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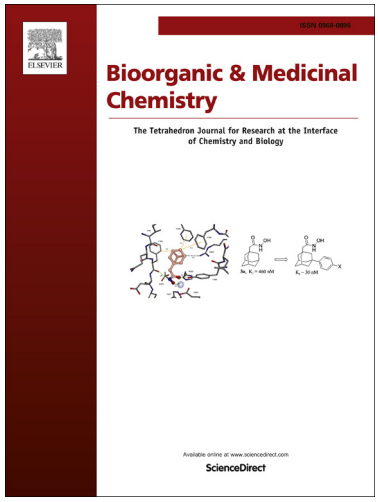

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Graphical abstract:

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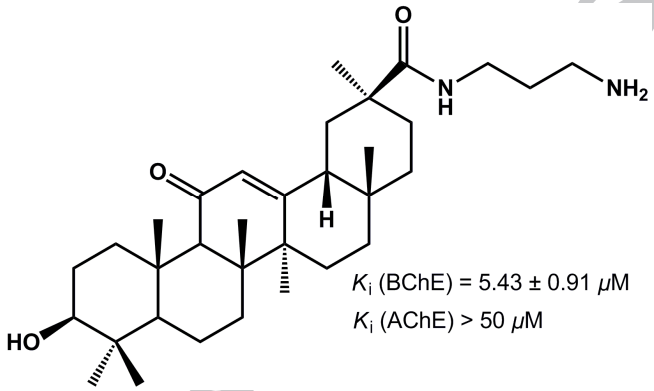

Amino derivatives of glycyrrhetinic acid as potential inhibitors of cholinesterases

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Abstract:

The development of remedies against the Alzheimer's disease (AD) is one of the biggest challenges in medicinal chemistry nowadays. Although not completely understood, there are several strategies fighting this disease or at least bringing some relief. During the progress of AD, the level of acetylcholine (ACh) decreases; hence, a therapy using inhibitors should be of some benefit to the patients. Drugs presently used for the treatment of AD inhibit the two ACh controlling enzymes, acetylcholinesterase as well as butyrylcholinesterase; hence, the design of selective inhibitors is called for. Glycyrrhetinic acid seems to be an interesting starting point for the development of selective inhibitors. Although its glycon, glycyrrhizinic acid is known for being an AChE activator, several derivatives, altered in position C-3 and C-30, exhibited remarkable inhibition constants in micro-molar range. Furthermore, five representative compounds were subjected to three more enzyme assays (on carbonic anhydrase II, papain and the lipase from *Candida antarctica*) to gain information about the selectivity of the compounds in comparison to other enzymes. In addition, photometric sulforhodamine B assays using murine embryonic fibroblasts (NiH 3T3) were performed to study the cytotoxicity of these compounds. Two derivatives, bearing either a 1,3-diaminopropyl or a 1*H*-benzotriazolyl residue, showed a BChE selective inhibition in the single-digit micro-molar range without being cytotoxic up to 30 µM. *In silico* molecular docking studies on the active sites of AChE and BChE were performed to gain a molecular insight into the mode of action of these compounds and to explain the pronounced selectivity for BChE.

Keywords: Glycyrrhetinic acid, Ellman's test, cholinesterase inhibitors, docking studies

1. Introduction

Leading a healthy life is *the* aim for most of the people living in the western hemisphere. However, a long lifespan as well as modern living standards go along with an increase of so-called "lifestyle diseases", *e.g.* cancer or cardiovascular problems. Neurodegenerative diseases, especially dementia gain more and more of importance in ageing societies. Especially the number of patients suffering from Alzheimer's disease (AD) increased continuously during the last decades. Meanwhile AD is regarded the most common illness of over-60-year old people[1].

One characteristic feature of AD is a decreased level of the neurotransmitter acetylcholine (ACh). This results in a decline in memory and recognition[2-4]. Usually, the level of ACh is controlled by the enzyme acetylcholinesterase (AChE, E.C. 3.1.1.7), which cleaves the transmitter in the postsynaptic area. Another enzyme, butyrylcholinesterase (BChE, E.C. 3.1.1.8) is also able to hydrolyze ACh, albeit it doesn't possess the same affinity for the transmitter as AChE[5] does. Less attention has been paid to this fact for a long time, since AChE and not BChE is the predominant ACh cleaving enzyme in the human brain[6]. Thus, drugs already introduced into an emerging market for the treatment of AD are non-selective (rivastigmine[7]) or AChE-selective inhibitors (galantamine[8] or donepezil[9, 10]). However, those drugs retard the progress of AD only in a very early stage; during the progress of AD, the loss of AChE-activity is compensated by BChE[11, 12]. Thus, the BChE/AChE-ratio changes from 0.2 in normal human brains to approximately 11 in AD brains[13]. Furthermore, a correlation between BChE-activity and the maturation of β-amyloid plaques has been suggested [14]. Consequently, the influence and role of BChE in AD and its progress is still under investigation[15].

There is growing academic interest in investigating pentacyclic triterpenoic acids as drugs for the treatment of AD in

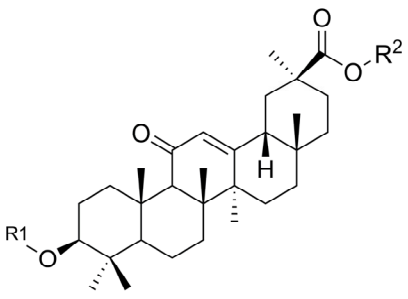
general and for the inhibition of cholinesterases in particular. Triterpenes possessing a hopyl[16], lanostyl[17] or lupyl[18] skeleton were shown to be inhibitors of AChE, and Mehmood *et al*. extracted triterpenes of the amyryl type that inhibited BChE on a micro-molar range[19]. Ursolic acid and its derivatives were determined to be potent cholinesterase inhibitors[18, 20]. Especially ursolic acid inhibited AChE in a mixed-type inhibition to an amount being of the same magnitude as the well-established drug tacrine[21]. Although glycyrrhetinic acid is well known for its anti-inflammatory[22, 23] and anti-viral[24, 25] activities, it was unforeseen that this triterpenoid might demonstrate a potential in acting as an inhibitor of cholinesterases. Glycyrrhizinc acid, the glycone and natural form of glycyrrhetinic acid, was determined to act as activator of AChE instead of being an inhibitor[26]. Other oleanolic-type triterpenes were neuroprotective (oleanolic acid) [27] as well as AChE inhibitors (*e.g.* oleanolic acid[28, 29], taraxerol[30] or kalopanaxsaponin A/B[31]) possessing IC_{50} values comparable to those of donepezil (lancemaside A and echinocystic acid[32]). Thus, a series of glycyrrhetinic acid derivatives differing in the substitution pattern at positions C-3 and C-30 were prepared and screened for their inhibitory action against AChE and BChE using Ellman's assay. Five representative compounds were selected and investigated for their ability to act as an inhibitor for the lipase from *candida antarctica* (a serine hydrolase), papain (a sulfhydryl enzyme) and carbonic anhydrase II (a metalloenzyme) to gain information about the selectivity of the compounds towards other enzymes. In addition, preliminary toxicity studies were performed employing murine embryonic fibroblasts (NiH 3T3) in photometric sulforhodamine B assays (SRB).

2. Results and discussion

2.1. Chemistry

The first group of compounds (**1**-**27**, Scheme 1) was synthesized *via* STEGLICH esterification[33] in DCM at room temperature using boc protected amino acids. These conditions turned out to be appropriate to esterify triterpenoic acids with amino acids while avoiding an amino acid racemization[34]. De-protection of these compounds was performed in almost quantitative yields following standard procedures using either trifluoroacetic acid or a solution of dry hydrogen chloride in DCM [35, 36].

1: R^1 = Gly-HCl, R^2 = H
2: R^1 = Gly-HCl, R^2 = Me
3: R^1 = L-Ala-HCl, R^2 = H
4: R^1 = L-Ala-HCl, R^2 = Me
5: R^1 = L-Ala, R^2 = Me
6: R^1 = D-Ala, R^2 = Me
7: R^1 = L-Val, R^2 = Me
8: R^1 = L-Ile, R^2 = Me
9: R^1 = L-Leu, R^2 = Me
10: R^1 = L-Pro, R^2 = Me
11: R^1 = L-Phe, R^2 = Me
12: R^1 = boc-L-Phe, R^2 = Et
13: R^1 = L-Phe, R^2 = Et

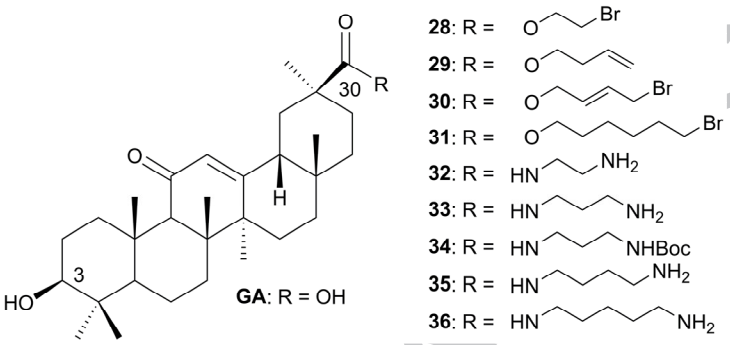


14: R^1 = Sar, R^2 = Me
15: R^1 = boc-D-Trp, R^2 = Et
16: R^1 = D-Trp, R^2 = Et
17: R^1 = boc-L-Cys(SBn), R^2 = Me
18: R^1 = L-Cys(SBn), R^2 = Me
19: R^1 = L-Dab, R^2 = Me
20: R^1 = L-Orn, R^2 = Me
21: R^1 = L-Lys, R^2 = Me
22: R^1 = L-Glu-HCl, R^2 = Me
23: R^1 = L-Glu(OBn), R^2 = Me
24: R^1 = L-Glu(OMe), R^2 = Me
25: R^1 = L-Asp-HCl, R^2 = Me
26: R^1 = L-Asp(OBn), R^2 = Me
27: R^1 = L-Asp(OMe), R^2 = Me

Scheme 1. Structure of the aminoacid derivatives **1**-**27** (Sar corresponds to a sarcosine residue, Dab stands for a 2,4-diamino-butyric acid moiety).

