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Research paper

# Novel triterpenoid pyrones, phthalimides and phthalates are selectively cytotoxic in CCRF-CEM cancer cells – Synthesis, potency, and mitochondrial mechanism of action

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ABSTRACT

A series of triterpenoid pyrones was synthesized and subsequently modified to introduce phthalimide or phthalate moieties into the triterpenoid skeleton. These compounds underwent *in vitro* cytotoxicity screening, revealing that a subset of six compounds exhibited potent activity, with $IC_{50}$ values in the low micromolar range. Further biological evaluations, including Annexin V and propidium iodide staining experiment revealed, that all compounds induce selective apoptosis in cancer cells. Measurements of mitochondrial potential, cell cycle analysis, and the expression of pro- and anti-apoptotic proteins confirmed, that apoptosis was mediated *via* the mitochondrial pathway. These findings were further supported by cell cycle modulation and DNA/RNA synthesis studies, which indicated a significant increase in cell accumulation in the G0/G1 phase and a marked reduction in S-phase cells, alongside a substantial inhibition of DNA synthesis. The activation of caspase-3 and the cleavage of PARP, coupled with a decrease in the expression of Bcl-2 and Bcl-XL proteins, underscored the induction of apoptosis through the mitochondrial pathway. Given their high activity and pronounced effect on mitochondria function, trifluoromethyl pyrones **1f** and **2f**, and dihydrophthalimide **2h** have been selected for further development.

## 1. Introduction

Pentacyclic triterpenoids, natural products found across a broad spectrum of living organisms, continue to capture scientific interest with new discoveries every year [1,2]. These compounds and their semi-synthetic analogs have a diverse range of interesting biological activities [3]. Among them, their antiviral [4–6], antiparasitic [7,8], antifungal [9], hepatoprotective [10], nephroprotective [11], or neuroprotective activities gained considerable attention [12]. Currently, a significant research effort is dedicated to exploring triterpenoids with strong selective cytotoxicity against various cancer cell lines [13–20]. Triterpenoids that incorporate a heterocycle fused to the A-ring play a significant role among the anticancer derivatives [21–23]. Our team has discovered several groups of cytotoxic triterpenoids each bearing a heterocycle fused to the A-ring, highlighting their promise in oncology [24–27]. Our recent publication detailed our findings on triterpenoid pyridines and pyrazines, which exhibited superior efficacy against leukemic cell lines, alongside a thorough investigation into their mechanism of action [28]. Inspired by these promising results we synthesized new variants of heterocycle-containing triterpenoids. Since the previous studies showed that most of the active compounds occur among triterpenoid acids containing a free carboxylate at the C-17 position, we selected betulinic acid **1a**, dihydrobetulinic acid **2a**, and ursolic acid **3a** as our primary substrates of interest. In addition, allobetulon **4** was chosen as a "training" compound for the optimization of the reaction conditions, as it typically does not undergo side-reactions due to its

