Accepted Manuscript

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PII: S0223-5234(19)30555-0

DOI: https://doi.org/10.1016/j.ejmech.2019.06.029

Reference: EJMECH 11432

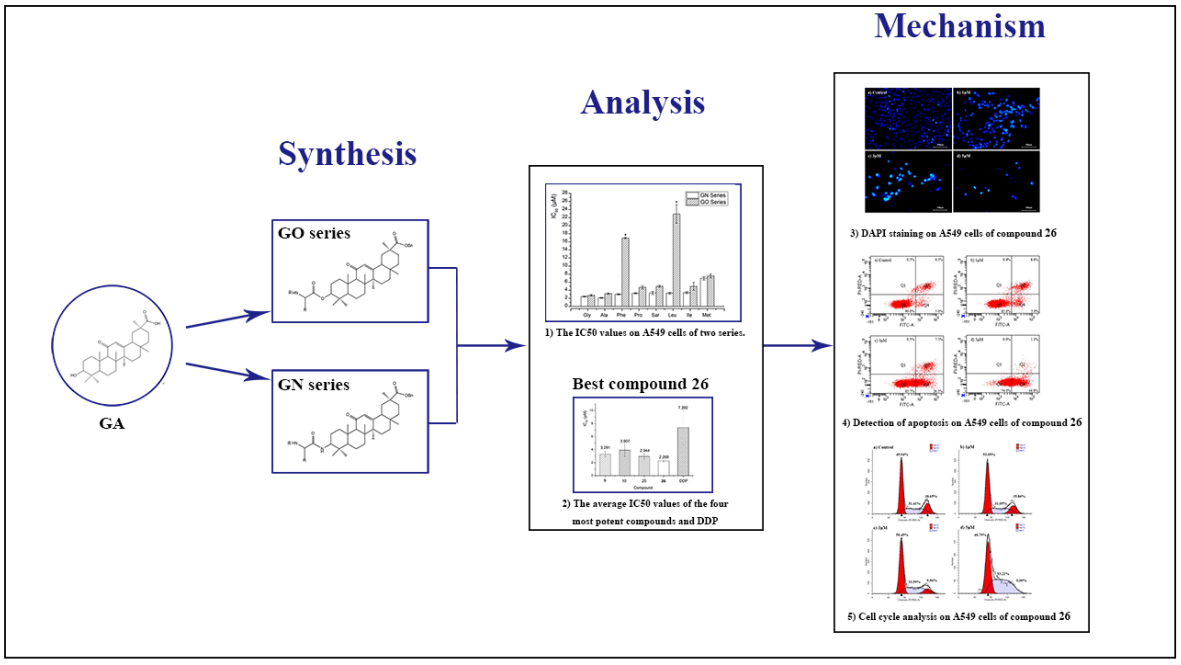
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 11 May 2019

Revised Date: 2 June 2019

Accepted Date: 10 June 2019

Please cite this article as: F. Zhou, G.-R. Wu, D.-S. Cai, B. Xu, M.-M. Yan, T. Ma, W.-B. Guo, W.-X. Zhang, X.-M. Huang, X.-h. Jia, Y.-Q. Yang, F. Gao, P.-L. Wang, H.-M. Lei, Synthesis and biological activity of glycyrrhetinic acid derivatives as antitumor agents, *European Journal of Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.ejmech.2019.06.029.



Synthesis and biological activity of glycyrrhetinic acid derivatives as antitumor agents

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Abstract: Glycyrrhetinic acid (GA) had been the star anticancer lead compound and appealed to many scientists all over the world; however, its antitumor activity was not potent enough. To improve GA's cytoxicity and explore the effect of bonding mode on antitumor activity, 32 compounds including GA-OH series (GO, esters in C-3 position) and GA-NH_2 series (GN, with amide linkages in C-3 position) had been designed and synthesized. All the compounds were screened for *in vitro*

cytotoxicity against A549, HepG2, MCF-7, Hela and MDCK cell lines. As a result, all the de-protected (without Boc group) derivatives showed much stronger cytotoxic activity than GA, and surprisingly enough, all the GN series of the compounds were more potent than GO series against various tumor cells. Among them, the compound **26** (amide linkages in C-3 position) exhibited stronger antitumor activity against A549 cell line (IC_{50} = 2.109 ± 0.11 μM) than the positive drug cisplatin (IC_{50} = 9.001 ± 0.37 μM). Further studies indicated that compound **26** could induce A549 apoptosis via nuclei fragmentation. The detection of apoptosis and cell cycle analysis indicated that compound **26** could induce the early apoptosis and prevent A549 cells transition from S to G2 phase. Furthermore, the structure-activity relationships were briefly discussed. Among which, current study displayed amide linkages in C-3 position could effectively enhance GA cytotoxicity, providing a new modification strategy for further study.

Keywords: Glycyrrhetinic; amino acid; antitumor; bonding mode; amide linkages.

1. Introduction

In recent years, increasing effort had been made to find new antitumor compounds, and natural products always appealed to most scientists [1-3]. Among them, pentacyclic triterpenoids were largely found in medicinal

plants and possessed a wide range of pharmacological activities [4-6]. Glycyrrhetinic acid (GA), the aglycone of glycyrrhizin found in the roots of licorice [7], had become a star anticancer compound as thousands of research papers published were carried around it. GA had been gotten into the focus of worldwide scientific interest because of its advantages such as being obtained easily and cheaply, good stability, high biosecurity and antiproliferative activity against various cancer cell lines. Nowadays, due to the influence of cancer, it was very common that people became poor or even went bankrupt in many developing countries. With the advantages of GA-like drugs, it had the prospect of developing affordable chemotherapy drugs that were acceptable for the poor people [8-12]. However, the weak antitumor activity of GA was a big weakness when compared to other triterpenes like triptolide and betulinic acid [13,14]. All the advantages mentioned and desire to improve the inadequacies of GA had made it meaningful to be modified for the discovery of potential antitumor compounds [15,16].

According to many other researches and previous work performed by our group, introduction of protected or de-protected amino acid could increase the antitumor activity as well as water solubility [17-19]. Structure activity relationship indicated that esterification at C-30 could enhance the activity of GA, and among methyl ester, ethyl ester and benzyl (Bn) ester at C-30, benzyl ester showed the best antitumor

activities [20,21]. In addition, according to the reports that bonding mode of the compound might strongly affect the activity, and amide linkages not only improved activity of compound but also more stable than esters in metabolism [22,23]. Based on the above, to improve GA's cytotoxicity and to explore the effect of bonding mode on antitumor activity, 32 GA derivatives including GO series and GN series had been designed and synthesized. The cytotoxic activity of all compounds was screened against A549 (human lung cancer), MCF-7 (human mammary cancer), HepG2 (human hepatocellular carcinoma), Hela (human cervical cancer) and MDCK (Madin-Darby canine kidney) cell lines *in vitro*. Moreover, fluorescence staining observations and flow cytometric analyses were performed to observe the preliminary antitumor mechanisms of the most selectivity compound. In addition, the structure-activity relationships of the two series of GA derivatives were briefly discussed. Notably, current study displayed amide linkages in C-3 position could effectively enhance the antitumor activity, providing a new modification strategy for the following study of GA.

2. Results and Discussion

2.1 Chemistry

The designed derivatives were prepared following the procedures in Schemes 1-3. In Scheme 1, GA derivative GA-BN were synthesized

through a combination of GA and benzyl bromide (Bn-Br) containing K_2CO_3 at 85□ in N,N-dimethylformamide (DMF). Then the intermediate GA-BN was further reacted with N-protected-L-amino acid in dry dichloromethane (DCM) in the presence of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) and 4-dimethylaminopyridine (DMAP) to yield compounds **1-8**. Subsequently, deprotection was performed with trifluoroacetic acid (TFA) in dry DCM at 0□ to afford compounds **9-16**.

In Scheme 2, the intermediate GO-BN was obtained through the oxidation with CrO_3/H_2SO_4 in acetone, the two epimers of GN-BN was obtained by Borch reduction with sodium cyanoborohydride and ammonium acetate in methanol [22]. Considering that the change of absolute stereochemistry in C3-NH_2 didn't evidently improve the antitumor activity which was reported by Czuk's group [24,25], we only chose the main product 3β-GN-BN to synthesize the GA derivatives. After 3β-GN-BN was produced, as shown in Scheme 3, compounds **17-24** were obtained through the combination of 3β-GN-BN and N-protected-L-amino acid under catalyzed by EDCI and 1-Hydroxybenzotriazole (HOBt) and N,N-diisopropylethylamine (DIPEA) in dry DCM, and deprotection was performed with TFA in dry DCM at 0 □ to yield compounds **25-32**. All reactions were carried out as detailed in the experimental section, and the structures of all target derivatives were

112 confirmed by spectral (^{1}H NMR, ^{13}C NMR and HRMS) analysis.

113

114 (Insert Scheme 1.)

115 (Insert Scheme 2.)

116 (Insert Scheme 3.)

117

118 **2.2 Biology**

119 **2.2.1 Cytotoxicity assay**

120 The cytotoxicity of glycyrrhetinic acid derivatives *in vitro* was
121 evaluated on four tumor cell lines (A549: human non-small-cell lung
122 cancer; MCF-7: human mammary cancer; HepG2: human hepatocellular
123 carcinoma; Hela: human cervical cancer cell) using the the
124 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)
125 assay. In addition, their toxicity was tested using one normal cell line
126 (MDCK: Madin-Darby canine kidney) cells. The IC_{50} values of these
127 compounds were summarized in Table 1. As shown, those protected GA
128 amino acid derivatives seemed not improved apparently, but all of the
129 de-protection GA amino acid derivatives were more potent against four
130 tumor cell lines than GA. The cytotoxicity detection also revealed that
131 most of GA amino acids derivatives, especially the amide linkages
132 derivatives (GN series), exhibited better antitumor activities than the
133 positive drug cisplatin (DDP). For A549 cell lines, compound **26**

exhibited stronger antitumor activities (IC_{50}=2.109±0.11μM) than DDP (IC_{50}=9.001±0.37μM), and was over 18 times better than GA (IC_{50} > 40μM); For HepG2 cell line, compound **28** (IC_{50}=2.143±0.09μM) was over 16 times better than GA (IC_{50} > 40μM) and stronger than DDP (IC_{50}=3.908 ± 0.17μM). Among the derivatives, four most potent compounds and DDP were listed and calculated their average IC_{50} values against four cancer cell lines as shown in Fig.1. Compound **26** showed the strongest antitumor activity (average IC_{50}=2.268μM) which was threefold than the positive drug DDP (average IC_{50}=7.392μM)

In the meantime, it could be obviously seen that the amide linkages derivatives (GN series) exhibited better antiproliferative activities against the cell lines than ester linkages derivatives (GO series). As shown in Fig.2, the IC_{50} values of all GN series were lower than GO series on A549 cells, especially for derivatives connected with phenylalanine and leucine, which in separate improved 5.64 times and 6.98 times, respectively. Among them, compound **26** was the most potent one against A549 cells. As described above, introduction of de-protected amino acid (without Boc group) could enhance antitumor activities and turning hydroxyl of C-3 into amino made all derivatives obtain better antitumor activities, Considering compound **26** had the lowest average of IC_{50} values, we chose it for further analysis to learn the mechanism of growth inhibition on A549 cells.

(Insert Table 1.)

(Insert Figure 1.)

(Insert Figure 2.)

2.2.2 Analyses of apoptosis

2.2.2.1 Morphological detection of apoptosis using DAPI staining

To further investigate the mechanism of apoptosis inducted by compound **26** on A549 cell line, DAPI staining was performed after treating A549 cells with 0, 1, 3 and 5 μM of compound **26** for 72 h. As shown in Fig.3, control groups of A549 cells showed intact cell bodies with clear round nuclei; while with increasing concentration of compound, the formation of apoptotic bodies appeared and A549 cells showed condensed chromatin, nuclear fragmentation and weak fluorescence. In summary, these results indicated that compound **26** could induced apoptosis in A549 cells.

(Insert Figure 3.)

2.2.2.2 Detection of apoptosis using Annexin V-FITC/PI staining

To substantiate the ability of compound **26** to induce apoptosis, further studies were carried out using Annexin V-FITC/PI staining

technique in A549 cells. As performed in Fig.4, when treated with different concentrations (0, 1, 3, and 5 μM) of compound **26**, the early apoptosis ratios increased from 3.0% of the control to 5.9%, 26.5%, 44.8%, respectively. These results indicated that compound **26** had the potential to induce A549 cell early apoptosis.

(Insert Figure 4.)

2.2.2.3Cell cycle analysis using PI staining

To analyze the cell cycle, A549 cells were stained with PI apoptosis detection kit As shown in Fig.5, with the increasing concentration of compound **26** (0, 1, 3, and 5 μM), the percentage of A549 cells in S phase increased dramatically (from 31.41% to 53.21%), which evidently indicated that compound **26** was able to induce cell cycle arrest in the S phase.

(Insert Figure 5.)

3. Conclusions

In this study, 32 Glycyrrhetinic acid derivatives had been designed and synthesized and all of them were characterized by ^{1}H NMR, ^{13}C NMR, HRMS. In addition, all these compounds were tested for cytotoxic

activity against MCF-7, A549, Hela, HepG2 and MDCK cell lines by standard MTT assay. From the obtained results, it could be known that all of the de-protection GA amino acid derivatives were more active against four tumor cell lines than GA, while those protection GA amino acid derivatives did not behave obvious antitumor activities. Meanwhile, what surprised us was that the amide linkages derivatives (GN series) were more potent against those tumor cells than ester linkages derivatives (GO series), which proved the bonding mode of GA derivatives indeed effectively affected the antitumor activity. Among them, compound **26** exhibited a wide range of antitumor activity and was better than cisplatin against A549 cells whose IC_{50} was 2.109±0.11μM and 9.001±0.37μM respectively. Through DAPI staining, it was confirmed that compound **26** could induce A549 apoptosis via nuclei fragmentation. The detection of apoptosis and cell cycle analysis proved that compound **26** could induce cell cycle arrest at the S phase. In summary, the introduction of amino and the formation of amide bond could effectively enhance the antitumor activity of GA, although that the selectivity of the compound was not improved, this kind of compounds with significantly increased activity were suitable for the prodrug design, the strategy of our group by introducing targeted pentapeptide which could effectively change the selective effect between the PSMA-expressing and the non-PSMA-expressing tumor cells and between the tumor and non-tumor

cells[26]. Furthermore, these results in this manuscript might prove meaningful for the chemical modification of GA and the further researches on GN series would be carried out on the following research.

4. Experimental section

4.1 Chemistry

Reagents were obtained from commercial suppliers and used without further purification. All melting points were determined on a micro melting point apparatus and were uncorrected. TLC was performed on silica gel coated aluminum sheets (Qingdao Haiyang Chemical Co., Qingdao, China) and visualized in UV light (254 nm). NMR spectra were recorded on a Bruker 400 spectrometer (Bruker, Germany) with tetramethylsilan (TMS) as an internal standard; chemical shifts δ were given in ppm and coupling constants *J* in Hz. HR-MS were acquired using a Thermo Scientific TM LTQ Orbitrap XL hybrid FTMS instrument (Thermo Technologies, New York, NY, USA).

