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# Synthesis and cytotoxicity of apoptosis-inducing *N*-heterocyclic triterpene amides

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ABSTRACT

The modification of lipophilic triterpenes to enhance their cytotoxicity, is a viable strategy for finding new anti-cancer agents. Herein we report the synthesis, analysis of 18 pentacyclic triterpenoic acid *N*-heterocyclic amides and their cytotoxicity, tumor cell/non-tumor cell selectivity, as well as their putative mode of action. $EC_{50}$ values were measured by SRB-assays, and found to be as low as 3.13 μM, with a selectivity as high as S = 5.05. Moreover, supportive assays were performed to further analyze their cytotoxicity; these experiments showed the compounds to act mainly by apoptosis.

## 1. Introduction

Phytochemicals, such as pentacyclic triterpenoic acids, are a cheap and diverse source of bioactive compounds; their cytotoxicity and anti-cancer activity have been determined [1,2]. Furthermore, for several of them chemo-preventive effects have been reported, too [3]. For some triterpenoic acid derived amides potent cytotoxicity for several human tumor cell lines have been reported; they induced apoptosis rather than necrosis in the tumor cells [4–6]. Recently, a platanic acid-derived homopiperazinyl amide [7] showed an impressive $EC_{50}$-value of 0.8–1.1 μM for epithelial melanoma cells, and an augustic acid-derived 4-isoquinolinyl amide showed cytotoxicity ($EC_{50}$ = 1.2–2.6 μM) for several tumor cell lines with excellent selectivity against non-malignant murine fibroblasts NIH 3T3 ($EC_{50}$ > 50 μM) [6]. As a consequence, the synthesis of acetylated triterpenoic acid amides seems to be a viable strategy to access new cytotoxic agents. To proof this concept, glycyrrhetinic (**1**, **GA**, Scheme 1) ursolic (**2**, **UA**), and oleanolic acid (**3**, **OA**) were chosen as starting materials, and *N*-heterocyclic amines **7**–**12** (Fig. 1) were selected as representative molecules for coupling; all of these are commercially available. The amines represent pyridine and indazole structural isomers as well as pyrazole amines; several of these scaffolds showed attractive chemotherapeutic activities and are part of approved chemotherapeutics such as Crizotinib, Bosutinib, Sorafenib, Pazopanib, Regorafenib, and Linifanib [8–10]. Herein, we report the design, synthesis, analysis, and biological evaluation of novel *N*-heterocyclic triterpenoic amides.

## 2. Results and discussion

### 2.1. Chemistry

First, the hydroxyl group of glycyrrhetinic (**1**), ursolic (**2**), and oleanic (**3**) at position C-3 was acetylated (Scheme 1) in excellent yields (86–90%) to access their respective acetates **4**–**6**. For the subsequent synthesis of the amides, acetates **4**–**6** were transformed with oxalyl chloride in the presence of catalytic amounts of DMF *in situ* into their respective acid chlorides followed by adding the amines **7**–**12** (Scheme 2) to afford amides **13**–**30**. These amides were then subjected to sulforhodamine B (SRB) assays to assess their cytotoxic activity.

### 2.2. Biology

SRB assays were used to evaluate the cytotoxicity of the compounds employing several human tumor cell lines (cut-off concentration 30 μM); the results of these SRB assays are summarized in Table 1. **UA** and **OA** derived acetates **5** and **6** were of minor cytotoxicity for the cell lines A375, MCF7, HT29, and A2780. A correlation between cytotoxicity (measured) and the respective octanol-water partition coefficient

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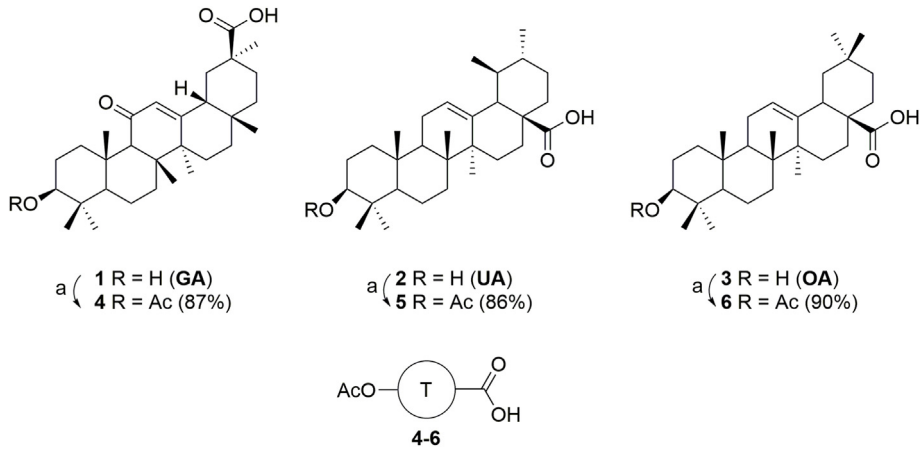



**Scheme 1.** Structure and synthesis of acetylated triterpenoic acids (**T**): a) cat. DMAP, $(Ac)_2O$, pyridine, 21 °C, 3 h.

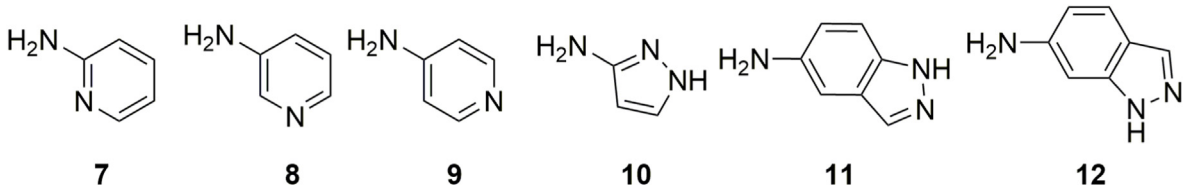
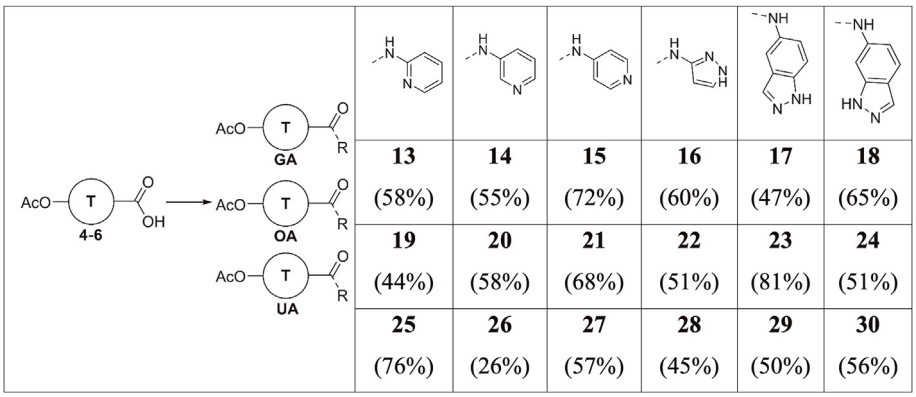



**Fig. 1.** *N*-heterocyclic amines selected for the synthesis of triterpenoid amides.




**Scheme 2.** Synthesis of N-heterocyclic triterpenoic amides: cat. DMF, $(COCl)_2$, 0 °C → 21 °C, 2 h → corresponding amine (**7**-**12**), THF/DCM, 21 °C, 24 h.

(calculated, SwissADME) could not be established neither for the starting materials, their acetates, nor for the amides **13**–**30**. The cytotoxicity of the amides seems to be primarily determined by their structure. As a result, almost all the amides **13**–**30** showed better $EC_{50}$ values than their acetylated precursors, thus proving the viability of the concept that amides are of superior cytotoxicity as compared to the corresponding carboxylic acids. This parallels previous findings for amides of the lupane series [11]. Interestingly, apart from two amides (**17** and **18**), the amides were more cytotoxic for the malignant A375 cells. Moreover, the position of the nitrogen in the pyridine subunit seems also to affect both cytotoxicity and selectivity. Again, this is incomplete agreement with previous results obtained for quinolinyl or isoquinolinyl amides [7,11]. Hereby, the **UA** derived pyridinyl amide **26** held the highest cytotoxicity with an $EC_{50}$ value of 3.13 μM for A375 epithelial melanoma cells. For the structural isomer **25** the best selectivity of all tested compounds (S = 5.05) was determined for this cell line. Interestingly, most of the amides showed diminished selectivity but **GA** derived amide **13** also held enhanced selectivity for all malignant cell lines as compared to non-malignant fibroblasts.

Further investigations of compounds **21** and **22** using Annexin V-FITC/PI assays showed these compounds to have – by and large – a smaller number of vital cells (−15/-17%) after 24 h which is probably due to cells being in an apoptotic rather than in a necrotic state (Fig. 2). The number of apoptotic ells increased drastically after 48 h of treatment showing an average of −65% (**21**) and −60% (**22**) less vital cells than counted in the untreated control group.

Moreover, investigation of the cell cycle of A2780 cells treated with **21** and **22** was performed by FACS-analysis applying incubation times of 24 h and 48 h, respectively. Notably, the average cell count difference between the control group and those cell populations having been treated with **21** or **22** in the G1-phase is 24–25% higher than for the control group after 24 h (Fig. 3). In reverse, this means that fewer cells are passing the checkpoint into the S-phase and counts in the S- and G2/M-phase decrease. There is no remarkable difference in the apoptosis between all analyzed cell groups, but this changes significantly to an increase of 52/53% after 48 h of incubation (Fig. 4). The comparison of these results (Figs. 5 and 6) shows that the treated cells stop cell division and are arrested in the G1-or G0-phase with an increasing effect at 48 h

**Table 1**
Cytotoxicity (from SRB assays, $EC_{50}$ values [μM] from SRB assays after 72 h of treatment, the values are averaged from three independent experiments performed each in triplicate, confidence interval CI = 95%; mean ± standard mean error) of starting materials **GA** (**1**), **UA** (**2**), **OA** (**3**) [12], their acetates (**4**–**6**) [13–15] and amides **13**–**30**. Human cancer cell lines: A375 (epithelial melanoma), HT29 (colorectal adenocarcinoma), MCF-7 (breast adenocarcinoma), A2780 (ovarian carcinoma), FaDu (hypopharyngeal carcinoma), HeLa (cervical carcinoma); non-malignant: NIH 3T3 (mouse fibroblasts); n.s. not soluble; n.d. not determined; S selectivity against NIH 3T3; doxorubicin (**DX**) and staurosporine (**ST**) were used as positive controls.

