] (Mixed type)	inactive
16	20.73 ± 0.17 [12.42 ± 1.33] (Mixed type)	inactive	**31**	17.01 ± 1.02 [36.51 ± 4.25](Mixed type)	inactive
18	1.72 ± 0.24 [1.28 ± 0.05] (Mixed type)	inactive	**32**	21.89 ± 5.93 [21.53 ± 3.57] (Mixed type)	inactive
19	4.43 ± 0.46 [5.26 ± 1.04] (Mixed type)	inactive	**34**	12,88 ± 0.77 [14.58 ± 0.92] (Mixed type)	inactive

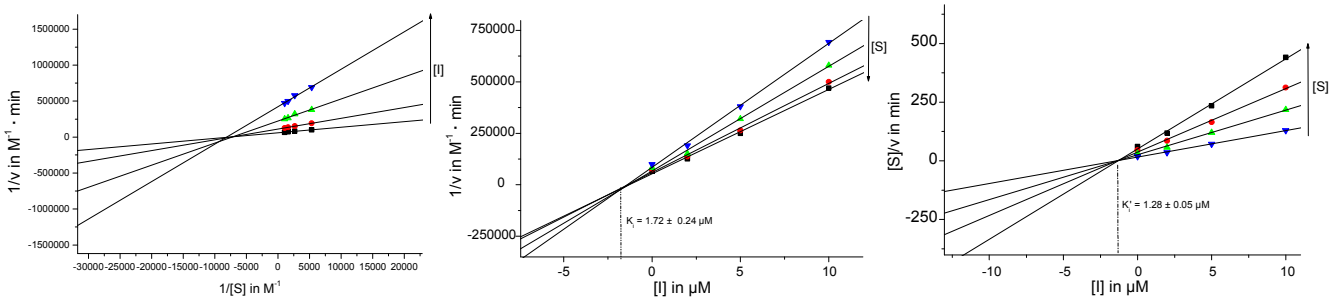


Fig. 2. Lineweaver-Burk (left), Dixon (middle) and Cornish-Bowden (right) plots for mixed-type inhibitor 18.

mixed-type inhibition (K_i = 8.54 ± 1.33 μM, K_i' = 38.16 ± 3.28 μM) while for oleanolic acid (**20**) a competitive inhibition (K_i = 11.62 ± 2.82 μM) was found. Inhibition of both compounds, however, was lower than the inhibition measured for standard galantamine hydrobromide (**GH**, K_i = 0.54 ± 0.01 μM). Esterification of **1** had no significant impact on the ability to act as an inhibitor, and the inhibition constants of **3** were similar to those of parent ursolic acid. Interestingly, the methyl ester of oleanolic acid **22** was a mixed-type inhibitor, and elimination of HO-C(3) provided compounds with rather lower K_i values. Furthermore, introduction of a 2-methyl-3-oxo-moiety increased the inhibition potential, and inhibition constants of K_i = 1.72 ± 0.24 μM and K_i' = 1.28 ± 0.05 μM were determined for **18**. Finally, introduction of a 3-hydroxyprop-1-ynyl moiety provided a selective BChE inhibitor; thus, ursolic acid derived **10** (K_i = 4.29 ± 0.29 μM, competitive inhibitor) was twice as active as galantamine hydrochloride (See Fig. 2).

2.3. Docking

Molecular modeling is a valuable tool to rationalize different biological outcomes from closely related compounds. Therefore, we used GOLD 5.2 docking software [40] to evaluate the preferred poses of some of the synthesized compounds in the active site of the targeted enzymes AChE and BChE. Compound **18** being the most active compound towards AChE showed a preferential U-shape pose facing the active site Ser203. This led to an acceptable positioning for subsequent ester hydrolysis and hence to an inactivation of the enzyme. Docking of several **UA** derived compounds revealed that their preferential pose did not allow the entrance into the AChE gorge and thus there was no inhibition of the targeted esterase. From the results of the biological assay it was known that the selectivity of the inhibitors to AChE is higher than to BChE. This can probably be explained by the fact that the inhibitors interact with the residues Phe 330, Trp 279, Tyr 121 in the AChE binding pocket via pi-pi interactions whereas the corresponding residues in BChE (Ala 328, Val 277, Gln 119 cannot. The active compounds could fit reasonably into the AChE hydrophobic binding pocket. In addition, the carboxyl groups of the compounds fit very well into the hydrophilic part of the pocket (See Fig. 3).

3. Conclusions

Several derivatives of ursolic and oleanolic acid varying in their substitution pattern were synthesized in this study and screened for their inhibitory potential for the enzymes AChE and BChE applying Ellman's assays. The inhibition constants and the type of inhibition were determined for each compound. While parent ursolic acid gave inhibition constants of about 10 μM for AChE, no inhibition for BChE was detected. However, several compounds showed a higher inhibition for AChE and two compounds inhibited BChE even in micromolar magnitude. The results obtained in the biological assay can be explained by appropriate molecular modelling calculations.

4. Experimental

4.1. Chemistry

4.1.1. General

Reagents were bought from commercial suppliers without any further purification. Instrumental analysis was performed as previously reported [42]. The purity of the compounds was checked by HPLC, and found to > 96%.

4.2. Syntheses

4.2.1. 3β-Hydroxy-urs-12-en-28-oic acid (ursolic acid, 1)

This compound was commercially obtained from Betulinines (Stříbrná Skalice, Czech Republic) and used as received.

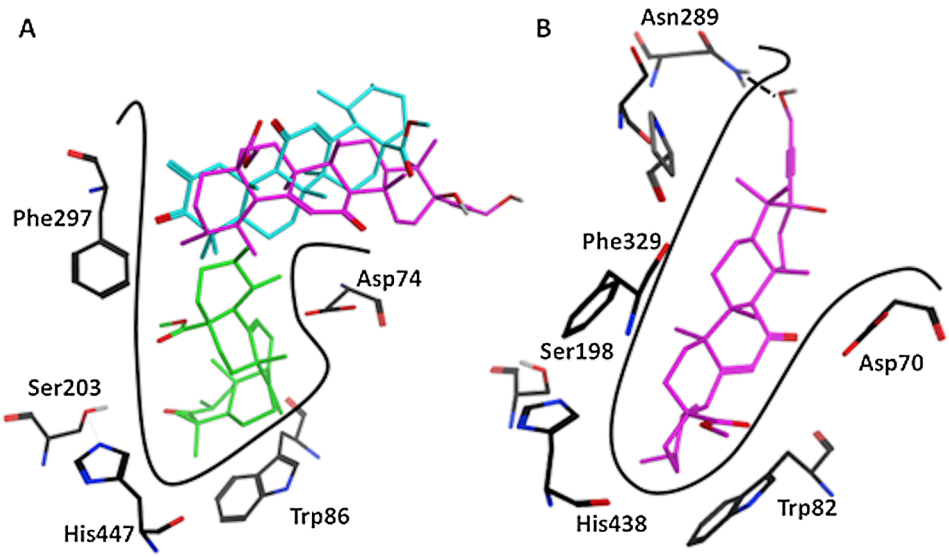


Fig. 3. Docked poses at the AChE and BChE active sites calculated with GOLD 5.2 [37]. (A) AChE: compounds 18 (green), 17 (cyano) and 10 (pink); (B) BChE, compound 10 (pink). Pictures made with MOE2014 software [41].