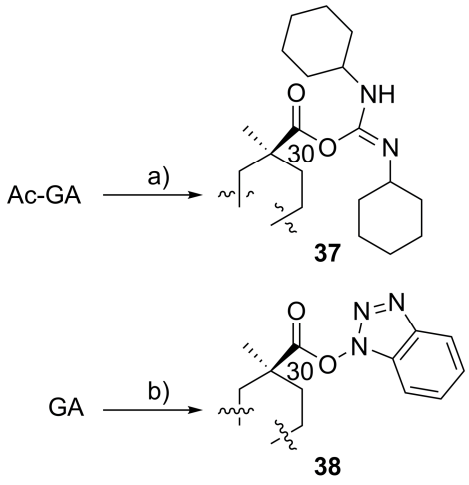
The synthesis of the second group of compounds (**28**-**31**, Scheme 2) started from potassium glycyrrhetinate (from the reaction of glycyrrhetinic acid **GA** with potassium carbonate in dry DMF) that was treated with an excess of the respective bromide[37, 38]. The esters were obtained with yields ranging between 58 % and 79 %.

Likewise, the preparation of the amides (**32**-**36**, Scheme 2) was performed starting from glycyrrhetinic acid by its reaction with DCC and 1-hydroxybenzotriazole in a 2:1-mixture of dry DCM and dry DMF at room temperature[37]; by this procedure reasonable yields (up to 54 %) of the products were obtained. Furthermore, the use of an excess of the respective α,ω-alkyl-diamine allowed a direct coupling without additional protection of position 3.



Scheme 2. Structures glycyrrhetinic acid (**GA**), esters **28**-**31** and amides **32**-**36**.

In addition, two derivatives bearing a bulky moiety in position 30 (Scheme 3) were synthesized. Acetylation of **GA** gave 3-*O*-acetyl-GA (**AcGA**) whose reaction with DCC gave quite nicely the corresponding *O*-acyl isoureate of 3-acetyl glycyrrhetinic acid **37**. Similarly, the 1*H*-benzotriazolyl ester (**38**) could be obtained from **GA**, DCC and 1-hydroxybenzotriazol in dry DCM at room temperature; no prior protection of HO-C(3) was required.



Scheme 3. Synthesis of the derivatives **37** and **38**: a) DCC, DCM/DMF, r.t., 12 h; b) DCC, 1-hydroxybenzotriazole, DCM, r.t., 24 h.

Compounds **37** and **38** could be characterized by their 1D and 2D ^{1}H and ^{13}C NMR spectra as well as by their ESI mass spectra (*m/z* = 719 for **37** and *m/z* = 587 for **38**); the isotopic pattern of the quasimolecular ions agreed perfectly with the calculated values. Esterification of position 30 resulted in a shift of the signal of C(30) as expected ($\Delta\delta$ = 6 ppm) to higher fields.

2.2. Biology

Glycyrrhitinic acid, galantamine hydrobromide as well as compounds **1**-**36** were subjected to Ellman's assays to determine their inhibitory activity towards AChE and BChE. The results from these measurements are compiled in Table 1.

Table 1

Compounds **1**-**38**, except those being not soluble under the conditions of the assay, are competitive inhibitors of AChE and BChE. For the amino acid substituted derivatives **3**-**27**, inhibition constants as low as K_i = 11.73 ± 1.79 µM (compound **16**) were obtained. Hence, this substance has a 22-times lower activity on AChE than galantamine possessing a K_i of 0.54 ± 0.01 µM. As compiled in Table 1, many of these aminoacid-derived compounds exhibited inhibitory constants in double-digit micro-molar range.

For none of the compounds, K_i values smaller than 100 µM could be determined. The selectivity (F) for AChE, [expressed by the quotient of K_i (AChE) / K_i (BChE)] is similar to galantamine hydrobromide (*cf.* **16** (F < 0.12) and galantamine hydrobromide: F = 0.06).

The esters **28**-**31**, representing a second group of compounds, gave similar results. Inhibitory constants of K_i = 13.02 to 29.63 µM for AChE were determined, while no K_i was detectable below 100 µM for BChE. As a consequence, selectivities ranging between F < 0.74 and F < 0.13 were obtained.

With the exception of **33**, none of the compounds of a third group (**32**-**38**) showed any remarkable activities for both enzymes. Compound **33**, however, represents the first derivative possessing not only an inhibitory constant in micro-molar range (5.43 ± 0.91 µM) for BChE, but also a K_i above 100 µM for AChE. Accordingly, selectivity concerning BChE was established, expressed by a selectivity factor of F > 18.42. While compound **37** was no good inhibitor at all, **38** was the second compound of this series exhibiting an inhibitory constant in the single-digit micro-molar range (K_i = 9.81 ± 0.32 µM) as well as a selectivity of F > 2.04 in favour of BChE.

In addition to these cholinesterase assays, five representative compounds were subjected to assays employing three other enzymes – the lipase from *Candida antarctica*, papain from *Carica papaya* and carbonic anhydrase II from bovine erythrocytes; each of these enzymes stands for a different mechanism taking place in the active site of the enzyme. From these experiments some information about the general selectivity of the inhibitors can be expected. Five derivatives (**19**, **23**, **31**, **33** and **38**) were selected and tested for their inhibitory action on these enzymes (using a concentration of 50 µM, except for **38** that was tested using a 10 µM concentration due to its limited solubility). The results from these studies are compiled in Table 2.

Table 2

None of these compounds showed any inhibitory action on papain. Almost the same results were obtained for the bovine carbonic anhydrase II (bCA II); the compounds did not inhibit this enzyme, except for compounds **23** and **38** being weak inhibitors (15 % inhibition for **23** and 27 % for **38**). All of the compounds, however, were inhibitors for the lipase from *C. antarctica*. This lipase is a serine hydrolase like the cholinesterases. With exception of compound **31**, inhibition of the fungal lipase was weaker than that of the cholinesterases. Thus, compounds **33** and **38** showed a reasonably high BChE/lipase and BChE/AChE-selectivity.

The same compounds were subjected to a sulforhodamine B assay using murine embryonic fibroblasts (NiH 3T3) to obtain IC_{50} values as an indicator for their cytotoxicity (Table 3).

Table 3

The AChE inhibitors **19**, **23** and **31** differ in their cytotoxicity: While compound **23** was not cytotoxic at concentrations lower than 30 μM (cut-off of the assay), compounds **31** and **19** showed IC_{50} values in a micro-molar range (for **31**: IC_{50} = 3.05 ± 0.17 μM; for **19**: IC_{50} = 19.73 ± 1.02 μM). Both BChE inhibitors **33** and **38**, however, did not show any cytotoxicity at concentrations lower than 30 μM.

2.3. Docking Studies

In order to have a molecular insight on the mode of action of **33** and **38** as BChE inhibitors and their selectivity towards AChE we performed *in silico* molecular docking studies in the active site of both enzymes, using GOLD 5.1 software[39]. The coordinates of the enzyme structure were obtained from Protein Data Bank selecting the structures with accession codes 1P0I and 4BDT, for BChE and AChE, respectively. AChE and BChE have a common catalytic triad, Ser-His-Glu, and for both enzymes the triad is located at the bottom of a deep and narrow gorge where the Ser residue acts as a nucleophile to attack the carbonyl groups of substrates or of pseudo-substrate inhibitors[40].

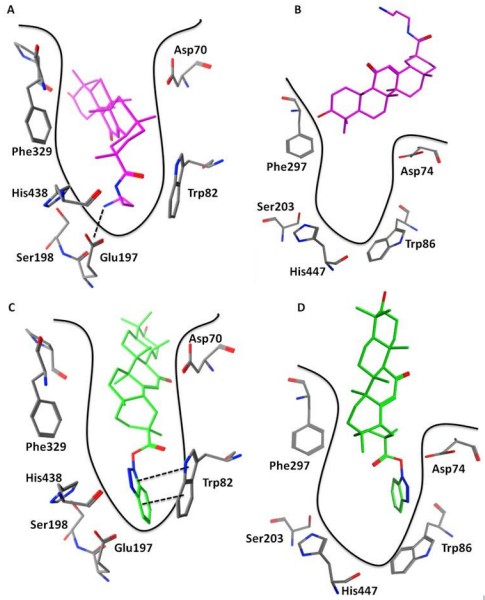


Figure 1. Illustration of the results from the docking calculations for compounds **33** (A: on the BChE gorge, B: on the AChE gorge) and **38** (C: on the BChE gorge, D: on the AChE gorge).

The docked poses for compounds **33** and **38** in BChE (A and C Figure 1) and AChE (B and D, Figure 1) showed for BChE the inhibitors very well accommodated in the enzyme gorge allowing compound **33** (A) to establish an ionic interaction between the terminal protonated amine with Glu197 and for compound **38** (C) the benzotriazole adopted a parallel fitting with Trp82 leading to a π-π interaction within the enzyme's active-site. On the other hand when these compounds were docked into the AChE active site, the compounds did not fit properly into the AChE gorge. While for compound **33** (B) the preferred pose sat on the entrance of the gorge and no interactions were observed with important residues, for compound **38** (D) the benzotriazole moiety was inside the gorge but still no interactions were observed with essential residues. Hence, the presence of interactions with residues in the bottom of the gorge observed for compounds **33** and **38** seem mandatory for a better recognition and/or to block the action of the catalytic triad and thus for an increased inhibitory activity.