| Compound | A375 | HT29 | MCF-7 | A2780 | HeLa | NIH 3T3 |
|---|---|---|---|---|---|---|
| ***1*** | n.d. | >30 | >30 | >30 | n.d. | 18.7 ± 4.2 |
| ***2*** | n.d. | 10.6 ± 0.7 | 12.7 ± 0.1 | 11.7 ± 0.6 | n.d. | 13.1 ± 1.1 |
| ***3*** | n.d. | >30 | >30 | >30 | n.d. | >30 |
| ***4*** | >30 | >30 | >30 | >30 | n.d. | >30 |
| ***5*** | 11.4 ± 1.4 | 17.3 ± 1.5 | 12.1 ± 1.2 | 8.3 ± 0.9 | n.d. | 16.4 ± 1.7 |
| ***6*** | 13.1 ± 1.1 | 20.5 ± 1.7 | 12.9 ± 1.9 | 9.4 ± 0.5 | n.d. | 17.5 ± 1.5 |
| ***13*** | 11.96 ± 0.9 (S = 2.51) | 21.0 ± 2.3 (S = 1.43) | 19.3 ± 1.9 (S = 1.55) | 16.0 ± 1.3 (S = 1.88) | 19.0 ± 2.9 (S = 1.58) | >30 |
| ***14*** | >30 | >30 | >30 | >30 | >30 | >30 |
| ***15*** | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| ***16*** | >20 | >30 | >30 | >30 | >30 | >30 |
| ***17*** | 14.03 ± 0.6 (S = 0.97) | 17.53 ± 0.4 (S = 0.78) | 12.82 ± 0.7 (S = 1.06) | 15.01 ± 0.7 (S = 0.91) | 16.20 ± 0.9 (S = 0.84) | 13.61 ± 0.5 |
| ***18*** | 22.1 ± 1.4 (S = 1.22) | 23.9 ± 2.0 (S = 1.13) | 19.4 ± 2.1 (S = 1.39) | 17.4 ± 1.7 (S = 1.55) | 23.6 ± 1.0 (S = 1.14) | 27.0 ± 1.3 |
| ***19*** | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| ***20*** | 8.50 ± 0.8 (S = 1.04) | 25.1 ± 3.2 (S = 0.35) | 10.52 ± 1.2 (S = 0.84) | 11.6 ± 1.2 (S = 0.76) | 15.52 ± 0.9 (S = 0.57) | 8.83 ± 0.5 |
| ***21*** | 4.3 ± 1.3 (S = 1.07) | 22.8 ± 3.3 (S = 0.20) | 7.57 ± 0.5 (S = 0.61) | 8.32 ± 0.4 (S = 0.55) | 11.43 ± 0.9 (S = 0.40) | 4.61 ± 0.7 |
| ***22*** | 3.56 ± 0.1 (S = 1.59) | 12.79 ± 0.8 (S = 0.44) | 10.61 ± 0.8 (S = 0.53) | 7.51 ± 0.4 (S = 0.75) | 8.04 ± 0.5 (S = 0.71) | 5.67 ± 0.9 |
| ***23*** | 8.77 ± 0.4 (S = 1.23) | >30 | 9.99 ± 0.6 (S = 1.08) | 10.75 ± 0.4 (S = 1.00) | 13.8 ± 1.0 (S = 0.78) | 10.75 ± 0.4 |
| ***24*** | >20 | >30 | >30 | >30 | >20 | >30 |
| ***25*** | 4.52 ± 0.4 (S = 5.05) | 17.02 ± 1.1 (S = 1.34) | 15.70 ± 1.3 (S = 1.45) | 8.18 ± 0.7 (S = 2.79) | 15.10 ± 2.0 (S = 1.51) | 22.83 ± 4.5 |
| ***26*** | 3.13 ± 0.3 (S = 2.20) | 7.3 ± 1.1 (S = 0.94) | 6.26 ± 0.7 (S = 1.10) | 5.56 ± 0.6 (S = 1.24) | 10.64 ± 0.4 (S = 0.65) | 6.88 ± 0.9 |
| ***27*** | 4.07 ± 0.2 (S = 1.34) | 9.4 ± 1.0 (S = 0.58) | 7.83 ± 0.7 (S = 0.70) | 6.90 ± 0.6 (S = 0.79) | 7.44 ± 0.4 (S = 0.73) | 5.45 ± 0.8 |
| ***28*** | 6.66 ± 0.1 (S = 1.53) | 16.56 ± 0.5 (S = 0.62) | 11.61 ± 0.8 (S = 0.88) | 11.01 ± 0.8 (S = 0.93) | 12.08 ± 0.8 (S = 0.85) | 10.22 ± 0.8 |
| ***29*** | 4.44 ± 0.1 (S = 0.95) | 11.06 ± 0.6 (S = 0.38) | 8.38 ± 0.9 (S = 0.50) | 7.91 ± 0.5 (S = 0.53) | 7.38 ± 0.6 (S = 0.57) | 4.22 ± 0.7 |
| ***30*** | 6.1 ± 1.7 (S = 1.93) | 11.1 ± 1.2 (S = 1.06) | 8.06 ± 0.3 (S = 1.46) | 7.88 ± 0.9 (S = 1.49) | 15.75 ± 1.5 (S = 0.75) | 11.76 ± 1.0 |
| ***ST*** | n.d. | 0.9 ± 0.01 | 1.1 ± 0.3. | 0.01 ± 0.01 | n.d. | 0.45 ± 0.04 |
| ***DX*** | n.d. | 0.2 ± 0.02 | 0.1 ± 0.01 | 0.1 ± 0.01 | n.d. | 0.01 ± 0.001 |

and thus leading to fewer cells in the S- and G2/M-phase and higher amounts of apoptotic cells. This is in accordance with the results obtained from the Annexin V-FITC/PI assay (Fig. 2); for the latter, the average apoptosis difference after 24 h was 46% for **21** and 37% for **22**, respectively.

As a further proof for inducing apoptosis some extra microscopic experiments were performed. Thereby, samples treated with **21** or **22** showed a significantly lower number of cells than the respective control groups (Fig. 7, a/d). For example, after treatment of the cells with compound **21** for 24 h, apoptotic cells were visible as indicated by blebbing and the occurrence of cell fragments (Fig. 7, b). Treatment of the cells for 48 h led to late apoptosis; propidium iodide (PI) inclusions in the shrunken parts of the nucleus of the cells was established which gave them an orange or red color (Fig. 7, e).

Treatment with compound **22** for 24 h led to cell shrinkage and blebbing, thus resulting in single cell fragments (Fig. 7, c). These still had an intact membrane, so that PI could not enter the cells. These observations strongly suggest an early stage of apoptosis. After 48 h, the presence of PI in some cells indicate late apoptosis (Fig. 7, f). The red fluorescence was visible in small spots signifying the nucleus fragmentation. Details can be found in the experimental part.

## 3. Conclusion

We successfully synthesized 18 *N*-heterocyclic triterpenoic acid amides and assayed them for their cytotoxicity and selectivity for several malignant human cell lines. Our findings highly suggest the viability of modifying lipophilic core structures with *N*-heterocyclic residues to further enhance the cytotoxicity and selectivity of these hybrids. While these compounds overall proved to be cytotoxic and selective especially for A375 epithelial melanoma cells, the selectivity for the HT29 and HeLa cell lines were diminished. However, these compounds induce favorable apoptotic cell death, with little to no necrosis, and $EC_{50}$ as low as 3.13 μM for the ursolic acid derived amide **26** was determined. In addition, the highest selectivity of S = 5.05 could be obtained for the structural analogue **25** thereby holding an $EC_{50}$ value of 4.52 μM for the same cell line. In conclusion, when aiming for selectivity and high cytotoxicity, 2-aminopyridine (**7**) seems to be a promising candidate for further lead optimization of cytotoxic drugs.

## 4. Experimental part

### 4.1. General

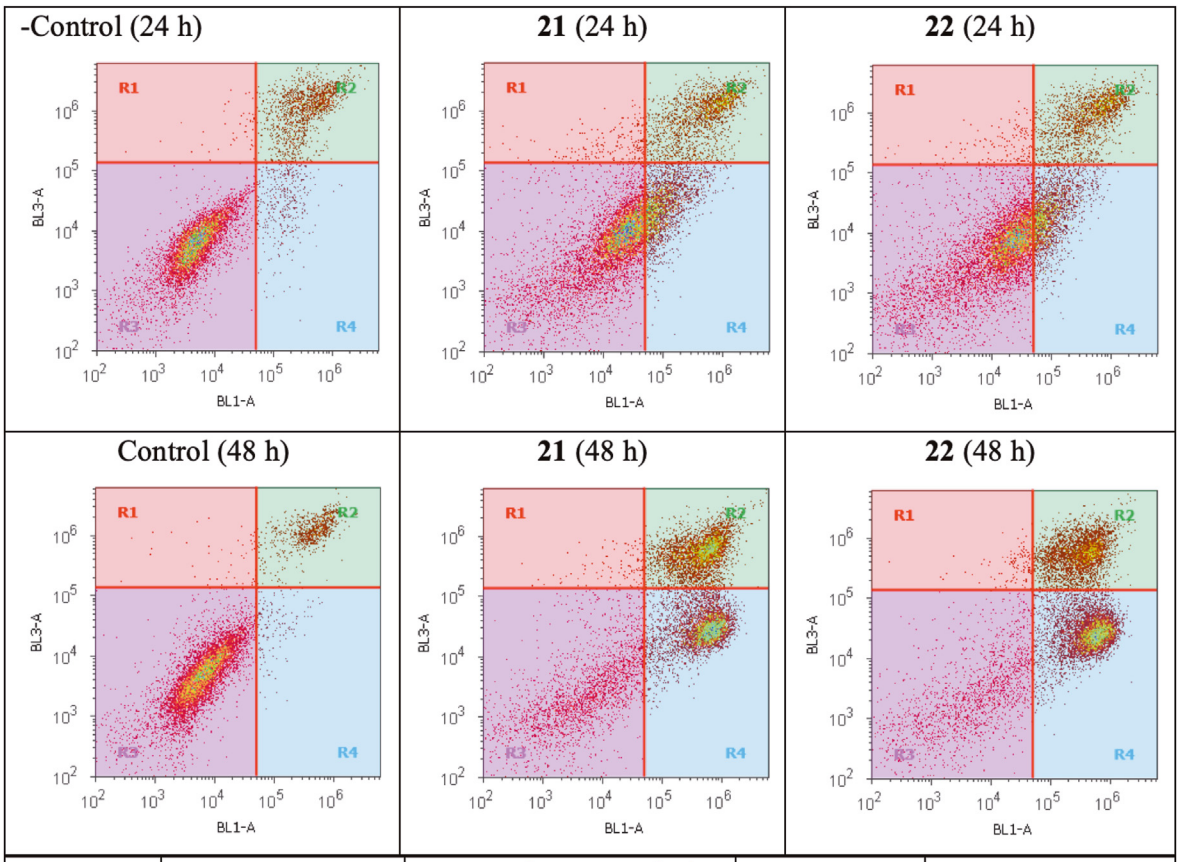
Ursolic and oleanolic acid were obtained from Betulinines (Stribrna Skalice, Czech Republic) and glycyrrhetinic acid was bought from Orgentis GmbH (Neugatersleben, Germany) and used as received. Amines were purchased from TCI, abcr, and Sigma Aldrich. Equipment and lab equipment was used as previously described.

### 4.2. Flow cytometry

For flow cytometry investigation, $4 \times 10^5$ A375 cells were seeded in a flask and allowed to grow for 24 h. The medium was removed, and the cells were treated with 10 mL fresh medium as control and medium mixed with compounds **21** and **22** (double $EC_{50}$ concentration), respectively. After 24 h and 48 h, the cells were harvested by trypsinization, and all solutions were collected in a tube. Each sample was centrifuged (1500 rpm, 5 min, 4 °C), the supernatant was discarded, the pellet was suspended in 1 mL PBS (with $Ca^{2+}$ and $Mg^{2+}$); this was repeated twice. Thereafter the cells were counted using the Attune® FACS machine (Life technologies™, Darmstadt, Germany).

### 4.3. Cell cycle

For Cell Cycle investigation [16] $1 \times 10^6$ cells were collected by centrifugation and fixed with ethanol (70%, 4 °C, 24 h). The samples were centrifuged (4500 rpm, 5 min, 4 °C), washed with PBS once and centrifuged again. Staining Solution (10 μL PI (1 mg/mL) in 1 mL PBS) with RNAse (100 μL, 100 μg/mL) was added. After incubation for at least




| Incubation time in h | necrotic (R1) | | secondary necrotic / late-stage apoptotic (R2) | | vital (R3) | | apoptotic (R4) | |
|---|---|---|---|---|---|---|---|---|
| | **21** | **22** | **21** | **22** | **21** | **22** | **21** | **22** |
| 24 | 1% | 1% | 1% | 1% | -15% | -17% | 13% | 15% |
| 48 | 0% | 0% | 19% | 19% | -65% | -60% | 46% | 37% |
| | average difference to control | | | | | | | |

**Fig. 2.** Representative Annexin V-FITC/PI assay density plots determined by flow cytometry after 24 h and 48 h. R1: necrotic (red), R2: secondary necrotic/late-stage apoptotic (green), R3: vital (pink), R4: apoptotic (blue). The difference to the control group was calculated from the arithmetic means of each three biological and two technical replicates. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

30 min at room temperature, the cells were analyzed. The data was collected from the BL-2A and BL-2H channel, plotting A against H for exclusion of doublet cells. For each cell cycle distribution 20,000 events were collected in technical triplicates, each sample was measured in duplicates/triplicates, depending on the sample volume. Cell cycle distribution was calculated using ModFit LT (Verity Software House, Topsham, US).

### 4.4. Annexin V/PI assay

For this assay [16] $1 \times 10^5$ cells were collected by centrifugation and re-suspended in Annexin V binding buffer (100 µL, BioLegend ®, San Diego, US). After treatment with propidium iodide solution (3 µL, 1 mg/mL) and Annexin V-FITC (5 µL, BioLegend ®, San Diego, US) for 15 min in the dark at room temperature, Annexin V binding buffer (400 µL) was added, und the samples were analyzed by Attune ® FACS machine. After gating for living cells, the data from detectors BL-1A and BL-3A were collected (10,000 events) in technical triplicates. The assay was performed in duplicates; cell distribution was calculated using Attune ® Software.