*4.2.2. Methyl 3β-hydroxy-urs-12-en-28-oate (**3**)*

This compound was synthesized according to literature from **1** [39]; m.p. 165–167 °C; $[\alpha]_D = +68°$ ($c = 0.53$, $CHCl_3$); MS (ESI): *m/z* (%) = 471.2 ($[M+H]^+$, 80), 493.4 ($[M+Na]^+$, 100).

*4.2.3. Methyl 3β-hydroxy-11-oxo-urs-12-en-28-oate (**4**)*

Esterification [39] of **2** [36] gave compound **4**; m.p. 132–135 °C; $[\alpha]_D = +87°$ ($c = 3.0$, $CHCl_3$); MS (ESI): *m/z* (%) = 485.5 ($[M+H]^+$, 100), 507.5 ($[M+Na]^+$, 50).

*4.2.4. Methyl 3β-acetyloxy-urs-12-en-28-oate (**5**)*

This compound was synthesized by acetylation of **3** [42]; m.p. 243–246 °C; $[\alpha]_D = 64.7°$ ($c = 0.30$, $CHCl_3$); MS (ESI): *m/z* (%) = 513.2 ($[M+H]^+$, 40), 535.5 ($[M+Na]^+$, 100).

*4.2.5. Methyl 3β-acetyloxy-11-oxo-urs-12-en-28-oate (**6**)*

Compound **5** (480 mg, 0.94 mmol) was dissolved in acetone (50 mL) and glacial acetic acid (5 mL). *N*-hydroxysuccinimide (1.20 g, 10.43 mmol) and potassium dichromate (1.05 g, 3.56 mmol) were added. After 2 days of continuous stirring at 40 °C, solutions of saturated potassium bisulfite solution and saturated sodium bicarbonate were added. The aqueous solution was extracted with dichloromethane (4 × 50 mL); the combined organic layers were washed with water (20 mL) and brine (20 mL), dried (Na_2SO_4) and evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexanes/ethyl acetate, 8:2) to afford **6** (0.32 g, 65%) as an colorless solid; m.p. 245–250 °C (lit.: 235 °C [43]; $R_F = 0.66$ (chloroform/diethyl ether, 95:5); $[\alpha]_D = +75°$ ($c = 3.3$, $CHCl_3$); UV–Vis (methanol): λ_{max} (log ε) = 274 nm (4.21); IR (KBr): ν = 3441*br*, 2971*s*, 2875*m*, 1731*s*, 1655*s*, 1620*w*, 1459*m*, 1427*w*, 1396*m*, 1371*m*, 1319*m*, 1307*m*, 1272*m*, 1239*s*, 1202*m*, 1142*m*, 1116*m*, 1101*w*, 1088*w*, 1028*m*, 1002*m*, 983*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.58 (*s*, 1H, H-12), 4.51 (*dd*, 1H, J = 11.6, 4.6 Hz, H-3), 3.59 (*s*, 3H, OMe), 2.77 (*d*, 1H, J = 13.8 Hz, H-1), 2.40 (*d*, 1H, J = 11.3 Hz, H-18), 2.30 (*s*, 1H, H-9), 2.11–2.01 (*m*, 1H, H-16), 2.03 (*s*, 3H, Ac)), 1.81–1.50 (*m*, 10H, H-2, H-6, H-7, H-15, H-16′, H-21, H-22), 1.44–1.20 (*m*, 4H, H-7′, H-19, H-15′, H-21′), 1.28 (*s*, 3H, H-27), 1.13 (*s*, 3H, H-25), 1.10–1.01 (*m*, 2H, H-1′, H-20), 0.95 (*d*, 3H, J = 6.4 Hz, H-30), 0.89 (*s*, 3H, H-26), 0.86 (*d*, 3H, J = 6.1 Hz, H-29), 0.85 (*s*, 3H, H-24), 0.85 (*s*, 3H, H-23), 0.76 (*d*, 1H, J = 11.4 Hz, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.6 (C-11), 177.1 (C-28), 170.9 (Ac), 162.8 (C-13), 130.6 (C-12), 80.6 (C-3), 61.3 (C-9), 55.0 (C-5), 52.7 (C-18), 51.8 (OMe), 47.6 (C-17), 44.6 (C-8), 43.7 (C-14), 38.8 (C-4), 38.6 (C-19), 38.5 (C-20), 38.0 (C-1), 37.0 (C-10), 35.9 (C-22), 32.9 (C-7), 30.3 (C-21), 28.3 (C-15), 28.0 (C-23), 23.9 (C-16), 23.5 (C-2), 21.3 (Ac), 21.0 (C-27), 20.9 (C-30), 18.8 (C-26), 17.3 (C-6), 17.1 (C-29), 16.7 (C-24), 16.2 (C-25) ppm; MS (ESI): *m/z* (%) = 527.5 ($[M+H]^+$, 85), 549.5 ($[M+Na]^+$, 100); anal. calcd. for $C_{33}H_{50}O_5$ (526.75): C, 75.25; H, 9.57; found: C, 75.07; H, 9.74.

*4.2.6. Methyl 3-oxo-urs-12-en-28-oate (**7**)*

This compound was synthesized according to literature from **3** [44]; m.p. 193–195 °C; $[\alpha]_D = +90°$ ($c = 5.90$, $CHCl_3$); MS (ESI): *m/z* (%) = 496.3 ($[M+H]^+$, 75), 523.0 ($[M+Na+MeOH]^+$,100).

*4.2.7. Methyl 3,11-dioxo-urs-12-en-28-oate (**8**)*

This amorphous compound was synthesized according to literature from **4** [44]; $[\alpha]_D = +108°$ ($c = 4.80$, $CHCl_3$); MS (ESI): *m/z* (%) = 483.5 ($[M+H]^+$, 100).