4.1.1 Preparation of Benzyl protected glycyrrhetinic acid (GA-BN)

Glycyrrhetinic acid (1 equiv.) was dissolved in DMF (40 mL), Benzyl bromide (1.2 equiv.) and anhydrous K_2CO_3 (3 equiv.) were added. The mixture was stirred at 80 □ for 3 h. After completion of the reaction (as monitored by TLC), the solvent was condensed to less than 20 mL under

reduced pressure, and then 100 mL water was added while the mixture was stirred, precipitated substances in water were filtered and dried them in the oven. Purification was performed by flash chromatography.

4.1.2 Preparation of Benzyl protected 3-aminoglycyrrhetinic acid (GN-BN)

To a solution of Benzyl protected glycyrrhetinic acid (1 equiv.) in CH_3COCH_3 (20 mL), a solution of chromium trioxide in dilute sulfuric acid (10 mL.) was added drop by drop. The reaction mixture was stirred at 0 □ for 1 h. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with 50 mL DCM, then successively washed with water and brine (20 mL each), dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated under vacuum. Purification was performed by flash chromatography to give GO-BN as white solid.

To a solution of the intermediate (GO-BN) (1 equiv.) and CH_3COONH_4 (10 equiv.）in CH_3OH (100 mL), $NaCNBH_3$ (1.5 equiv.) was added and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was concentrated, H_2O (100 mL) was added. The pH of the mixture was adjusted to 9 with NaOH solution (1M), and filtered to give crude product. The crude product was purified using flash chromatography to give Benzyl protected 3β-aminoglycyrrhetinic

acid as white solid.

4.1.3. General procedure for esterification at C3-OH of Benzyl protected glycyrrhetinic acid (method A)

The corresponding intermediate (GA-BN) (1 equiv.) was dissolved in dry DCM (25 mL), then DMAP (0.2 equiv.), EDCI (1.5 equiv.) and the protected amino acid with Boc group (1.2 equiv.) were added. The mixture was stirred at room temperature for 12 h. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with 50 mL DCM, then successively washed with water and brine (20 mL each), dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated under vacuum. Purification was performed by flash chromatography.

4.1.4. General procedure for de-protection (method B)

TFA (2 mL) was added slowly to the solution of the Boc-protected compound in dry DCM (6 mL). The mixture was stirred at 0 □ for 4 h. After completion of the reaction (as monitored by TLC), the solvent was removed under reduced pressure and washed with a saturated sodium bicarbonate solution (10 mL). The aqueous layer was extracted with DCM (3×25 mL) and the combined organic extracts were dried over anhydrous sodium sulfate, filtrated and evaporated. Then the crude

product was purified by flash chromatography.

4.1.5. General procedure for esterification at C3-NH2 of Benzyl protected 3-aminoglycyrrhetinic acid (method C)

The corresponding intermediate (GN-BN) (1 equiv.) was dissolved in dry DCM (25 mL), then HOBt (1.5 equiv.), EDCI (1.5 equiv.), DIPEA (2.5 equiv.) and the protected amino acid with Boc group (1.2 equiv.) were added. The mixture was stirred at room temperature for 12 h. After completion of the reaction (as monitored by TLC), the reaction mixture was diluted with 50 mL DCM, then successively washed with water and brine (20 mL each), dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated under vacuum. Purification was performed by flash chromatography.

4.1.3.1.Benzyl 3β-(*N*-Boc-glycyl)-11-oxo-olean-12-en-30-oate*(Compound 1)*.

According to Method A, compound **1** was obtained as white powder; yield: 79.6%; m.p.: 89.8 □, $[a]_D$ =+140 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.40-7.30 (m, 5H, H-Ar), 5.55 (s, 1H, H-12), 5.18, 5.10 (d, each, 1H, Bn-CH_2, J = 12.4 Hz), 4.59 (dd, 1H, H-3, J = 11.6 Hz, 4.8 Hz), 3.90 (m, 2H, Gly-C$\underline{H}_2$NH), 2.80 (dt, 1H, H-1, J = 13.6

Hz, 3.6 Hz), 2.33 (s, 1H, H-9), 2.05-1.91, 1.80-1.51, 1.42-1.35, 1.31-1.18, 1.04-0.96, 0.80-0.77 (19H, methylene and methine of triterpenoid structure), 1.45 (brs, 9H, Boc-CH_3), 1.34 (s, 3H, H-27), 1.16-1.15 (br, 6H, H-29 and H-25), 1.10 (s, 3H, H-26), 0.87 (brs, 6H, H-23 and H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3 (C-13), 170.3(Gly-COO), 169.3(C-13), 155.8 (Boc-COO), 136.3 (C_{ar}), 128.8 (C_{ar}), 128.8 (C_{ar}), 128.6 (C-12), 128.4 (C_{ar}), 128.3 (C_{ar}), 128.3 (C_{ar}), 82.1 (C-3), 80.0 (Boc-*q*.C), 66.4 (Bn-CH_2), 61.8 (C-9), 55.1 (C-5), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 42.8 (Gly-CH_2NH), 41.2 (C-19), 38.9 (C-1), 38.3 (C-4), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.6 (C-29), 28.5 (C-23), 28.4 (Boc-CH_3), 28.2 (C-28), 26.6 (C-16), 26.5 (C-15), 23.7 (C-2), 23.4 (C-27), 18.8 (C-26), 17.5 (C-6), 16.8 (C-24), 16.5 (C-25). HRMS (ESI) *m/z*: 740.4481 $[M+Na]^+$, calcd. for $C_{44}H_{63}NNaO_7$ 740.4502.

4.1.3.2.Benzyl 3β-(*N*-Boc-L-alanyl)-11-oxo-olean-12-en-30-oate*(Compound 2)*.

According to Method A, compound **2** was obtained as white powder; yield: 65.4%; m.p.: 192.4 □, $[a]_D$ =+140 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): *δ* (ppm) 7.40-7.29 (m, 5H, H-Ar), 5.55 (d, 1H, H-12, *J* =8.8 Hz), 5.18, 5.09 (d, each, 1H, Bn-CH_2, *J* = 12.0 Hz), 4.94 (m, 1H, H-3), 4.11 (m, 1H, Ala-CHN<u>H</u>), 3.64 (m, 1H, Ala-C<u>H</u>NH), 2.78-2.66 (br,

1H, H-1), 2.35 (s, 1H, H-9), 2.07-1.91, 1.83-1.48, 1.42-1.39, 1.30-1.25, 1.03-0.96, 0.86-0.84 (19H, methylene and methine of triterpenoid structure), 1.44 (s, 9H, Boc-CH_3), 1.36-1.32 (br, 6H, H-27 and -CH_3 of Alanine), 1.16-1.12 (br, 6H, H-29 and H-25), 1.10 (s, 3H, H-26), 0.89 (s, 3H, H-24), 0.78 (m, 3H, H-23), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1(C-11), 176.3 (C-30), 173.2 (Ala-COO), 169.3 (C-13), 155.2 (Boc-COO), 136.3 (C_{ar}), 128.8 (C_{ar}), 128.8 (C_{ar}), 128.6 (C-12), 128.4 (C_{ar}), 128.3 (C_{ar}), 128.3 (C_{ar}), 81.8 (C-3), 79.8 (Boc-*q*.C), 66.4 (Bn-CH_2), 61.8 (C-9), 55.1 (C-5), 49.70 (Ala-CHNH), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 38.9 (C-1), 38.4 (C-4), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.6 (C-29), 28.5 (C-23), 28.4 (Boc-CH_3), 28.2 (C-28), 26.6 (C-16), 26.5 (C-15), 23.7 (C-2), 23.4 (C-27), 19.1 (Ala-CH_3), 18.8 (C-26), 17.5 (C-6), 16.9 (C-24), 16.5 (C-25). HRMS (ESI) *m/z*: 754.4686 $[M+Na]^+$, calcd. for $C_{45}H_{65}NNaO_7$ 754.4659.

4.1.3.3.Benzyl 3β-(*N*-Boc-L-phenylalanine)-11-oxo-olean-12-en-30-oate*(Compound 3)*.

According to Method A, compound **3** was obtained as white powder; yield: 66.9%; m.p.: 99.0 □, $[a]_D$ =+132 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.40-7.27, 7.25-7.15 (br, 10H, H-Ar), 5.54 (s, 1H, H-12), 5.19, 5.10 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 4.90 (d, 1H,

Phe-CHNH, J = 8.4 Hz), 4.54 (m, 2H, Phe-CHNH and H-3), 3.12 (m, 1H, Phe-CHH′), 3.03 (m, 1H, Phe-CHH′), 2.81 (dt, 1H, H-1, *J* = 13.6 Hz, 3.6 Hz), 2.32 (s, 1H, H-9), 2.08-1.90, 1.84-1.43, 1.32-1.18, 1.05-0.95, 0.76 (19H, methylene and methine of triterpenoid structure), 1.40 (brs, 9H, Boc-CH_3), 1.34 (s, 3H, H-27), 1.16 (s, 3H, H-29), 1.14 (s, 3H, H-25), 1.10 (s, 3H, H-26), 0.82 (s, 3H, H-24), 0.80 (s, 3H, H-23), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1(C-11), 176.3 (C-30), 171.9 (Phe-COO), 169.2 (C-13), 155.2 (Boc-COO), 136.3 (Phe-C_{ar}), 136.2 (Bn-C_{ar}), 129.5 (Phe-C_{ar}), 129.5 (Phe-C_{ar}), 128.8 (Bn-C_{ar}), 128.8 (Bn-C_{ar}), 128.7 (Bn -C_{ar}), 128.6 (C-12), 128.5 (Phe -C_{ar}), 128.5 (Phe-C_{ar}), 128.4 (Bn-C_{ar}), 128.4 (Bn-C_{ar}), 127.1 (Phe-C_{ar}), 82.3 (C-3), 79.9 (Boc-*q*.C), 66.4 (Bn-CH_2), 61.8 (C-9), 55.2 (C-5), 54.8 (Phe-CHNH), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 38.9 (C-1), 38.6 (C-4), 38.2 (Phe-CH_2), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.6 (C-29), 28.5 (C-23), 28.4(Boc-CH_3), 28.2 (C-28), 26.6 (C-16), 26.5 (C-15), 23.6 (C-2), 23.4 (C-27), 18.8 (C-26), 17.5 (C-6), 16.8 (C-24), 16.5 (C-25). HRMS (ESI) *m/z*: 830.4996 $[M+Na]^+$, calcd. for $C_{51}H_{69}NNaO_7$ 830.4972.

4.1.3.4.Benzyl

3β-(*N*-Boc-L-proline)-11-oxo-olean-12-en-30-oate*(Compound 4)*. According to Method A, compound **4** was obtained as white powder;

yield: 75.7%; m.p.: 102.5 □, $[a]_D$ =+148 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.41-7.29 (m, 5H, H-Ar), 5.55 (d, 1H, H-12, J = 5.2 Hz), 5.18, 5.10 (d, each, 1H, Bn-CH_2, J = 12.4 Hz), 4.53 (m, 1H, H-3), 4.31 (m, 1H, Pro-CH), 3.48 (m, 2H, Pro-CH_2), 2.79 (m, 1H, H-1), 2.33 (s, 1H, H-9), 2.21 (m, 1H, Pro-C<u>H</u>H′), 2.04-1.52, 1.42-1.36, 1.32-1.18, 1.05-0.93, 0.83-0.78 (22H, methylene and methine of triterpenoid structure and proline), 1.45-1.42 (br, 9H, Boc-CH_3), 1.32 (s, 3H, H-27), 1.16 (brs, 6H, H-29 and H-25), 1.10 (s, 3H, H-26), 0.88 (m, 6H, H-23 and H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3 (C-30), 173.0 (Pro-COO), 169.3 (C-13), 154.0 (Boc-COO), 136.3 (C_{ar}), 128.8 (C_{ar}), 128.8 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 81.2 (C-3), 80.1 (Boc-*q*.C), 66.4 (Bn-CH_2), 61.8 (C-9), 59.6 (Pro-CH), 55.2 (C-5), 48.4 (C-18), 46.4 (Pro-CH_2), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 38.8 (C-1), 38.3 (C-4), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.7 (Pro-CH_2), 28.6 (C-29), 28.5 (C-23), 28.4 (Boc-CH_3), 28.3 (C-28), 26.6 (C-16), 26.5 (C-15), 24.5 (Pro-CH_2), 23.6 (C-2), 23.4 (C-27), 18.8 (C-26), 17.5 (C-6), 16.9 (C-24), 16.5 (C-25). HRMS (ESI) *m/z:* 780.4837 $[M+Na]^+$, calcd. for $C_{47}H6_7NNaO_7$ 780.4815.

4.1.3.5.Benzyl 3β-(*N*-Boc-L-sarcosine)-11-oxo-olean-12-en-30-oate*(Compound 5).*

According to Method A, compound **5** was obtained as white powder; yield: 81.2%; m.p.: 82.6 □, $[a]_D$ =+140 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.40-7.30 (m, 5H, H-Ar), 5.54 (s, 1H, H-12), 5.18, 5.10 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 4.57 (m, 1H, H-3), 3.90 (m, 2H, Sar-CH_2), 2.94 (m, 3H, Sar-CH_3), 2.80 (m, 1H, H-1), 2.33 (s, 1H, H-9), 2.07-1.89, 1.83-1.52, 1.38, 1.32-1.18, 1.06-0.95, 0.83-0.78 (19H, methylene and methine of triterpenoid structure), 1.45-1.43 (br, 9H, Boc-CH_3), 1.34 (s, 3H, H-27), 1.16 (brs, 6H, H-29 and H-25), 1.10 (s, 3H, H-26), 0.87 (m, 6H, H-23 and H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3 (C-30), 169.9 (Sar-COO), 169.3 (C-13), 155.6 (Boc-COO), 136.3 (C_{ar}), 128.8 (C_{ar}), 128.8 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 81.7 (C-3), 80.3 (Boc-*q*.C), 66.4 (Bn-CH_2), 61.8 (C-9), 55.1 (C-5), 51.5 (Sar-CH_2), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 38.9 (C-1), 38.2 (C-4), 37.8 (C-22), 37.0 (C-10), 35.8 (Sar-CH_3), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.6 (C-29), 28.5 (C-23), 28.4 (Boc-CH_3), 28.3(C-28), 26.6 (C-16), 26.5 (C-15), 23.7 (C-2), 23.4 (C-27), 18.8 (C-26), 17.5 (C-6), 16.9 (C-24), 16.5 (C-25). HRMS (ESI) *m/z*: 754.4682 $[M+Na]^+$, calcd. for $C_{45}H_{65}NNaO_7$ 754.4659.