### 4.5. Microscopy

On the first day, $4 \times 10^5$ A375 cells were seeded in a flask. After 24 h, the medium was removed, and the cells were treated with 10 mL fresh medium as control and medium mixed with compounds **21** and **22** (double $EC_{50}$ concentration), respectively. After 24 h and 48 h, the cells were harvested by trypsinization, and all solutions were collected in a tube. Each sample was centrifuged (1500 rpm, 5 min, 4 °C), the supernatant was discarded, the pellet was suspended in 1 mL PBS (with $Ca^{2+}$ and $Mg^{2+}$), and centrifuged again. The cells were now taken up in 150 µL PBS. For the microscopic investigation, 10 µL cell suspension was mixed with 10 µL AO/PI solution (5 µg/mL each in PBS), placed on a slide, and measured directly with the microscope.

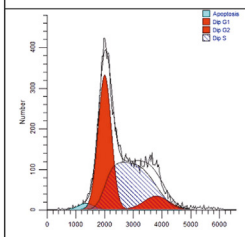
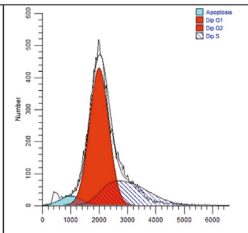
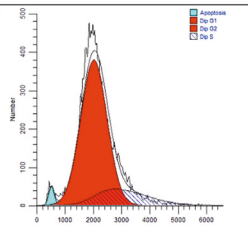
| Cell cycle analysis of A2780 after 24 h | | | |
|---|---|---|---|
| |  |  |  |
| | control | difference to **21** | difference to **22** |
| G1 | 44% | 24% | 25% |
| S | 38% | -13% | -13% |
| G2/M | 14% | -12% | -12% |
| Apop. | 1% | 1% | 1% |

**Fig. 3.** FACS cell cycle analysis after 24 h of incubation of A2780 cell line highlighting the differences between the control group and the tested compounds (**21** and **22**). Average values are displayed and were taken from at least two biological and two technical replicates. Representative graphs were chosen from one biological replicate group.

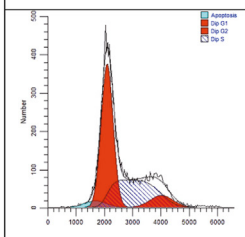
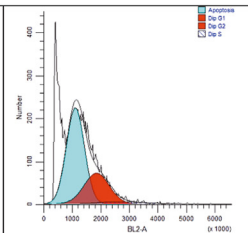
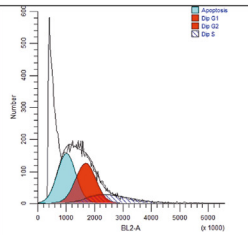
| Cell cycle analysis of A2780 after 48 h | | | |
|---|---|---|---|
| |  |  |  |
| | average control | difference to **21** | difference to **22** |
| G1 | 61% | 30% | 25% |
| S | 28% | -22% | -7% |
| G2/M | 12% | -8% | -8% |
| Apop. | 1% | 53% | 52% |

**Fig. 4.** FACS cell cycle analysis after 48 h of incubation of A2780 cell line highlighting the differences between the control group and the tested compounds (**21** and **22**). Average values are displayed and were taken from at least two biological and technical replicates. Representative graphs were chosen from one biological replicate group.

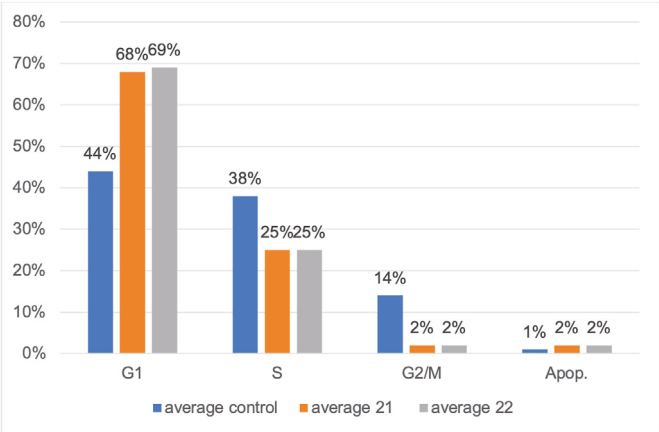



**Fig. 5.** Cell cycle analysis of A2780 cells after incubation with **21** or **22** for after 24 h.

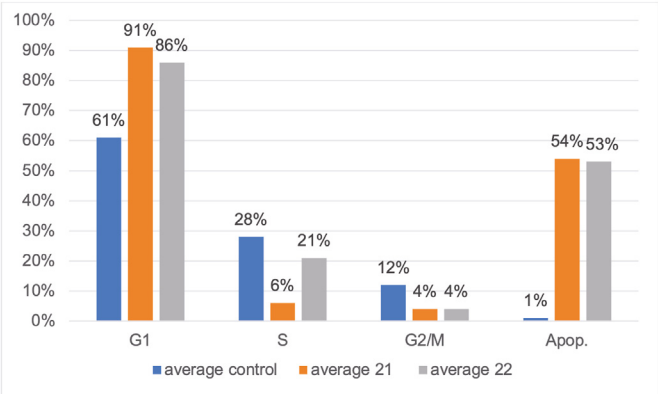



**Fig. 6.** Cell cycle analysis of A2780 cells after incubation with **21** or **22** for 48 h.

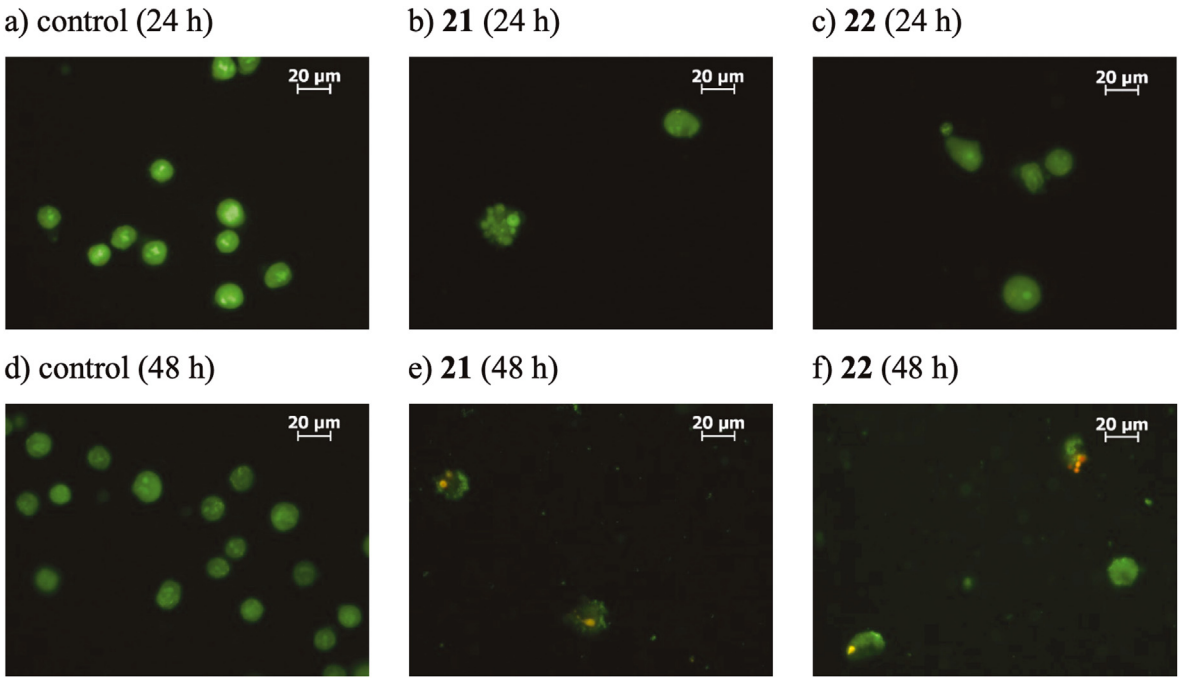



**Fig. 7.** Microscopic investigations of cells stained with AO/PI. The cells were treated with and without compounds **21** and **22**.

### *4.6. Syntheses*

#### *4.6.1. General procedure (GP1) for the acetylation of triterpene carboxylic acids*

The triterpene carboxylic acids (**1**–**3**, 5.0 g) were dissolved in dry pyridine (20 mL). Under stirring, acetic anhydride (2.0 mL, 18.1 mmol) and cat. amounts of DMAP were added. After stirring at 21 °C for 3 h, the reaction solution was checked with TLC and poured into HCl (0.1 M, 50 mL). Then the product was filtered off and dried under reduced pressure. Analytical samples were obtained by re-crystallization or column chromatography.

#### *4.6.2. General procedure (GP2) for the synthesis of the amides*

The acetylated triterpene carboxylic acid (**4**–**6**, 5.0 g) was dissolved in dry DCM (20 mL), and the mixture was cooled to 0 °C. Under stirring, oxalyl chloride (20 eq, 1.62 mL, 19 mmol) and cat. amounts of dry DMF were added. The reaction was allowed to warm to 21 °C, and stirring was continued until the gas evolution had ceased (2 h). Excessive oxalyl chloride and solvent were removed under reduced pressure while re-dissolving the obtained solid twice in dry THF. Part of the obtained acid chloride (0.5 g) was dissolved in dry DCM (20 mL) and triethylamine (0.12 mL 0.94 mmol) was added. Then the corresponding amine (**7**–**12**, 1 eq) dissolved in dry THF/DCM was added to the acid chloride solution under stirring. The reaction mixture was stirred at 21 °C for 24 h, quenched with HCl (1 M, 30 mL), extracted with DCM (3 × 30 mL), the combined organic phases were dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to obtain a solid which was purified by column chromatography ($SiO_2$, hexanes/ethyl acetate, 11:9).

#### *4.6.3. 3-O-Acetyl-glycyrrhetinic acid (4)*

Following GP1, **4** (4.77 g; 87%) was obtained as a white solid; m.p. 308–310 °C (lit.: [17]: 310–313 °C); $R_F = 0.56$ (hexanes/ethyl acetate, 11:9); $[\alpha]_D = +165.1°$ (*c* 1.0, $CHCl_3$) [lit.: [18]: $[\alpha]_D = +163.3°$ (*c* 1.0, $CHCl_3$)]; IR (ATR): $\tilde{\nu} = 2952br$, 1731*s*, 1654*s*, 1293 *m*, 1227*s*, 1075*s*, 753 s $cm^{-1}$; MS (ESI, MeOH): $m/z = 511.1$ ($[M - H]^-$, 100%), 1023.9 ($[2M - H]^-$, 64%).

#### *4.6.4. 3-O-Acetyl-oleanolic acid (5)*

Following GP1, **5** (4.7 g, 86%) was obtained as a white solid; m.p. 261 °C (lit.: [19]: 260–261 °C); $R_F = 0.6$ (hexanes/ethyl acetate, 6:4); $[\alpha]_D = +72.5°$ (*c* 1.0, $CHCl_3$) [lit.: [20]: $[\alpha]_D = +74°$ (*c* 1.0, $CHCl_3$)]; IR (ATR): $\tilde{\nu} = 2946br$, 1679*s*, 1455*s*, 1380 *m*, 1034*s*, 748 m $cm^{-1}$; MS (ESI, MeOH): $m/z = 497.0$ ($[M - H]^-$, 100%).

#### *4.6.5. 3-O-Acetyl-ursolic acid (6)*

Following GP1, **6** (4.95 g; 90%) was obtained as a white solid; m.p. 264–266 °C (lit.: [21]: 242.7–244.1 °C); $[\alpha]_D = +72.5°$ (*c* 1.0, $CHCl_3$) [lit.: [21]: $[\alpha]_D = +71.2°$ (*c* 1.0, $CHCl_3$)]; $R_F = 0.65$ (hexanes/ethyl acetate, 6:4); IR (ATR): $\tilde{\nu} = 2948br$, 1683*s*, 1467 *m*, 1371 *m*, 1034*s*, 938 *m*, 748 m $cm^{-1}$; MS (ESI, MeOH): $m/z = 497.1$ ($[M - H]^-$, 100%).