*4.2.8. Methyl 3β-hydroxy-3α-hydroxy-1-propynyl)-urs-12-en-28-oate (**9**)*

To a freshly prepared solution of lithium diisopropylamide [made from diisopropylamine (605 mg, 5.99 mmol) and *n*-butyllithium (1.6 M in hexane, 3.78 mL, 6.05 mmol) in dry THF (20 mL)] at −78 °C, 2-propyn-1-ol (223 mg, 4 mmol) in dry THF (4 mL) was added, and the mixture was stirred for 30 min. Compound **7** (360 mg, 0.74 mmol) in dry THF (5 mL) was added dropwise, and the mixture was allowed to warm to room temperature. Usual aqueous workup followed by column chromatography (silica gel, hexanes/ethyl acetate, 7:3) gave **9** (207 mg, 53%) as an amorphous solid; $R_F = 0.35$ (hexanes/ethyl acetate, 7:3); $[\alpha]_D = 32°$ ($c = 3.0$, $CHCl_3$); UV–Vis (methanol): λ_{max} (log ε) = 213 nm (3.86); IR (KBr): ν = 3433*br*, 2948*s*, 2870*m*, 1725*m*, 1455*m*, 1388*m*, 1308*w*, 1231*m*, 1200*w*, 1144*w*, 1114*w*, 1076*w*, 1033*m*, 994*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.19 (*m*, 1H, H-12), 4.26 (*s*, 2H), 3.54 (*s*, 3H, OMe), 2.17 (*d*, 1H, H-18, J = 11.0 Hz), 1.97–1.83 (*m*, 4H, H-11, H-11′, H-16, H-2), 1.70 (*ddd*, 1H, H-15, J = 13.8, 13.8, 4.6 Hz), 1.63–1.57 (*m*, 3H, H-22, H-16′, H-2′), 1.55–1.47 (*m*, 3H, H-9, H-22′, H-1), 1.45–1.39 (*m*, 3H, H-6, H-21, H-7), 1.32–1.18 (*m*, 5H, H-19, H-6′, H-1′, H-7′, H-21′), 1.06 (*d*, 1H, H-5, J = 11.0 Hz), 1.03–0.92 (*m*, 2H, H-15′, H-20), 1.02 (*s*, 3H, H-27), 0.98 (*s*, 3H, H-23), 0.87 (*d*, 3H, H-30, J = 5.6 Hz), 0.86 (*s*, 3H, H-25), 0.81 (*s*, 3H, H-24), 0.80 (*d*, 3H, H-29, J = 6.0 Hz), 0.67 (*s*, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.0 (C-28), 138.1 (C-13), 125.5 (C-12), 88.9 (C-31, *C*≡C), 83.7 (C-32, C≡*C*), 75.8 (C-3), 53.1 (C-5), 52.8 (C-18), 51.4 (OMe), 51.2 (C-33, H_2C-O), 48.1 (C-17), 47.6 (C-9), 41.9 (C-14), 41.4 (C-4), 39.4 (C8),

39.0 (C-19), 38.8 (C-20), 37.8 (C-1), 36.9 (C-10), 36.6 (C-22), 32.8 (C-7), 32.4 (C-2), 30.6 (C-21), 28.0 (C-15), 25.8 (C-23), 24.2 (C-16), 23.7 (C-27), 23.3 (C-11), 21.1 (C-30), 18.5 (C-6), 17.8 (C-24), 17.0 (C-29), 16.8 (C-26), 15.7 (C-25) ppm; MS (ESI): *m/z* (%) = 547.5 ($[M+Na]^+$, 100); anal. calcd. for $C_{34}H_{52}O_4$ (524.77): C, 77.82; H, 9.99; found: C, 77.52; H 10.19.

4.2.9. Methyl 3β-hydroxy-3α-hydroxy-1-propynyl)-11-oxo-urs-12-en-28-oate (10)

Following procedure described for the synthesis of **9**, compound **10** (282 mg, 63%) was obtained as an amorphous solid from the reaction of **8** (400 mg, 0.83 mmol) followed by column chromatography (silica gel, hexanes/ethyl acetate, 7:3); R_F = 0.54 (hexanes/ethyl acetate, 1:1); $[\alpha]_D$ = 66° (*c* = 3.5, $CHCl_3$); UV–Vis (MeOH): λ_{max} (log ε) = 269 nm (4.04); IR (KBr): ν = 3432*br*, 2950*s*, 2871*m*, 1728*s*, 1661*s*, 1457*m*, 1388*w*, 1323*w*, 1272*w*, 1231*w*, 1202*m*, 1167*w*, 1145*w*, 1112*w*, 1083*w*, 1036*m*, 993*m* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.59 (*s*, 1H, H-12), 4.29 (*s*, 2H), 3.59 (*s*, 3H, OMe), 2.75 (*dd*, 1H, H-1, *J* = 14.0, 2.8 Hz), 2.40 (*d*, 1H, H-18, *J* = 11.9 Hz), 2.34 (*s*, 1H, H-9), 2.09–1.95 (*m*, 2H, H-2, H-16), 1.81–1.71 (*m*, 3H, H-16′, H-22, H-15), 1.68–1.49 (*m*, 5H, H-2′, H-7, H-22′, H-6, H-21), 1.43–1.34 (*m*, 5H, H-19, H-7′, H-16′, H-1′, H-15′, H-21′), 1.30 (*s*, 3H, H-27), 1.11 (*s*, 3H, H-25), 1.08–1.00 (*m*, 2H, H-5, H-20), 1.03 (*s*, 3H, H-23), 0.95 (*d*, 3H, H-30, *J* = 6.4 Hz), 0.89 (*s*, 3H, H-26), 0.87 (*s*, 3H, H-24), 0.85 (*d*, 3H, H-29, *J* = 6.7 Hz) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.8 (C-11), 177.2 (C-28), 162.9 (C-13), 130.6 (C-12), 88.9 (C-31, *C*≡C), 83.7 (C-32, C≡*C*), 75.5 (C-3), 61.5 (C-9), 52.8 (C-5), 52.7 (C-18), 51.8 (OMe), 51.1 (C-33, H_2C-O), 47.6 (C-17), 44.6 (C-8), 43.6 (C-14), 41.5 (C-4), 38.6 (C-19), 38.5 (C-20), 38.3 (C-1), 37.1 (C-10), 36.0 (C-22), 32.9 (C-7), 32.5 (C-2), 30.3 (C-21), 28.4 (C-15), 25.9 (C-23), 23.9 (C-16), 21.0 (C-27), 20.9 (C-30), 18.8 (C-26), 17.8 (C-24), 17.7 (C-6), 17.0 (C-29), 16.5 (C-25) ppm; MS (ESI): *m/z* (%) = 539.5 ($[M+H]^+$, 100); anal. calcd. for $C_{34}H_{50}O_5$ (538.76): C, 75.80; H, 9.35; found: C, 75.63; H, 9.51.