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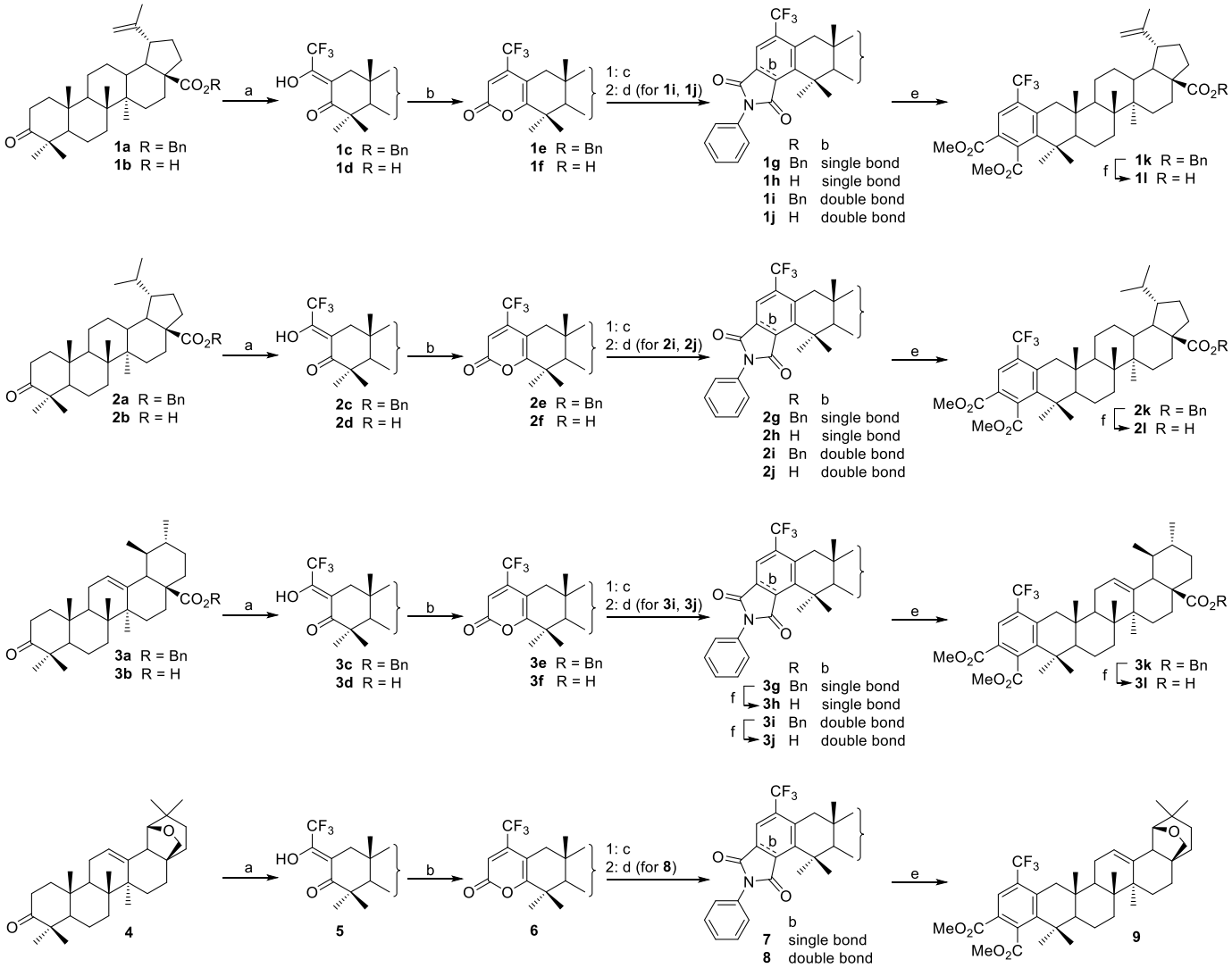
stability. Since the last step contains an alkylation with dimethyl sulfate, all reaction sequence were performed with benzyl-protected carboxylic functions.

## 2. Results and discussion

### 2.1. Chemistry

The chemistry part started from three triterpenoid acids betulonic acid **1b**, dihydrobetulonic acid **2b**, and ursonic acid **3b**, their benzyl esters **1a**, **2a**, **3a**, and allobetulon **4**. The procedures (Scheme 1) were performed in tandem and did not differ for each reaction pathway unless otherwise commented later. Benzyl esters were necessary as protection for some of the following steps. The first reaction step was the alkylation of the position C-2 with ethyl trifluoroacetate under basic conditions generated using NaH. The reaction proceeded smoothly, however, the products **1c**, **1d**, **2c**, **2d**, **3c**, **3d**, and **5** were rather unstable on silica gel during purification and therefore were used crude for the following reaction steps. It's worth mentioning that all products are predominantly in their enol forms according to the spectral data and acted as hydroxyl ketones during the next reaction. The following step was performed according to the literature [29] with slight modifications of the reaction conditions (benzene was replaced with toluene and the reaction temperature increased from 65 °C to 90 °C). The procedure used an intramolecular Wittig reaction of hydroxy ketones – in our case compounds **1c**, **1d**, **2c**, **2d**, **3c**, **3d**, and **5** with (triphenylphosphoranylidene)ethenone. The resulting lactones **1e**, **1f**, **2e**, **2f**, **3e**, **3f**, and **6** were subjected to a Diels-Alder reaction with N-phenylmaleimide to give dihydrophthalates **1g**, **1h**, **2g**, **2h**, **3g**, and **7**. Compound **3h** was obtained alternatively by the cleavage of benzyl ester **3g**. The following aromatization of the benzene ring was performed according to Ref. [30] using elemental sulfur in dry 1,3-dimethyl-3,4,5,6-tetrahydro-2(*1H*)-pyrimidinone at 220 °C under an inert atmosphere. Compounds **1i**, **1j**, **2**, **2hj**, **3i**, and **7** were used by this procedure while compound **3j** was obtained alternatively by the cleavage of benzyl ester **3i**. The alternative route to compounds **3h** and **3i** was used only due to a lack of the unprotected material within this pathway. The last step consisted of a basic cleavage of the phthalimide ring in compounds **1i**, **2i**, **3i** and **8**. The reaction was performed using KOH in a mixture of MeOH and $H_2O$ which, unfortunately, did not yield pure phthalic acids but complex and