4.1.3.6.Benzyl 3β-(*N*-Boc-L-leucine)-11-oxo-olean-12-en-30-oate*(Compound 6).*

421 According to Method A, compound **6** was obtained as white powder;
422 yield: 77.8%; m.p.: 166.1 □, $[a]_D$ =+132 (c 0.3 mg/mL, MeOH); ^{1}H NMR
423 (400MHz, $CDCl_3$): δ (ppm) 7.40-7.31 (m, 5H, H-Ar), 5.54 (s, 1H, H-12),
424 5.18, 5.10 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 4.88 (m, 1H, Leu-CHNH),
425 4.55 (dd, 1H, H-3, J = 12.0 Hz, 4.8 Hz), 4.29 (m, 1H, Leu-CHNH), 2.82
426 (dt, 1H, H-1, J = 13.6 Hz, 3.6 Hz), 2.33 (s, 1H, H-9), 2.05-1.92, 1.84-1.48,
427 1.42-1.36, 1.32-1.18, 1.07-0.98, 0.83-0.77 (22H, methylene and methine
428 of triterpenoid structure and leucine), 1.44 (brs, 9H, Boc-CH_3), 1.34 (s,
429 3H, H-27), 1.16 (brs, 6H, H-29 and H-25), 1.10 (s, 3H, H-26), 0.95-0.94
430 (br, 6H, Leu-CH_3), 0.89-0.87 (m, 6H, H-23 and H-24), 0.73 (s, 3H, H-28).
431 ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3 (C-30), 173.3
432 (Leu-COO), 169.2 (C-13), 155.5 (Boc-COO), 136.3 (C_{ar}), 128.8 (C_{ar}),
433 128.8 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 81.8 (C-3),
434 79.8 (Boc-*q*.C), 66.4 (Bn-CH_2), 61.8 (C-9), 55.2 (C-5), 52.7 (Leu-CHNH),
435 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 42.2 (Leu-CH_2), 41.2
436 (C-19), 38.9 (C-1), 38.3 (C-4), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9
437 (C-17), 31.3 (C-21), 28.6 (C-29), 28.5 (C-23), 28.4 (Boc-CH_3), 28.3
438 (C-28), 26.6 (C-16), 26.5 (C-15), 25.0 (Leu-CH), 23.7 (C-2), 23.4 (C-27),
439 23.1 (Leu-CH_3), 18.8 (C-26), 17.5 (C-6), 16.9 (C-24), 16.5 (C-25).
440 HRMS (ESI) *m/z*: 796.5153 $[M+Na]^+$, calcd. for $C_{48}H_{71}NNaO_7$ 796.5128.
441

4.1.3.7.Benzyl

3β-(*N*-Boc-L-isoleucine)-11-oxo-olean-12-en-30-oate*(Compound 7).* According to Method A, compound **7** was obtained as white powder; yield: 70.9%; m.p.: 162.8 □, $[a]_D$ =+132 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.40-7.28 (m, 5H, H-Ar), 5.54 (s, 1H, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 5.01 (m, 1H, Ile-CHN<u>H</u>), 4.56 (dd, 1H, H-3, J = 11.6 Hz, 5.2 Hz), 4.26 (m, 1H, Ile-C<u>H</u>NH), 2.81 (dt, 1H, H-1, J = 13.6 Hz, 3.2 Hz), 2.33 (s, 1H, H-9), 2.08-1.87, 1.84-1.46, 1.42-1.37, 1.30-1.18, 1.07-0.98, 0.83-0.77 (22H, methylene and methine of triterpenoid structure and isoleucine), 1.44 (brs, 9H, Boc-CH_3), 1.34 (s, 3H, H-27), 1.15 (brs, 6H, H-29 and H-25), 1.10 (s, 3H, H-26), 0.96-0.91 (m, 6H, Ile-CH_3), 0.89-0.88 (m, 6H, H-23 and H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3 (C-30), 172.2 (Ile-COO), 169.2 (C-13), 155.8 (Boc-COO), 136.3 (C_{ar}), 128.8 (C_{ar}), 128.8 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 82.1 (C-3), 79.8 (Boc-*q*.C), 66.4 (Bn-CH_2), 61.8 (C-9), 58.6 (Ile-CHNH), 55.2 (C-5), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 38.9 (C-1), 38.2 (C-4), 38.1 (Ile-CH), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.6 (C-29), 28.5 (C-23), 28.4 (Boc-CH_3), 28.2 (C-28), 26.6 (C-16), 26.5 (C-15), 24.9 (Ile-CH_2), 23.7 (C-2), 23.4 (C-27), 18.8 (C-26), 17.5 (C-6), 17.0 (C-24), 16.5 (C-25), 15.9 (Ile-<u>C</u>H_3CH_2),

11.8 (Ile-$\underline{C}H_3CH$). HRMS (ESI) *m/z*: 796.5156 $[M+Na]^+$, calcd. for $C_{48}H_{71}NNaO_7$ 796.5128.

4.1.3.8.Benzyl

3β-(*N*-Boc-L-methionine)-11-oxo-olean-12-en-30-oate*(Compound **8**).* According to Method A, compound **8** was obtained as white powder; yield: 66.9%; m.p.: 152.9 □, $[a]_D$ =+120 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.41-7.29 (m, 5H, H-Ar), 5.54 (s, 1H, H-12), 5.18, 5.10 (d, each, 1H, Bn-CH_2, *J* = 12.0 Hz), 4.57 (dd, 1H, H-3, *J* = 11.6 Hz, 4.8 Hz), 4.46-4.34 (br, 1H, Met-C$\underline{H}$NH), 2.80 (dt, 1H, H-1, *J* = 13.6 Hz, 3.2 Hz), 2.55 (m, 2H, Met-CH_2S), 2.33 (s, 1H, H-9), 2.26-2.11 (m, 2H, Met-C$\underline{H_2}$CH), 2.10 (s, 3H, Met-CH_3), 2.04-1.92, 1.79-1.53, 1.42-1.37, 1.32-1.18, 1.07-0.96, 0.83-0.76 (19H, methylene and methine of triterpenoid structure), 1.44 (brs, 9H, Boc-CH_3), 1.34 (s, 3H, H-27), 1.16 (brs, 6H, H-29 and H-25), 1.10 (s, 3H, H-26), 0.89-0.87 (m, 6H, H-23 and H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3 (C-30), 172.1 (Met-COO), 169.3 (C-13), 155.5 (Boc-COO), 136.3 (C_{ar}), 128.8 (C_{ar}), 128.8 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 82.3 (C-3), 80.1 (Boc-*q*.C), 66.4 (Bn-CH_2), 61.8 (C-9), 55.1 (C-5), 53.4 (Met-CHNH), 48.4(C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 38.8 (C-1), 38.3 (C-4), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 30.2 (Met-$\underline{C}H_2$CH),

28.6 (C-29), 28.5 (C-23), 28.4 (Boc-CH_3), 28.3 (C-28), 28.2 (Met-CH_2S), 26.6 (C-16), 26.5 (C-15), 23.7 (C-2), 23.4 (C-27), 18.8 (C-26), 17.5 (C-6), 17.0 (C-24), 16.5 (C-25), 15.6 (Met-CH_3). HRMS (ESI) *m/z*: 814.4712 $[M+Na]^+$, calcd. for $C_{47}H_{69}NNaO_7S$ 814.4692.

4.1.4.1.Benzyl 3β-(glycyl)-11-oxo-olean-12-en-30-oate*(Compound 9).* According to Method B, compound **9** was obtained as white powder; yield: 79.8%; m.p.: 189.0 □, $[a]_D$ =+144 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.40-7.30 (m, 5H, H-Ar), 5.54 (s, 1H, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 4.58 (dd, 1H, H-3, J = 11.6 Hz, 4.8 Hz), 3.44 (s, 2H, Gly-CH_2), 2.80 (dt, 1H, H-1, J = 13.6 Hz, 3.6 Hz), 2.36 (s, 1H, H-9), 2.04-1.92, 1.84-1.37, 1.32-1.18, 1.05-0.95, 0.83-0.76 (19H, methylene and methine of triterpenoid structure), 1.34 (s, 3H, H-27), 1.15 (brs, 6H, H-29 and H-25), 1.10 (s, 3H, H-26), 0.87 (brs, 6H, H-23 and H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3 (C-30), 173.9 (Gly-COO), 169.2 (C-13), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.4 (C_{ar}), 128.3 (C_{ar}), 128.3 (C_{ar}), 81.6 (C-3), 66.4 (Bn-CH_2), 61.8 (C-9), 55.1 (C-5), 48.4 (C-18), 45.5 (C-8), 44.1 (Gly-CH_2), 44.1(C-20), 43.3 (C-14), 41.2 (C-19), 38.9 (C-1), 38.3 (C-4), 37.8(C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.5 (C-29), 28.4 (C-23), 28.2 (C-28), 26.6 (C-16), 26.5 (C-15), 23.7 (C-2), 23.4 (C-27), 18.8 (C-26), 17.5 (C-6), 16.8 (C-24), 16.5 (C-25).

HRMS (ESI) *m/z*: 618.4159 $[M+H]^+$, calcd. for $C_{39}H_{56}NO_5$ 618.4158.

4.1.4.2.Benzyl 3β-(L-alanyl)-11-oxo-olean-12-en-30-oate*(Compound **10**).* According to Method B, compound **10** was obtained as white powder; yield: 71.7%; m.p.: 199.1 □, $[a]_D$ =+164 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.42-7.28 (m, 5H, H-Ar), 5.54 (s, 1H, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 4.56 (dd, 1H, H-3, J = 11.6 Hz, 4.8 Hz), 3.52 (q, 1H, Ala-CH, J = 7.2 Hz), 2.79 (dt, 1H, H-1, J = 13.6 Hz, 3.6 Hz), 2.34 (s, 1H, H-9), 2.08-1.89, 1.80-1.41, 1.31-1.18, 1.05-0.95, 0.81-0.79 (19H, methylene and methine of triterpenoid structure), 1.37-1.34 (br, 6H, H-27 and Ala-CH_3), 1.16 (brs, 6H, H-29 and H-25), 1.10 (s, 3H, H-26), 0.89 (s, 3H, H-24), 0.87 (s, 3H, H-23), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.4 (C-30), 176.3 (Ala-COO), 169.2 (C-13), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.4 (C_{ar}), 128.3 (C_{ar}), 128.3 (C_{ar}), 81.2 (C-3), 66.3 (Bn-CH_2), 61.8 (C-9), 55.1 (C-5), 50.5 (Ala-CH), 48.3 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 38.9 (C-1), 38.4 (C-4), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.5 (C-29), 28.4 (C-23), 28.2 (C-28), 26.6 (C-16), 26.5 (C-15), 23.7 (C-2), 23.4 (C-27), 20.9 (Ala-CH_3), 18.8 (C-26), 17.5 (C-6), 16.9 (C-24), 16.5 (C-25). HRMS (ESI) *m/z*: 632.4277 $[M+H]^+$, calcd. for $C_{40}H_{58}NO_5$ 632.4315.

4.1.4.3.Benzyl

3β-(L-phenylalanine)-11-oxo-olean-12-en-30-oate*(Compound* ***11****).*

According to Method B, compound **11** was obtained as white powder; yield: 80.9%; m.p.: 192.8 □, $[a]_D$ =+140 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.40-7.28, 7.24-7.18 (br, 10H, H-Ar), 5.54 (s, 1H, H-12), 5.19, 5.10 (d, each, 1H, Bn-CH_2, J = 12.4 Hz), 4.56 (dd, 1H, H-3, J = 11.6 Hz, 4.8 Hz), 3.74 (m, 1H, Phe-C<u>H</u>NH_2), 3.14 (dd, 1H, Phe-C<u>H</u>H′, J = 13.6 Hz, 4.4 Hz), 2.82 (m, 1H, H-1), 2.78 (m, 1H, Phe-CH<u>H</u>′), 2.34 (s, 1H, H-9), 2.05-1.91, 1.81-1.41, 1.32-1.18, 1.08-0.96, 0.78 (19H, methylene and methine of triterpenoid structure), 1.35 (s, 3H, H-27), 1.16 (brs, 6H, H-29 and H-25), 1.10 (s, 3H, H-26), 0.86 (s, 3H, H-24), 0.80 (s, 3H, H-23), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3 (C-30), 174.8 (Phe-COO), 169.2 (C-13), 137.6 (Phe-C_{ar}), 136.3(Bn-C_{ar}), 129.5 (Phe-C_{ar}), 129.5 (Phe-C_{ar}), 128.8(Bn-C_{ar}), 128.8(Bn-C_{ar}), 128.7(Bn-C_{ar}), 128.6 (C-12), 128.5 (Phe-C_{ar}), 128.5 (Phe-C_{ar}), 128.4(Bn-C_{ar}), 128.4(Bn-C_{ar}), 126.9 (Phe-C_{ar}), 81.6 (C-3), 66.4 (Bn-CH_2), 61.8 (C-9), 56.4 (Phe-$CHNH_2$), 55.2 (C-5), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 38.9 (C-1), 38.6 (C-4), 38.3 (Phe-CH_2), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.6 (C-29), 28.4 (C-23), 28.2 (C-28), 26.6 (C-16), 26.5 (C-15), 23.7 (C-2), 23.4 (C-27), 18.8 (C-26), 17.5 (C-6), 16.9 (C-24),

16.5 (C-25). HRMS (ESI) *m/z*: 708.4593 $[M+H]^+$, calcd. for $C_{46}H_{62}NO_5$ 708.4628.

4.1.4.4.Benzyl 3β-(L-proline)-11-oxo-olean-12-en-30-oate*(Compound 12)*. According to Method B, compound **12** was obtained as white powder; yield: 70.8%; m.p.: 152.9 □, $[a]_D$ =+164 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.40-7.28 (m, 5H, H-Ar), 5.54 (s, 1H, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12.4 Hz), 4.58 (dd, 1H, H-3, J = 11.6 Hz, 4.8 Hz), 3.76 (m, 1H, Pro-CH), 3.10 (m, 1H, Pro-C<u>H</u>H′NH), 2.92 (m, 1H, Pro-CH<u>H</u>′NH), 2.81 (dt, 1H, H-1, J = 14.0 Hz, 3.2 Hz), 2.43(m, 1H, Pro-C<u>H</u>H′(CH)), 2.33 (s, 1H, H-9), 2.15 (m, 1H, Pro-CH<u>H</u>′(CH)), 2.04-1.38, 1.32-1.18, 1.07-0.95, 0.81-0.78 (21H, methylene and methine of triterpenoid structure and proline), 1.34 (s, 3H, H-27), 1.15 (brs, 6H, H-29 and H-25), 1.10 (s, 3H, H-26), 0.89 (s, 3H, H-23), 0.87 (s, 3H, H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3 (C-30), 175.2 (Pro-COO), 169.2 (C-13), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 81.2 (C-3), 66.3 (Bn-CH_2), 61.8 (C-9), 60.3 (Pro-CH), 55.1 (C-5), 48.3 (C-18), 47.0 (Pro-CH_2), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 38.9 (C-1), 38.4 (C-4), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 30.5 (Pro-CH_2), 28.5 (C-29), 28.4 (C-23), 28.3 (C-28), 26.6 (C-16), 26.5 (C-15), 25.6

572 (Pro-CH_2), 23.7 (C-2), 23.4 (C-27), 18.8 (C-26), 17.5 (C-6), 16.9 (C-24),
573 16.5 (C-25). HRMS (ESI) *m/z*: 658.4461 $[M+H]^+$, calcd. for $C_{42}H_{60}NO_5$
574 658.4471.