3. Conclusions

In this study 36 derivatives of glycyrrhetinic acid, altered in positions 3 and/or 30, were measured in Ellman's assays employing acetyl- and butyrylcholinesterases. Nearly half of these compounds exhibited competitive inhibition of either AChE or BChE showing inhibition constants K_i = 5.4 to 41.8 μM. Two derivatives (**33** and **38**) inhibited BChE selectively with K_i = 5.43 μM (for **33**) and K_i = 9.81 μM (for **38**) as well as selectivity factors of F > 18.42 (for **33**) and F > 2.04 (for **38**). Thus, both compounds represent the first derivatives of a triterpenoic acid showing a selective inhibition of BChE as compared to AChE. Docking studies revealed interactions (for **33**: ionic interaction with Glu197, for **38**: π-π interaction with Trp82) between the compounds and the active site of BChE. In addition, both of these derivatives did not fit properly into the AChE gorge, hence giving an explanation for the observed BChE/AChE selectivity. Furthermore, **33** and **38** were subjected to three more enzyme assay using bCA II, a lipase and papain. The results from these experiments emphasized the observed selectivity of compounds for BChE. In addition, neither **33** nor

38 showed any cytotoxic effect on NiH 3T3 cells.

In conclusion, glycyrrhetinic acid derivatives represent a novel class of possible cholinesterase inhibitors with inhibitions constants in the micro-molar range. Two of these compounds were selective BChE inhibitors thus being possible leads for further investigations and, consequently, for new therapeutic approaches for the treatment of AD.

4. Experimental

4.1. Chemistry

4.1.1 General

Reagents were bought from commercial suppliers without any further purification. NMR spectra were measured on Varian Gemini 2000 or Unity 500 spectrometers at 27 °C with trimethylsilane as an internal standard, δ are given in ppm and *J* in Hertz. Mass spectra were taken on a Finnigan MAT TSQ 7000 (electronspray, voltage 4.5 kV, sheath gas nitrogen) instrument. IR spectra were recorded on a Perkin–Elmer FT-IR spectrometer Spectrum 1000, optical rotations on a Perkin–Elmer 341 polarimeter (1 cm micro cell) and UV–vis spectra on a Perkin–Elmer unit, Lambda 14. Melting points were measured with a Leica hot stage microscope and are uncorrected. Elemental analysis was done on a Foss-Heraeus Vario EL unit. TLC was performed on silica gel (Merck 5554, detection by UV absorption). Solvents were dried before use according to usual procedures.

The following compounds were synthesized as previously published: **1** to **6**[38], **7** to **11**, **14** and **19** to **21**[36], **22** to **27**[35], **31** to **33** and **35** to **38**[37]. The purity of the compounds was checked by HPLC (> 98 %).

4.1.2 General method for esterifications at position C(3) (method A)

The starting material (1 equiv) was dissolved in dry DCM, DMAP (20 mg, 0.16 mmol) and the protected aminoacid (1.2 equiv) were added. After addition of DCC (1.2 equiv), the mixture was stirred at room temperature for 12 h, filtered, and the filtrate was washed with water and brine, dried over sodium sulfate, filtered, and the solvents were evaporated. Purification was performed by flash chromatography (silica gel, chloroform/ether, 9:1).

4.1.3 General method for deprotection (method B)

To a solution of the Boc-protected compound in dry DCM, trifluoroacetic acid (1 mL per 10 mL DCM) was added. The mixture was allowed to stir at room temperature for 12 h. After completion of the reaction (as monitored by TLC) the solution was washed with a satd aqueous solution of sodium hydrogen carbonate. The aqueous layer was extracted with DCM, the combined organic extracts were washed with brine, dried over sodium sulfate, filtrated and evaporated to yield the amine.

4.1.4 General method for amidation (method C)

GA (1 equiv), DCC (1.1 equiv) and 1-hydroxybenzotriazole (1.2 equiv) were dissolved in 8 mL of dry DMF and stirred at room temperature for 1 h. The alkyl-1,ω-diamine (2 equiv), dissolved in 1 mL of dry DMF, was added, and stirring at room temperature was continued for 14 h. The mixture was poured into ice-cooled water; the precipitate was collected, dried in vacuum and subjected to column chromatography (silica gel, methanol) to yield the product.

4.1.5.1. Ethyl (3β) 3-([*N*-Boc-L-phenylalanyl]oxy)-11-oxo-olean-12-en-30-oate (12)

Compound **12** was obtained from methyl glycyrrhetinate (518 mg, 1.09 mmol) by method A as a colorless powder, using boc-L-phenylalanine (348 mg, 1.31 mmol) and DCC (270 mg, 1.31 mmol); yield: 769 mg, 99 %; m.p. 90-92 °C; R_F = 0.66 (hexane/ethyl acetate, 7:3); $[\alpha]_D$ = 89.08° (*c* 0.48, $CHCl_3$); UV-Vis (MeOH): λ_{max} (log ε) = 267 nm (4.09); IR (KBr): ν = 3446 (br), 2976 (s), 1725 (s), 1661 (s), 1498 (m), 1456 (m), 1389 (m), 1366 (m), 1249 (m), 1216 (m), 1173 (s), 1086 (w), 1020 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.28 (m, 2H, Ph-H-3 and Ph-H-5), 7.22 (m, 1H, Ph-H-4), 7.17 (m, 2H, Ph-H-2 and Ph-H-5), 5.64 (s, 1H, H-12), 4.88 (d, 1H, Phe-CHN*H*, *J* = 8.7 Hz), 4.55 (m, 1H, Phe-C*H*NH), 4.52 (dd, 1H, H-3, *J* = 10.8, 5.6 Hz), 4.18 (dq, 1H, Et-C*H*H′, *J* = 10.8, 7.2 Hz), 4.12 (dq, 1H, Et-CH*H′*, *J* = 10.8, 7.2 Hz), 3.10 (m, 1H, Phe-C*H*H′), 3.04 (m, 1H, Phe-CH*H′*), 2.79 (m, 1H, H-1), 2.34 (s, 1H, H-9), 2.09 (dd, 1H, H-18, *J* = 13.3, 3.5 Hz), 2.02 (ddd, 1H, H-15, *J* = 13.7, 13.7, 4.6 Hz), 1.98 (m, 1H, H-21), 1.92 (ddd, 1H, H-19, *J* = 13.4, 4.0, 2.7 Hz), 1.81 (ddd, 1H, H-16, *J* = 13.4, 13.4, 4.3 Hz), 1.62 (m, 1H, H-7), 1.60 (dd, 1H, H-19′, *J* = 13.4, 13.4 Hz), 1.56 (m, 1H, H-6), 1.42 - 1.37 (br, 3H, H-6′ and H-7′ and H-22), 1.39 (s, 9H, Boc-CH_3), 1.35 (s, 3H, H-27), 1.32 (m, 1H, H-22′), 1.31 (m, 1H, H-21′), 1.25 (t, 3H, $COOCH_2CH_3$, *J* = 7.2 Hz), 1.17 (m, 1H, H-16′), 1.13 (s, 6H, H-28 and H-25), 1.11 (s, 3H, H-26), 1.03 (m, 1H, H-1′), 1.00 (m, 1H, H-15′), 0.80 (s, 3H, H-23), 0.80 (m, 1H, H-5), 0.79 (s, 6H, H-24 and H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.3 (C-30), 171.8 (COO), 169.3 (C-13), 155.0 (Boc-COO), 136.1 (Ph-C-1), 129.3 (Ph-C-2, Ph-C-6), 128.6 (C-12), 128.5 (Ph-C-3), 128.4 (Ph-C-5), 126.9 (Ph-C-4), 82.1 (C-3), 79.7 (Boc-*q*.C), 61.7 (C-9), 60.3 (Et-CH_2), 55.0 (C-5), 54.6 (Phe-CHNH), 48.4 (C-18), 45.4 (C-14), 43.8 (C-20), 43.2 (C-8), 41.0 (C-19), 38.7 (C-1), 38.4 (Phe-CH_2), 38.0 (C-10), 37.7 (C-22), 36.9 (C-4), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (Boc-CH_3), 26.5 (C-16), 26.4 (C-15), 23.4 (C-2), 23.3 (C-27), 18.6 (C-26), 17.3 (C-6), 16.7 (C-24), 16.3 (C-25), 14.3 (Et-CH_3) ppm; MS (ESI, MeOH): *m/z* (%) = 746.1 ($[M+H]^+$, 28), 768.5 ($[M+Na]^+$, 100); analysis calculated for $C_{46}H_{67}NO_7$ (746.03): C 74.06, H 9.05, N 1.88; found: C 73.91, H 9.14, N 1.72.