4.2.10. Methyl 3β-acryloyloxy-urs-12-en-28-oate (11)

Compound **3** (233 mg, 0.49 mmol) was dissolved in dry chloroform (30 mL), triethylamine (184 mg, 1.81 mmol), acryloyl chloride (113 mg, 1.25 mmol) and catalytic amounts of 4-dimethylaminopyridine were added successively. After 30 min of continuous stirring, water (40 mL) was added, and the layers were separated. The aqueous solution was extracted with chloroform (2 × 30 mL); the combined organic layers were washed with water (20 mL) and brine (20 mL), dried (Na_2SO_4) and evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexanes/ethyl acetate, 9:1) to afford **11** (115 mg, 45%) as an amorphous solid; R_F = 0.78 (hexanes/ethyl acetate, 8:2); $[\alpha]_D$ = 67° (*c* = 5.0, $CHCl_3$); UV–Vis (methanol): λ_{max} (log ε) = 221 nm (4.19); IR (KBr): ν = 2935*s*, 2872*s*, 1728*s*, 1634*m*, 1456*m*, 1409*m*, 1390*w*, 1370*w*, 1295*m*, 1272*w*, 1225*m*, 1204*s*, 1187*s*, 1146*m*, 1114*w*, 1034*w*, 1012*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 6.36 (*dd*, 1H, acryloyl, *J* = 17.2, 1.5 Hz), 6.10 (*dd*, 1H, acryloyl, *J* = 17.2, 10.4 Hz), 5.79 (*dd*, 1H, acryloyl, *J* = 10.6, 1.5 Hz), 5.23 (*m*, 1H, H-12), 4.56 (*dd*, 1H, H-3, *J* = 9.8, 7.3 Hz), 3.59 (*s*, 3H, OMe), 2.22 (*d*, 1H, H-18, *J* = 11.0 Hz), 1.99 (*ddd*, 1H, H-16, *J* = 13.6, 13.4, 4.8 Hz), 1.91 (*d*, 1H, H-11, *J* = 3.7 Hz), 1.89 (*d*, 1H, H-11′, *J* = 3.7 Hz), 1.76 (*ddd*, 1H, H-15, *J* = 13.9, 13.6, 4.8 Hz), 1.69–1.56 (*m*, 6H, H-22, H-22′, H-1, H-16′, H-2, H-2′), 1.54–1.44 (*m*, 4H, H-9, H-6, H-21, H-7), 1.37–1.23 (*m*, 4H, H-19, H-6′, H-7′, H-21), 1.12–1.03 (*m*, 2H, H-15′, H-1′), 1.06 (*s*, 3H, H-27), 0.97–0.91 (*m*, 1H, H-20), 0.94 (*s*, 3H, H-25), 0.93 (*d*, 3H, H-30, *J* = 6.2 Hz), 0.89–0.83 (*m*, 1H, H-5), 0.88 (*s*, 3H, H-23), 0.87 (*s*, 3H, H-26), 0.85 (*d*, 3H, H-29, *J* = 6.6 Hz), 0.74 (*s*, 3H, H-24) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.0 (C-28), 166.0 (C-31), 138.1 (C-13), 130.0 (C-33, H_2C═CH), 129.1 (C-32, H*C*═CH_2), 125.4 (C-12), 81.0 (C-3, H*C*-O), 55.3 (C-5), 52.9 (C-18), 51.4 (OMe), 48.0 (C-17), 47.5 (C-9), 42.0 (C-14), 39.5 (C-8), 39.0 (C-19), 38.8 (C-20), 38.2 (C-1), 37.8 (C-4), 36.8 (C-22), 36.6 (C-10), 32.9 (C-7), 30.6 (C-21), 28.1 (C-23), 28.0 (C-15), 24.2 (C-16), 23.6 (C-27), 23.5 (C-2), 23.3 (C-11), 21.1 (C-30), 18.2 (C-6), 17.0 (C-29), 16.9 (C-24), 16.7 (C-26), 15.4 (C-25) ppm; MS (ESI): *m/z* (%) = 525.3 ($[M+H]^+$, 60), 547.3 ($[M+Na]^+$, 100); anal. calcd. for $C_{34}H_{52}O_4$ (524.77): C, 77.82; H, 9.99; found: C, 77.66; H, 10.15.

4.2.11. Methyl 3β-[(aminosulfonyl)oxy]-urs-12-en-28-oate (12)

This compound was synthesized from **3** as previously reported [39]; m.p. 115–118 °C; $[\alpha]_D$ = +62.10° (*c* = 0.32, $CHCl_3$); MS (ESI): *m/z* (%) = 548.3 ($[M-H]^-$, 90), 1097.1 ($[2M-H]^-$, 100).

4.2.12. Methyl 3β-{[[(aminocarbonyl)amino]sulfonyl]oxy}-urs-12-en-28-oate (13)

This compound was synthesized from **12** as previously reported [45]; m.p. 49–53 °C; $[\alpha]_D$ = +81.4 (*c* = 0.3, $CHCl_3$); MS (ESI): *m/z* (%)) = 591.5 ($[M-H]^-$, 32), 1183.1 ($[2M-H]^-$, 31).

4.2.13. Methyl 2,2-dibromo-3-oxo-urs-12-en-28-oate (14)

This amorphous compound was synthesized from **7** as previously reported [44]; $[\alpha]_D$ = +43° (*c* = 5.70, $CHCl_3$); MS (ESI): *m/z* (%) = 647.1 ($[M\ (2\times {}^{79}Br)+Na]^+$, 56), 649.2 ($[M\ ({}^{79}Br, {}^{81}Br)+Na]^+$, 100), 651.2 ($[M\ (2\times {}^{81}Br)+Na]^+$,46).

4.2.14. Methyl 2,2-dibromo-3,11-dioxo-urs-12-en-28-oate (15)

This compound was synthesized from **8** as previously reported [41]; m.p. 225–227 °C; $[\alpha]_D$ = +48° (*c* = 5.90, $CHCl_3$); MS (ESI): *m/z* (%) = 639.3 ($[M\ (2\times {}^{79}Br)+H]^+$, 23), 641.3 ($[M\ ({}^{79}Br, {}^{81}Br)+H]^+$, 51), 643.3 ($[M\ (2\times {}^{81}Br)+H]^+$,27), 661.1 ($[M\ (2\times {}^{79}Br)+Na]^+$, 46), 663.1 ($[M\ ({}^{79}Br, {}^{81}Br)+Na]^+$, 100), 665.0 ($[M\ (2\times {}^{81}Br)+Na]^+$,52).