575

576 **4.1.4.5.Benzyl 3β-(L-sarcosine)-11-oxo-olean-12-en-30-oate***(Compound*
577 ***13)***. According to Method B, compound **13** was obtained as white powder;
578 yield: 68.4%; m.p.: 182.9 □, $[a]_D$ =+172 (c 0.3 mg/mL, MeOH); ^{1}H NMR
579 (400MHz, $CDCl_3$): δ (ppm) 7.42-7.29 (m, 5H, H-Ar), 5.53 (s, 1H, H-12),
580 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 4.62 (dd, 1H, H-3, J =
581 11.6 Hz, 4.4 Hz), 3.37 (brs, 2H, Sar-CH_2), 2.81 (dt, 1H, H-1, J = 13.6 Hz,
582 3.2 Hz), 2.45 (s, 3H, Sar-CH_3), 2.33 (s, 1H, H-9), 2.04-1.92, 1.86-1.38,
583 1.32-1.18, 1.06-0.94, 0.81-0.78 (19H, methylene and methine of
584 triterpenoid structure and sarcosine), 1.34 (s, 3H, H-27), 1.16 (brs, 6H,
585 H-29 and H-25), 1.10 (s, 3H, H-26), 0.88 (brs, 6H, H-23 and H-24), 0.73
586 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3
587 (C-30), 172.2 (Sar-COO), 169.2 (C-13), 136.3 (C_{ar}), 128.8 (C_{ar}), 128.8
588 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 81.4 (C-3), 66.4
589 (Bn-CH_2), 61.8 (C-9), 55.1 (C-5), 52.9 (Sar-CH_2NH), 48.4 (C-18), 45.5
590 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 38.9 (C-1), 38.3 (C-4), 37.8
591 (C-22), 37.0 (C-10), 36.2 (Sar-CH_3), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21),
592 28.6 (C-29), 28.4 (C-23), 28.3 (C-28), 26.6 (C-16), 26.5 (C-15), 23.8
593 (C-2), 23.4 (C-27), 18.8 (C-26), 17.5 (C-6), 16.9 (C-24), 16.5 (C-25).

HRMS (ESI) *m/z*: 632.4287 $[M+H]^+$, calcd. for $C_{40}H_{58}NO_5$ 632.4315.

4.1.4.6.Benzyl 3β-(L-leucine)-11-oxo-olean-12-en-30-oate*(Compound **14**)*. According to Method B, compound **14** was obtained as white powder; yield: 65.4%; m.p.: 186.1 □, $[a]_D$ =+172 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.40-7.30 (m, 5H, H-Ar), 5.54 (s, 1H, H-12), 5.18, 5.10 (d, each, 1H, Bn-CH_2, J = 12.4 Hz), 4.56 (dd, 1H, H-3, J = 11.6 Hz, 4.8 Hz), 3.47 (m, 1H, Leu-C<u>H</u>NH_2), 2.82 (dt, 1H, H-1, J = 13.6 Hz, 3.6 Hz), 2.34 (s, 1H, H-9), 2.04-1.92, 1.83-1.38, 1.32-1.18, 1.08-0.98, 0.81-0.78 (22H, methylene and methine of triterpenoid structure and leucine),

1.34 (s, 3H, H-27), 1.16 (brs, 6H, H-29 and H-25), 1.10 (s, 3H, H-26), 0.96-0.93 (br, 6H, Leu-CH_3), 0.89-0.88 (m, 6H, H-23 and H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3 (C-30), 176.2 (Leu-COO), 169.2 (C-13), 136.3 (C_{ar}), 128.8 (C_{ar}), 128.8 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 81.3 (C-3), 66.4 (Bn-CH_2), 61.8 (C-9), 55.2 (C-5), 53.3 (Leu-$CHNH_2$), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 42.3 (Leu-CH_2), 41.2 (C-19), 38.9 (C-1), 38.3 (C-4), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.6 (C-29), 28.4 (C-23), 28.3 (C-28), 26.6 (C-16), 26.5 (C-15), 25.0 (Leu-CH), 23.7 (C-2), 23.4 (C-27), 23.2 (Leu-CH_3), 18.8 (C-26), 17.5 (C-6), 17.0 (C-24), 16.5 (C-25). HRMS (ESI) *m/z:* 674.4768 $[M+H]^+$,

calcd. for $C_{43}H_{64}NO_5$ 674.4784.

4.1.4.7.Benzyl

3β-(L-isoleucine)-11-oxo-olean-12-en-30-oate*(Compound* ***15****).*

According to Method B, compound **15** was obtained as white powder; yield: 73.9%; m.p.: 169.5 □, $[a]_D$ =+180 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.42-7.29 (m, 5H, H-Ar), 5.54 (s, 1H, H-12), 5.18, 5.10 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 4.57 (dd, 1H, H-3, J = 11.6 Hz, 4.8 Hz), 3.36 (m, 1H,Ile-C<u>H</u>NH_2), 2.82 (dt, 1H, H-1, J = 13.6 Hz, 3.2 Hz), 2.34 (s, 1H, H-9), 2.08-1.92, 1.84-1.37, 1.30-1.18, 1.08-1.01, 0.82-0.79 (23H, methylene and methine of triterpenoid structure and isoleucine), 1.34 (s, 3H, H-27), 1.16 (brs, 6H, H-29 and H-25), 1.11 (s, 3H, H-26), 1.00-0.98 (br, 3H, Ile-CH_3), 0.93-0.89 (m, 9H, Ile-CH_3 and H-23 and H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3 (C-30), 173.5 (Ile-COO), 169.2 (C-13), 136.3 (C_{ar}), 128.8 (C_{ar}), 128.8 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 81.4 (C-3), 66.4 (Bn-CH_2), 61.8 (C-9), 59.9 (Ile-$CHNH_2$), 55.2 (C-5), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 39.5 (Ile-CH), 38.9 (C-1), 38.2 (C-4), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.6 (C-29), 28.4 (C-23), 28.3 (C-28), 26.6 (C-16), 26.5 (C-15), 24.4 (Ile-CH_2), 23.8 (C-2), 23.4 (C-27), 18.8 (C-26), 17.5 (C-6), 17.1 (C-24), 16.5 (C-25), 16.1 (Ile-<u>C</u>H_3CH_2), 11.9

638 (Ile-$\underline{C}H_3CH$). HRMS (ESI) *m/z*: 674.4763 $[M+H]^+$, calcd. for $C_{43}H_{64}NO_5$
639 674.4784.
640
641 **4.1.4.8.Benzyl**
642 **3β-(L-methionine)-11-oxo-olean-12-en-30-oate***(Compound* ***16****).*
643 According to Method B, compound **16** was obtained as white powder;
644 yield: 62.1%; m.p.: 179.6 □, $[a]_D$ =+116 (c 0.3 mg/mL, MeOH); ^{1}H NMR
645 (400MHz, $CDCl_3$): δ (ppm) 7.41-7.29 (m, 5H, H-Ar), 5.54 (s, 1H, H-12),
646 5.18, 5.10 (d, each, 1H, Bn-CH_2, *J* = 12.4 Hz), 4.57 (dd, 1H, H-3, *J* =
647 11.6 Hz, 4.8 Hz), 3.62 (q, 1H, Met-C$\underline{H}NH_2$, *J* = 4.4 Hz), 2.80 (dt, 1H,
648 H-1, *J* = 13.6 Hz, 3.2 Hz), 2.65 (t, 2H, Met-CH_2, *J* = 8.0 Hz), 2.34 (s, 1H,
649 H-9), 2.11 (brs, 5H, Met-CH_2 and Met-CH_3), 2.04-1.92, 1.83-1.39,
650 1.31-1.18, 1.05-0.96, 0.81-0.79 (19H, methylene and methine of
651 triterpenoid structure), 1.34 (s, 3H, H-27), 1.16 (brs, 6H, H-29 and H-25),
652 1.10 (s, 3H, H-26), 0.89-0.88 (m, 6H, H-23 and H-24), 0.73 (s, 3H, H-28).
653 ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.1 (C-11), 176.3 (C-30), 175.4
654 (Met-COO), 169.3 (C-13), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6
655 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 81.7 (C-3), 66.4 (Bn-CH_2),
656 61.8 (C-9), 55.1 (C-5), 53.8 (Met-$CHNH_2$), 48.4 (C-18), 45.5 (C-8), 44.1
657 (C-20), 43.3 (C-14), 41.2 (C-19), 38.9 (C-1), 38.3 (C-4), 37.8 (C-22),
658 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 33.9 (Met-$\underline{C}H_2CH$), 31.3 (C-21),
659 30.7 (Met-CH_2S), 28.5 (C-29), 28.4 (C-23), 28.3 (C-28), 26.6 (C-16),

26.5 (C-15), 23.7 (C-2), 23.4 (C-27), 18.8 (C-26), 17.5 (C-6), 17.0 (C-24), 16.5 (C-25), 15.5 (Met-CH_3). HRMS (ESI) *m/z*: 692.4328 $[M+H]^+$, calcd. for $C_{42}H_{62}NO_5S$ 692.4349.

4.1.5.1.Benzyl 3β-[(*N*-Boc-glycyl)amino]-11-oxo-olean-12-en-30-oate*(Compound **17**)*.

According to Method C, compound **17** was obtained as white powder; yield: 89.2%; m.p.: 136.1 □, $[a]_D$ =+136 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.39-7.29 (m, 5H, H-Ar), 6.11 (m, 1H, NHCO), 5.53 (s, 1H, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 5.15 (m, 1H, Gly-CH_2N<u>H</u>), 3.81-3.64 (m, 3H, H-3 and Gly-C<u>H</u>$_2$NH), 2.76 (dt, 1H, H-1, J = 13.6 Hz, 3.6 Hz), 2.35 (s, 1H, H-9), 2.03-1.92, 1.83-1.51, 1.40-1.36, 1.32-1.15, 1.05-0.95, 0.86-0.82 (19H, methylene and methine of triterpenoid structure), 1.45 (brs, 9H, Boc-CH_3), 1.34 (s, 3H, H-27), 1.15 (s, 3H, H-29), 1.12 (s, 3H, H-25), 1.09 (s, 3H, H-26), 0.88 (s, 3H, H-23), 0.78 (s, 3H, H-24), 0.72 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.2 (C-11), 176.3 (C-30), 169.7 (Gly-COO), 169.2 (C-13), 156.2 (Boc-COO), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 80.5 (Boc-*q*.C), 66.3 (Bn-CH_2), 61.8 (C-9), 56.6 (C-3), 55.6 (C-5), 48.4 (C-18), 45.5 (C-8), 45.1 (Gly-CH_2NH), 44.1 (C-20), 43.4 (C-14), 41.2 (C-19), 39.8(C-1), 38.3 (C-4), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17),

682 31.3 (C-21), 28.6 (C-29), 28.5 (C-23), 28.4 (Boc-CH_3), 28.4 (C-28), 26.6
683 (C-16), 26.5 (C-15), 25.4 (C-2), 23.4 (C-27), 18.8 (C-26), 17.8 (C-6),
684 16.6 (C-24), 16.4 (C-25). HRMS (ESI) *m/z*: 717.4821 $[M+H]^+$, calcd. for
685 $C_{44}H_{65}N_2O_6$ 717.4843.
686
687 **4.1.5.2.Benzyl**
688 **3β-[(*N*-Boc-L-alanyl)amino]-11-oxo-olean-12-en-30-oate***(Compound*
689 ***18)***. According to Method C, compound **18** was obtained as white powder;
690 yield: 80.3%; m.p.: 116.2 □, $[a]_D$ =+132 (c 0.3 mg/mL, MeOH); ^{1}H NMR
691 (400MHz, $CDCl_3$): δ (ppm) 7.41-7.29 (m, 5H, H-Ar), 5.55 (d, 1H, *J* =8.0
692 Hz, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, *J* = 12.0 Hz), 4.96 (brs, 1H,
693 Ala-CHNH), 4.11 (m, 1H, Ala-CHNH), 3.83-3.60 (m, 1H, H-3),
694 2.78-2.66 (m, 1H, H-1), 2.35 (s, 1H, H-9), 2.06-1.91, 1.85-1.48,
695 1.42-1.36, 1.33-1.30, 1.16, 1.04-0.96, 0.86-0.82 (19H, methylene and
696 methine of triterpenoid structure), 1.44 (brs, 9H, Boc-CH_3), 1.35 (s, 3H,
697 H-27), 1.25 (m, 3H, Ala-CH3), 1.16 (m, 3H, H-29), 1.12-1.10 (s, 6H,
698 H-25 and H-26), 0.89 (s, 3H, H-23), 0.78 (m, 3H, H-24), 0.73 (m, 3H,
699 H-28). uu^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.3 (C-11), 176.3
700 (C-30), 172.2 (Ala-COO), 169.2 (C-13), 155.6 (Boc-COO), 136.3 (C_{ar}),
701 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}),
702 80.3 (Bn-CH_2), 66.3 (Bn-CH_2), 61.8 (C-9), 56.5 (C-3), 55.6 (C-5), 53.8
703 (Ala-CHNH), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2

(C-19), 39.8 (C-1), 38.3 (C-4), 37.8 (C-22), 37.1 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.6 (C-29), 28.5 (C-23), 28.4 (Boc-CH_3), 28.4 (C-28), 26.6 (C-16), 26.5 (C-15), 25.4 (C-2), 23.4 (C-27), 18.8 (C-26), 17.8 (Ala-CH_3), 17.5 (C-6), 16.6 (C-24), 16.4 (C-25). HRMS (ESI) *m/z*: 731.4980 $[M+H]^+$, calcd. for $C_{45}H_{67}N_2O_6$ 731.4999.

4.1.5.3.Benzyl 3β-[(*N*-Boc-L-phenylalanine)amino]-11-oxo-olean-12-en-30-oate*(Compound **19**).* According to Method C, compound **19** was obtained as white powder; yield: 78.4%; m.p.: 112.8 □, $[a]_D$ =+120 (c 0.3 mg/mL, MeOH); ^1H NMR (400MHz, $CDCl_3$): δ (ppm) 7.38-7.28, 7.23-7.18 (m, 10H, H-Ar), 5.88 (m, 1H, NHCO), 5.53 (s, 1H, H-12), 5.18, 5.10 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 5.02 (m, 1H, Phe-CHNH), 4.31 (m, 1H, Phe-CHNH), 3.61 (m, 1H, H-3), 3.14-2.99 (m, 2H, Phe-CH_2), 2.72 (dt, 1H, H-1, J = 13.6 Hz, 3.6 Hz), 2.33 (s, 1H, H-9), 2.04-1.92, 1.82-1.43, 1.37, 1.30-1.18, 1.00-0.78 (19H, methylene and methine of triterpenoid structure), 1.41 (brs, 9H, Boc-CH_3), 1.35 (s, 3H, H-27), 1.16 (brs, 3H, H-29), 1.07 (s, 3H, H-25 and H-26), 0.72 (m, 6H, H-23 and H-24), 0.56 (m, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.3 (C-11), 176.4 (C-30), 171.0 (Phe-COO), 169.3 (C-13), 155.6 (Boc-COO), 136.3 (Phe-C_{ar}), 136.3 (Bn-C_{ar}), 129.4 (Phe-C_{ar}), 129.4 (Phe-C_{ar}), 128.9 (Bn-C_{ar}), 128.9 (Bn-C_{ar}), 128.7 (Phe-C_{ar}), 128.7 (Phe-C_{ar}), 128.6 (C-12), 128.4

(Bn-C_{ar}), 128.3 (Bn-C_{ar}), 128.3 (Bn-C_{ar}), 127.1 (Phe-C_{ar}), 80.5 (Boc-*q*.C), 66.4 (Bn-CH_2), 61.8 (C-9), 56.8 (C-3), 56.3 (Phe-CHNH), 55.6 (C-5), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 39.7 (C-1), 38.2 (C-4), 38.0 (Phe-CH_2), 37.8 (C-22), 37.0 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.6 (C-29), 28.5 (C-23), 28.4 (Boc-CH_3), 28.4(C-28), 26.6 (C-16), 26.5 (C-15), 25.3(C-2), 23.4(C-27), 18.8 (C-26), 17.8 (C-6), 16.4 (C-24), 16.3 (C-25). HRMS (ESI) *m/z*: 807.5293 $[M+H]^+$, calcd. for $C_{51}H_{71}N_2O_6$ 807.5312.