4.1.5.2. Ethyl (3β) 3-([L-phenylalanyl]oxy)-11-oxo-olean-12-en-30-oate (13)

Compound **13** was obtained from compound **12** (622 mg, 0.83 mmol) by method B as a colorless powder; yield: 480 mg, 90 %. m.p. 171-175 °C; R_F = 0.40 (DCM/MeOH, 9:1); $[\alpha]_D$ = 113.50° (*c* 0.51, $CHCl_3$); UV-Vis (MeOH): λ_{max} (log ε) = 267 nm (4.07); IR (KBr): ν = 3432 (br), 2950 (s), 2362 (w), 1727 (s), 1655 (s), 1455 (m), 1388 (w), 1324 (w), 1215 (m), 1176 (m), 1086 (w), 1021 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.28 (m, 2H, Ph-H-2 and Ph-H-6), 7.22 (m, 1H, Ph-H-4), 7.20 (m, 2H, Ph-H-3 and Ph-H-5), 5.63 (s, 1H, H-12), 4.54 (dd, 1H, H-3, *J* = 11.6, 5.0 Hz), 4.17 (dq, 1H, Et-C*H*H′, *J* = 10.8, 7.1 Hz), 4.11 (dq, 1H, Et-CH*H′*, *J* = 10.8, 7.1 Hz), 3.71 (dd, 1H, Phe-C*H*NH_2, *J* = 8.3, 5.4 Hz), 3.13 (dd, 1H, Phe-C*H*H′, *J* = 13.3, 5.4 Hz), 2.80 (m, 1H, H-1), 2.78 (m, 1H, Phe-CH*H′*), 2.34 (s, 1H, H-9), 2.09 (dd, 1H, H-18, *J* = 13.7, 3.3), 2.02 (ddd, 1H, H-15, *J* = 13.3, 13.3, 4.2 Hz), 1.97 (m, 1H, H-21), 1.91 (ddd, 1H, H-19, *J* = 13.7, 4.2, 2.5 Hz), 1.81 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.6 Hz), 1.69 (m, 1H, H-2), 1.63 (m, 1H, H-7), 1.59 (dd, 1H, H-19′, *J* = 13.3, 13.3 Hz), 1.57 (m, 1H, H-2′), 1.45 (m, 1H, H-6′), 1.40 (m, 1H, H-7′), 1.37 (m, 1H, H-22), 1.35 (s, 3H, H-27), 1.31 (m, 1H, H-22′), 1.30 (m, 1H, H- 21′), 1.25 (t, 3H, Et-CH_3, *J* = 7.1), 1.16 (m, 1H, H-16′), 1.15 (s, 3H, H-25), 1.13 (s, 3H, H-28), 1.11 (s, 3H, H-26), 1.04 (ddd, 1H, H-1′, *J* = 13.7, 13.7, 4.2 Hz), 1.00 (m, 1H, H-15′), 0.85 (s, 3H, H-24), 0.79 (m, 1H, H-5), 0.79 (s, 6H, H-23 and H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.3 (C-30), 174.7 (Phe-COO), 169.4 (C-13), 137.5 (Ph-C-1), 129.3 (Ph-C-3 and Ph-C-5), 128.5 (Ph-C-2 and Ph-C-6), 128.4 (C-12), 126.7 (Ph-C-4), 81.4 (C-3), 61.7 (C-9), 60.3 (Et-CH_2), 56.2 (Phe-CNH), 55.0 (C-5), 48.4 (C-18), 45.4 (C-14), 43.8 (C-20), 43.2 (C-8), 41.3 (Phe-CH_2), 41.1 (C-19), 38.7 (C-1), 38.1 (C-10), 37.7 (C-22), 36.9 (C-4), 32.7 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-23), 28.0 (C-28), 26.5 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.3 (C-25), 14.3 (Et-CH_3) ppm; MS (ESI, MeOH): *m/z* = 646.5 ($[M+H]^+$, 100); analysis calculated for

$C_{41}H_{59}NO_5$ (645.91): C 76.24, H 9.21, N 2.17; found: 76.10, H 9.37, N 2.09.

4.1.5.3. Ethyl (3β) 3-([Boc-D-tryptophanyl]oxy)-11-oxo-olean-12-en-30-oate (15)

Compound **15** was obtained from **GA-Et** (466 mg, 0.94 mmol) by method A as a colorless powder, using boc-D-tryptophane (230 mg, 1.13 mmol) and DCC (233 mg, 1.13 mmol); m.p. 106-110 °C; R_F = 0.49 (hexane/ethyl acetate, 7:3); $[\alpha]_D$ = 98.62° (*c* 0.52, $CHCl_3$); UV-Vis (MeOH): λ_{max} (log ε) = 241 nm (4.62), 269 nm (4.22); IR (KBr): ν = 3386 (br), 2976 (s), 1726 (s), 1659 (s), 1498 (m), 1458 (m), 1390 (m), 1366 (m), 1251 (m), 1216 (s), 1173 (s), 1086 (m), 1049 (m), 1020 (m), 987 (m), 740 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.09 (br, 1H, Trp-NH), 7.60 (d, 1H, indole-7, *J* = 7.8 Hz), 7.33 (d, 1H, indole-4, *J* = 8.1 Hz), 7.17 (dd, 1H, indole-5, *J* = 8.1, 7.8 Hz), 7.10 (dd, 1H, indole-6, *J* = 7.6, 7.6 Hz), 7.00 (s, 1H, indole-2), 5.63 (s, 1H, H-12), 5.08 (d, 1H, Trp-C*H*NH, *J* = 7.7 Hz), 4.65 (m, 1H, Trp-CHN*H*), 4.45 (dd, 1H, H-3, *J* = 9.8, 6.7 Hz), 4.18 (dq, 1H, Et-C*H*H′, *J* = 10.7, 7.2 Hz), 4.12 (dq, 1H, Et-CH*H′*, *J* = 10.7, 7.2 Hz), 3.27 (m, 2H, Trp-CH_2), 2.75 (ddd, 1H, H-1, *J* = 13.4, 3.4, 3.4 Hz), 2.33 (s, 1H, H-9), 2.10 (dd, 1H, H-18, *J* = 12.9, 3.6 Hz), 2.02 (m, 1H, H-15), 1.99 (m, 1H, H-21), 1.92 (ddd, 1H, H-19, *J* = 13.6, 3.8, 2.5 Hz), 1.81 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.6 Hz), 1.64 (m, 1H, H-7), 1.60 (dd, 1H, H-19′, *J* = 13.5, 13.5 Hz), 1.52 (m, 1H, H-6), 1.50 (m, 1H, H-2), 1.43 (s, 12H, Boc-CH_3 and H-28), 1.41 (m, 1H, H-6′), 1.38 (m, 1H, H-7′), 1.36 (m, 1H, H-22), 1.35 (s, 3H, H-27), 1.34 (m, 1H, H- 22′), 1.30 (m, 1H, H-21′), 1.26 (t, 3H, Et-CH_3, *J* = 7.2), 1.17 (m, 1H, H-16′), 1.14 (s, 3H, H-23), 1.11 (s, 6H, H-25 and H-26), 1.00 (m, 1H, H-15′), 0.98 (m, 1H, H-1′), 0.80 (s, 3H, H-29), 0.79 (m, 1H, H-5), 0.77 (s, 3H, H-24) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 176.4 (C-30), 172.1 (Trp-COO), 169.5 (C-13), 153.1 (Boc-COO), 136.1 (indole-*q*.C), 128.4 (C-12), 128.3 (indole-*q*.C), 122.6 (indole-2), 122.1 (indole-5), 119.5 (indole-6), 119.1 (indole-7), 111.0 (indole-4), 110.5 (indole-*q*.C), 81.9 (C-3), 79.6 (Boc-*q*.C), 61.6 (C-9), 60.4 (Et-CH_2), 55.0 (C-5), 54.3 (Trp-CHNH), 48.4 (C-18), 45.4 (C-14), 43.8 (C-20), 43.2 (C-8), 41.0 (C-19), 38.6 (C-1), 38.0 (C-10), 37.7 (C-22), 36.8 (C-4), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-29), 28.3 (C-28), 28.3 (C-23), 27.9 (Trp-CH_2), 26.4 (C-16), 26.4 (C-15), 23.3 (C-27), 23.2 (C-2), 18.6 (C-26), 17.3 (C-6), 16.5 (C-24), 16.3 (C-25), 14.3 (Et-CH_3) ppm; MS (ESI, MeOH): *m/z* = 785.3 ($[M+H]^+$, 23), 807.3 ($[M+Na]^+$, 100); analysis calculated for $C_{48}H_{68}N_2O_7$ (785.06): C 73.44, H 8.73, N 3.57; found: 72.29, H 8.91, N 3.42.

4.1.5.4. Ethyl (3β) 3-([D-tryptophanyl]oxy)-11-oxo-olean-12-en-30-oate (16)

Compound **16** was obtained from compound **15** (127 mg, 0.16 mmol) by method B as a colorless powder; yield: 105 mg, 96 %. m.p. 91-95 °C; R_F = 0.37 (DCM/MeOH, 9:1); $[\alpha]_D$ = 96.48° (*c* 0.61, $CHCl_3$); UV-Vis (MeOH): λ_{max} (log ε) = 226 nm (4.62), 240 nm (4.61), 266 nm (4.18); IR (KBr): *v* = 3407 (br), 2950 (s), 1727 (s), 1658 (s), 1458 (m), 1388 (m), 1280 (w), 1215 (s), 1176 (m), 1087 (m), 1020 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.21 (s, 1H, Trp-NH), 7.61 (d, 1H, indole-7, *J* = 7.7 Hz), 7.35 (d, 1H, indole-4, *J* = 7.9 Hz), 7.18 (m, 1H, indole-5), 7.12 (m, 1H, indole-6), 7.09 (s, 1H, indole-2), 5.64 (s, 1H, H-12), 4.50 (dd, 1H, H-3, *J* = 11.4, 5.0 Hz), 4.18 (dq, 1H, Et-C*H*H′, *J* = 10.9, 7.1 Hz), 4.12 (dq, 1H, Et-CH*H′*, *J* = 10.9, 7.1 Hz), 3.89 (m, 1H, Trp-$CHNH_2$), 3.31 (dd, 1H, Trp-C*H*H′, *J* = 14.9, 4.6 Hz), 3.09 (dd, 1H, Trp-CH*H′*, *J* = 14.8, 7.8 Hz), 2.78 (ddd, 1H, H-1, *J* = 13.6, 3.7, 3.7 Hz), 2.42 (br, 2H, NH_2), 2.34 (s, 1H, H-9), 2.10 (dd, 1H, H-18, *J* = 13.6, 3.8 Hz), 2.03 (m, 1H, H-15), 1.99 (m, 1H, H-21), 1.92 (ddd, 1H, H-19, *J* = 13.4, 3.9, 2.7 Hz), 1.82 (ddd, 1H, H-16, *J* = 13.7, 13.7, 4.2 Hz), 1.65 (m, 1H, H-7), 1.63 (m,1H, H-2), 1.61 (dd, 1H, H-19′, *J* = 13.6, 13.6 Hz), 1.59 (m, 1H, H-2′), 1.56 (m, 1H, H-6), 1.43 (m, 1H, H-6′), 1.40 (m, 1H, H-7′), 1.38 (m, 1H, H-22), 1.36 (s, 3H, H-27), 1.34 (m, 1H, H-22′), 1.31 (m, 1H, H-21′), 1.26 (t, 3H, Et-CH_3, *J* = 7.1 Hz), 1.17 (m, 1H, H-16′), 1.14 (s, 3H, H-28), 1.13 (s, 3H, H-25), 1.11 (s, 3H, H-26), 1.02 (m, 1H, H-15′), 1.00 (m, 1H, H-1′), 0.83 (s, 3H, H-24), 0.82 (s, 3H, H-23), 0.80 (s, 3H, H-29), 0.79 (m, 1H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.4 (C-30), 169.4 (C-13),