#### *4.6.6. N-2-pyridinyl-3β-acetoxy-11-oxoolean-12-en-30-ic acid amide (13)*

Following GP2, **13** (326 mg, 58%) [22–24] was obtained as a white solid; m.p. 265–268 °C (lit.: [22]: 266–267 °C); $R_F = 0.12$ (hexanes/ethyl acetate, 11:9); $[\alpha]_D = +168.0°$ (*c* 0.124, $CHCl_3$) [lit.: [22]: $[\alpha]_D = +178°$ (*c* 0.12, $CHCl_3$)]; IR (ATR): $\tilde{\nu} = 2950br$, 1727 *m*, 1656*s*, 1511 *m*, 1428*s*, 1293 *m*, 1244*s*, 753 s $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta = 8.40$ (dt, $J = 8.5$, 1.0 Hz, 1H, 37-H), 8.24 (ddd, $J = 5.2$, 1.9, 0.9 Hz, 1H, 34-H), 7.84 (ddd, $J = 8.8$, 7.4, 1.9 Hz, 1H, 35-H), 7.13 (ddd, $J = 7.4$, 5.2, 1.0 Hz, 1H, 36-H) 5.75 (s, 1H, 12-H), 4.52 (dd, $J = 11.7$, 4.7 Hz, 1H, 3-H), 2.81 (m, 1H, 1-$H_a$), 2.37 (s, 1H, 9-H), 2.28 (m, 1H, 18-H), 2.14 (m, 1H, 16-$H_a$), 2.12–1.98 (m, 2H, 15-$H_a$+19-$H_a$), 2.06 (s, 3H, 32-$H_3$) 1.90–1.79 (m, 1H, 15-$H_b$), 1.78–1.54 (m, 5H, 19-$H_b$, 2-$H_a$+2-$H_b$+6-$H_a$+7-$H_a$), 1.52–1.36 (m, 5H, 16-$H_b$+7-$H_b$+21-$H_a$+12-$H_b$+ 22-$H_a$), 1.39 (s, 3H, 27-$H_3$), 1.31 (s, 3H, 32-$H_3$), 1.28–1.18 (m, 1H, 6-$H_b$), 1.17 (s, 3H, 25-$H_3$), 1.13 (s, 3H, 26-$H_3$), 1.07 (m, 2H, 1-$H_b$+22-$H_b$), 0.89 (s, 3H, 23-$H_3$), 0.88 (s, 3H, 24-$H_3$), 0.82 (s, 3H, 28-$H_3$), 0.81 (s, 1H, 5-H) ppm; $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ = 199.9 (C-11), 174.7 (C-30), 170.9 (C-31), 168.8 (C-13), 140.8 (C-33), 138.4 (C-37), 136.8 (C-35). 130.5 (C-36) 128.5 (C-21), 124.7 (C-34) 80.5 (C-3), 61.7 (C-9), 54.9 (C-5), 47.8 (C-18), 45.4 (C-8), 45.0 (C-17), 43.2 (C-14), 41.3 (C-19), 38.8 (C-1), 38.0 (C-4), 37.5 (C-21), 36.9 (C-10), 32.7 (C-7), 31.9 (C-22), 31.4 (C-16), 28.9 (C-29), 28.3 (C-28), 28.0 (C-24), 26.4 (C-15), 23.6 (C-2), 23.3 (C-27), 21.3 (C-32), 18.7 (C-26), 17.3 (C-6), 16.7 (C-23), 16.3 (C25) ppm; MS (ESI, MeOH): $m/z = 589.0$ ($[M - H]^+$, 57%), 1199.2 ($[2 M + Na^+]^+$, 100%); analysis

calcd for $C_{37}H_{52}N_2O_4$ (588.83): C 75.47, H 8.90, N 4.76; found: C 75.19, H 9.03, N 4.57.

#### 4.6.7. N-3-pyridinyl-3β-acetoxy-11-oxoolean-12-en-30-ic acid amide (14)

Following GP2, **14** (306 mg, 55%) was obtained as a white solid; m.p. 190–192 °C; $R_F$ = 0.11 (hexanes/ethyl acetate, 11:9); $[\alpha]_D$ = +155.0° (*c* 0.136, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2950*br*, 1727 *m*, 1655 *m*, 1479 *m*, 1244*s*, 1027 *m*, 751*s*, 706 m $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 9.03 (d, *J* = 2.8 Hz, 1H, 37-H), 8.70–8.52 (m, 1H, 36-H), 8.32 (dd, *J* = 5.0, 1.4 Hz, 1H, 34-H), 7.46 (dd, *J* = 8.5, 5.0 Hz, 1H, 35-H), 5.73 (s, 1H, 12-H), 4.53 (dd, *J* = 11.7, 4.7 Hz, 1H, 3-H), 2.80 (m, 1H, 1-$H_a$), 2.38 (s, 1H, 9-H), 2.27 (m, 2H, 18-H), 2.21 (m, 1H, 19-$H_a$), 2.12–2.04 (m, 2H, H-15-$H_a$+19-$H_a$), 2.06 (s, 3H, 32-$H_3$) 1.90–1.80 (m, 1H, 15-$H_b$), 1.78–1.56 (m, 5H, 19-$H_b$, 2-$H_a$+2-$H_b$+6-$H_a$+7-$H_a$), 1.56–1.38 (m, 5H, 16-$H_b$+7-$H_b$+21-$H_a$+21-$H_b$+22-$H_a$), 1.40 (s, 3H, 27-$H_3$), 1.32 (s, 3H, 29-$H_3$), 1.28–1.18 (m, 1H, 6-$H_b$), 1.16 (s, 3H, 25-$H_3$), 1.13 (s, 3H, 26-$H_3$), 1.10–1.02 (m, 2H, 1-$H_b$+22-$H_b$), 0.89 (s, 3H, 23-$H_3$), 0.88 (s, 3H, 24-$H_3$), 0.82 (s, 3H, 28-$H_3$), 0.81 (s, 1H, 5-H) ppm; $^{13}C$ NMR (101 MHz, $CDCl_3$): δ = 199.9 (C-11), 175.5 (C-30), 170.9 (C-31), 168.6 (C-13), 150.9 (C-36), 140.4 (C-37), 128.7 (C-12), 119.5 (C-34), 114.8 (C-35) 80.6 (C-3), 61.7 (C-9), 55.1 (C-5), 47.9 (C-18), 45.3 (C-8), 44.9 (C-17), 43.2 (C-14), 41.4 (C-19), 38.8 (C-1), 38.0 (C-4), 37.5 (C-21), 36.9 (C-10), 32.7 (C-7), 31.9 (C-22), 31.5 (C-16), 28.9 (C-29), 28.5 (C-28), 28.1 (C-24), 26.4 (C-15), 23.5 (C-2), 23.4 (C-27), 21.3 (C-32), 18.7 (C-26), 17.3 (C-6), 16.7 (C-23), 16.4 (C25) ppm; MS (ESI, MeOH): *m/z* = 587.2 ([M − H]⁻, 100%), 623.2 ([M + $Cl^-$]⁻, 34%)%); analysis calcd for $C_{37}H_{52}N_2O_4$ (588.83): C 75.47, H 8.90, N 4.76; found: C 75.26, H 8.15, N 4.63.

#### 4.6.8. N-4-pyridinyl-3β-acetoxy-11-oxoolean-12-en-30-ic acid amide (15)

Following GP2, **15** (403 mg, 72%) was obtained as a white solid; m.p. 296–297 °C; $R_F$ = 0.14 (hexanes/ethyl acetate, 11:9); $[\alpha]_D$ = +157.2° (*c* 0.117, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2949*br*, 1727 *m*, 1655 *m*, 1588*s*, 1505 *m*, 1245*s*, 751 s $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 8.50–8.43 (m, 2H, 36-H+35-H), 7.96 (d, *J* = 6.3 Hz, 2H, 34-H+37-H), 5.71 (s, 1H, H-12), 4.52 (dd, *J* = 11.7, 4.7 Hz, 1H, 3-H), 2.79 (m, 1H, 1-$H_a$), 2.38 (s, 1H, 9-H), 2.24 (m, 2H, 18-H+16-$H_a$), 2.12–2.04 (m, 2H, 15-$H_a$+19-$H_a$), 2.05 (s, 3H, 32-$H_3$) 1.90–1.80 (m, 1H, 15-$H_b$), 1.78–1.56 (m, 3H, 19-$H_b$, 6-$H_a$+7-$H_a$), 1.56–1.38 (m, 7H, 16-$H_b$+7-$H_b$+21-$H_a$+21-$H_b$+22-$H_a$+2-$H_a$+2-$H_b$), 1.40 (s, 3H, 27-$H_3$), 1.32 (s, 3H, 29-$H_3$), 1.28–1.18 (m, 1H, 6-$H_b$), 1.16 (s, 3H, 25-$H_3$), 1.13 (s, 3H, 26-$H_3$), 1.10–1.0 (m, 2H, 1-$H_b$+22-$H_b$), 0.89 (s, 3H, 23-$H_3$), 0.88 (s, 3H, 26-$H_3$), 0.81 (s, 3H, 28-$H_3$), 0.8 (s, 1H, 5-H) ppm; $^{13}C$ NMR (125 MHz, $CDCl_3$): δ = 199.9 (C-11), 175.8 (C-30), 170.8 (C-31), 146.6 (C-35+C-36), 128.6 (C-12), 114.5 (C-34+C-37) 80.3 (C-3),61.8 (C-9), 54.9 (C-5), 48.0 (C-18), 45.5 (C-8), 45.3 (C-17), 43.2 (C-14), 41.3 (C-19), 38.9 (C-1), 37.9 (C-4), 37.5 (C-21), 36.9 (C-10), 32.6 (C-7), 31.9 (C-22), 31.4 (C-16), 28.6 (C-29), 28.4 (C-28), 28.0 (C-24), 26.3 (C-15), 23.5 (C-2), 23.4 (C-27), 21.3 (C-32), 18.7 (C-26), 17.3 (C-6), 16.7 (C-23), 16.4 (C-25) ppm; MS (ESI, MeOH): *m/z* = 587.0 ([M − H]⁻, 100%), 623.1 ([M + $Cl^-$]⁻, 16%); analysis calcd for $C_{37}H_{52}N_2O_4$ (588.83): C 75.47, H 8.90, N 4.76; found: C 75.29, H 9.13, N 4.58.

#### 4.6.9. N-3-pyrazolyl-3β-acetoxy-11-oxoolean-12-en-30-ic acid amide (16)

Following GP2, **16** (348 mg, 60%) was obtained as a white solid; m.p. 257–259 °C; $R_F$ = 0.15 (hexanes/ethyl acetate, 11:9); $[\alpha]_D$ = +136.0° (*c* 0.115, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2950*br*, 1729 *m*, 1652 *m*, 1465 *m*, 1364 *m*, 1243*s*, 985 *m*, 755 s $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 7.67 (d, *J* = 2.6 Hz, 1H, 35-H), 6.73 (d, *J* = 2.6 Hz, 1H, 34-H), 5.8 (s, 1H, 12-H), 4.52 (dd, *J* = 11.7, 4.7 Hz, 1H, 3-H), 2.80 (m, 1H, 1-$H_a$), 2.38 (s, 1H, 9-H), 2.28–2.19 (m, H, 18-H), 2.16–1.95 (m, 3H, 16-$H_a$+15-$H_a$+19-$H_a$), 2.06 (s, 3H, 32-$H_3$) 1.90–1.80 (m, 1H, 15-$H_b$), 1.79–1.58 (m, 5H, 19-$H_b$, 2-$H_a$+2-$H_b$+6-$H_a$+7-$H_a$), 1.54–1.34 (m, 4H, 16-$H_b$+21-$H_a$+21-$H_b$+22-$H_a$), 1.40 (s, 3H, 27-$H_3$), 1.27 (s, 3H, 29-$H_3$), 1.28–1.18 (m, 2H, 6-$H_b$+7-$H_b$), 1.16 (s, 3H, 25-$H_3$), 1.12 (s, 3H, 26-$H_3$), 1.10–1.00 (m, 2H, 1-$H_b$+15-$H_b$), 0.89 (s, 3H, 23-$H_3$), 0.88 (s, 3H, 24-$H_3$), 0.83 (s, 1H, 5-H), 0.81 (s, 3H, 28-H) ppm; $^{13}C$ NMR (125 MHz, $CDCl_3$): δ = 200.3 (C-11), 174.0 (C-30), 171.1 (C-31), 168.6 (C-13), 145.1 (C-33), 131.2 (C-35), 128.8 (C-12), 96.7 (C-34), 80.6 (C-3), 61.8 (C-9), 54.9 (C-5), 47.8 (C-18), 45.3 (C-8), 44.5 (C-17), 43.2 (C-14), 41.2 (C-19), 38.9 (C-1), 38.0 (C-4), 37.5 (C-21), 36.9 (C-10), 32.7 (C-7), 31.9 (C-22), 31.4 (C-16), 29.0 (C-29), 28.4 (C-28), 28.0 (C-24), 26.4 (C-15), 23.6 (C-2), 23.4 (C-27), 21.3 (C-32), 18.7 (C-26), 17.4 (C-6), 16.7 (C-23), 16.4 (C-25) ppm; MS (ESI, MeOH): *m/z* = 576.1 ([M − H]⁻, 100%), 639.1 ([M+2-propanol + H]⁻, 17%); analysis calcd for $C_{35}H_{51}N_3O_4$ (577.81): C 72.75, H 8.90, N 7.27; found: C 72.55, H 9.01, N 7.06.