4.1.5.4.Benzyl 3β-[(*N*-Boc-L-proline)amino]-11-oxo-olean-12-en-30-oate*(Compound 20).* According to Method C, compound **20** was obtained as white powder; yield: 81.2%; m.p.: 102.5 □, $[a]_D$ = +104 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.38-7.29 (m, 5H, H-Ar), 5.53 (s, 1H, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12.4 Hz), 4.28 (m, 1H, Pro-CH), 3.63 (m, 1H, H-3), 3.42 (m, 2H, Pro-CH_2), 2.77 (m, 1H, H-1), 2.35 (s, 1H, H-9), 2.18 (m, 1H, Pro-C<u>H</u>H′), 2.07-1.51, 1.43-1.37, 1.32-1.18, 1.05-0.94, 0.87-0.84 (22H, methylene and methine of triterpenoid structure and proline), 1.46 (brs, 9H, Boc-CH_3), 1.35 (s, 3H, H-27), 1.15 (s, 3H, H-29), 1.12 (s, 3H, H-25), 1.09 (s, 3H, H-26), 0.91 (s, 3H, H-23), 0.78 (s, 3H, H-24), 0.72 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.3 (C-11), 176.3 (C-30), 171.6 (Pro-COO), 169.2

(C-13), 155.5 (Boc-COO), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 80.5 (Boc-*q*.C), 66.3 (Bn-CH_2), 61.8 (C-9), 60.4 (Pro-CH), 56.5 (C-3), 55.6 (C-5), 48.4 (C-18), 47.3 (Pro-CH_2), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 39.8 (C-1), 38.4 (C-4), 37.8 (C-22), 37.1 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.5 (Pro-CH_2), 28.5 (C-29), 28.5 (C-23), 28.5 (Boc-CH_3), 28.4 (C-28), 26.6 (C-16), 26.5 (C-15), 25.6 (C-2), 23.4 (Pro-CH_2), 23.4 (C-27), 18.8 (C-26), 17.8 (C-6), 16.6 (C-24), 16.4 (C-25). HRMS (ESI) *m/z*: 757.5137 $[M+H]^+$, calcd. for $C_{47}H_{69}N_2O_6$ 757.5156.

4.1.5.5.Benzyl

3β-[(*N*-Boc-L-sarcosine)amino]-11-oxo-olean-12-en-30-oate

*(Compound **21**).* According to Method C, compound **21** was obtained as white powder; yield: 76.3%; m.p.: 119.5 □, $[a]_D$ =+140 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.39-7.29 (m, 5H, H-Ar), 5.53 (s, 1H, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12,0 Hz), 3.88-3.77 (m, 2H, Sar-CH_2), 3.68 (m, 1H, H-3), 2.94 (s, 3H, Pro-CH_3), 2.79 (m, 1H, H-1), 2.35 (s, 1H, H-9), 2.05-1.91, 1.83-1.48, 1.42-1.37, 1.32-1.17, 1.04-0.94, 0.85 (19H, methylene and methine of triterpenoid structure), 1.46 (s, 9H, Boc-CH_3), 1.34 (s, 3H, H-27), 1.15 (s, 3H, H-29), 1.12 (s, 3H, H-25), 1.09 (s, 3H, H-26), 0.88 (s, 3H, H-23), 0.85 (m, 1H, H-5), 0.76 (s, 3H, H-24), 0.72 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$):

δ (ppm) 200.2 (C-11), 176.3 (C-30), 174.2 (Sar-COO), 169.2 (C-13), 156.8 (Boc-COO), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 80.9 (Boc-*q*.C), 66.3 (Bn-CH_2), 61.8 (C-9), 56.4 (C-3), 55.6 (C-5), 53.8 (Sar-CH_2), 48.4 (C-18), 45.4 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 39.8 (C-1), 38.2 (C-4), 37.8 (C-22), 37.0 (C-10), 35.9 (Sar-CH_3), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.6 (C-29), 28.5 (C-23), 28.5(Boc-CH_3), 28.4 (C-28), 26.6 (C-16), 26.5 (C-15), 25.5 (C-2), 23.4 (C-27), 18.9 (C-26), 17.9 (C-6), 16.6 (C-24), 16.4 (C-25). HRMS (ESI) *m/z*: 731.4986 $[M+H]^+$, calcd. for $C_{45}H_{67}N_2O_6$ 731.4999.

4.1.5.6.Benzyl

3β-[(*N*-Boc-L-leucine)amino]-11-oxo-olean-12-en-30-oate *(Compound **22**)*. According to Method C, compound **22** was obtained as white powder; yield: 65.8%; m.p.: 116.5 □, $[a]_D$ =+112 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.39-7.31 (m, 5H, H-Ar), 6.06 (m, 1H, NHCO), 5.53 (s, 1H, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 4.85 (m, 1H, Leu-CHN<u>H</u>), 4.04 (m, 1H, Leu-C<u>H</u>NH), 3.64 (m, 1H, H-3), 2.77 (m, 1H, H-1), 2.35 (s, 1H, H-9), 2.04-1.91, 1.82-1.47, 1.41-1.37, 1.30-1.17, 1.07-0.96, 0.86-0.83 (22H, methylene and methine of triterpenoid structure and leucine), 1.44 (brs, 9H, Boc-CH_3), 1.35 (s, 3H, H-27), 1.15 (s, 3H, H-29), 1.12 (s, 3H, H-25), 1.10 (s, 3H, H-26),

792 0.95-0.92 (m, 6H, Leu-CH_3), 0.88 (s, 3H, H-23), 0.78 (s, 3H, H-24), 0.72
793 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.2 (C-11), 176.3
794 (C-30), 172.0 (Leu-COO), 169.2 (C-13), 155.8 (Boc-COO), 136.3 (C_{ar}),
795 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}),
796 80.2 (Boc-*q*.C), 66.3 (Bn-CH_2), 61.8 (C-9), 56.5 (C-3), 55.6 (C-5), 53.7
797 (Leu-CHNH), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 42.0
798 (Leu-CH_2), 41.2 (C-19), 39.8 (C-1), 38.2 (C-4), 37.8 (C-22), 37.1 (C-10),
799 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.7 (C-29), 28.6 (C-23), 28.4
800 (Boc-CH_3), 28.4(C-28), 26.6 (C-16), 26.5 (C-15), 25.4 (C-2), 25.0
801 (Leu-CH), 23.4 (C-27), 23.0 (Leu-CH_3), 18.8 (C-26), 17.9 (C-6), 17.0
802 (C-24), 16.4 (C-25). HRMS (ESI) *m/z*: 773.5449 $[M+H]^+$, calcd. for
803 $C_{48}H_{73}N_2O_6$ 773.5469.
804
805 **4.1.5.7.Benzyl**
806 **3β-[(*N*-Boc-L-isoleucine)amino]-11-oxo-olean-12-en-30-oate***(Compoun*
807 *d **23**)*. According to Method C, compound **23** was obtained as white
808 powder; yield: 67.7%; m.p.: 116.0 □, $[a]_D$ =+84 (c 0.3 mg/mL, MeOH);
809 ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.41-7.30 (m, 5H, H-Ar), 5.92 (m,
810 1H, NHCO), 5.54 (s, 1H, H-12), 5.18, 5.10 (d, each, 1H, Bn-CH_2, J =
811 12.4 Hz), 5.01 (m, 1H, Ile-CHN<u>H</u>), 3.86 (m, 1H, Ile-C<u>H</u>NH), 3.66 (m, 1H,
812 H-3), 2.77 (dt, 1H, H-1, J = 13.2 Hz, 3.2 Hz), 2.35 (s, 1H, H-9), 2.05-1.46,
813 1.41-1.37, 1.32-1.28, 1.19, 1.04-0.97, 0.86-0.83 (22H, methylene and

methine of triterpenoid structure and isoleucine), 1.44 (brs, 9H, Boc-CH_3), 1.35 (s, 3H, H-27), 1.25 (s, 3H, Ile-CH_3), 1.16 (s, 3H, H-29), 1.12 (s, 3H, H-25), 1.10 (s, 3H, H-26), 0.95 (s, 3H, Ile-CH_3), 0.88 (s, 3H, H-23), 0.78 (s, 3H, H-24), 0.72 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.3 (C-11), 176.4 (C-30), 171.3 (Ile-COO), 169.3 (C-13), 156.1 (Boc-COO), 136.3 (C_{ar}), 128.8 (C_{ar}), 128.8 (C_{ar}), 128.6, 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 80.2 (Boc-*q*.C), 66.4 (Bn-CH_2), 61.8 (C-9), 60.1 (Ile-CHNH), 56.7 (C-3), 55.6 (C-5), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.4 (C-14), 41.2 (C-19), 39.8 (C-1), 38.1 (C-4), 37.8 (C-22), 37.1 (C-10), 36.8 (Ile-CH), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.7 (C-29), 28.6 (C-23), 28.5(Boc-CH_3), 28.5 (C-28), 26.7 (C-16), 26.6 (C-15), 25.5 (C-2), 24.9 (Ile-CH_2), 23.4 (C-27), 18.8 (C-26), 17.9 (C-6), 16.8 (C-24), 16.4 (C-25), 16.0 (Ile-$\underline{C}H_3CH_2$), 11.6 (Ile-$\underline{C}H_3CH$). HRMS (ESI) *m/z*: 773.5450 $[M+H]^+$, calcd. for $C_{48}H_{73}N_2O_6$ 773.5469.

4.1.5.8.Benzyl 3β-[(*N*-Boc-L-methionine)amino]-11-oxo-olean-12-en-30-oate*(Compound **24**)*. According to Method C, compound **24** was obtained as white powder; yield: 80.9%; m.p.: 129.5 □, $[a]_D$ =+116 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.40-7.30 (m, 5H, H-Ar), 6.11 (m, 1H, NHCO), 5.53 (s, 1H, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 5.16 (m, 1H, Met-CHN$\underline{H}$), 4.21 (m, 1H, Met-C$\underline{H}$NH), 3.64 (m,

836 1H, H-3), 2.78 (dt, 1H, H-1, *J* = 13.6 Hz, 3.2 Hz), 2.57 (m, 2H,
837 Met-CH_2S), 2.35 (s, 1H, H-9), 2.11 (m, 5H, Met-CH_2 and Met-CH_3),
838 2.04-1.92, 1.83-1.47, 1.42-1.37, 1.32-1.17, 1.05-0.95, 0.86-0.83 (19H,
839 methylene and methine of triterpenoid structure), 1.44 (brs, 9H,
840 Boc-CH_3), 1.34 (s, 3H, H-27), 1.32 (m, 1H, H-21′), 1.15 (s, 3H, H-29),
841 1.12 (s, 3H, H-25), 1.10 (s, 3H, H-26), 0.88 (s, 3H, H-23), 0.79 (s, 3H,
842 H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.2
843 (C-11), 176.3 (C-30), 171.0 (Met-COO), 169.2 (C-13), 155.7 (Boc-COO),
844 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}),
845 128.4 (C_{ar}), 80.4 (Boc-*q*.C), 66.3 (Bn-CH_2), 61.8 (C-9), 56.8 (C-3), 55.6
846 (C-5), 54.1 (Met-CHNH), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3
847 (C-14), 41.2 (C-19), 39.8 (C-1), 38.2 (C-4), 37.8 (C-22), 37.1 (C-10),
848 32.8 (C-7), 31.9 (C-17), 31.4 (C-21), 31.3 (Met-$\underline{C}H_2CH$), 30.5
849 (Met-CH_2S), 28.7 (C-29), 28.6 (C-23), 28.5 (Boc-CH_3), 28.4 (C-28), 26.6
850 (C-16), 26.5 (C-15), 25.5 (C-2), 23.4 (C-27), 18.8 (C-26), 17.9 (C-6),
851 16.7 (C-24), 16.4 (C-25), 15.4 (Met-CH_3). HRMS (ESI) *m/z*: 791.5023
852 $[M+H]_+$, calcd. for $C_{47}H_{71}N_2O_6S$ 791.5033.

853

854 **4.1.4.9.Benzyl**

855 **3β-[(glycyl)amino]-11-oxo-olean-12-en-30-oate***(Compound* ***25****).*
856 According to Method B, compound **25** was obtained as white powder;
857 yield: 75.7%; m.p.: 215.9 □, $[a]_D$ =+160 (c 0.3 mg/mL, MeOH); ^{1}H NMR

(400MHz, $CDCl_3$): δ (ppm) 7.39-7.29 (m, 5H, H-Ar), 5.53 (s, 1H, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12.4 Hz), 3.68 (m, 1H, H-3), 3.36 (s, 2H, Gly-CH_2), 2.77 (dt, 1H, H-1, J = 13.6 Hz, 3.2 Hz), 2.36 (s, 1H, H-9), 2.07-1.88, 1.80-1.43, 1.32-1.18, 1.07-0.94, 0.87 (19H, methylene and methine of triterpenoid structure), 1.34 (s, 3H, H-27), 1.15 (s, 3H, H-29), 1.14 (s, 3H, H-25), 1.10 (s, 3H, H-26), 0.88 (s, 3H, H-23), 0.82 (s, 3H, H-24), 0.72 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.3 (C-11), 176.3 (C-30), 172.0 (Gly-COO), 169.1 (C-13), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 66.3 (Bn-CH_2), 61.9 (C-9), 56.0 (C-3), 55.6 (C-3), 48.4 (C-18), 45.5 (C-8), 44.9 (Gly-CH_2NH_2), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 39.8 (C-1), 38.3 (C-4), 37.8 (C-22), 37.1 (C-10), 32.9 (C-7), 31.9 (C-17), 31.3 (C-21), 28.7 (C-29), 28.5 (C-23), 28.4 (C-28), 26.6 (C-16), 26.4 (C-15), 25.5 (C-2), 23.4 (C-27), 18.8 (C-26), 17.9 (C-6), 16.7 (C-24), 16.4 (C-25). HRMS (ESI) *m/z*: 617.4299 $[M+H]^+$, calcd. for $C_{39}H_{57}N_2O_4$ 617.4318.

4.1.4.10.Benzyl 3β-[(L-alanyl)amino]-11-oxo-olean-12-en-30-oate*(Compound 26)*.

According to Method B, compound **26** was obtained as white powder; yield: 78.1%; m.p.: 176.5 □, $[a]_D$ =+152 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.40-7.29 (m, 5H, H-Ar), 5.53 (s, 1H, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12.4 Hz), 3.62 (m, 1H, H-3), 3.53 (q,

1H, Ala-CH, *J* = 6.8 Hz), 2.77 (dt, 1H, H-1, *J* = 13.6 Hz, 3.2 Hz), 2.36 (s, 1H, H-9), 2.07-1.91, 1.80-1.43, 1,32-1.25, 1.18, 1.07-0.94, 0.85 (19H, methylene and methine of triterpenoid structure), 1.35 (s, 3H, H-27), 1.25 (m, 3H, Ala-CH3), 1.15 (s, 3H, H-29), 1.14 (s, 3H, H-25), 1.10 (s, 3H, H-26), 0.87 (s, 3H, H-23), 0.85 (m, 1H, H-5), 0.81 (s, 3H, H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.3 (C-11), 176.3 (C-30), 175.0 (Ala-COO), 169.1 (C-13), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 66.3 (Bn-CH_2), 61.8 (C-9), 56.1 (C-3), 55.6 (C-5), 50.9 (Ala-$CHNH_2$), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 39.8 (C-1), 38.3 (C-4), 37.8 (C-22), 37.1 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.7 (C-29), 28.5 (C-23), 28.4 (C-28), 26.5 (C-16), 25.5 (C-2), 23.4 (C-27), 18.8 (C-26), 17.9 (Ala-CH_3), 16.8 (C-24), 16.4 (C-25). HRMS (ESI) *m/z*: 631.4465 $[M+H]^+$, calcd. for $C_{40}H_{59}N_2O_4$ 631.4475.