136.3 (indole-*q*.C), 128.4 (C-12), 127.4 (indole-*q*.C), 123.3 (indole-2), 122.2 (indole-5), 119.5 (indole-6), 118.8 (indole-7), 111.2 (indole-4), 110.5 (indole-*q*.C), 81.9 (C-3), 61.6 (C-9), 60.3 (Et-CH_2), 55.0 (C-5), 54.8 (Trp-CHNH), 48.4 (C-18), 45.4 (C-14), 43.8 (C-20), 43.2 (C-8), 41.1 (C-19), 38.7 (C-1), 38.1 (C-10), 37.7 (C-22), 36.9 (C-4), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 30.0 (Trp-CH_2), 28.5 (C-29), 28.3 (C-28), 28.0 (C-23), 26.5 (C-16), 26.4 (C-15), 23.3 (C-27), 23.3 (C-2), 18.6 (C-26), 17.3 (C-6), 16.6 (C-24), 16.3 (C-25), 14.3 (Et-CH_3) ppm; MS (ESI, MeOH): *m/z* = 685.3 ($[M+H]^+$, 100); analysis calculated for $C_{43}H_{60}N_2O_5$ (684.95): C 75.40, H 8.83, N 4.09; found: C 75.26, H 8.99, N 3.87.

4.1.5.5. Methyl (3β) 3-([*S*-benzyl-*N*-boc-L-cysteinyl]oxy)-11-oxo-olean-12-en-30-oate (17)

Compound **17** was obtained from methyl glycyrrhetinate (730 mg, 1.51 mmol) by method A as a colorless powder, using *S*-benzyl-*N*-boc-L-cysteine (562 mg, 1.81 mmol) and DCC (373 mg, 1.81 mmol); yield: 670 mg, 57 %. m.p. 112-115 °C; R_F = 0.64 (hexane/ethyl acetate, 7:3); $[\alpha]_D$ = 57.94° (*c* 0.43, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 248 nm (4.01); IR (KBr): ν = 3329 (br), 2930 (s), 2852 (m), 1719 (s), 1654 (s), 1627 (s), 1576 (m), 1508 (m), 1455 (m), 1390 (m), 1367 (m), 1341 (m), 1246 (m), 1216 (s), 1167 (s), 1088 (w), 1063 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.32-7.20 (br, 5H, H-Ar), 5.67 (s, 1H, H-12), 5.29 (d, 1H, NH, *J* = 7.9), 4.57 (dd, 1H, H-3, *J* = 11.7, 4.8 Hz), 4.51 (m, 1H, Cys-CHNH), 3.75 (s, 2H, Bn-CH_2), 3.69 (s, 3H, OCH_3), 2.88 (m, 1H, Cys-C*H*H′), 2.84 (m, 1H, Cys-CH*H*′), 2.82 (m, 1H, H-1), 2.35 (s, 1H, H-9), 2.09 (dd, 1H, H-18, *J* = 13.5, 3.5 Hz), 2.03 (m, 1H, H-15), 1.99 (m, 1H, H-21), 1.93 (m, 1H, H-19), 1.83 (ddd, 1H, H-16, *J* = 13.7, 13.7, 3.7 Hz), 1.74 (m, 1H, H-2), 1.72 (m, 1H, H-7), 1.66 (m, 1H, H-6), 1.64 (m, 1H, H-2′), 1.61 (dd, 1H, H-19′, *J* = 13.6, 13.6 Hz), 1.58 (m, 1H, H-6′), 1.46 (s, 9H, Boc-CH_3), 1.43 (m, 1H, H-7′), 1.38 (m, 1H, H-22), 1.36 (s, 3H, H-27), 1.31 (m, 1H, H-22′), 1.31 (m, 1H, H-21′), 1.18 (m, 1H, H-16′), 1.16 (s, 3H, H-25), 1.15 (s, 3H, H-29), 1.13 (s, 3H, H-26), 1.08 (m, 1H, H-15′), 1.02 (m, 1H, H-1′), 0.87 (s, 3H, H-23), 0.85 (s, 3H, H-24), 0.81 (s, 3H, H-28), 0.79 (m, 1H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.0 (C-11), 176.9 (C-30), 170.8 (Cys-COO), 169.3 (C-13), 157.6 (Boc-COO), 137.7 (C_{ar}), 130.1 (C_{ar}), 128.9 (C_{ar}), 128.6 (C_{ar}), 128.5 (C-12), 128.4 (C_{ar}), 127.1 (C_{ar}), 82.4 (C-3), 79.7 (Boc-*q*.C), 61.6 (C-9), 55.0 (C-5), 53.5 (Cys-CHNH), 51.7 (OCH_3), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.7 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 36.8 (Bn-CH_2), 33.7 (Cys-CH_2), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (Boc-CH_3), 28.3 (C-29), 28.1 (C-23), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.3 (C-27), 18.7 (C-26), 17.3 (C-6), 16.7 (C-24), 16.3 (C-25) ppm; MS (ESI): *m/z* (%) = 778.1 ($[M+H]^+$, 10), 795.3 ($[M+NH_4]^+$, 11), 800.4 ($[M+Na]^+$, 100), 816.3 ($[M+K]^+$, 20), 832.7 ($[M+Na+MeOH]^+$, 13), 1189.7 ($[3M+2Na]^{2+}$, 12), 1578.3 ($[2M+H+Na]^+$, 20); analysis calculated for $C_{46}H_{67}NO_7S$ (778.09): C 71.01, H 8.68, N 1.80; S, 4.12; found: C 70.86, H 8.74, N 1.69, S 3.98.

4.1.5.6. Methyl (3β) 3-([*S*-benzyl-L-cysteinyl]oxy)-11-oxo-olean-12-en-30-oate (18)

Compound **18** was obtained from compound **16** (190 mg, 0.24 mmol) by method B as a colorless powder, using trifluoroacetic acid (1 mL, 12.98 mmol); yield: 140 mg, 86 %. m.p. 128-131 °C; R_F = 0.66 ($CHCl_3$/MeOH, 9:1); [α] = 62.38° (*c* 0.43, $CHCl_3$); UV-vis (MeOH): λ_{max} (log ε) = 249 nm (4.31); IR (KBr): *v* = 3406 (br), 2929 (s), 1732 (s), 1660 (s), 1570 (w), 1454 (m), 1387 (m), 1324 (w), 1217 (s), 1155 (m), 1087 (w), 1028 (w) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.36-7.20 (m, 5H, H-Ar), 5.66 (s, 1H, H-12), 4.58 (dd, 1H, H-3, *J* = 11.8, 4.7 Hz), 3.93 (m, 1H, Cys-CHNH), 3.82 (s, 2H, Bn-CH_2), 3.69 (s, 3H, OCH_3), 3.02 (dd, 1H, Cys-C*H*H′, *J* = 13.9, 4.4 Hz), 2.91 (dd, 1H, Cys-CH*H*′, *J* = 13.9, 7.1 Hz), 2.81 (m, 1H, H-1), 2.34 (s, 1H, H-9), 2.09 (dd, 1H, H-18, *J* = 13.5, 3.7 Hz), 2.03 (m, 1H, H-15), 1.99 (m, 1H, H-21), 1.93 (m, 1H, H-19), 1.82 (ddd, 1H, H-16, *J* = 13.5, 13.5, 3.9 Hz), 1.71 (m, 1H, H-2), 1.68 (m, 1H, H-7), 1.66 (m, 1H, H-6), 1.63 (m, 1H, H-2′), 1.61 (dd, 1H, H-19′, *J* = 13.5, 13.5 Hz), 1.56 (m, 1H, H-6′), 1.42 (m, 1H, H-7′), 1.38 (m, 1H, H-22), 1.36 (s, 3H, H-27), 1.31 (m, 1H, H-22′), 1.31 (m, 1H, H-21′), 1.18 (m, 1H, H-16′), 1.15

(s, 3H, H-25), 1.15 (s, 3H, H-29), 1.12 (s, 3H, H-26), 1.09 (m, 1H, H-15′), 1.02 (m, 1H, H-1′), 0.85 (s, 3H, H-23), 0.83 (s, 3H, H-24), 0.81 (s, 3H, H-28), 0.77 (m, 1H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.8 (C-11), 176.9 (C-30), 169.2 (Cys-COO), 169.2 (C-13), 137.6 (C_{ar}), 129.0 (C_{ar}), 129.0 (C_{ar}), 128.6 (C_{ar}), 128.5 (C-12), 128.5 (C_{ar}), 127.2 (C_{ar}), 83.1 (C-3), 61.6 (C-9), 55.0 (C-5), 51.7 (OCH_3), 49.2 (Cys-CHNH), 48.4 (C-18), 45.4 (C-8), 44.0 (C-20), 43.2 (C-14), 41.1 (C-19), 38.6 (C-1), 38.1 (C-4), 37.7 (C-22), 36.9 (C-10), 36.7 (Bn-CH_2), 33.9 (Cys-CH_2), 32.6 (C-7), 31.8 (C-17), 31.1 (C-21), 28.5 (C-28), 28.3 (C-29), 28.2 (C-23), 26.4 (C-16), 26.4 (C-15), 23.5 (C-2), 23.4 (C-27), 18.7 (C-26), 17.3 (C-6), 16.8 (C-24), 16.3 (C-25) ppm; MS (ESI): *m/z* (%) = 678.3 ($[M+H]^+$, 100), 1017.2 ($[3M+2H]^{2+}$, 6), 1355.6 ($[2M+H]^+$, 2); analysis calculated for $C_{41}H_{59}NO_5S$ (677.98): C 72.63, H 8.77, N 2.07; S, 4.73; found: c 72.57, H 8.96, N 1.94, S 4.57.