#### 4.6.10. N-5-indazolyl-3β-acetoxy-11-oxoolean-12-en-30-ic acid amide (17)

Following GP2, **17** (278 mg, 47%) was obtained as a yellow solid; m.p. 226–228 °C; $R_F$ = 0.18 (hexanes/ethyl acetate, 11:9); $[\alpha]_D$ = +184.5° (*c* 0.131, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2950*br*, 1715 *m*, 1651*s*, 1465 *m*, 1245*s*, 942 *m*, 753 s $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 8.28–8.24 (m, 1H, 37-H), 8.16 (s, 1H, 39-H), 7.50 (dd, *J* = 9.0, 1.9 Hz, 1H, 34-H), 7.44 (d, *J* = 9.0 Hz, 1H, 35-H), 5.75 (s, 1H, 12-H), 4.5 (dd, *J* = 11.7, 4.7 Hz, 1H, 3-H), 2.81 (m, 1H, 1-$H_a$), 2.38 (s, 1H, 9-H), 2.23 (m, 2H, 18-H+16-$H_a$), 2.16–2.01 (m, 2H, 15-$H_a$+19-$H_a$), 2.06 (s, 3H, 32-$H_3$) 1.90–1.80 (m, 1H, 15-$H_b$), 1.78–1.56 (m, 5H, 19-$H_b$+6-$H_a$+7-$H_a$+2-$H_a$+2-$H_b$), 1.56–1.38 (m, 5H, 16-$H_b$+7-$H_b$+21-$H_a$+21-$H_b$+22-$H_a$), 1.42 (s, 3H, 27-$H_3$), 1.32 (s, 3H, 29-$H_3$), 1.28–1.18 (m, 1H, 6-$H_b$), 1.16 (s, 3H, 25-$H_3$), 1.14 (s, 3H, 26-$H_3$), 1.10–0.92 (m, 2H, 1-$H_b$+22-$H_b$), 0.89 (s, 3H, 23-$H_3$), 0.88 (s, 3H, 24-$H_3$), 0.84 (s, 3H, 28-$H_3$), 0.81 (s, 1H, 5-H) ppm; $^{13}C$ NMR (125 MHz, $CDCl_3$): δ = 200.3 (C-11), 174.7 (C-30), 171.1 (C-31), 170.4 (C-33), 133.4 (C-37), 128.3 (C-12), 125.6 (C-34), 112.3 (C-39), 111.3 (C-35), 80.7 (C-3),61.8 (C-9), 54.9 (C-5), 48.5 (C-18), 45.5 (C-8), 44.5 (C-17), 43.3 (C-14), 41.5 (C-19), 38.9 (C-1), 38.1 (C-4), 37.5 (C-21), 36.9 (C-10), 32.7 (C-7), 31.9 (C-22), 31.6 (C-16), 29.2 (C-29), 28.6 (C-28), 27.9 (C-24), 26.5 (C-15), 23.5 (C-2), 23.3 (C-27), 21.3 (C-32), 18.7 (C-26), 17.3 (C-6), 16.6 (C-23), 16.4 (C-25) ppm; MS (ESI, MeOH): *m/z* = 626.1 ([M − H]⁻, 100%), 662.0 ([M + $Cl^-$]⁻, 15%); analysis calcd for $C_{39}H_{53}N_3O_4$ (627.87): C 74.61, H 8.51, N 6.69; found: C 74.48, H 8.78, N 6.51.

#### 4.6.11. N-6-indazolyl-3β-acetoxy-11-oxoolean-12-en-30-ic acid amide (18)

Following GP2, **18** (386 mg, 65%) was obtained as a yellow; m.p. 217–219 °C; $R_F$ = 0.29 (hexanes/ethyl acetate, 11:9); $[\alpha]_D$ = 166.6° (*c* 0.139, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2952*br*, 1712 *m*, 1650*s*, 1503 *m*, 1364w, 1249s, 985*w*, 944*w*, 752 s $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): δ = 8.58–8.49 (m, 1H, 36-H), 7.48 (d, *J* = 8.8 Hz, 1H, 34-H), 6.81 (s, 1H, 38-H), 6.67 (dd, *J* = 8.7, 1.9 Hz, 1H, 39-H), 5.33 (s, 1H, 12-H), 4.5 (dd, *J* = 11.7, 4.7 Hz, 1H, 3-H), 2.81 (m, 1H, 1-$H_a$), 2.38 (s, 1H, 9-H), 2.29 (m, 2H, 18-H+16-$H_a$), 2.17–2.01 (m, 2H, 15-$H_a$+19-$H_a$), 2.07 (s, 3H, 32-$H_3$) 1.90–1.80 (m, 2H, 15-$H_b$+ 19-$H_b$), 1.78–1.56 (m, 4H, 6-$H_a$+7-$H_a$+2-$H_a$+2-$H_b$), 1.56–1.38 (m, 5H, 16-$H_b$+7-$H_b$+21-$H_a$+21-$H_b$+22-$H_a$), 1.41 (s, 3H, 27-$H_3$), 1.32 (s, 3H, 29-$H_3$), 1.25–1.18 (m, 1H, 6-$H_b$), 1.16 (s, 3H, 25-$H_3$), 1.13 (s, 3H, 26-$H_3$), 1.10–0.99 (m, 2H, 1-$H_b$+22-$H_b$), 0.89 (s, 3H, 23-$H_3$), 0.88 (s, 3H, 24-$H_3$), 0.82 (s, 3H, 28-$H_3$), 0.75 (s, 1H, 5-H) ppm; $^{13}C$ NMR (125 MHz, $CDCl_3$): δ = 200.1 (C-11), 174.7 (C-30), 171.1 (C-31), 169.5 (C-33), 121.3 (C-38), 128.5 (C-12), 80.6 (C-3),61.7 (C-9), 54.9 (C-5), 48.4 (C-18), 45.3 (C-8), 44.76 (C-17), 43.3 (C-14), 41.6 (C-19), 38.7 (C-1), 38.0 (C-2), 37.3 (C-21), 36.9 (C-10), 32.6 (C-7), 31.9 (C-22), 31.5 (C-16), 29.1 (C-29), 28.4 (C-28), 28.0 (C-24), 26.5 (C-15), 23.5 (C-2), 23.4 (C-27), 21.3 (C-32), 18.7 (C-26), 17.4 (C-6), 16.7 (C-23), 16.4 (C-25) ppm; MS (ESI, MeOH): *m/z* = 626.2 ([M − H]⁻, 100%), 662.0 ([M + $Cl^-$]⁻, 10%); analysis calcd for $C_{39}H_{53}N_3O_4$ (627.87): C 74.61, H 8.51, N 6.69; found: C 74.40, H 8.77, N 6.41.

#### 4.6.12. N-2-pyridinyl-3β-acetoxyolean-12-en-28-ic acid amide (19)

Following GP2, **19** (253 mg, 44%) [25] was obtained as a white solid; m.p. 218.9 °C; $R_F$ = 24 (hexanes/ethyl acetate, 9:1); $[\alpha]_D$ = +42.7° (*c* 0.19, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 3360 *w*, 2955 *m*, 2937 *m*, 2930 *m*, 2860 *w*, 1729 *s*, 1684 *s*, 1595 *w*, 1575 *m*, 1515 *s*, 1459 *m*, 1430 *s*, 1367 *m*, 1302 *m*, 1293 *m*, 1244 *vs*, 1172 *m*, 1148 *m*, 1097 *w*, 1024 *m*, 1010 *m*, 991 *m*, 968

*m*, 898 *w*, 780 *s*, 743 *w*, 654 *s*, 594 *m*, 516 *m*, 468 *w*, 410 m $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ 9.46 (s, 1H, N*H*), 8.45 (d, $J$ = 8.6 Hz, 1H, 37-H), 8.21 (ddd, $J$ = 5.4, 1.9, 0.9 Hz, 1H, 34-H), 7.84 (ddd, $J$ = 8.9, 7.3, 1.9 Hz, 1H, 35-H), 7.11 (ddd, $J$ = 7.4, 5.4, 1.1 Hz, 1H, 36-H), 5.52 (t, $J$ = 3.7 Hz, 1H, 12-H), 4.51–4.41 (m, 1H, 3-H), 2.92–2.79 (m, 1H, 18-$H_a$), 2.15 (td, $J$ = 14.1, 4.0 Hz, 1H, 16-$H_a$), 2.03 (s, 3H, 32-$H_3$), 2.01–1.82 (m, 3H, 2-$H_a$+11-$H_a$+16-$H_b$), 1.82–1.67 (m, 3H, 7-$H_2$), 1.66–1.53 (m, 5H, 1-$H_a$+2-$H_b$+9-H+11-$H_b$+15-$H_a$), 1.52–1.35 (m, 3H, 6-$H_a$+21-$H_a$+22-$H_a$), 1.34–1.18 (m, 4H, 6-$H_b$+19-$H_b$+21-$H_b$+22-$H_b$), 1.17 (s, 3H, 27-$H_3$), 1.12 (dt, $J$ = 14.0, 3.5 Hz, 1H, 15-$H_b$), 1.09–0.98 (m, 1H, 1-$H_b$), 0.94 (s, 3H, 29-$H_3$), 0.92 (s, 3H, 30-$H_3$), 0.88 (s, 3H, 25-$H_3$), 0.84 (s, 3H, 24-$H_3$), 0.81 (s, 3H, 23-$H_3$), 0.85–0.78 (m, 1H, 5-H), 0.64 (s, 3H, 26-$H_3$) ppm; $^{13}C$ NMR (126 MHz, $CDCl_3$): 177.4 (C-28), 170.9 (C-31), 150.9 (C-33), 143.6 (C-13), 143.6 (C-34), 141.1 (C-35), 123.3 (C-12), 119.2 (C-36), 115.1 (C-37), 80.8 (C-3), 55.2 (C-5), 47.9 (C-17), 47.5 (C-9), 46.3 (C-19), 41.9 (C-14), 41.5 (C-18), 39.4 (C-8), 38.1 (C-1), 37.7 (C-4), 36.9 (C-10), 34.1 (C-21), 33.0 (C-30), 32.4 (C-7+C-22), 30.6 (C-20), 28.0 (C-24), 27.5 (C-15), 25.8 (C-27), 23.6 (C-16), 23.6 (C-29), 23.5 (C-11), 23.5 (C-2), 21.3 (C-32), 18.1 (C-6), 16.6 (C-26), 16.6 (C-23), 15.4 (C-25) ppm; MS (ESI, MeOH): $m/z$ = 575.1 ($[M+H]^+$, 100%), 1171.0 ($[2\,M + Na^+]^+$, 12%); analysis calcd for $C_{37}H_{54}N_2O_3$ (574.85): C 77.31, H 9.47, N 4.87; found: C 77.05, H 9.61, N 4.57.