4.2.15. Methyl 2-methylene-3-oxo-urs-12-en-28-oate (16)

This amorphous compound was synthesized from **7** as previously reported [44]; $[\alpha]_D$ = +100° (*c* = 3.20, $CHCl_3$); MS (ESI): *m/z* (%) = 481.1 ($[M+H]^+$, 100).

4.2.16. Methyl 2-methylene-3,11-dioxo-urs-12-en-28-oate (17)

This compound was synthesized from **8** as previously reported [44]; m.p. 219–222 °C; $[\alpha]_D$ = +126° (*c* = 4.40, $CHCl_3$); MS (ESI): *m/z* (%) = 495.5 ($[M+H]^+$, 100).

4.2.17. Methyl 2β-methyl-3-oxo-urs-12-en-28-oate (18)

To a solution of lithium diisopropylamide [prepared from diisopropylamine (85 mg, 0.84 mmol) and *n*-butyllithium (1.6 M in hexane, 0.60 mL, 0.96 mmol) in dry THF (10 mL)] at −78 °C methyl 3-oxo-urs-12-en-28-oate (300 mg, 0.64 mmol) in dry THF (5 mL) was added followed by iodomethane (200 mg, 1.41 mmol) in dry THF (3 mL) after additional 30 min. The mixture was allowed to reach room temperature, and water (30 mL) was added. Usual aqueous work-up and extraction with dichloromethane (3 × 20 mL) followed by column chromatography (silica gel, hexanes/ethyl acetate, 9:1) afforded **18** (220 mg, 71%) as an amorphous solid; R_F = 0.4 (hexanes/ethyl acetate, 9:1); $[\alpha]_D$ = 49° (*c* = 2.0; $CHCl_3$); UV–Vis (MeOH): λ_{max} (log ε) = 216 nm (3.80); IR (KBr): ν = 3440*br*, 2929*s*, 1725*m*, 1704*m*, 1654*w*, 1456*w*, 1388*w*, 1230*w*, 1200*w*, 1146*w* cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.24 (*m*, 1H, H-12), 3.60 (*s*, 3H, OMe), 2.77–2.71 (*m*, 1H, H-2), 2.21 (*d*, 1H, H-18, *J* = 11.3 Hz), 2.02–1.89 (*m*, 4H, H-11, H-11′, H-16, H-1), 1.75 (*ddd*, 1H, H-15, *J* = 13.8, 13.8, 4.6 Hz), 1.69–1.62 (*m*, 2H, H-22, H-16′), 1.56 (*ddd*, 1H, H-22′, *J* = 13.4, 13.4, 4.0 Hz), 1.52–1.41 (*m*, 5H, H-6, H-6′, H-21, H-7, H-9), 1.35–1.22 (*m*, 3H, H-19, H-7′, H-21′), 1.19 (*s*, 3H, H-31), 1.12 (*d*, 1H, H-5, *J* = 12.2 Hz), 1.08–1.01 (*m*, 2H, H-15′, H-1′), 1.05 (*s*, 3H, H-23), 1.04 (*s*, 3H, H-27), 1.03 (*s*, 3H, H-24), 1.00–0.95 (*m*, 1H, H-20), 0.99 (*s*, 3H, H-25), 0.91 (*d*, 3H, H-30, *J* = 6.1 Hz), 0.83 (*d*, 3H, H-29, *J* = 6.4 Hz), 0.78 (*s*, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 218.0 (C-3), 177.9 (C-28), 138.3 (C-13), 125.2 (C-12), 57.2 (C-5), 52.8 (C-18), 51.4 (OMe), 49.5 (C-1), 48.0 (C-17), 48.0 (C-4), 47.0 (C-9), 42.0 (C-14), 39.5

(C-8), 38.9 (C-19), 38.8 (C-20), 37.2 (C-10), 36.6 (C-22), 36.4 (C-2), 32.7 (C-7), 30.6 (C-21), 28.0 (C-15), 25.4 (C-23), 24.1 (C-16), 23.5 (C-27), 23.4 (C-11), 22.0 (C-24), 21.1 (C-30), 19.3 (C-6), 17.0 (C-29), 16.9 (C-26), 15.5 (C-25), 15.5 (C-31) ppm; MS (ESI): *m/z* (%) = 483.3 ([M + H]$^+$, 65), 537.0 ([M + Na + MeOH]$^+$, 100); anal. calcd. for $C_{32}H_{50}O_3$ (482.74): C, 79.62; H, 10.44; found: C 79.51; H, 10.58.

4.2.18. Methyl 11-oxo-urs-2,12-dien-28-oate (19)

This compound was synthesized from **4** as previously reported [37]; m.p. 179–187 °C; $[\alpha]_D$ = +141° (*c* = 1.8, $CHCl_3$); MS (ESI): *m/z* (%) = 467.5 ([M + H]$^+$, 100), 489.5 [M + Na]$^+$, 30).

4.2.19. 3β-Hydroxy-olean-12-en-28-oic acid (oleanolic acid, 20)

This compound was commercially obtained from Betulinines (Stříbrná Skalice, Czech Republic) and used as received.

4.2.20. Methyl 3β-hydroxy-olean-12-en-28-oate (22)

This compound was synthesized from **20** as previously reported [42]; m.p. 198–200 °C; $[\alpha]_D$ = +70° (*c* = 0.43, $CHCl_3$); MS (ESI): *m/z* (%) = 493.5 ([M + Na]$^+$, 100).

4.2.21. Methyl 3β-hydroxy-11-oxo-olean-12-en-28-oate (23)

Esterification of **21** as previously reported [42] gave **21** as a colorless solid; m.p. 184–188 °C; $[\alpha]_D$ = +82 (*c* = 0.14, $CHCl_3$); MS (ESI): *m/z* (%) = 485.6 ([M + H]$^+$, 100), 507.5 ([M + Na]$^+$, 35).

4.2.22. Methyl 3β-acetyloxy-olean-12-en-28-oate (24)

The acetylation of **22** using acetic anhydride gave **24** [37]; m.p. 219–221 °C 224–225 °C; $[\alpha]_D$ = +69.3 (*c* = 3.8, $CHCl_3$); MS (ESI): *m/z* (%) = 535.5 ([M + Na]$^+$, 100).