4.1.5.9. (3β) *N*-(2-Aminoethyl) 3-hydroxy-11-oxo-olean-12-en-30-amide (32)

Compound **32** was obtained from GA (400 mg, 0.85 mmol) by method C as a colorless powder, using DCC (194 mg, 0.94 mmol), 1-hydroxybenzotriazole (138 mg, 1.02 mmol) and 1,2-diaminoethane (102 mg, 1.70 mmol); yield: 150 mg, 34 %. m.p. 193-199°C (decomp.); R_F = 0.05 ($MeOH/CHCl_3$, 8:2); $[\alpha]_D$ = 82.93° (*c* 0.34, MeOH); UV-vis (MeOH): λ_{max} (log ε) = 249 nm (3.77); IR (KBr): ν = 3403 (br), 2950 (m), 2870 (m), 1642 (s), 1534 (m), 1384 (s), 1040 (w), 994 (w), 826 (w), 726 (w), 668 (m), 590 (m), 544 (m) cm^{-1}; ^{1}H NMR (500 MHz, MeOH-d_4): δ = 5.62 (s, 1H, H-12), 3.46 (m, 2H, chain-1-CH_2), 3.17 (dd, 1H, H-3, *J* = 11.8, 4.5 Hz), 3.03 (dd, 2H, chain-2-CH_2, *J* = 6.6, 6.6 Hz), 2.72 (ddd, 1H, H-1, *J* = 13.3, 3.3, 3.3 Hz), 2.46 (s, 1H, H-9), 2.16 (m, 1H, H-15), 2.15 (dd, 1H, H-18, *J* = 13.4, 4.0 Hz), 1.93 (m, 1H, H-21), 1.88 (m, 1H, H-19), 1.88 (m, 1H, H-16), 1.74 (m, 1H, H-19′), 1.72 (m, 1H, H-7), 1.67 (m, 1H, H-2), 1.63 (m, 1H, H-6), 1.54 (m, 1H, H-2′), 1.49 (m, 1H, H-21′), 1.47 (m, 1H, H-6′), 1.45 (m, 1H, H-7′), 1.43 (s, 3H, H-27), 1.42 (m, 1H, H-22), 1.37 (m, 1H, H-22′), 1.25 (m, 1H, H-16′), 1.15 (s, 3H, H-29), 1.14 (s, 3H, H-25), 1.14 (s, 3H, H-26), 1.04 (m, 1H, H-15′), 1.02 (m, 1H, H-1′), 1.00 (s, 3H, H-23), 0.83 (s, 3H, H-28), 0.80 (s, 3H, H-24), 0.77 (m, 1H, H-5) ppm; ^{13}C NMR (100 MHz, MeOH-d_4): δ = 202.7 (C-11), 180.3 (C-30), 172.6 (C-13), 129.0 (C-12), 79.4 (C-3), 63.2 (C-9), 56.2 (C-5), 49.7 (C-18), 46.7 (C-8), 44.8 (C-20), 44.6 (C-14), 42.5 (C-19), 40.8 (chain-2), 40.3 (C-1), 40.2 (C-4), 38.7 (C-22), 38.7 (chain-1), 38.3 (C-10), 33.8 (C-7), 32.9 (C-17), 31.9 (C-21), 29.5 (C-29), 29.1 (C-28), 28.7 (C-23), 27.8 (C-2), 27.6 (C-16), 27.4 (C-15), 23.8 (C-27), 19.3 (C-26), 18.6 (C-6), 16.9 (C-25), 16.3 (C-24) ppm; MS (ESI): *m/z* (%) = 513.5 ($[M+H]^+$, 100), 1025.6 ($[2M+H]^+$, 3); analysis calculated for $C_{32}H_{52}N_2O_3$ (512.77): C 74.95, H 10.22, N 5.46; found: C 74.79, H10.37, N 5.31.

4.1.5.10. (3β) *N*-(5-Aminopentyl) 3-hydroxy-11-oxo-olean-12-en-30-amide (36)

Compound **34** was obtained from GA (400 mg, 0.85 mmol) by method C as a colorless powder, using DCC (194 mg, 0.94 mmol), 1-hydroxybenzotriazole (138 mg, 1.02 mmol) and 1,2-diaminopentane (174 mg, 1.70 mmol); yield: 165 mg, 35 %. m.p. 187-192°C (decomp.); R_F = 0.05 ($MeOH/CHCl_3$, 8:2); $[\alpha]_D$ = 94.12° (*c* 0.52, MeOH); UV-vis (MeOH): λ_{max} (log ε) = 249 nm (3.89); IR (KBr): ν = 3424 (br), 2946 (m), 2868 (w), 1638 (m), 1540 (w), 1384 (s), 1214 (w), 1040 (w) cm^{-1}; ^{1}H NMR (500 MHz, MeOH-d_4): δ = 5.61 (s, 1H, H-12), 3.27 (m, 1H, chain-1-C*H*H′), 3.20 (m, 1H, chain-1-CH*H′*) 3.17 (m, 1H, H-3), 2.94 (m, 2H, chain-5-CH_2), 2.71 (ddd, 1H, H-1, *J* = 13.3, 3.4, 3.4 Hz), 2.46 (s, 1H, H-9), 2.17 (m, 1H, H-15), 2.13 (m, 1H, H-18), 1.96 (m, 1H, H-21), 1.90 (m, 1H, H-19), 1.88 (m, 1H, H-16), 1.74 (m, 1H, H-19′), 1.74 (m, 1H, H-7), 1.70 (m, 2H, chain-4-CH_2), 1.67 (m, 1H, H-2), 1.63 (m, 1H, H-6), 1.57 (m, 2H, chain-2-CH_2) 1.55 (m, 1H, H-2′), 1.49 (m, 1H, H-6′), 1.45 (m, 1H, H-7′), 1.43 (m, 1H, H-21′), 1.43 (s, 3H, H-27), 1.42 (m, 2H, chain-3-CH_2), 1.42 (m, 2H, H-22 and H-22′), 1.25 (m, 1H, H-16′), 1.14 (s, 3H, H-25), 1.14 (s, 3H, H-26), 1.12 (s, 3H, H-29), 1.05 (m, 1H, H-15′), 1.02 (m, 1H, H-1′), 1.00 (s, 3H, H-23), 0.82 (s, 3H, H-28), 0.80 (s, 3H, H-24), 0.77 (m, 1H,

H-5) ppm; ^{13}C NMR (100 MHz, MeOH-d_4): δ = 202.6 (C-11), 178.8 (C-30), 172.7 (C-13), 128.9 (C-12), 79.3 (C-3), 63.1 (C-9), 56.1 (C-5), 49.9 (C-18), 46.7 (C-8), 44.8 (C-20), 44.6 (C-14), 42.6 (C-19), 40.7 (chain-5), 40.3 (C-1), 40.2 (C-4), 39.9 (chain-1), 38.8 (C-22), 38.3 (C-10), 33.8 (C-7), 32.9 (C-17), 31.9 (C-21), 30.1 (chain-2), 29.5 (C-29), 29.3 (C-28), 28.7 (C-23), 28.1 (chain-4), 27.8 (C-2), 27.6 (C-16), 27.4 (C-15), 24.8 (chain-3), 23.7 (C-27), 19.3 (C-26), 18.6 (C-6), 16.9 (C-25), 16.3 (C-24) ppm; MS (ESI): *m/z* (%) = 555.5 ([M+H]$^+$, 100), 1109.5 ([2M+H]$^+$, 2); analysis calculated for $C_{35}H_{58}N_2O_3$ (554.85): C 75.76, H 10.54, N 5.05; found: C 75.51, H 10.65, N 4.98.