#### 4.6.13. N-3-pyridinyl-3β-acetoxyolean-12-en-28-ic acid amide (20)

Following GP2, **20** (325 mg, 58%) was obtained as a white solid; m.p. 193–194 °C; $R_F$ = 0.15 (hexanes/ethyl acetate, 3:2); $[\alpha]_D$ = 24.3° (*c* 0.109, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2924*br*, 1732 *m*, 1586*s*, 1505*s*, 1369 *m*, 1325 *m*, 1244*s*, 1026 *m*, 826 *m*, 581*w*, 536 m $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ = 8.59 (d, $J$ = 3.0 Hz, 1H, 37-H), 7.72 (d, $J$ = 2.8 Hz, 3H, 36-H), 7.14 (d, $J$ = 3.1 Hz, 1H, 34-H), 6.93 (d, $J$ = 2.8 Hz, 3H, 35-H), 5.58 (s, 1H, 12-H), 4.49 (dd, $J$ = 11.7, 4.7 Hz, 1H, 3-H), 2.89 (m, 1H, 18-H), 2.21–2.1 (m, 1H, 16-$H_a$), 2.05 (s, 3H, 32-$H_3$), 1.98 (m, 1H, 16-$H_b$), 1.88 (m, 2H, 11-$H_a$+11-$H_b$), 1.84–1.69 (s, 2H, 15-$H_a$, 22-$H_a$) 1.68–1.55 (m, 6H, 2-$H_a$+2-$H_b$+6-$H_a$+1-$H_a$+19-$H_a$+9-H), 1.50–1.32 (m, 4H, 7-$H_a$+7-$H_b$+6-$H_b$+21-$H_a$), 1.32–1.15 (m, 4H, 21-$H_b$+19-$H_b$+1-$H_b$+15-$H_b$), 1.17 (s, 3H, 27-$H_3$), 0.98 (s, 3H, 25-$H_3$), 0.95 (s, 3H, 30-$H_3$), 0.90 (s, 3H, 29-$H_3$), 0.86 (s, 3H, 24-$H_3$), 0.83 (s, 3H, 23-$H_3$), 0.68–0.63 (m, 1H, 5-H), 0.66 (s, 3H, 26-$H_3$) ppm; $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ = 176.5 (C-36), 174.7 (C-37), 170.8 (C-31), 165.4 (C-33), 142.7 (C-13), 123.9 (C-12), 80.8 (C-3), 55.1 (C-5), 47.6 (C-9), 47.5 (C-19), 47.5 (C-17), 41.7 (C-14), 41.3 (C-18), 39.4 (C-8), 39.3 (C-1), 37.7 (C-4), 36.9 (C-10), 36.8 (C-21), 36.8 (C-29), 32.9 (C-7), 32.3 (C-22), 30.7 (C-20), 30.7 (C-24), 27.9 (C-15), 23.7 (C-27), 23.6 (C-30), 21.3 (C-32), 18.2 (C-6), 16.7 (C-26), 16.7 (C-23), 15.4 (C-25) ppm; MS (ESI, MeOH): $m/z$ = 598.1 ($[M + Na–2H]^-$, 100%); analysis calcd for $C_{37}H_{54}N_2O_3$ (574.85): C 77.31, H 9.47, N 4.87; found: C 77.17, H 9.63, N 4.62.

#### 4.6.14. N-4-pyridinyl-3β-acetoxyolean-12-en-28-ic acid amide (21)

Following GP2, **21** (376 mg, 68%) was obtained as a white solid; m.p. 155–158 °C; $R_F$ = 0.25 (hexanes/ethyl acetate, 3:2); $[\alpha]_D$ = +24.5° (*c* 0.132, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2924*br*, 1733 *m*, 1680*w*, 1532 *m*, 1479 *m*, 1369 *m*, 1245*s*, 1026 *m*, 706 m $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ = 8.26 (dd, $J$ = 5.2, 1.3 Hz, 2H, 36-H+35-H), 7.53 (dd, $J$ = 8.6, 5.2 Hz, 2H, 34-H +37-H), 5.5 (s, 1H, 12-H), 4.51 (dd, $J$ = 11.7, 4.7 Hz, 1H, 3-H), 2.34 (m, 1H, 18-H), 2.19–2.11 (m, 1H, 16-$H_a$), 2.05 (s, 3H, 32-$H_3$), 2.07–1.84 (m, 3H, 11-$H_a$+16-$H_b$+11-$H_b$), 1.85–1.73 (s, 1H, 15-$H_a$) 1.68–1.56 (m, 7H, 2-$H_a$+2-$H_b$+6-$H_a$+1-$H_a$+19-$H_a$+9-H+22-$H_a$), 1.44–1.21 (m, 4H, 7-$H_a$+7-$H_b$+6-$H_b$+21-$H_a$), 1.19–1.05 (m, 2H, 21-$H_b$+19-$H_b$), 1.14 (s, 3H, 27-$H_3$), 1.02–0.92 (m, 2H, 1-$H_b$+15-$H_b$) 0.99 (s, 3H, 25-$H_3$), 0.95 (s, 3H, 30-$H_3$), 0.90 (s, 3H, 29-$H_3$), 0.86 (s, 3H, 24-$H_3$), 0.83 (s, 3H, 23-$H_3$), 0.88–0.80 (m, 1H, 5-H), 0.68 (s, 3H, 26-$H_3$) ppm; $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ = 177.6 (C-36+C-35), 170.8 (C-31), 139.4 (C-34+C-37), 126.0 (C-12), 80.8 (C-3), 55.2 (C-5), 49.1 (C-19), 47.5 (C-9), 42.4 (C-17), 39.7 (C-18), 39.6 (C-8), 38.3 (C-1), 37.7 (C-4), 36.8 (C-10), 36.7 (C-21), 32.6 (C-29), 30.8 (C-20), 28.0 (C-24), 27.9 (C-15), 24.5 (C-27), 23.6 (C-30), 23.5 (C-11), 21.3 (C-32), 18.2 (C-6), 17.1 (C-26), 16.7 (C-23), 15.5 (C-25) ppm; MS (ESI, MeOH): $m/z$ = 673.1 ($[M - H]^-$, 100%), 609.1 ($[M + Cl^-]^-$, 89%); analysis calcd for $C_{37}H_{54}N_2O_3$ (574.85): C 77.31, H 9.47, N 4.87; found: C 77.19, H 9.54, N 4.30.

#### 4.6.15. N-3-pyrazolyl-3β-acetoxyolean-12-en-28-ic acid amide (22)

Following GP2, **22** (282 mg, 51%) was obtained as a white solid; m.p. 138–141 °C; $R_F$ = 0.19 (hexanes/ethyl acetate, 6:4); $[\alpha]_D$ = 36.3° (*c* 0.128, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2944*br*, 1731 *m*, 1524 *m*, 1479 *m*, 1367 *m*, 1246*s*, 1026 *m*, 730*s*, 706 m $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ = 8.28 (d, $J$ = 5.1 Hz, 1H, 35-H), 7.51–7.45 (m, 1H, 34-H), 5.54 (s, 1H, 12-H), 4.49 (dd, $J$ = 11.7, 4.7 Hz, 1H, 3-H), 2.85 (m, 1H, 18-H), 2.19–2.1 (m, 1H, 16-$H_a$), 2.05 (s, 3H, 32-$H_3$), 2.01–1.94 (m, 2H, 11-$H_a$ + H-16$_b$), 1.92–1.85 (m, 1H, 11-$H_b$), 1.85–1.69 (s, 2H, 15-$H_a$+22-$H_a$) 1.68–1.56 (m, 6H, 2-$H_a$+2-$H_b$+6-$H_a$+1-$H_a$+19-$H_a$+9-H), 1.54–1.38 (m, 4H, 7-$H_a$+7-$H_b$+6-$H_b$+21-$H_a$), 1.32–1.15 (m, 2H, 21-$H_b$+19-$H_b$), 1.20 (s, 3H, 27-$H_3$), 1.18–1.01 (m, 2H, 1-$H_b$+15-$H_b$) 0.97 (s, 3H, 25-$H_3$), 0.95 (s, 3H, 30-$H_3$), 0.91 (s, 3H, 29-$H_3$), 0.86 (s, 3H, 24-$H_3$), 0.84 (s, 3H, 23-$H_3$), 0.88–0.81 (m, 1H, 5-H), 0.70 (s, 3H, 26-$H_3$) ppm; $^{13}C$ NMR (101 MHz, $CDCl_3$): $\delta$ = 177.6 (C-35), 171.1 (C-31), 144,1 (C-13), 124.9 (C-34), 122.8 (C-12), 80.3 (C-3), 55.1 (C-5), 47.7 (C-19), 47.4 (C-9), 46.4 (C-17), 42.0 (C-14), 41.9 (C-18), 39.4 (C-8), 38.1 (C-1), 37.7 (C-4), 36.9 (C-10), 34.2 (C-21), 32.9 (C-29), 32.3 (C-7), 32.2 (C-22), 30.7 (C-20), 27.9 (C-24), 27.4 (C-15), 25.7 (C-27), 23.7 (C-30), 23.6 (C-2), 23.5 (C-11), 21.3 (C-32), 18.1 (C-6), 16.9 (C-26), 16.6 (C-23), 15.4 (C-25) ppm; MS (ESI, MeOH): $m/z$ = 609.2 ($[M + FA-H]^-$, 100%); analysis calcd for $C_{35}H_{53}N_3O_3$ (563.83): C 74.56, H 9.48, N 7.45; found: C 74.33, H 9.67, N 7.46.

#### 4.6.16. N-5-indazolyl-3β-acetoxyolean-12-en-28-ic acid amide (23)

Following GP2, **23** (480 mg, 81%) was obtained as a yellow solid; m.p. 206–208 °C; $R_F$ = 0.20 (hexanes/ethyl acetate, 2:1); $[\alpha]_D$ = 39.5° (*c* 0.131, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2945*br*, 1717 *m*, 1656 *m*, 1502*s*, 1463 *m*, 1366 *m*, 1245*s*, 1027 *m*, 753 s $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ = 8.33 (dd, $J$ = 2.0, 0.8 Hz, 1H, 37-H), 8.17 (d, $J$ = 1.0 Hz, 1H, 39-H), 7.62 (dt, $J$ = 9.0, 0.9 Hz, 1H, 34-H), 7.33 (ddd, $J$ = 9.0, 4.6, 2.0 Hz, 1H, 35-H), 5.6 (s, 1H, 12-H), 4.5 (dd, $J$ = 11.7, 4.7 Hz, 1H, 3-H), 2.72 (m, 1H, 18-H), 2.17–2.07 (m, 1H, 16-$H_a$), 2.05 (s, 3H, 32-$H_3$), 2.07–1.90 (m, 2H, 11-$H_a$+16-$H_b$), 1.89–1.70 (m, 4H, 15-$H_a$+11-$H_b$+2-$H_a$+2-$H_b$) 1.68–1.56 (m, 5H, 6-$H_a$+1-$H_a$+19-$H_a$+9-H+22-$H_a$), 1.55–1.39 (m, 3H, 7-$H_a$+7-$H_b$+6-$H_b$), 1.38–1.19 (m, 3H, 21-$H_b$+19-$H_b$+21-$H_a$), 1.2 (s, 3H, 27-$H_3$), 1.17–1.03 (m, 2H, 1-$H_b$+15-$H_b$) 0.98 (s, 3H, 25-$H_3$), 0.96 (s, 3H, 30-$H_3$), 0.91 (s, 3H, 29-$H_3$), 0.86 (s, 3H, 24-$H_3$), 0.82 (s, 3H, 23-$H_3$), 0.88–0.75 (m, 1H, 5-H), 0.71 (s, 3H, 26-$H_3$) ppm; $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ = 176.7 (C-35), 171.2 (C-31), 145.3 (C-13), 136.9 (C-33), 133.3 (C-37), 124,1 (C-38), 123.4 (C-34), 121.8 (C-12), 111.5 (C-39), 110.9 (C-35), 80.8 (C-3), 55.2 (C-5), 47.5 (C-9), 47.2 (C-19), 46.7 (C-17), 42.6 (C-18), 42.2 (C-14), 39.5 (C-8), 38.2 (C-1), 37.7 (C-4), 36.8 (C-10), 34.2 (C-21), 32.9 (C-29), 32.4 (C-7), 32.2 (C-22), 30.8 (C-20), 28.0 (C-24), 27.4 (C-15), 25.7 (C-27), 24.2 (C-30),23.7 (C-2), 23.6 (C-11), 23.5 (C-16), 21.3 (C-32), 18.1 (C-6), 16.9 (C-26), 16.6 (C-23), 15.5 (C-25) ppm; MS (ESI, MeOH): $m/z$ = 612.2 ($[M - H]^-$, 100%), 648.2 ($[M + Cl^-]^-$, 10%); analysis calcd for $C_{39}H_{55}N_3O_3$ (613.89): C 76.31, H 9.03, N 6.85; found: C 76.05, H 9.27, N 6.66.