4.1.4.11.Benzyl 3β-[(L-phenylalanine)amino]-11-oxo-olean-12-en-30-oate*(Compound 27).* According to Method B, compound **27** was obtained as white powder; yield: 71.9%; m.p.: 139.5 □, $[a]_D$ =+156 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.40-7.29, 7.24-7.19 (m, 10H, H-Ar), 5.54 (s, 1H, H-12), 5.19, 5.10 (d, each, 1H, Bn-CH_2, *J* = 12.0 Hz), 3.71-3.60 (m, 2H, Phe-C<u>H</u>NH_2 and H-3), 3.27 (dd, 1H, Phe-C<u>H</u>H′, *J* = 13.6 Hz, 4.0 Hz),

2.78-2.71 (m, 2H, H-1 and Phe-CHH′), 2.37 (s, 1H, H-9), 2.05-1.93, 1.79-1.42, 1.31-1.18, 1.08-0.95, 0.89 (19H, methylene and methine of triterpenoid structure), 1.35 (s, 3H, H-27), 1.15 (s, 3H, H-29), 1.13 (s, 3H, H-25), 1.10 (s, 3H, H-26), 0.86 (s, 3H, H-23), 0.76 (s, 3H, H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.3 (C-11), 176.3 (C-30), 173.6 (Phe-COO), 169.1 (C-13), 138.1 (Phe-C_{ar}), 136.3 (Bn-C_{ar}), 129.5 (Phe-C_{ar}), 129.5 (Phe-C_{ar}), 128.8 (Bn-C_{ar}), 128.8 (Bn-C_{ar}), 128.7 (Phe-C_{ar}), 128.7 (Phe-C_{ar}), 128.6 (C-12), 128.4 (Bn-C_{ar}), 128.3 (Bn-C_{ar}), 128.3 (Bn-C_{ar}), 126.9 (Phe-C_{ar}), 66.3 (Bn-CH_2), 61.8 (C-9), 56.5 (C-3), 56.2 (Phe-$CHNH_2$), 55.6 (C-5), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 39.8 (C-1), 38.4 (Phe-CH_2), 38.2 (C-4), 37.8 (C-22), 37.1 (C-10), 32.9 (C-7), 31.9 (C-17), 31.3 (C-21), 28.7 (C-29), 28.6 (C-23), 28.4 (C-28), 26.6 (C-16), 25.5 (C-2), 23.4 (C-27), 18.8 (C-26), 17.9 (C-6), 16.7 (C-24), 16.4 (C-25). HRMS (ESI) *m/z:* 707,4779 $[M+H]^+$, calcd. for $C_{46}H_{63}N_2O_4$ 707.4788.

4.1.4.12.Benzyl 3β-[(L-proline)amino]-11-oxo-olean-12-en-30-oate*(Compound **28**).* According to Method B, compound **28** was obtained as white powder; yield: 75.5%; m.p.: 116.2 □, $[a]_D$ =+148 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.63 (d, 1H,-NH-, J = 10.4 Hz), 7.39-7.29 (m, 5H, H-Ar), 5.52 (s, 1H, H-12), 5.18, 5.09 (d, each, 1H, Bn-CH_2, J = 12.4

Hz), 3.79 (dd, 1H, Pro-CHNH, *J* = 8.8 Hz, 5.6 Hz), 3.59 (m, 1H, H-3), 3.02 (m, 1H, Pro-CHH′NH), 2.90 (m, 1H, Pro-CHH′NH), 2.76 (dt, 1H, H-1, *J* = 13.2 Hz, 3.2 Hz), 2.35 (s, 1H, H-9), 2.14 (m, 1H, Pro-CHH′), 2.03-1.87, 1.83-1.37, 1.29-1.17, 1.06-0.96, 0.85 (22H, methylene and methine of triterpenoid structure and proline), 1.34 (s, 3H, H-27), 1.15 (s, 3H, H-29), 1.13 (s, 3H, H-25), 1.10 (s, 3H, H-26), 0.86 (s, 3H, H-23), 0.81 (s, 3H, H-24), 0.72 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.3 (C-11), 176.3 (C-30), 174.1 (Pro-COO), 169.1 (C-13), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 66.3 (Bn-CH_2), 61.9 (C-9), 60.8 (Pro-CH), 56.1 (C-3), 55.6 (C-5), 48.4 (C-18), 47.3 (Pro-CH_2), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 39.8 (C-1), 38.3 (C-4), 37.8 (C-22), 37.1 (C-10), 32.9 (C-7), 31.9 (C-17), 31.3 (C-21), 30.9 (Pro-CH_2), 28.7 (C-29), 28.5 (C-23), 28.4 (C-28), 26.5 (C-16), 26.5 (C-15), 26.2 (Pro-CH_2), 25.5 (C-2), 23.4 (C-27), 18.8 (C-26), 17.9 (C-6), 16.8 (C-24), 16.4 (C-25). HRMS (ESI) *m/z*: 657.4622 $[M+H]^+$, calcd. for $C_{42}H_{61}N_2O_4$ 657.4631.

4.1.4.13.Benzyl 3β-[(L-sarcosine)amino]-11-oxo-olean-12-en-30-oate *(Compound **29**).* According to Method B, compound **29** was obtained as white powder; yield: 69.3%; m.p.: 182.9 □, $[a]_D$ =+156 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.21 (d, 1H, -NH-, *J* = 10.4Hz), 7.40-7.30 (m, 5H, H-Ar), 5.53 (s, 1H, H-12), 5.18, 5.09 (d, each,

1H, Bn-CH_2, J = 12.0 Hz), 3.69 (td, 1H, H-3, J = 11.2 Hz, 4.4 Hz), 3.25 (m, 2H, Sar-CH_2), 2.76 (dt, 1H, H-1, J = 13.6 Hz, 3.2 Hz), 2.43 (s, 3H, Sar-NH$\underline{CH_3}$), 2.36 (s, 1H, H-9), 2.05-1.91, 1.81-1.40, 1.32-1.18, 1.07-0.94, 0.87-0.84 (19H, methylene and methine of triterpenoid structure), 1.35 (s, 3H, H-27), 1.16 (s, 3H, H-29), 1.14 (s, 3H, H-25), 1.10 (s, 3H, H-26), 0.89 (s, 3H, H-23), 0.81 (s, 3H, H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.3 (C-11), 176.3 (C-30), 170.7 (Sar-COO), 169.1 (C-13), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 66.3 (Bn-CH_2), 61.9 (C-9), 55.6 (C-5), 54.8 (Sar-CH_2), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 39.9 (C-1), 38.3 (C-4), 37.8 (C-22), 37.1 (C-10), 36.9 (Sar-CH_3), 32.9 (C-7), 31.9 (C-17), 31.3 (C-21), 28.7 (C-29), 28.6 (C-23), 28.4 (C-28), 26.6 (C-16), 26.5 (C-15), 25.6 (C-2), 23.4 (C-27), 18.8 (C-26), 17.9 (C-6), 16.8 (C-24), 16.4 (C-25). HRMS (ESI) *m/z*: 631.4480 $[M+H]^+$, calcd. for $C_{40}H_{59}N_2O_4$ 631.4475.

4.1.4.14.Benzyl 3β-[(L-leucine)amino]-11-oxo-olean-12-en-30-oate*(Compound 30).* According to Method B, compound **30** was obtained as white powder; yield: 78.9%; m.p.: 139.5 □, $[a]_D$ =+156 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.39-7.29 (m, 5H, H-Ar), 5.53 (s, 1H, H-12), 5.18, 5.10 (d, each, 1H, Bn-CH_2, J = 12.0 Hz), 3.79 (m, 1H,

Leu-CHNH2), 3.62 (m, 1H, H-3), 2.77 (m, 1H, H-1), 2.35 (s, 1H, H-9), 2.08-1.89, 1.79-1.37, 1.32-1.18, 1.07-0.98. 0.81 (22H, methylene and methine of triterpenoid structure and leucine), 1.35 (s, 3H, H-27), 1.16 (s, 3H, H-29), 1.12 (s, 3H, H-25), 1.10 (s, 3H, H-26), 0.96-0.93 (m, 6H, Leu-CH_3), 0.86 (s, 3H, H-23), 0.81 (s, 3H, H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.3 (C-11), 176.4 (C-30), 172.0 (Leu-COO), 169.3 (C-13), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 66.4 (Bn-CH_2), 61.9 (C-9), 57.0 (C-3), 55.6 (C-5), 53.2 (Leu-$CHNH_2$), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 42.5 (Leu-CH_2), 41.2 (C-19), 39.8 (C-1), 38.4 (C-4), 37.8 (C-22), 37.1 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (C-21), 28.8 (C-29), 28.6 (C-23), 28.4 (C-28), 26.6 (C-16), 26.6 (C-15), 25.0 (C-2), 24.9 (Leu-CH), 23.4 (C-27), 22.8 (Leu-CH_3), 18.8 (C-26), 17.9 (C-6), 16.8 (C-24), 16.4 (C-25). HRMS (ESI) *m/z*: 673.4933 $[M+H]^+$, calcd. for $C_{43}H_{65}N_2O_4$ 673.4944.

4.1.4.15.Benzyl 3β-[(L-isoleucine)amino]-11-oxo-olean-12-en-30-oate *(Compound **31**).* According to Method B, compound **31** was obtained as white powder; yield: 80.2%; m.p.: 122.5 □, $[a]_D$ =+120 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.41-7.28 (m, 5H, H-Ar), 5.54 (s, 1H, H-12), 5.18, 5.10 (d, each, 1H, J = 12.0 Hz, -OCH_2-), 3.68-3.56 (m, 2H, H-3 and Ile-CH), 2.77 (m, 1H, H-1), 2.35 (s, 1H, H-9),

2.08-1.37, 1.32-1.30, 1.18, 1.02, 0.91, 0.86-0.83 (22H, methylene and methine of triterpenoid structure and isoleucine), 1.35 (s, 3H, H-27), 1.25 (s, 3H, Ile-CH_3), 1.16 (s, 3H, H-29), 1.12 (s, 3H, H-25), 1.10 (s, 3H, H-26), 0.99-0.97 (m, 3H, Ile-CH_3), 0.91 (m, 1H, H-15′), 0.87 (s, 3H, H-23), 0.85 (m, 1H, H-5), 0.81 (s, 3H, H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.3 (C-11), 176.4 (C-30), 176.4 (Ile-COO), 169.3 (C-13), 136.3 (C_{ar}), 128.8 (C_{ar}), 128.8 (C_{ar}), 128.6 (C-12), 128.5 (C_{ar}), 128.4 (C_{ar}), 128.4 (C_{ar}), 66.4 (Bn-CH_2), 61.8 (C-9), 59.3 (Ile-$CHNH_2$), 57.1 (C-3), 55.7 (C-5), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 39.8 (C-1), 38.3 (C-4), 37.8 (C-22), 37.6 (Ile-CH), 37.1 (C-10), 32.9 (C-7), 31.9 (C-17), 31.3 (C-21), 28.9 (C-29), 28.6 (C-23), 28.4 (C-28), 26.6 (C-16), 26.6 (C-15), 25.2 (C-2), 24.4 (Ile-CH_2), 23.4 (C-27), 18.8 (C-26), 17.9 (C-6), 16.8 (C-24), 16.3 (C-25), 15.6 (Ile-$\underline{C}H_3CH_2$), 11.8 (Ile-$\underline{C}H_3CH$). HRMS (ESI) *m/z*: 673.4940 $[M+H]^+$, calcd. for $C_{43}H_{65}N_2O_4$ 673.4944.

4.1.4.16.Benzyl 3β-[(L-methionine)amino]-11-oxo-olean-12-en-30-oate *(Compound **32**).* According to Method B, compound **32** was obtained as white powder; yield: 71.0%; m.p.: 102.9 □, $[a]_D$ =+144 (c 0.3 mg/mL, MeOH); ^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.40-7.30 (m, 5H, H-Ar), 7.15 (d, 1H, -NH-, *J* = 10.0 Hz), 5.53 (s, 1H, H-12), 5.18, 5.10 (d, each, 1H, Bn-CH_2, *J* = 12.4 Hz), 3.71-3.61 (m, 2H, Met-$C\underline{H}NH_2$ and H-3), 2.76

(dt, 1H, H-1, J = 13.6 Hz, 3.2 Hz), 2.61 (m, 2H, Met-CH_2S), 2.36 (s, 1H, H-9), 2.11 (m, 1H, Met-C<u>H</u>H′), 2.10 (s, 3H, Met-CH_3), 2.07-1.88, 1.82-1.37, 1.30-1.18, 1.06-0.95, 0.85-0.84 (20H, methylene and methine of triterpenoid structure and methionine), 1.35 (s, 3H, H-27), 1.16 (s, 3H, H-29), 1.13 (s, 3H, H-25), 1.10 (s, 3H, H-26), 0.87 (s, 3H, H-23), 0.82 (s, 3H, H-24), 0.73 (s, 3H, H-28). ^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 200.2 (C-11), 176.3 (C-30), 172.5 (Met-COO), 169.2 (C-13), 136.3 (C_{ar}), 128.7 (C_{ar}), 128.7 (C_{ar}), 128.6 (C-12), 128.4 (C_{ar}), 128.3 (C_{ar}), 128.3 (C_{ar}), 66.3 (Bn-CH_2), 61.8 (C-9), 56.7 (C-3), 55.6 (C-5), 54.2 (Met-$CHNH_2$), 48.4 (C-18), 45.5 (C-8), 44.1 (C-20), 43.3 (C-14), 41.2 (C-19), 39.8 (C-1), 38.3 (C-4), 37.8 (C-22), 37.1 (C-10), 32.8 (C-7), 31.9 (C-17), 31.3 (Met-<u>C</u>H_2CH), 30.5 (Met-CH_2S), 28.8 (C-29), 28.6 (C-23), 28.4 (C-28), 26.6 (C-16), 26.6 (C-15), 25.4 (C-2), 23.4 (C-27), 18.8 (C-26), 17.9 (C-6), 16.8 (C-24), 16.4 (C-25), 15.3 (Met-CH_3). HRMS (ESI) *m/z*: 691.4500 $[M+H]^+$, calcd. for $C_{42}H_{63}N_2O_4S$ 691.4509.

4.2 Bio-evaluation methods

4.2.1 Cell culture

A549, MCF-7, HepG2, Hela and MDCK cells were obtained from the Chinese Academy of Medical Sciences and Peking Union Medical College. Cultures were maintained as monolayer in RPMI-1640/DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v)

penicillin/streptomycin (Thermo Technologies, New York, NY, USA) under a humidified atmosphere containing 5% CO_2 at 37 □. The GA derivatives under study were dissolved in DMSO (Sigma, St. Louis, MO, USA) and added at required concentrations to the cell culture. Cells incubated without the preparations served as the control.