4.1.5.7. (Cyclohexylamino)-(cyclohexylimino)-methyl (3β) 3-acetyloxy-11-oxo-olean-12-en-30-oate (37)

3β-Acetyloxy-11-oxo-olean-12-en-30-oic acid (400 mg, 0.78 mmol) was dissolved in a mixture of dry DMF (10 mL) and dry dichloromethane (15ml) and DCC (270 mg, 0.82 mmol) was added. After 12 h of continous stirring at room temperature, the solvents were removed under reduced pressure, and the residue was dissolved in mixture of dichlormethane and water (20 mL, 1:1). The mixture was extracted with dichloromethane (3 x 15 mL), the combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), filtered and evaporated. The residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 7:3) to yield **37** (280 mg, 50 %) as a colorless powder. m.p. 141-145 °C; R_F = 0.18 (hexane/ethyl acetate, 8:2); $[\alpha]_D$ = 83.43° (*c* 0.58, $CHCl_3$); UV-Vis (MeOH): λ_{max} (log ε) = 269 nm (4.10); IR (KBr): ν = 3360 (br), 2932 (s), 2856 (s), 2669 (m), 1740 (s), 1700 (s), 1660 (s), 1509 (s), 1453 (s), 1374 (s), 1343 (s), 1320 (s), 1246 (s), 1153 (s), 1085 (m), 1029 (s), 1001 (m), 985 (s) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.74 (s, 1H, H-12), 5.49 (d, 1H, NH, *J* = 7.9 Hz), 4.51 (dd, 1H, H-3, *J* = 11.7, 4.4 Hz), 4.25 (dddd, 1H, imino-CH, *J* = 11.8, 11.8, 3.1, 3.1 Hz), 3.66 (br, 1H, amino-CH), 2.78 (ddd, 1H, H-1, *J* = 13.5, 3.1, 3.1 Hz), 2.34 (s, 1H, H-9), 2.15 (dd, 1H, H-18, *J* = 12.3, 3.7 Hz), 2.08 (m, 1H, H-19), 2.06 (m, 1H, H-21), 2.04 (s, 3H, Ac-CH_3), 2.03-1.94 (br, 4H, 2×CH_2 cyclo), 1.72 (ddd, 1H, H-16, *J* = 11.7, 3.3, 3.3 Hz), 1.84-1.52 (br, 15H, H-2 and H-2′ and H-15 and H-7 and H-21′ and H-6 and H-19′ and 5 × CH_2 cyclo), 1.39-1.30 (br, 10H, H-6′ and H-7′ and H-22 and H-22′ and 3×CH_2 cyclo), 1.35 (s, 3H, H-27), 1.28 (s, 3H, H-23), 1.19 (m, 1H, H-16′), 1.11 (m, 1H, H-15′), 1.16 (s, 3H, H-25), 1.12 (s, 3H, H-26), 1.07 (m, 1H, H-1′), 0.87 (s, 3H, H-24), 0.87 (s, 3H, H-28), 0.80 (m, 1H, H-5), 0.80 (s, 3H, H-29) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.8 (C-11), 175.4 (C-30), 170.9 (Ac-CO), 168.9 (C-13), 154.5 (C=N), 128.6 (C-12), 80.6 (C-3), 61.6 (C-9), 55.6 (CHN=), 55.0 (C-5), 50.3 (CHNH), 48.2 (C-18), 45.3 (C-20), 43.8 (C-19), 43.2 (C-14), 43.2 (C-8), 38.8 (C-1), 38.4 (C-22), 38.0 (C-10), 36.9 (C-4), 32.8 (C-7), 32.6 (CH_2 cyclo), 32.5 (CH_2 cyclo), 31.9 (C-17), 31.6 (CH_2 cyclo), 31.6 (CH_2 cyclo), 30.9 (C-21), 28.7 (C-28), 28.6 (C-29), 28.0 (C-23), 27.0 (CH_2 cyclo), 27.0 (CH_2 cyclo), 26.7 (C-16), 26.4 (C-15), 26.2 (CH_2 cyclo), 25.5 (CH_2 cyclo), 25.3 (CH_2 cyclo), 24.7 (CH_2 cyclo), 23.5 (C-2), 23.0 (C-27), 21.3 (Ac-Me), 18.6 (C-26), 17.4 (C-6), 16.7 (C-24), 16.4 (C-25); MS (ESI, MeOH): *m/z* = 719.4 ([M+H]$^+$, 34), 741.5 ([M+Na]$^+$, 100), 1460.4 ([2M+Na]$^+$, 42); analysis calculated for $C_{45}H_{70}N_2O_5$ (719.05): C 75.17, H 9.81, N 3.90; found: C 74.14, H 10.01, N 3.87.

4.1.5.8. 1*H*-Benzotriazol-1-yl (3β) 3-hydroxy-11-oxo-olean-12-en-30-oate (38)

To a solution of **GA** (500 mg, 1.06 mmol) and 1-hydroxybenzotriazole (157 mg, 1.19 mmol) in dry dichloromethane (40 mL), DCC (240 mg, 1.17 mmol) was added. After continous stirring at room temperature for 24 h, the mixture was filtered, and the filtrate was washed with hydrochloric acid (1 M, 20 mL). The mixture was extracted with dichlormethane (3 x 20 mL), the combined organic layers were washed with brine (20 mL), dried (Na_2SO_4), filtered and evaporated to dryness. The residue was subjected to column chromatography (silica gel, hexane/ethyl acetate, 1:1) to yield **38** (170 mg, 27 %) as a colorless powder. m.p. 192-195 °C (decomp.); R_F = 0.13 (hexane/ethyl acetate, 5:3); $[\alpha]_D$ = 120.21° (*c* 0.32, $CHCl_3$); UV-Vis (MeOH): λ_{max} (log ε) = 214 nm (4.48), 269 nm (4.12); IR (KBr): ν = 3619 (br),

3328 (s), 2931 (s), 2851 (s), 1795m, 1655 (s), 1626 (s), 1576m, 1535 (w), 1445 (w), 1387 (w), 1312 (w), 1266 (w), 1244 (w), 1203 (w), 1090 (m), 1036 (m), 1014 (m) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.08 (m, 1H, H-Ar), 7.56 (m, 1H, H-Ar), 7.43 (m, 1H, H-Ar), 7.33 (m, 1H, H-Ar), 5.70 (s, 1H, H-12), 4.52 (dd, 1H, *J* = 10.7, 5.5 Hz), 3.22 (dd, 1H, H-3, *J* = 10.7, 5.5 Hz), 2.77 (ddd, 1H, H-1, *J* = 13.5, 3.3, 3.3 Hz), 2.35 (dd, 1H, H-18, *J* = 13.0, 3.9 Hz), 2.34 (s, 1H, H-9), 2.27 (ddd, 1H, H-21, *J* = 10.3, 2.8, 2.8 Hz), 2.15 (ddd, 1H, H-19, *J* = 13.9, 3.9, 3.0 Hz), 2.09 (ddd, 1H, H-15, *J* = 13.9, 13.9, 4.2 Hz), 1.87 (dd, 1H, H-19′, *J* = 13.9, 13.9 Hz), 1.70 (ddd, 1H, H-16, *J* = 13.6, 3.9, 3.9 Hz), 1.69 (m, 1H, H-7), 1.65-1.59 (br, 4H, H-6 and H-22 and H-21′ and H-16′), 1.57 (s, 3H, H-23), 1.46 (m, 1H, H-6′), 1.43 (m, 1H, H-7′), 1.41 (s, 3H, H-27), 1.34 (m, 1H, H-15′), 1.28 (m, 1H, H-2), 1.16 (m, 1H, H-2′), 1.15 (s, 3H, H-26), 1.13 (s, 3H, H-25), 1.01 (s, 3H, H-29), 0.98 (m, 1H, H-1′), 0.94 (s, 3H, H-28), 0.81 (s, 3H, H-24), 0.70 (m, 1H, H-5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 199.7 (C-11), 172.4 (C-30), 167.4 (C-13), 143.6 (C_{ar}), 129.1 (C_{ar}), 128.7 (C_{ar}), 128.6 (C_{ar}), 124.8 (C_{ar}), 120.7 (C_{ar}), 128.8 (C-12), 78.8 (C-3), 61.9 (C-9), 55.0 (C-5), 48.3 (C-18), 45.4 (C-8), 44.4 (C-20), 43.2 (C-14), 41.0 (C-19), 39.2 (C-1), 39.2 (C-4), 37.8 (C-22), 37.2 (C-10), 32.8 (C-7), 32.0 (C-17), 31.3 (C-21), 28.6 (C-28), 28.1 (C-29), 28.1 (C-23), 27.4 (C-2), 26.5 (C-16), 25.6 (C-15), 23.5 (C-27), 18.8 (C-26), 17.5 (C-6), 16.4 (C-25), 15.6 (C-24) ppm; MS (ESI, MeOH): *m/z* = 588.5 ($[M+H]^+$, 10), 610.4 ($[M+Na]^+$, 100), 626.4 ($[M+K]^+$, 6), 1175.2 ($[2M+H]^+$, 8), 1197.2 ($[2M+Na]^+$, 64), 1213.2 ($[2M+K]^+$, 2); analysis calculated for $C_{36}H_{49}N_3O_4$ (587.79): C 73.56, H 8.40, N 7.15; found: C 73.38, H 8.57, N 6.97.

4.2. Biology

4.2.1. Cell lines and Culture Conditions

The NiH3T3 cells were included in this study. Cultures were maintained as monolayer in RPMI 1640 (PAA Laboratories, Pasching, Germany) supplemented with 10 % heat inactivated fetal bovine serum (Biochrom AG, Berlin, Germany) and penicillin / streptomycin (PAA Laboratories) at 37 °C in a humidified atmosphere of 5 % CO_2 / 95 % air.