4.2.23. Methyl 3β-acryloyloxy-olean-12-en-28-oate (25)

Following the procedure given for the synthesis of **11**, from **22** (198 mg, 0.42 mmol) and acryloyl chloride followed by chromatography (silica gel, chloroform/diethyl ether, 98:2) **25** (138 mg, 63%) was obtained as a colorless solid; m.p. 227–233 °C; R_F = 0.62 (hexanes/ethyl acetate, 9:1); $[\alpha]_D$ = 70° (*c* = 4.2, $CHCl_3$); UV–Vis (MeOH): λ_{max} (log ε) = 219 nm (4.28); IR (KBr): ν = 3425*br*, 2940*s*, 2862*m*, 1722*s*, 1471*m*, 1404*m*, 1388*w*, 1363*w*, 1272*m*, 1240*w*, 1204*m*, 1191*s*, 1163*m*, 1124*w*, 1096*w*, 1046*m*, 1014*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.30 (*dd*, 1H, acryloyl, *J* = 17.4, 1.5 Hz), 6.04 (*dd*, 1H, acryloyl, *J* = 17.2, 10.6 Hz), 5.73 (*dd*, 1H, acryloyl, *J* = 10.7, 1.5 Hz), 5.21 (*m*, 1H, H-12), 4.50 (*dd*, 1H, H-3, *J* = 8.6, 7.3 Hz), 3.56 (*s*, 3H, OMe), 2.82 (*dd*, 1H, H-18, *J* = 13.8, 4.2 Hz), 1.90 (*ddd*, 1H, H-16, *J* = 14.4, 14.5, 4.0 Hz), 1.85–1.80 (*m*, 2H, H-11, H-11′), 1.65–1.42 (*m*, 11H, H-9, H-1, H-19, H-6, H-7, H-15, H-22, H-22′, H-16′, H-2, H-2′), 1.39–1.09 (*m*, 4H, H-6′, H-21, H-21′, H-7′, H-19′), 1.07 (*s*, 3H, H-27), 1.02–0.96 (*m*, 2H, H-15′, H-1′), 0.88 (*s*, 3H, H-25), 0.86 (*s*, 3H, H-30), 0.84–0.77 (*m*, 1H, H-5), 0.83 (*s*, 3H, H-29), 0.83 (*s*, 3H, H-26), 0.81 (*s*, 3H, H-23), 0.66 (*s*, 3H, H-24) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.2 (C-28), 166.0 (C-31), 143.8 (C-13), 130.0 (C-33, H_2C = C), 129.9 (C-32), 122.2 (C-12), 81.1 (C-3, HC-O), 55.3 (C-5), 51.5 (OMe), 47.4 (C-9), 46.7 (C-17), 45.8 (C-19), 41.6 (C-14), 41.2 (C-18), 39.3 (C-8), 38.1 (C-1), 37.8 (C-4), 36.9 (C-10), 33.8 (C-21), 33.1 (C-29), 32.6 (C-7), 32.3 (C-22), 30.7 (C-20), 28.0 (C-23), 27.6 (C-15), 25.8 (C-27), 23.5 (C-30), 23.4 (C-11), 23.4 (C-2), 23.0 (C-16), 18.2 (C-6), 16.7 (C-24), 16.6 (C-26), 15.3 (C-25) ppm; MS (ESI): *m/z* (%) = 547.3 ([M + Na]$^+$, 100); anal. calcd. for $C_{34}H_{52}O_4$ (524.77): C, 77.82; H, 9.99; found: C, 77.64; H, 10.13.

4.2.24. Methyl 3,11-dioxo-olean-12-en-28-oate (26)

This amorphous compound was synthesized from **21** as previously reported [44]; $[\alpha]_D$ = +119° (*c* = 3.30, $CHCl_3$); MS (ESI): *m/z* (%) = 483.5 ([M + H]$^+$, 100).

4.2.25. Methyl 3β-hydroxy-3α-(3-hydroxy-1-propynyl)-11-oxo-olean-12-en-28-oate (27)

As described for **10**, compound **27** was obtained from methyl 3,11-dioxo-olean-12-en-28-oate (**26**, 300 mg, 0.62 mmol) as an amorphous solid (175 mg, 52%); R_F = 0.54 (hexanes/ethyl acetate, 1:1); $[\alpha]_D$ = 42° (*c* = 4.4, $CHCl_3$); UV–Vis (MeOH): λ_{max} (log ε) = 269 nm (4.02); IR (KBr): ν = 3423*br*, 2950*s*, 2867*m*, 1727*s*, 1655*s*, 1464*m*, 1385*s*, 1366*w*, 1328*w*, 1264*m*, 1206*m*, 1163*w*, 1081*w*, 1036*m*, 994*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.62 (*s*, 1H, H-12), 4.27 (*s*, 2H), 3.61 (*s*, 3H, OMe), 2.98 (*dd*, 2H, H-18, *J* = 13.4, 3.7 Hz), 2.77 (*dd*, 1H, H-1, *J* = 13.4, 3.7 Hz), 2.36 (*s*, 1H, H-9), 2.07–1.94 (*m*, 2H, H-2, H-16), 1.75–1.56 (*m*, 7H, H-19, H-16′, H-2, H-7, H-15, H-22, H-22′), 1.54–1.49 (*m*, 1H, H-6), 1.41–1.27 (*m*, 4H, H-1′, H-7′, H-21, H-6′), 1.37 (*s*, 3H, H-27), 1.26–1.15 (*m*, 4H, H-5, H-19′, H-21′, H-15′), 1.09 (*s*, 3H, H-25), 1.03 (*s*, 3H, H-23), 0.91 (*s*, 3H, H-30), 0.91 (*s*, 3H, H-29), 0.88 (*s*, 3H, H-26), 0.86 (*s*, 3H, H-24) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.4 (C-11), 177.5 (C-28), 168.8 (C-13), 127.7 (C-12), 88.9 (C-31, *C*≡C), 83.7 (C-32, C≡*C*), 75.5 (C-3), 61.9 (C-9), 52.7 (C-5), 51.9 (OMe), 51.0 (C-33), 46.2 (C-17), 45.0 (C-14), 44.3 (C-19), 43.4 (C-8), 41.6 (C-18), 41.6 (C-4), 38.2 (C-1), 37.2 (C-10), 33.7 (C-21), 32.8 (C-29), 32.7 (C-7), 32.5 (C-2), 31.6 (C-22), 30.6 (C-20), 27.7 (C-15), 25.9 (C-23), 23.6 (C-27), 23.4 (C-30), 22.9 (C-16), 18.9 (C-26), 17.7 (C-24), 17.6 (C-6), 16.5 (C-25) ppm; MS (ESI): *m/z* (%) = 539.5 ([M + H]$^+$, 100); anal. calcd. for $C_{34}H_{50}O_5$ (538.76): C, 75.80; H, 9.35; found: C, 75.56; H, 9.51.