4.2.2 Cytotoxicity assay

The cytotoxicity of these compounds was tested on five cells lines in vitro using the MTT method. In short, exponentially growing cells were seeded into 96-well plates at a density 3×10^3 cells/well and incubated for 24 h at 37 ℃ with 5% CO_2. Then the cells were exposed to various concentrations of the tested drugs and incubated for 72 h. Later, the MTT solution (20 μL, 5 mg/mL) was added to each well and incubated for another 4 h. After throwing away the supernatant medium, formazan crystals were dissolved with DMSO (150 μL). The absorbance was measured at 490 nm with a plate reader (BIORAD 550 spectrophotometer, Bio-rad Life Science Development Ltd., Beijing, China). Wells without drugs were used to be blanks. The IC_{50} values were defined as the concentration of compounds that produced a 50% proliferation inhibition of surviving cells and calculated using the GraphPad Prism 5. The inhibitory rate was calculated in the following Equation (1):

% inhibition = [1 – (Sample group OD-Blank group OD)/(Control group OD-Blank group OD) × 100% (1)

4.2.3 Morphological detection of apoptosis using DAPI staining

Exponentially growing A549 cells were seeded in 12-well plates (2.4 $\times 10^4$ cells/well). After incubation for 24 h at 37 ℃ with 5% CO_2, certain concentrations of compound **26** (1, 3, 5 μM) were added to each well, cells were incubated for another 72 h. Then the cell culture medium was discarded and cells were washed twice with PBS and fixed with 4% paraformaldehyde for 10 min. Then the liquid was discarded and the cells were stained with DAPI (DAPI, 10μg/mL, Molecular Probes/Invitrogen Life Technologies, Carlsbad, CA, USA) for 1 min in dark. The cell morphological observation of nuclear changes were observed using inverted phase-contrast microscope.

4.2.4 Detection of apoptosis using Annexin V-FITC/PI staining

Exponentially growing A549 cells were seeded in 12-well plates (1.2 $\times 10^4$ cells/well). After incubation for 24 h at 37 ℃ with 5% CO_2, certain concentrations of compound **26** (2, 4, 8 μM) were added to each well, cells were incubated for another 72 h. Then cells were collected and washed twice with cold PBS and centrifuged at 1000 rpm for 5 min. The harvested cells were resuspended in 200 μL binding buffer, which

contained 10 μL Annexin V-FITC. After 15 min, the cells were washed twice and resuspended in 300 μL binding buffer. At the same time, 10 μL PI was added. After avoiding light reaction for 15 min, the cells were analyzed with a flow cytometer.

4.2.5 Cell cycle analysis using PI staining

Exponentially growing A549 cells were seeded in 12-well plates (1.2 $\times 10^4$ cells/well). After incubation for 24 h at 37 ℃ with 5% CO_2, certain concentrations of **26** (2, 4, 8 μM) were added to each well, cells were incubated for another 72 h. Then cells were collected and washed twice with cold PBS and centrifuged at 1000 rpm for 5 min, the supernatant was discarded. The resulting pellet was resuspended and fixed in 1mL 70% cold ethanol for 12 h at 4 ℃. And 0.5 mL of PI staining solution was added before cells were washed with PBS, after incubation at 37℃ for 30 min, the cells were analyzed with a flow cytometer.

4.3 Statistical analysis

All data were expressed as the means ± standard deviation (SD) of three replications. The statistical analysis was performed by SPSS software (Version 20.0) to analyze the variance. One-way analysis of variance (ANOVA) followed by the least significant difference (LSD) post hoc test for multiple comparisons. A p-value of less than 0.05 was

considered significant.

Author contributions

Ideas and experiment design: Fei Zhou, Peng-Long Wang and Hai-Min Lei; Chemistry and Biology: Fei Zhou, Gao-Rong Wu, De-Sheng Cai, Bing Xu, Meng-Meng Yan; Analysis and interpretation of data: Wen-Bo Guo, Wen-Xi Zhang, Tao Ma, Xue-Mei Huang, Xiao-hui Jia, Yu-Qin Yang, Feng Gao; Writing and review of the manuscript: All the authors; Study supervision: Hai-Min Lei, Peng-Long Wang.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This research was funded by the National Natural Science Foundation of China (No.81173519 and 81603256), the Fundamental Research Funds for the Central Universities (BUCM-2019-JCRC002, 2019-JYB-TD005 and BUCM-2018-2020), Beijing Key Laboratory for Basic and Development Research on Chinese Medicine (Beijing, 100102), project of China Association of Chinese Medicine (CACM-2018-QNRC2-B08).

Reference

1121 [1] Cragg, Gordon M., and David J. Newman, Natural products: a
1122 continuing source of novel drug leads, Biochimica et Biophysica Acta
1123 (BBA)-General Subjects. 1830 (2013) 3670-3695.
1124 [2] Wenzel, S. C., Hoffmann, H., Zhang, J., Debussche, L., Haag‐
1125 Richter, S., Kurz, M., ... & Schummer, D, Production of the bengamide
1126 class of marine natural products in myxobacteria: biosynthesis and
1127 structure–activity relationships, Angewandte Chemie International
1128 Edition. 54 (2015) 15560-15564.
1129 [3] Petersen F, Natural products based molecules for target and drug
1130 discovery in pharmaceutical research, Planta Medica International Open.
1131 4.S (2017) We-PL.
1132 [4] Salminen, A., Lehtonen, M., Suuronen, T., Kaarniranta, K., &
1133 Huuskonen, J, Terpenoids: natural inhibitors of NF-κB signaling with
1134 anti-inflammatory and anticancer potential, Cellular and Molecular Life
1135 Sciences. 65 (2008) 2979-2999.
1136 [5] Shanmugam, M. K., Dai, X., Kumar, A. P., Tan, B. K., Sethi, G., &
1137 Bishayee, A, Ursolic acid in cancer prevention and treatment: molecular
1138 targets, pharmacokinetics and clinical studies, Biochemical pharmacology.
1139 85 (2013) 1579-1587.
1140 [6] Song, J., Ko, H. S., Sohn, E. J., Kim, B., Kim, J. H., Kim, H. J., ... &
1141 Kim, S. H., Inhibition of protein kinase C α/βII and activation of c-Jun
1142 NH2-terminal kinase mediate glycyrrhetinic acid induced apoptosis in

1143 non-small cell lung cancer NCI-H460 cells, Bioorganic & medicinal
1144 chemistry letters. 24 (2014) 1188-1191.
1145 [7] Asl M N, Hosseinzadeh H, Review of pharmacological effects of
1146 Glycyrrhiza sp. and its bioactive compounds, Phytotherapy Research: An
1147 International Journal Devoted to Pharmacological and Toxicological
1148 Evaluation of Natural Product Derivatives. 22 (2008) 709-724.
1149 [8] Shanmugam, M. K., Nguyen, A. H., Kumar, A. P., Tan, B. K., & Sethi,
1150 G, Targeted inhibition of tumor proliferation, survival, and metastasis by
1151 pentacyclic triterpenoids: potential role in prevention and therapy of
1152 cancer, Cancer letters. 320 (2012) 158-170.
1153 [9] Li, Y., Feng, L., Song, Z. F., Li, H. B., & Huai, Q. Y., Synthesis and
1154 anticancer activities of glycyrrhetinic acid derivatives, Molecules. 21
1155 (2016) 199.
1156 [10] Tatsuzaki, J., Taniguchi, M., Bastow, K. F., Nakagawa-Goto, K.,
1157 Morris-Natschke, S. L., Itokawa, H., ... & Lee, K. H, Anti-tumor agents
1158 255: Novel glycyrrhetinic acid–dehydrozingerone conjugates as cytotoxic
1159 agents, Bioorganic & medicinal chemistry. 15 (2007) 6193-6199.
1160 [11] Lee, C. S., Kim, Y. J., Lee, M. S., Han, E. S., & Lee, S. J.,
1161 18β-Glycyrrhetinic acid induces apoptotic cell death in SiHa cells and
1162 exhibits a synergistic effect against antibiotic anti-cancer drug toxicity,
1163 Life sciences. 83 (2008) 481-489.
1164 [12] Hibasami, H., Iwase, H., Yoshioka, K., & Takahashi, H.,

Glycyrrhetic acid (a metabolic substance and aglycon of glycyrrhizin) induces apoptosis in human hepatoma, promyelotic leukemia and stomach cancer cells, International journal of molecular medicine. 17 (2006) 215-219.

[13] Schwarz S, Csuk R. Synthesis and antitumour activity of glycyrrhetinic acid derivatives, Bioorganic & medicinal chemistry. 18 (2010) 7458-7474.

[14] Csuk, R., Schwarz, S., Kluge, R., & Ströhl, D, Synthesis and biological activity of some antitumor active derivatives from glycyrrhetinic acid, European journal of medicinal chemistry. 45 (2010) 5718-5723.

[15] Yan, T. L., Bai, L. F., Zhu, H. L., Zhang, W. M., & Lv, P. C., Synthesis and Biological Evaluation of Glycyrrhetic Acid Derivatives as Potential VEGFR2 Inhibitors, ChemMedChem. 12 (2017) 1087-1096.

[16] Xu, B., Wu, G. R., Zhang, X. Y., Yan, M. M., Zhao, R., Xue, N. N., ... & Wang, P. L., An overview of structurally modified glycyrrhetinic acid derivatives as antitumor agents, Molecules. 22 (2017) 924.

[17] Wu, G. R., Xu, B., Yang, Y. Q., Zhang, X. Y., Fang, K., Ma, T., ... & Jia, X. H., Synthesis and biological evaluation of podophyllotoxin derivatives as selective antitumor agents, European journal of medicinal chemistry. 155 (2018) 183-196.

[18] Chu, F., Xu, X., Li, G., Gu, S., Xu, K., Gong, Y., ... & Wang, P.,

Amino acid derivatives of ligustrazine-oleanolic acid as new cytotoxic agents, Molecules. 19 (2014) 18215-18231.

[19] Drag-Zalesinska, M., Kulbacka, J., Saczko, J., Wysocka, T., Zabel, M., Surowiak, P., & Drag, M., Esters of betulin and betulinic acid with amino acids have improved water solubility and are selectively cytotoxic toward cancer cells, Bioorganic & medicinal chemistry letters. 19 (2009) 4814-4817.

[20] Wang R., Design, synthesis and anticancer activity study of derivatives of 18β-Glycyrrhetinic acid and Oleanolic acid, Shandong University. 2018.

[21] Alho, D. P., Salvador, J. A., Cascante, M., & Marin, S., Synthesis and Antiproliferative Activity of Novel Heterocyclic Glycyrrhetinic Acid Derivatives, Molecules. 24 (2019) 766.

[22] Cui, H. W., He, Y., Wang, J., Gao, W., Liu, T., Qin, M., ... & Yi, Z., Synthesis of heterocycle-modified betulinic acid derivatives as antitumor agents, European journal of medicinal chemistry. 95 (2015) 240-248.

[23] Zhang, C., Yan, W., Zhao, R., Xu, B., Fang, X., Yan, M., ... & Lei, H., Design, synthesis and evaluation of new ligustrazine derivatives as potential plasma-stable neuroprotective agents, MedChemComm. 8 (2017) 652-656.

[24] Csuk, R., Schwarz, S., Siewert, B., Kluge, R., & Ströhl, D., Synthesis and antitumor activity of ring A modified glycyrrhetinic acid

derivatives, European journal of medicinal chemistry. 46 (2011) 5356-5369.

[25] Heller, L., Sommerwerk, S., Tzschöckell, F., Wiemann, J., Schwarz, S., Siewert, B., ... & Csuk, R., First Occurrence of a Furano - glycyrrhetinoate and Its Cytotoxicity, Archiv der Pharmazie. 348 (2015) 889-896.

Captions to the Tables, Figures and Schemes.

Table 1. The *in vitro* cytotoxicity of compounds against various cell lines. GA —Glycyrrhetinic acid; DDP —cisplatin

Figure.1. The average IC_{50} values of the four most potent compounds and DDP on four cancer cell lines

Figure.2. The IC_{50} values of GN series and GO series on A549 cells. *P < 0.05, vs. GN series.

Figure.3. Morphological detection of apoptosis using DAPI staining (200 ×) on A549 cells treated with compound **26**: (a) control group; (b) 1 μM; (c) 3 μM; (d) 5 μM.

Figure. 4. Detection of apoptosis using Annexin V-FITC/PI staining on A549 cells treated with compound **26**: (a) control group; (b) 1 μM; (c) 3 μM; (d) 5 μM.

Figure. 5. Cell cycle analysis using PI staining on A549 cells treated with compound **26**: (a) control group; (b) 1 μM; (c) 3 μM; (d) 5 μM.

Scheme 1. Synthesis of the GA derivatives **1-16**. Reagents and Conditions: (a) Bn-Br, dry DMF, dry K_2CO_3, 80 □, reflux, 3 h; (b) Boc-amino acids, DCM, DMAP, EDCI, rt,12 h; (c) TFA in dry DCM.

Scheme 2. Synthesis of the GA derivative GN-BN. Reagents and Conditions: (a) CrO_3/H_2SO_4, CH_3COCH_3, 0 □, 1 h; (b) $NaCNBH_3$, CH_3COONH_4, CH_3OH, rt, 12 h.

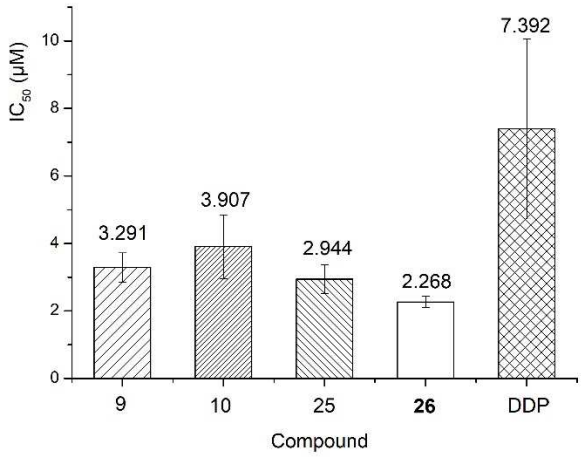
Scheme 3. Synthesis of the GA derivatives **17-32**. Reagents and Conditions: (a) Boc-amino acids, DCM, HOBt, EDCI, DIPEA, rt, 12 h; (b) TFA in dry DCM, 0 □, 4 h.

Table 1.