#### 4.6.17. N-6-indazolyl-3β-acetoxyolean-12-en-28-ic acid amide (24)

Following GP2, **24** (306 mg, 51%) was obtained as a yellow solid; m.p. 194–197 °C; $R_F$ = 0.6 (hexanes/ethyl acetate, 3:2); $[\alpha]_D$ = +27.3° (*c* 0.107, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2944*br*, 1732 *m*, 1573 *m*, 1464 *m*, 1364 *m*, 1244*s*, 1026 *m*, 754 s $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ = 8.57–8.51 (m, 1H, 36-H), 7.48 (d, $J$ = 8.8 Hz, 1H, 34-H), 6.81 (s, 1H, 38-H), 6.67 (dd, $J$ = 8.7, 1.9 Hz, 1H, 39-H), 5.33 (s, 1H, 12-H), 4.49 (dd, $J$ = 11.7, 4.7 Hz, 1H, 3-H), 2.84 (m, 1H, 18-H), 2.22–2.13 (m, 1H, 16-$H_a$), 2.05 (s, 3H, 32-$H_3$), 2.10–1.98 (m, 2H, 11-$H_a$), 1.85–1.77 (m, 6H, 15-$H_a$+11-$H_b$+2-$H_a$+2-$H_b$+16-$H_b$) 1.77–1.51 (m, 5H, 6-$H_a$+1-$H_a$+19-$H_a$+9-H+22-$H_a$), 1.51–1.24 (m, 3H, 7-$H_a$+7-$H_b$+6-$H_b$), 1.23–1.12 (m, 2H, 19-$H_b$+21-$H_a$), 1.2 (s, 3H, 27-$H_3$), 1.11–0.90 (m, 3H, 21-$H_b$+1-$H_b$+15-$H_b$) 1.01 (s, 3H,

25-$H_3$), 0.95 (s, 3H, 30-$H_3$), 0.92 (s, 3H, 29-$H_3$), 0.87 (s, 3H, 24-$H_3$), 0.84 (s, 3H, 23-$H_3$), 0.89–0.78 (m, 1H, 5-H), 0.68 (s, 3H, 26-$H_3$) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 175.1 (C-33), 170.8 (C-31), 143.8 (C-13), 140.2 (C-36), 132.2 (C-38), 123.7 (C-39), 122.2 (C-12), 119.7 (C-34), 80.9 (C-3), 55.3 (C-5), 47.6 (C-9), 47.5 (C-19), 42.3 (C-18), 41.9 (C-14), 39.2 (C-8), 38.1 (C-1), 37.7 (C-4), 36.9 (C-10), 34,1 (C-21), 33.1 (C-29), 32.6 (C-7), 32.5 (C-22), 30.6 (C-20), 27.9 (C-24), 27.4 (C-15), 25.8 (C-27), 24.0 (C-2), 23.5 (C-11), 23.4 (C-16), 21.3 (C-32), 18.1 (C-6), 16.7 (C-26), 16.6 (C-23), 15.4 (C-25) ppm; MS (ESI, MeOH): *m/z* = 612.4 ([M − H]$^-$, 74%); analysis calcd for $C_{39}H_{55}N_3O3$ (613.89): C 76.31, H 9.03, N 6.85; found: C 76.13, H 9.24, N 6.63.

#### 4.6.18. N-2-pyridinyl-3β-acetoxyolean-12-en-28-ic acid amide (25)

Following GP2, **25** (440 mg, 76%) [25] was obtained as a white solid; m.p. 221 °C; $R_F$ = 0.16 (hexanes/ethyl acetate, 9:1); $[\alpha]_D$ = +27.1° (*c* 16.3, $CHCl_3$); IR (ATR): 3424 *vw*, 2975 *w*, 2967 *w*, 2937 *w*, 2927 *w*, 2846 *w*, 1729 *s*, 1679 *m*, 1592 *w*,1576 *m*,1514 *s*, 1455 *m*, 1430 *s*, 1393 *w*, 1368 *m*, 1302 *m*, 1296 *m*, 1280 *w*, 1249 *vs*, 1167 *w*, 1146 *w*, 1096 *w*, 1049 *w*, 1025 *m*, 1004 *w*, 991 *m*, 971 *w*, 900 *w*, 803 *w*, 784 *s*, 667 *w*, 654 *w*, 609 *w*, 602 *w*, 582 *m*, 525 *w*, 516 *m*, 410 *w* cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 10.28 (s, 1H, NH), 8.56 (dd, *J* = 8.8, 5.5 Hz, 1H, 37-H), 8.17 (ddd, *J* = 5.6, 1.9, 0.8 Hz, 1H, 34-H), 7.97 (ddd, *J* = 9.0, 7.3, 1.8 Hz, 1H, 35-H-H), 7.20 (ddd, *J* = 7.2, 4.5, 1.1 Hz, 1H, 36-H), 5.49 (t, *J* = 3.7 Hz, 1H, 12-H), 4.48 (dd, *J* = 11.0, 5.0 Hz, 1H, 3-H), 2.36 (d, *J* = 10.7 Hz, 1H, 18-H), 2.18 (td, *J* = 14.1, 4.5 Hz, 1H, 16-$H_a$), 2.03 (s, 3H, 32-$H_3$), 2.01–1.95 (m, 2H, 2-$H_a$+16-$H_b$), 1.93 (dq, *J* = 8.0, 3.3, 2.8 Hz, 1H, 11-$H_a$), 1.90–1.87 (m, 1H, 22-$H_a$), 1.78 (td, *J* = 14.0, 4.7 Hz, 1H, 15-$H_a$), 1.72–1.39 (m, 9H, 1-$H_a$+2-$H_b$+6-$H_a$+7-$H_a$+9-H+11-$H_b$+19-H+21-$H_a$+22-$H_b$), 1.39–1.20 (m, 3H, 6-$H_b$+7-$H_b$+21-$H_b$), 1.15 (ddd, *J* = 13.0, 5.2, 2.6 Hz, 1H, 15-$H_b$), 1.10 (s, 3H, 27-$H_3$), 1.12–0.98 (m, 2H, 1-$H_b$+20-H), 0.96 (d, *J* = 6.4 Hz, 3H, 30-$H_3$), 0.92 (d, *J* = 6.4 Hz, 3H, 29-$H_3$), 0.88 (s, 3H, 25-$H_3$), 0.84 (s, 3H, 24-$H_3$), 0.87–0.73 (m, 1H, 5-H), 0.81 (s, 3H, 23-$H_3$), 0.64 (s, 3H, 26-$H_3$) ppm; $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 177.6 (C-28), 170.9 (C-31), 150.5 (C-33), 143.3 (C-35), 140.5 (C-34), 138.0 (C-13), 126.3 (C-12), 118.9 (C-36), 116.1 (C-37), 80.8 (C-3), 55.2 (C-5), 52.7 (C-18), 49.6 (C-17), 47.4 (C-9), 42.1 (C-14), 39.6 (C-19), 39.5 (C-8), 38.6 (C-20), 38.2 (C-1), 37.6 (C-4), 36.8 (C-10), 36.6 (C-22), 32.7 (C-7), 30.7 (C-21), 28.0 (C-24), 27.8 (C-15), 24.3 (C-16), 23.5 (C-27), 23.5 (C-11), 23.3 (C-2), 21.3 (C-32), 21.1 (C-30), 18.1 (C-6), 17.0 (C-29), 16.7 (C-26), 16.7 (C-23), 15.4 (C-25) ppm; MS (ESI, MeOH): *m/z* = 575.0 ([M+H]$^+$, 100%), 1171.1 ([2 M + Na$^+$]$^+$, 30%)%); analysis calcd for $C_{37}H_{54}N_2O_3$ (574.85): C 77.31, H 9.47, N 4.87; found: C 77.03, H 9.65, N 4.65.

#### 4.6.19. N-3-pyridinyl-3β-acetoxyurs-12-en-28-ic acid amide (26)

Following GP2, **26** (509 mg, 89%) was obtained as a white solid; m.p. 176–179 °C; $R_F$ = 0.5 (hexanes/ethyl acetate, 4:3); $[\alpha]_D$ = +28.1° (*c* 0.185, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2925 *m,* 1732 *m,* 1681 *m,* 1586*w*, 1524 *m,* 1480 *m,* 1455 *m,* 1416 *m,* 1390 *m,* 1370 *m,* 1326*w,* 1245*vs,* 1194 *m,* 1145*w,* 1026 *m,* 1006 *m,* 985 *m,* 967 *m,* 902*w,* 796 *m,* 752*s,* 707*s,* 665 m cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.99 (s, 1H, 37-H), 8.79 (s, 1H, 33-NH), 8.68 (d, *J* = 8.6 Hz, 1H, 36-H), 8.26 (dd, *J* = 5.1, 1.4 Hz, 1H, 34-H), 7.44 (dd, *J* = 8.5, 5.1 Hz, 1H, 35-H), 5.49 (t, *J* = 3.6 Hz, 1H, 12-H), 4.48 (dd, *J* = 10.9, 5.3 Hz, 1H, 3-H), 2.28–2.22 (m, 1H, 18-H), 2.17–2.08 (m, 2H, 2-$H_a$+16-$H_a$), 2.03 (s, 3H, 32-$H_3$), 2.02–1.89 (m, 3H, 11-$H_a$+16-$H_b$+22-$H_a$), 1.80–1.70 (m, 2H, 2-$H_b$+15-$H_a$), 1.69–1.59 (m, 3H, 1-$H_a$+11-$H_b$+22-$H_b$), 1.59–1.43 (m, 5H, 6-$H_a$+7-$H_a$+9-H+19-H+21-$H_a$), 1.43–1.30 (m, 1H, 21-$H_b$), 1.30–1.22 (m, 2H, 6-$H_b$+7-$H_b$), 1.12 (s, 3H, 27-$H_3$), 1.16–1.01 (m, 3H, 1-$H_b$+15-$H_b$+20-H), 0.97 (d, *J* = 6.3 Hz, 3H, 30-$H_3$), 0.93 (d, *J* = 6.4 Hz, 3H, 29-$H_3$), 0.89 (s, 3H, 25-$H_3$), 0.84 (s, 3H, 24-$H_3$), 0.83 (dd, *J* = 29.0, 2.4 Hz, 1H, 5-H), 0.82 (s, 3H, 32-$H_3$), 0.66 (s, 3H, 26-$H_3$) ppm; $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 177.7 (C-28), 171.0 (C-31), 139.7 (C-34), 139.4 (C-13), 137.5 (C-37), 130.4 (C-36), 129.0 (C-33), 126.1 (C-12), 124.9 (C-35), 80.8 (C-3), 55.2 (C-5), 53.4 (C-18), 49.0 (C-17), 47.4 (C-9), 42.4 (C-14), 39.7 (C-19), 39.6 (C-8), 38.8 (C-20), 38.3 (C-1), 37.6 (C-4), 36.8 (C-10), 36.7 (C-22), 32.6 (C-7), 30.8 (C-21), 28.0 (C-24), 27.9 (C-15), 24.6 (C-16), 23.5 (C-2), 23.5 (C-11), 23.4 (C-27), 21.3 (C-32), 21.2 (C-30), 18.1 (C-6), 17.2 (C-29), 16.9 (C-26), 16.7 (C-23) 15.5 (C-25) ppm; MS (ESI, MeOH/$CHCl_3$): *m/z* = 574.1 ([M − H]$^-$, 100%); analysis calcd for $C_{37}H_{54}N_2O_3$ (574.85): C 77.31, H 9.47, N 4.87; found: C 77.17, H 9.61, N 4.59.