4.2.26. Methyl 11-oxo-olean-2,12-dien-28-oate (28)

This compound was synthesized according to literature from **23** [37]; m.p. 177–183 °C; $[\alpha]_D$ = +147° (*c* = 1.5, $CHCl_3$); MS (ESI): *m/z* (%) = 467.5 ([M + H]$^+$, 100).

4.2.27. Methyl 3β-[(aminosulfonyl)oxy]-olean-12-en-28-oate (29)

As described for **12**, compound **29** was obtained from **22** [39]; m.p. 128–129 °C; $[\alpha]_D$ = 73.69° (*c* = 0.3, $CHCl_3$); MS (ESI): *m/z* (%) = 548.3 ([M-H]$^-$, 96), 1098.2 ([2M–H]$^-$, 100).

4.2.28. Methyl 3β-{[[(aminocarbonyl)amino]sulfonyl]oxy}-olean-12-en-28-oate (30)

As described for **13**, this compound was obtained from **29** [45]; m.p. 77–82 °C; $[\alpha]_D$ = +79.2° (*c* = 0.28, $CHCl_3$); MS (ESI): *m/z* (%) = 91.5 ([M − H]$^-$, 95), 1183.1 ([2M − H]$^-$, 97).

4.2.29. Methyl 2,3-dihydro-1α,9α-peroxo-11-oxo-olean-12-en-28-oate (31)

This compound was synthesized according to literature from **28**; [37]; m.p. 160–165 °C; $[\alpha]_D$ = -19° (*c* = 1.7, $CHCl_3$); MS (ESI): *m/z* (%) = 497.4 ([M + H]$^+$, 25), 519.6 ([M + Na]$^+$, 100).

4.2.30. Methyl 3α-epoxy-11-oxo-olean-12-en-28-oate (32)

To a mixture of **28** (391 mg, 0.84 mmol) in DCM (40 mL) *m*-CPBA (550 mg, 3,19 mmol) was added, and the mixture was stirred at 25 °C for 2 h. Cold saturated solution of sodium bicarbonate (100 mL) was added, and the reaction mixture was extracted with DCM (2 × 100 mL). The solvent was evaporated under diminished pressure. The residue was subjected to chromatography (silica gel, hexane/ethyl acetate, 9:1) to afford **32** (227 mg, 55%) as an colorless solid; m.p. 205–207 °C; R_F = 0.60 (hexanes/ethyl acetate, 4:1); $[\alpha]_D$ = +70° (*c* = 3.1, $CHCl_3$); UV–Vis (methanol): λ_{max} (log ε) = 269 nm (4.00); IR (KBr): ν = 3424*br*, 2948*s*, 1720*s*, 1653*s*, 1576*m*, 1461*m*, 1438*m*, 1387*m*, 1366*m*, 1329*m*, 1308*m*, 1259*m*, 1236*m*, 1214*m*, 1169*m*, 1129*w*, 1064*w*, 1012*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.64 (*s*, 1H, H-12), 3.62 (*s*, 3H, OMe), 3.21–3.14 (*m*, 2H, H-2, H-1), 2.99 (*dd*, 1H, *J* = 13.8, 3.7 Hz, H-18), 3.05 (*dd*, 1H, *J* = 17.8, 5.9 Hz, H-1′), 2.80 (*d*, 1H, *J* = 3.7 Hz, H-3), 2.28 (*s*, 1H, H-9), 2.02 (*ddd*, 1H, *J* = 13.8, 13.8, 4.3 Hz, H-16), 1.74–1.66 (*m*, 2H, H-16′, H-22), 1.64–1.51 (*m*, 5H, H-6H-7, H-15, H-19, H-22′), 1.49–1.20 (*m*, 6H, H-6′, H-7′ H-15′, H-19′, H-21), 1.29 (*s*, 3H, H-27), 1.10 (*s*, 3H, H-25), 1.08 (*s*, 3H, H-23), 1.01 (*s*, 3H, H-26), 0.96–0.90 (*m*,

1H, H-5), 0.92 (*s*, 3H, H-30), 0.91 (*s*, 3H, H-29), 0.87 (*s*, 3H, H-24) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.9 (C11), 177.5 (C-28), 169.1 (C-13), 127.8 (C-12), 61.3 (C-3), 60.4 (C-9), 52.6 (C-2, H*C*-O), 51.8 (OMe), 46.6 (C-5), 46.2 (C-17), 44.8 (C-14), 44.2 (C-19), 43.5 (C-8), 41.6 (C-18), 40.6 (C-1), 36.1 (C-4), 33.7 (C-21), 32.8 (C-29), 32.6 (C-10), 32.0 (C-7), 31.5 (C-22), 30.6 (C-20), 28.2 (C-23), 27.7 (C-15), 23.4 (C-27), 23.4 (C-26), 22.9 (C-16), 22.0 (C-30), 18.2 (C-25), 18.1 (C-24), 17.8 (C-6) ppm; MS (ESI): *m/z* (%) = 483.5 ($[M+H]^+$, 100); anal. calcd. for $C_{31}H_{46}O_4$ (482.69): C, 77.14; H, 9.61; found: C, 76.91; H, 9.72.