4.2.2. Cytotoxicity Assay[41]

The cytotoxicity of the compounds was evaluated using the sulforhodamine-B (SRB) (Sigma Aldrich) microculture colorimetric assay. In short, exponentially growing cells were seeded into 96-well plates on day 0 at the appropriate cell densities to prevent confluence of the cells during the period of experiment. After 24 h, the cells were treated with serial dilutions of the compounds (0-30 µM) for 96 h. The final concentration of DMSO or DMF solvent never exceeded 0.5 %, which was non-toxic to the cells. The percentages of surviving cells relative to untreated controls were determined 96 h after the beginning of drug exposure. After a 96 h treatment, the supernatant medium from the 96 well plates was discarded, and the cells were fixed with 10 % TCA. For a thorough fixation, the plates were allowed to rest at 4 °C. After fixation, the cells were washed in a strip washer. The washing was done four times with water using alternate dispensing and aspiration procedures. The plates were then dyed with 100 µl of 0.4 % SRB (sulforhodamine B) for about 20 min. After dying the plates were washed with 1 % acetic acid to remove the excess of the dye and allowed to air dry overnight. 100 µl of 10 mM Tris base solution were added to each well and absorbance was measured at λ = 570 nm (using a 96 well plate reader, Tecan Spectra, Crailsheim, Germany). The IC_{50} was estimated by linear regression between the value before and after the 50 % line is crossed in a dose-response curve.

4.2.3. Enzymatic Studies

4.2.3.1. Spectrophometer and Chemicals

A TECAN SpectraFluorPlus working on the kinetic mode and measuring the absorbance at $\lambda = 415$ nm was used for the enzymatic studies. Acetylcholinesterase (from *electrophorus electricus*), Papain (from *carica papaya*), Lipase (from *candida antarctica*), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and acetylthiocholine iodide were purchased from Fluka. Butyrylcholinesterase (from equine serum), carbonic anhydrase II (from bovine erythrocytes) as well as 4-nitrophenyl acetate (4-NA) were purchased from Sigma and butyrylthiocholine idioide was bought from Aldrich.

4.2.3.2. Solutions Preparation

Preparation of 50 mM Tris-HCl buffer solutions: Tris(hydroxymethyl)-aminomethan (606 mg) was dissolved in bi-distilled water (100 mL) and adjusted with HCl to a pH of 8.0 ± 0.1 (for AChE, BChE, bCA II and Lipase) and 6.2 ± 0.1 (for Papain), respectively. Buffers was freshly prepared and stored in the refrigerator. AChE solution 2.005 U/ml: the enzyme (271 U/mg, 0.037 mg) was dissolved in freshly prepared buffer pH 8.0 (5 mL) containing NaN_3 (0.98 mg). BChE solution 2.040 U/ml: the enzyme (7.54 U/mg, 1.353 mg) was dissolved in freshly prepared buffer pH 8.0 (5 mL) containing NaN_3 (0.98 mg). bCA II solution 798 W/A-units/ml: the enzyme (≥3,000 W-A units/mg, 0.87 mg) was dissolved in freshly prepared buffer pH 8.0 (5 mL). Papain solution 3.889 U/ml: the enzyme (2.1 U/mg, 9.52 mg) was dissolved in freshly prepared buffer pH 6.2 (5 mL). Lipase solution 2.003 U/ml: the enzyme (3.1 U/mg, 3.23 mg) was dissolved in freshly prepared buffer pH 8.0 (5 mL). DTNB solution 3 mM: DTNB (23.8 mg) was dissolved in freshly prepared buffer pH 8.0 (20 mL) containing NaCl (116.8 mg) and $MgCl_2$ (38.0 mg). ATChI solution 15 mM: ATChI (43.4 mg) was dissolved in bi-distilled water (10 mL). BTChI solution 15 mM: BTChI (47.6 mg) was dissolved in bi-distilled water (10 mL). 4-NA solution 6 mM: 4-NA (21.6 mg) was dissolved in methanol (2.2 mg) and bi-distilled water (17.8 mL). All solutions were stored in Eppendorf caps in the refrigerator or freezer, if necessary. The pure compounds were initially dissolved in DMSO, galantamine hydrobromide as standard for AChE and BChE was dissolved in bi-distilled water. The final concentrations for the enzymatic assay were yielded by diluting the stock solution with bi-distilled water. No inhibition was detected by residual DMSO (<0.5 %).

4.2.3.3. Enzyme Assay

Cholinesterase assay. A mixture of the DTNB solution (125 µL), enzyme (25 μL) and compounds solutions (25 µL, 3 different concentrations and once water) was prepared and incubated at 30 °C for 20 min. The substrate (25 µL, 4 different concentrations) was added to start the enzymatic reaction. The absorbance data (l = 415 nm) was recorded under a controlled temperature of 30 °C for 30 min at 1 min intervals. All measurements were performed as triplicates. The used substrate concentrations in the test were as follows: [ATChI] = [BTChI] = 0.9375 mM, 0.625 mM, 0.325 mM, 0.1875 mM. The mode of inhibition as well as K_i and K_i' were determined using Lineweaver-Burk plot[42], Dixon plot[43] and Cornish-Bowden plot[44].

bCA II-assay and lipase-assay. A mixture of buffer solution pH 8.0 (125 µL), enzyme (25 µL) and compounds solutions (25 µL, 50 µM) was prepared and incubated at 37 °C for 20 min. 4-NA (25 µL, 0.75 µM) was added to start the enzymatic reaction. The absorbance data (l = 415 nm) was recorded under a controlled temperature of 37 °C for 10 min

at 1 min intervals. All measurements were performed in duplicate. The relative inhibition was determined as the quotient of the slopes (compound divided by blank) of the linear ranges.

Papain-assay. The assay was performed like the bCA II and Lipase-Assay using a buffer solution of pH 6.2 instead.

4.2.4. Docking Studies

The 3D structure coordinates of BChE were obtained from the Protein Data Bank, PDB code 1P0I with a 2.00 Å resolution, and for AChE the PDB code 4BDT with 3.10 Å resolution was used. To prepare the enzymes for the docking studies, the co-crystallized inhibitor as well as crystallographic water molecules included in the PDB files were removed. Hydrogen atoms were added and the protonation states were correctly assigned using the Protonate-3D tool within the Molecular Operating Environment (MOE) 2011.10 software package[45], and energy was minimized using MMFF94x force field. Molecular docking studies were then performed using the GoldScore scoring function from GOLD 5.1 software package, and each ligand was subjected to 500 docking runs, using Trp82 and Trp86 N atom as active site center coordinate for 1P0I and 4BDT, respectively. Docking radius was considered 15 Å from the active site center. Docking protocol was validated by the docking of the co-crystallized inhibitors for each enzyme, respectively, and RMSD values between docked and crystallographic poses were below 1.5 Å.

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Table 1. Inhibitory constants for galantamine hydrochloride, **GA** and compounds **1**-**38** (K_i in μM), determined using Ellman's assays, employing acetylcholinesterase and butyrylcholinesterase with galantamine hydrochloride and **GA** as standards; four different substrate concentrations and four different inhibitor concentrations were used. Each experiment was performed in triplicate. F is the quotient of [K_i (AChE) / K_i (BChE)]; n.sol. - not soluble.

compound	K_i (AChE)	K_i (BChE)	F	compound	K_i (AChE)	K_i (BChE)	F
galantamine hydrobromide	0.54 ± 0.01	9.37 ± 0.67	0.06	**GA**	> 100	> 100	
1	> 100	> 100		**21**	19.98 ± 3.16	> 100	< 0.20
2	19.79 ± 1.13	> 100	< 0.20	**22**	18.94 ± 1.09	> 100	< 0.19
3	23.98 ± 0.81	> 100	< 0.24	**23**	16.05 ± 3.35	> 100	< 0.16
4	20.76 ± 1.54	> 100	< 0.21	**24**	n.sol.	n.sol.	
5	n.sol.	n.sol.		**25**	16.23 ± 0.16	> 100	< 0.16
6	17.67 ± 0.34	> 100	< 0.18	**26**	28.08 ± 4.43	> 100	< 0.28
7	n.sol.	n.sol.		**27**	n.sol.	n.sol.	
8	n.sol.	n.sol.		**28**	23.83 ± 5.74	> 100	< 0.24
9	n.sol.	n.sol.		**29**	14.87 ± 3.31	> 100	< 0.15
10	23.51 ± 8.45	> 100	< 0.24	**30**	29.63 ± 1.77	> 40	< 0.74
11	20.69 ± 0.78	> 100	< 0.21	**31**	13.02 ± 3.51	> 100	< 0.13
12	> 2	> 2		**32**	> 100	> 100	
13	13.74 ± 4.60	> 100	< 0.14	**33**	> 100	5.43 ± 0.91	> 18.42
14	20.39 ± 5.24	> 100	< 0.21	**34**	> 10	> 10	
16	11.73 ± 1.79	> 100	< 0.12	**35**	41.76 ± 7.37	74.30 ± 16.96	0.56
18	40.58 ± 12.78	> 100	< 0.41	**36**	> 100	> 100	
19	18.72 ± 2.34	> 100	< 0.19	**37**	> 10	> 10	
20	n.sol.	n.sol.		**38**	> 20	9.81 ± 0.32	> 2.04

Table 2. Relative inhibition of the enzymes papain, carbonic anhydrase II (bovine erythrocytes), lipase (*candida antarctica*), AChE as well as BChE by **19**, **23**, **31**, **33** and **38**; the concentration of each compound was 50 µM, except for **38**, where a concentration of c = 10 µM was used due to its limited solubility.

enzyme	19	23	31	33	38
papain	0 %	0 %	0 %	0 %	0 %
bCA II	0 %	15 %	0 %	0 %	27 %
lipase	38 %	51 %	45 %	51 %	32 %
AChE	68 %	58 %	43 %	35 %	49 %
BChE	16 %	16 %	30 %	79 %	68 %

Table 3. IC_{50} values (in μM) for selected derivatives, determined in photometric SRB assays, using non-malignant murine embryonic fibroblasts (NiH 3T3). Each experiment was performed in triplicate.

compound	**19**	**23**	**31**	**33**	**38**[a]
IC_{50} (NiH 3T3)	3.05 ± 0.17	> 30	> 30	> 30	> 30

[a] experiment was limited due to the solubility