#### 4.6.20. N-4-pyridinyl-3β-acetoxyursol-12-en-28-ic acid amide (27)

Following GP2, **27** (108 mg, 19%) was obtained as a white solid; m.p. 168–171 °C; $R_F$ = 0.09 (hexanes/ethyl acetate, 3:2); $[\alpha]_D$ = +34.4° (*c* 0.138, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2944*br*, 1731 *m*, 1586*s*, 1504*s*, 1366 *m*, 1326 *m*, 1245*s*, 1026 *m*, 824 *m*, 751*s*, 579 *m*, 537 m cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.42 (d, *J* = 6.0 Hz, 2H, 35-H+36-H), 7.80 (d, *J* = 6.0 Hz, 2H, 34-H+37-H), 5.50 (t, *J* = 3.6 Hz, 1H, 12-H), 4.47 (dd, *J* = 11.7, 4.7 Hz, 1H, 3-H), 2.16 (s, 3H, 32-$H_3$), 2.19–2.08 (m, 1H, 16-$H_a$), 2.08–1.84 (m, 5H, 11-$H_a$+11-$H_b$+15-$H_a$+22-$H_a$+22-$H_b$), 1.76–1.43 (m, 6H, 2-$H_a$+2-$H_b$+1-$H_a$+6-$H_a$+21-$H_a$+7-$H_a$), 1.42–1.21 (m, 2H, 16-$H_b$+8-$H_b$) 1.12 (s, 3H, 23-$H_3$), 1.15–1.01 (m, 1H, 22-$H_b$), 0.97 (s, 3H, 31-$H_3$), 0.92 (s, 3H, 30-$H_3$), 0.87 (s, 3H, 23-$H_3$), 0.86 (s, 3H, 24-$H_3$), 0.86 (s, 3H, 29-$H_3$), 0.88–0.79 (m, 1H, 5-H), 0.62 (s, 3H, 26-$H_3$) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 177.1 (C-28), 170.8 (C-31), 148.1 (C-1), 146.9 (C-33), 139.7 (C-35+C-36), 126.9 (C-12), 114,1 (C-34+C-37), 80.7 (C-3), 55.2 (C-5), 53.7 (C-18), 49.3 (C-17), 47.4 (C-9), 42.4 (C-14), 39.7 (C-8), 39.5 (C-19), 38.8 (C-1), 38.3 (C-20), 37.6 (C-4), 36.8 (C-10), 36.5 (C-22), 32.6 (C-7), 30.7 (C-21), 28.0 (C-24), 27.9 (C-15), 24.8 (C-16), 23.5 (C-2), 21.3 (C-32), 21.1 (C-30), 18.0 (C-6), 17.2 (C-26), 16.7 (C-23), 15.5 (C-25) ppm; MS (ESI, MeOH): *m/z* = 573.2 ([M − H]$^-$, 100%), 609.2 ([M + Cl$^-$]$^-$, 21%); analysis calcd for $C_{37}H_{54}N_2O_3$ (574.85): C 77.31, H 9.47, N 4.87; found: C 77.01, H 9.67, N 4.68.

#### 4.6.21. N-3-pyrazolyl-3β-acetoxyursol-12-en-28-ic acid amide (28)

Following GP2, **28** (245 mg, 45%) was obtained as a white solid using GP2 and **10**; m.p. 188–191 °C; $R_F$ = 0.1 (hexanes/ethyl acetate, 3:2); $[\alpha]_D$ = +27.5° (*c* 0.115, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2925*br*, 1732 *m*, 1565 *m*, 1455 *m*, 1369 *m*, 1244*s*, 1026*s*, 754 s cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 7.51 (d, *J* = 2.4 Hz, 1H, 35-H), 6.50 (d, *J* = 2.4 Hz, 1H, 34-H), 5.48 (t, *J* = 3.6 Hz, 1H, 12-H), 4.47 (dd, *J* = 11.7, 4.7 Hz, 1H, 3-H), 2.17–2.05 (m, 2H, 16-$H_a$+16-$H_b$), 2.03 (s, 3H, 32-$H_3$), 2.01–1.87 (m, 3H, 11-$H_a$+11-$H_b$+15-$H_a$), 1.87–1.77 (m, 1H, 22-$H_a$), 1.77–1.65 (m, 1H, 22-$H_b$), 1.65–1.41 (m, 6H, 2-$H_a$+2-$H_b$+1-$H_a$+6-$H_a$+21-$H_a$+7-$H_a$), 1.41–1.21 (m, 3H, 9-H+6-$H_b$+ 21-$H_b$) 1.11 (s, 3H, 27-$H_3$), 1.15–1.01 (m, 2H, 15-$H_b$+1-$H_b$), 0.97 (s, 3H, 25-$H_3$), 0.91 (s, 3H, 30-$H_3$), 0.88 (s, 3H, 23-$H_3$), 0.85 (s, 3H, 24-$H_3$), 0.82 (s, 3H, 29-$H_3$), 0.86–0.76 (m, 1H, 5-H), 0.65 (s, 3H, 26-$H_3$) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 176.2 (C-28), 170.8 (C-31), 151.9 (C-33), 145.2 (C-13), 139.0 (C-35), 126.4 (C-12), 96.1 (C-34), 80.7 (C-3), 55.1 (C-5), 53.6 (C-18), 48.4 (C-17), 47.4 (C-9), 42.3 (C-14), 39.7 (C-19), 39.5 (C-1), 38.9 (C-20), 37.6 (C-4), 36.9 (C-10), 36.7 (C-22), 32.6 (C-7), 30.8 (C-21), 27.9 (C-24), 27.8 (C-15), 25.0 (C-16), 23.5 (C-16), 21.3 (C-32), 21.1 (C-30), 18.1 (C-6), 17.2 (C-7), 16.7 (C-23), 15.5 (C-25) ppm; MS (ESI, MeOH): *m/z* = 562.0 ([M − H]$^-$, 100%), 598.1 ([M + Cl$^-$]$^-$, 96%); analysis calcd for $C_{35}H_{53}N_3O_3$ (563.83): C 74.56, H 9.48, N 7.45; found: C 74.41, H 9.65, N 7.35.

#### 4.6.22. N-6-indazolyl-3β-acetoxyurs-12-en-28-ic acid amide (29)

Following GP2, **29** (292 mg, 50%) was obtained as an off-white solid; m.p. 189–191 °C; $R_F$ = 0.48 (hexanes/ethyl acetate, 3:2); $[\alpha]_D$ = +41.6° (*c* 0.115, $CHCl_3$); IR (ATR): $\tilde{\nu}$ = 2925*br*, 1718 *m*, 1653 *m*, 1509 *m*, 1465 *m*, 1359 *m*, 1244*s*, 1026 *m*, 942 *m*, 841*w*, 753 s cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 8.18 (d, *J* = 1.0 Hz, 1H, 36-H), 8.03 (s, 1H, 34-H), 7.71 (dd, *J* = 8.7, 4.9 Hz, 1H, 38-H), 7.09 (dd, *J* = 8.8, 1.7 Hz, 1H, 39-H), 5.55 (t, *J* = 3.6 Hz, 1H, 12-H), 4.48 (dd, *J* = 11.7, 4.7 Hz, 1H, 3-H), 2.21–1.95 (m, 3H, 16-$H_a$+16-$H_b$+22-$H_a$), 2.03 (s, 3H, 32-$H_3$), 1.95–1.83 (m, 2H, 11-$H_a$+11-$H_b$), 1.83–1.68 (m, 1H, 22-$H_b$), 1.68–1.41 (m, 6H, 2-$H_a$+2-$H_b$+1-$H_a$+6-$H_a$+21-$H_a$+7-$H_a$), 1.41–1.21 (m, 2H, 9-H+6-$H_b$) 1.14 (s, 3H, 27-$H_3$), 1.19–1.02 (m, 3H, 15-$H_b$+1-$H_b$+21-$H_b$), 0.99 (s, 3H, 25-$H_3$), 0.95 (s, 3H, 30-$H_3$), 0.88 (s, 3H, 23-$H_3$), 0.83 (s, 3H, 24-$H_3$), 0.81 (s, 3H, 29-$H_3$), 0.91–0.78 (m, 1H, 5-H), 0.66 (s, 3H, 26-$H_3$) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ = 176.9 (C-28), 171.1 (C-31), 140.2 (C-13), 139.0 (C-33),

126.4 (C-12), 121.8 (C-36), 118.8 (C-38), 116.9 (C-39), 100.1 (C-34), 80.8 (C-3), 55.1 (C-5), 54.3 (C-18), 48.9 (C-17), 47.4 (C-9), 42.6 (C-14), 39.9 (C-19), 39.6 (C-1), 39.1 (C-20), 38.3 (C-4), 37.7 (C-10), 36.7 (C-22), 32.6 (C-7), 30.8 (C-21), 28.0 (C-24), 27.9 (C-15), 25.2 (C-16), 23.6 (C-2), 21.3 (C-32), 21.2 (C-30), 18.0 (C-6), 17.3 (C-26), 16.6 (C-23), 15.5 (C-25) ppm; MS (ESI, MeOH): $m/z = 612.0$ ([M − H]$^-$, 100%), 648.1 ([M + Cl$^-$]$^-$, 18%); analysis calcd for $C_{39}H_{55}N_3O_3$ (613.89): C 76.31, H 9.03, N 6.85; found: C 76.19, H 9.24, N 6.61.

#### 4.6.23. N-5-indazolyl-3β-acetoxyurs-12-en-28-ic acid amide (30)

Following GP2, **30** (333 mg, 56%) was obtained as a white solid; m.p. 238–241 °C; $R_F = 0.35$ ($CHCl_3$/MeOH/$NH_4OH$, 98:1.8:0.2); $[\alpha]_D = +30.1°$ (*c* 0.170, $CHCl_3$); IR (ATR): $\tilde{\nu} = 2946$ *m,* 2925 *m,* 2872*w,* 1731 *m,* 1717 *m,* 1650 *m,* 1593*w,* 1535 *m,* 1502*s,* 1453 *m,* 1390 *m,* 1369 *m,* 1311*w,* 1245*vs,* 1146*w,* 1104*w,* 1078*w,* 1027 *m,* 1006 *m,* 985 *m,* 967 *m,* 944 *m,* 901*w,* 875*w,* 831*w,* 806 *m,* 753*vs,* 665 *m,* 608 *m,* 557*w,* 536*w,* 426 m cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 8.50$ (d, $J = 1.9$ Hz, 1H, 39-H), 8.30 (s, 1H, 37-H), 7.92 (s, 1H, NH), 7.80 (d, $J = 9.1$ Hz, 1H, 34-H), 7.34 (dd, $J = 9.2$, 1.9 Hz, 1H, 35-H), 5.57–5.53 (m, 1H, 12-H), 4.48 (dt, $J = 11.6$, 3.6 Hz, 1H, 3-H), 2.16–1.94 (m, 5H, 2-$H_a$+11-$H_a$+16-$H_a$+18-H), 2.03 (s, 3H, 32-$H_3$), 1.86 (d, $J = 13.3$ Hz, 1H, 16-$H_b$), 1.78–1.67 (m, 2H, 2-$H_b$+15-$H_a$), 1.68–1.54 (m, 5H, 1-$H_a$+9-H+11-$H_b$+21-$H_a$+22-$H_b$), 1.54–1.45 (m, 3H, 6-$H_a$+7-$H_a$+19-H), 1.43–1.21 (m, 3H, 6-$H_b$+7-$H_b$+21-$H_b$), 1.14 (s, 3H, 27-$H_3$), 1.21–1.03 (m, 2H, 1-$H_b$+15-$H_b$), 1.01–0.98 (m, 4H, 20-H+30-$H_3$), 0.95 (t, $J = 5.8$ Hz, 3H, 29-$H_3$), 0.90–0.87 (m, 3H, 25-$H_3$), 0.84 (s, 3H, 24-$H_3$), 0.84–0.79 (m, 4H, 5-H+23-$H_3$), 0.66–0.62 (m, 3H, 26-$H_3$) ppm; $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta = 176.9$ (C-28), 171.0 (C-31), 140.3 (C-13), 136.4 (C-38), 134.5 (C-33), 128.0 (C-37), 126.4 (C-35), 126.2 (C-12), 120.8 (C-36), 112.8 (C-34), 110.3 (C-39), 80.8 (C-3), 55.2 (C-5), 54.3 (C-18), 48.8 (C-17), 47.4 (C-9), 42.7 (C-14), 39.9 (C-19), 39.6 (C-8), 39.1 (C-20), 38.3 (C-1), 37.6 (C-4), 37.0 (C-22), 36.8 (C-10), 32.6 (C-7), 30.8 (C-21), 28.0 (C-15), 27.9 (C-24), 25.2 (C-16), 23.6 (C-11), 23.5 (C-2), 23.3 (C-27), 21.3 (C-32), 21.1 (C-30), 18.0 (C-6), 17.3 (C-29), 16.9 (C-26), 16.7 (C-23), 15.6 (C-25) ppm; MS (ESI, MeOH/$CHCl_3$): $m/z = 613.1$ ([M − H]$^-$, 100%); analysis calcd for $C_{39}H_{55}N_3O_3$ (613.89): C 76.31, H 9.03, N 6.85; found: C 76.18, H 9.31, N 6.58.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmcr.2022.100085.

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