4.2.31. Methyl 2β-bromo-3α-hydroxy-11-oxo-olean-12-en-28-oate (33)

To a solution of **32** (95 mg, 0.20 mmol) in THF (10 mL), HBr (47%, 100 mg) was added dropwise at 10 °C. After stirring for 10 min, the solvent was removed *in vacuo*, water (10 mL) was added, and the reaction mixture was extracted with DCM (2 × 20 mL). Chromatographic purification (silica gel, hexane/ethyl acetate, 4:1) gave **33** (85 mg, 77%) as an amorphous solid; R_F = 0.50 (hexanes/ethyl acetate, 4:1); $[\alpha]_D$ = +102° (*c* = 4.2, $CHCl_3$); UV–Vis (MeOH): λ_{max} (log ε) = 268 nm (4.08); IR (KBr): ν = 3447*br*, 2949*s*, 2869*m*, 1727*s*, 1657*s*, 1463*m*, 1388*m*, 1367*m*, 1329*w*, 1304*w*, 1262*m*, 1210*m*, 1164*m*, 1104*w*, 1080*w*, 1052*w*, 1032*w*, 1009*w*, 757*m* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.61 (*s*, 1H, H-12), 4.23 (*ddd*, 1H, *J* = 11.9, 8.9, 7.9 Hz, H-2), 3.80 (*d*, 1H, *J* = 12.2 Hz, H-3), 3.56 (*s*, 3H, OMe), 2.96 (*dd*, 1H, *J* = 13.7, 4.3 Hz, H-18), 2.79 (*dd*, 1H, *J* = 15.3, 11.0 Hz, H-1), 2.43 (*s*, 1H, H-9), 2.19 (*dd*, 1H, *J* = 15.3, 7.9 Hz, H-1′), 1.97 (*ddd*, 1H, *J* = 13.7, 13.7, 4.3 Hz, H-16), 1.70–1.61 (*m*, 2H, H-16′, H-22), 1.60–1.50 (*m*, 4H, H-7, H-15, H-19, H-22′), 1.45–1.39 (*m*, 2H, H-6), 1.31–1.11 (*m*, 5H, H-7′, H-15′, H-19′H-21), 1.28 (*s*, 3H, H-27), 1.21 (*s*, 3H, H-25), 1.02–0.96 (*m*, 1H, H-5), 1.00 (*s*, 3H, H-24), 0.88 (*s*, 3H, H-23), 0.87 (*s*, 3H, H-30), 0.87 (*s*, 3H, H-29), 0.80 (*s*, 3H, H-26) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 198.7 (C-11), 177.4 (C-28), 169.6 (C-13), 127.6 (C-12), 76.2 (C-2), 62.9 (C-9), 58.7 (C-3), 52.4 (C-1), 51.9 (OMe), 50.5 (C-5), 46.1 (C-17), 44.8 (C-14), 44.3 (C-19), 43.6 (C-8), 41.6 (C-18), 39.2 (C-4), 39.0 (C-10), 33.7 (C-21), 32.8 (C-29), 31.7 (C-7), 31.6 (C-22), 30.7 (C 20), 27.7 (C-15), 23.6 (C 27), 23.4 (C-23), 23.4 (C-24), 23.0 (C-30), 22.8 (C-16), 21.9 (C 25), 19.1 (C-6), 18.5 (C-26) ppm; MS (ESI): *m/z* (%) = 585.2 ($[M(^{79}Br)+Na]^+$, 94), 587.2 ($[M(^{81}Br)+Na]^+$, 100); anal. calcd. for $C_{31}H_{47}BrO_4$ (563.61): C, 66.06; H, 8.41; found: C, 65.83; H, 8.62.

4.2.32. Methyl 2β-bromo-3,11-dioxo-olean-12-en-28-oate (34)

To a solution of **33** (84 mg, 0.15 mmol) in acetone (10 mL) at 0 °C CrO_3 (40 mg, 0.40 mmol) in sulfuric acid (2 M, 0.28 mL) was added. The mixture was stirred for 30 min and then evaporated to dryness. Saturated potassium disulfite solution (10 mL) and saturated sodium bicarbonate solution (10 mL) were added, and the mixture was extracted with DCM (2 × 20 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4, 10 mL), filtered and evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 4:1) to yield **34** (45 mg, 53%) as a colorless solid; m.p. 221–223 °C; R_F = 0.36 (hexane/ ethyl acetate, 4:1); $[\alpha]_D$ = +121° (*c* = 5.1, $CHCl_3$); UV–Vis (methanol): λ_{max} (log ε) = 269 nm (4.08); IR (KBr): ν = 2950*s*, 2868*m*, 1728*s*, 1659*s*, 1461*m*, 1388*m*, 1366*w*, 1329*w*, 1262*w*, 1190*w*, 1163*m*, 1125*w*, 1062*w*, 1012*w* cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.70 (*s*, 1H, H-12), 5.05 (*dd*, 1H, *J* = 10.4, 10.4 Hz, H-2), 3.62 (*s*, 3H, OMe), 3.04 (*dd*, 1H, *J* = 13.4, 4.3 Hz, H-18), 2.89 (*dd*, 1H, *J* = 14.7, 10.4 Hz, H-1), 2.59 (*s*, 1H, H-9), 2.53 (*dd*, 1H, *J* = 15.0, 11.0 Hz, H-1′), 2.06 (*ddd*, 1H, *J* = 14.4, 13.6, 4.8 Hz, H-16), 1.74–1.47 (*m*, 9H, H-5, H-6, H-7, H-15, H-16′, H-19, H-22), 1.41–1.33 (*m*, 2H, H-7′, H-21), 1.38 (*s*, 3H, H-27), 1.25–1.09 (*m*, 3H, H-15′, H-19′, H-21′), 1.15 (*s*, 3H, H-25), 1.13 (*s*, 3H, H-23), 1.04 (*s*, 3H, H-26), 0.93 (*s*, 3H, H-30), 0.93 (*s*, 3H, H-29), 0.87 (*s*, 3H, H-24) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 208.6 (C-3), 198.1 (C-11), 177.3 (C-28), 169.8 (C-13), 127.5 (C-12), 65.8 (C-9), 54.2 (C-1,*C*H2), 52.2 (C-5), 51.9 (OMe), 51.0 (C-2, H*C*-Br), 47.5 (C-4), 46.1 (C-17), 44.6 (C-14), 44.3 (C-19), 43.7 (C-8), 41.6 (C-18), 38.5 (C-10), 33.6 (C-21), 32.3 (C-29), 31.5 (C-7), 31.5 (C-22), 30.7 (C-20), 29.3 (C-23), 27.8 (C-15), 23.6 (C-27), 23.4 (C-30), 22.8 (C-16), 20.0 (C-25), 19.1 (C-6), 19.0 (C-26), 18.1 (C-24) ppm; MS (ESI): *m/z* (%) = 561.4 ($[M(^{79}Br)+H]^+$, 93), 563.4 ($[M(^{81}Br)+H]^+$, 100), 583.3 ($[M(^{79}Br)+Na]^+$, 34), 585.2 ($[M(^{81}Br)+Na]^+$, 40); anal. calcd. for $C_{31}H_{45}BrO_4$ (561.59): C, 66.30; H, 8.08; found: C, 66.11; H, 8.26.

4.3. Enzymatic studies

4.3.1. Spectrometer and chemicals

The Ellman assays were performed as previously described [35] using a TECAN SpectraFluorPlus working in the kinetic mode and measuring the absorbance at λ = 415 nm was used for the enzymatic studies. Acetylcholinesterase (from *Electrophorus electricus*), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and acetylthiocholine iodide were purchased from Fluka. Butyrylcholinesterase (from equine serum) was purchased from Sigma, and butyrylthiocholine iodide was bought from Aldrich. Details can be found in the Supplementary Material.

5. Modelling

A detailed description can be found in the Supplementary Material.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.12.013.

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