Half Maximal Inhibitory Concentration (IC_{50}) Values (μM)					
Compound	**A549**	**MCF-7**	**HepG2**	**Hela**	**MDCK**
1-8	>40	>40	>40	>40	>40
9	2.751±0.17	3.811±0.13	3.306±0.26	3.296±0.21	4.431±0.35
10	3.17±0.16	4.393±0.19	3.068±0.09	4.996±0.43	3.398±0.17
11	16.96±1.14	14.43±2.01	>40	18.84±0.93	14.41±1.31
12	4.731±0.42	5.218±0.36	6.39±0.55	9.051±0.52	9.63±0.71
13	4.995±0.29	3.891±0.12	4.564±0.26	7.995±0.29	5.765±0.35
14	22.91±2.29	11.94±1.56	14.94±2.01	43.09±1.13	19.33±1.88
15	4.966±0.97	10.0±1.03	8.146±0.88	9.647±0.64	9.011±0.72
16	7.532±0.46	8.070±0.82	9.958±0.69	10.56±1.07	39.25±3.53
17-24	>40	>40	>40	>40	>40
25	2.442±0.13	2.853±0.21	3.472±0.26	3.01±0.18	3.749±0.09
26	2.109±0.11	2.135±0.18	2.439±0.07	2.39±0.09	4.645±0.12
27	3.006±0.12	3.281±0.18	5.048±0.29	2.239±0.07	5.024±0.22

28	3.261±0.14	7.623±0.56	2.143±0.09	2.209±0.12	2.528±0.06
29	3.345±0.37	3.412±0.33	3.795±0.39	3.197±0.26	3.171±0.19
30	3.281±0.21	6.096±0.39	6.213±0.31	2.297±0.29	4.645±0.13
31	3.43±0.23	4.686±0.32	5.146±0.35	2.944±0.14	4.127±0.26
32	6.974±0.42	7.004±0.51	4.089±0.25	3.102±0.18	6.247±0.38
GA	>40	>40	>40	>40	>40
DDP	9.001±0.37	6.78±0.42	3.908±0.17	9.88±0.59	6.921±0.33

1242 **Figure 1.**



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1244 **Figure 2.**

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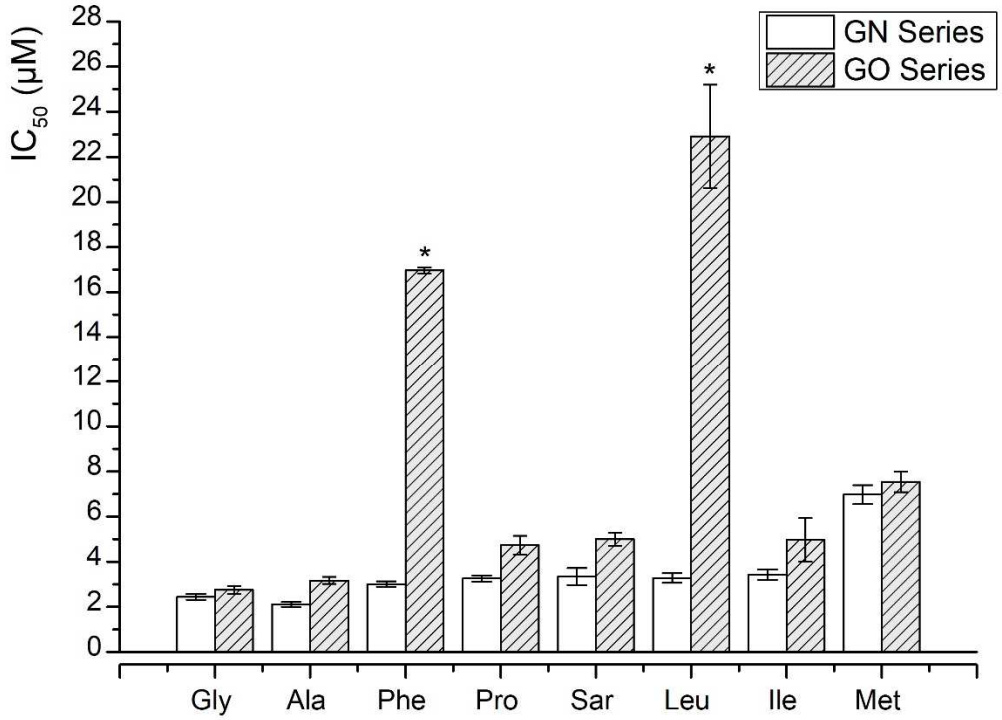
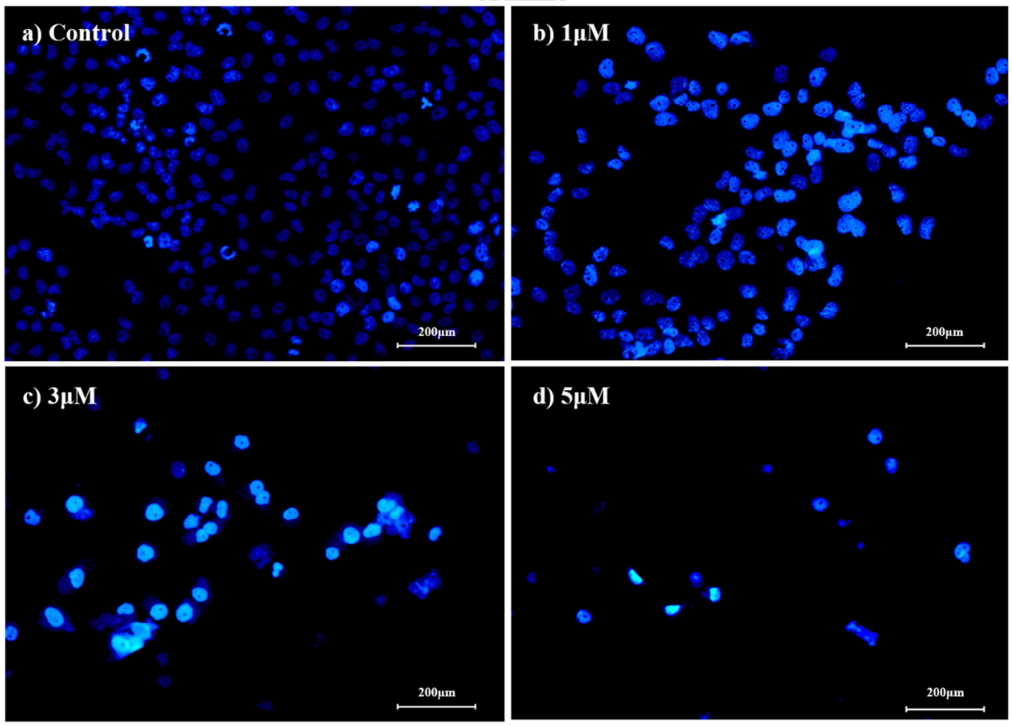


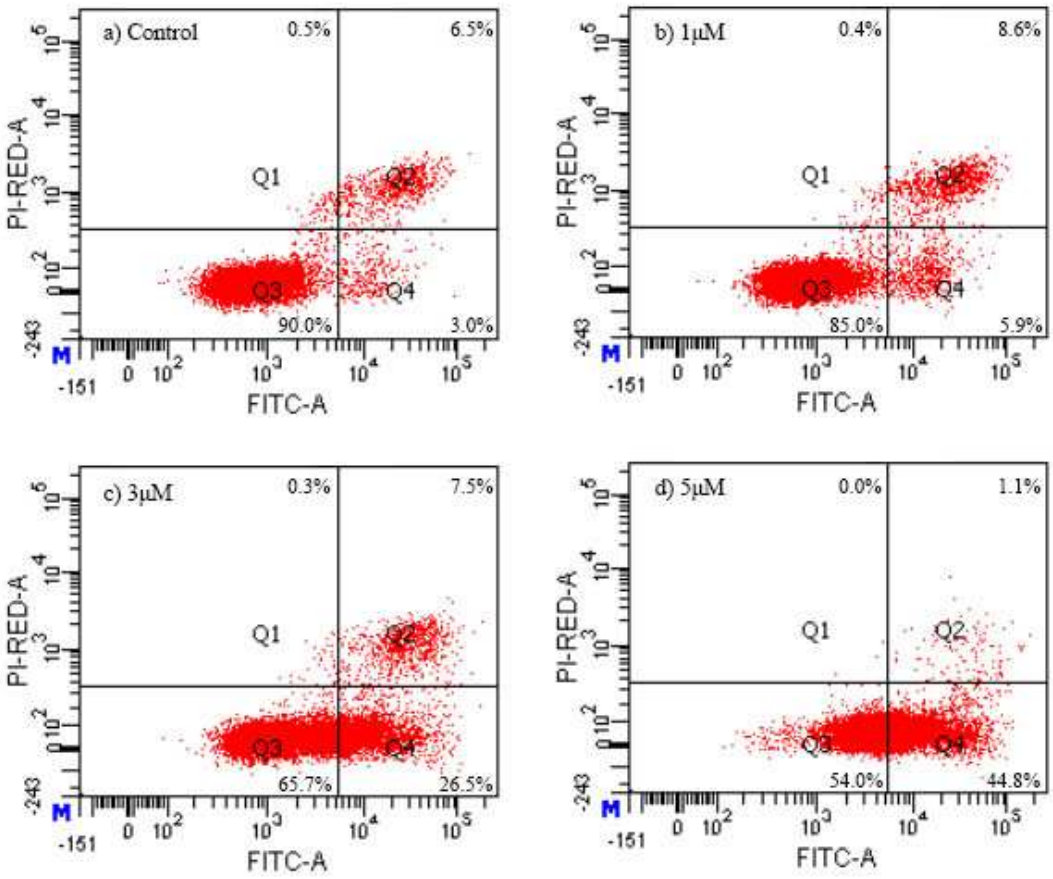
1247

1248 **Figure 3.**



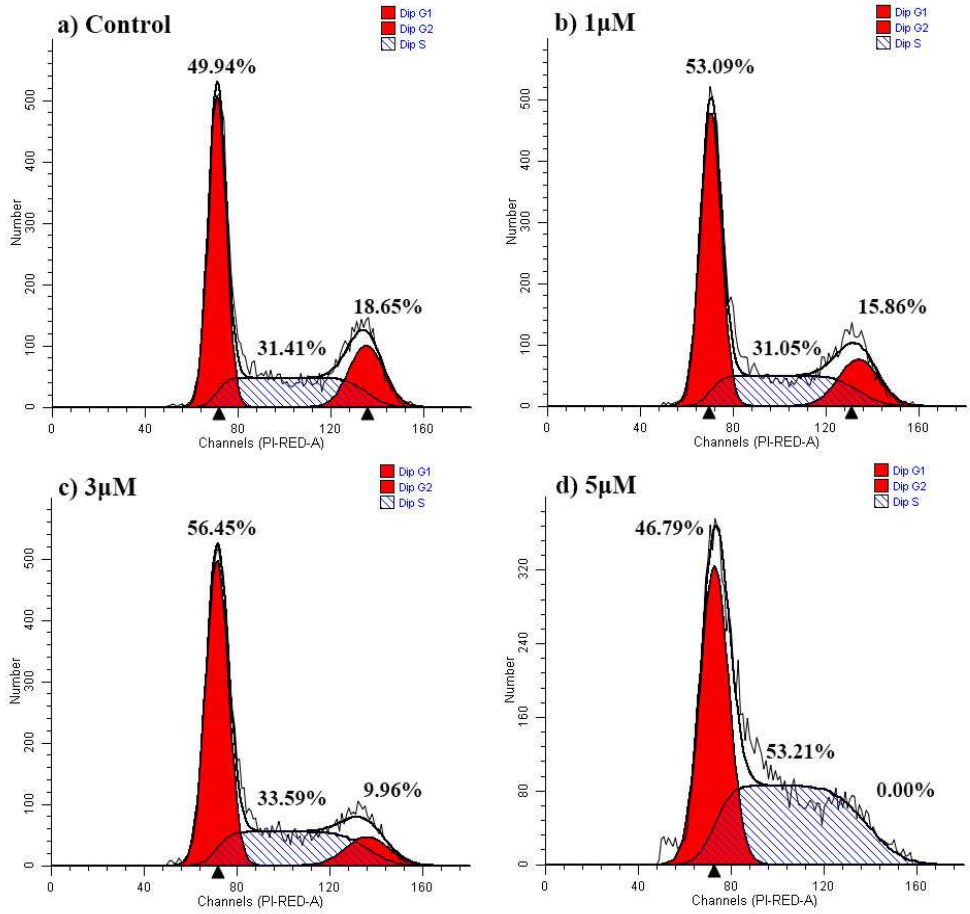
1249

1250 **Figure 4.**

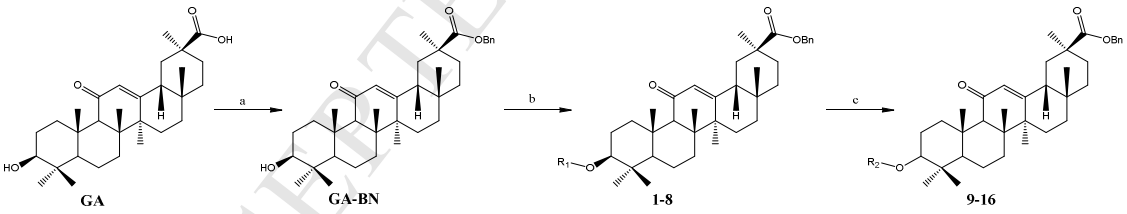


1251

1252 **Figure 5.**



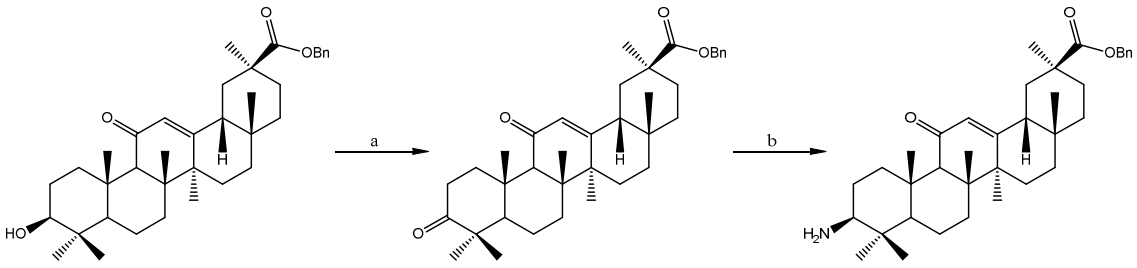
Scheme 1.



1 R_1=L-Boc-gly
2 R_1=L-Boc-ala
3 R_1=L-Boc-phe
4 R_1=L-Boc-pro
5 R_1=L-Boc-sar
6 R_1=L-Boc-leu
7 R_1=L-Boc-ile
8 R_1=L-Boc-met

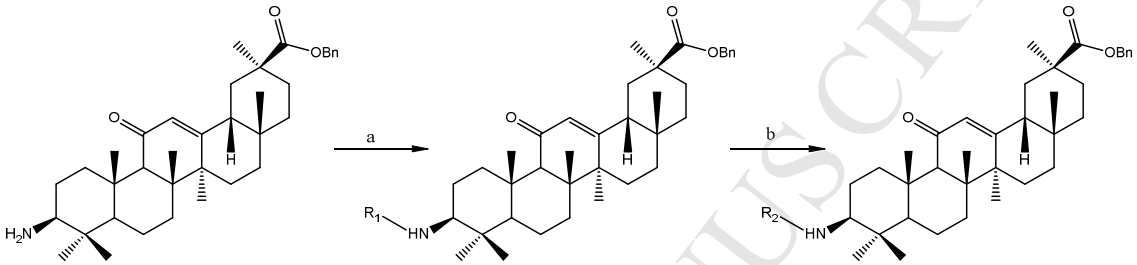
9 R_1=L-gly
10 R_1=L-ala
11 R_1=L-phe
12 R_1=L-pro
13 R_1=L-sar
14 R_1=L-leu
15 R_1=L-ile
16 R_1=L-met

Scheme 2.



GA-BN **GO-BN** **GN-BN**

Scheme 3.



GN-BN **17-24** **25-32**

17 R_1=L-Boc-gly
18 R_1=L-Boc-ala
19 R_1=L-Boc-phe
20 R_1=L-Boc-pro
21 R_1=L-Boc-sar
22 R_1=L-Boc-leu
23 R_1=L-Boc-ile
24 R_1=L-Boc-met

25 R_1=L-gly
26 R_1=L-ala
27 R_1=L-phe
28 R_1=L-pro
29 R_1=L-sar
30 R_1=L-leu
31 R_1=L-ile
32 R_1=L-met

Highlights

a. Thirty-two glycyrrhetinic acid (GA) derivatives had been designed and synthesized.

b. Most of the compounds were more potent than GA and positive drug cisplatin.

c. All the GN series were more potent than GO series against various tumor cells.

d. Compound **26** showed the most potent antitumor activity among all derivatives.