**Scheme 1.** Preparation of triterpenoid pyrones, phthalimides and phthalates. Reagents and conditions: a) i: NaH (60% in mineral oil, 5 equiv.), THF, 5 min; ii: $CF_3COOEt$, 20 °C, 2 h; b) (triphenylphosphoranylidene)ethenone (1.2–1.9 equiv.), toluene, 90 °C under $N_2$, 3–12 h (ref. [29]); c) *N*-phenylmaleimide (3 equiv.), diphenyl ether, 180–200 °C under $N_2$, 3 h; d) $S_8$ (2 equiv.), 1,3-dimethyl-3,4,5,6-tetrahydro-2(*1H*)-pyrimidinone, 220 °C under $N_2$, 2 h (ref. [30]); e) i: KOH (4.5 equiv.), MeOH/$H_2O$ (10:1), 60 °C, 13 h; ii: $Me_2SO_4$ (2 equiv.), $K_2CO_3$ (2 equiv.), acetone, 60 °C, 13 h; f) 1,3-cyclohexadiene (7.6 equiv.), 10% Pd/C (0.6 equiv.), THF/EtOAc (1:1), r.t., 2–24 h.

inseparable mixtures of these acids with partly methylated compounds. Therefore, the cleavage intermediates were dried and treated with dimethyl sulfate to obtain pure dimethylphthalates **1k**, **2k**, **3k**, and **9**. Benzyl esters were then transformed to free acids **1l**, **2l**, and **3l** by catalytic hydrogenation. All following attempts to obtain free tricarboxylic acids by ester cleavage were unsuccessful due to the low solubility of these compounds in both polar and lipophilic solvents.

Low solubility of triterpenoids is often one of the biggest hurdle for their biological testing and potential use in therapy. It is especially important to deal with this issue, since media for these experiments are based on water. We always carefully check, if the tested sample is fully dissolved at the beginning and if it does not precipitate during the experiments. In this set of compounds, all benzyl esters **1c**, **1e**, **1g**, **1i**, **1k**, **2c**, **2e**, **2g**, **2i**, **2k**, **3c**, **3e**, **3g**, **3i**, **3k**, and allobetulon derivatives **5**–**9** were not soluble enough in various mixtures of DMSO in water (0.5%, 5%, and 10%) and therefore we did not test them. We even did not expect these compounds to be active, since we use benzyl ester as a protection for harsh chemical reactions and allobetulon derivatives for model compound (cheap and very stable) to optimize some harsh reaction conditions. In our earlier work, we have tested solubility of various triterpenoids and found that compounds with the free carboxylic acid and a heterocycle fused to the A-ring are suluble in all concentration that we use in the biological tests [25]. Special attention was given to compounds **1f**, **1h**, **1l**, **2h**, **2f**, and **3h** during the measurement of pharmacological parameters (Table 2) where they had to be fully dissolved.

## 2.2. Biology

### 2.2.1. Cytotoxicity assay

The cytotoxic activity of all new derivatives was assessed *in vitro* against eight human cancer cell lines and two non-tumor fibroblast lines using the standard MTS test (Table 1) [31]. The rationale for selecting these cancer cell lines is detailed in Borková et al. [32] The cell lines included T-lymphoblastic leukemia (CCRF-CEM), myeloid leukemia (K562) and their multidrug-resistant analogs (CEM-DNR, K562-TAX), solid tumors represented by lung (A549) and colon (HCT116, HCT116p53−/−) carcinomas, osteosarcoma (U2OS), and, for comparison, two human non-cancer fibroblast lines (BJ, MRC-5). It was noted that all benzyl esters **1c**, **1e**, **1g**, **1i**, **1k**, **2c**, **2e**, **2g**, **2i**, **2k**, **3c**, **3e**, **3g**, **3i**, **3k** and allobetulon derivatives **5**–**9** did not fully dissolved under the experimental conditions, and thus, were not tested. Within the derivatives prepared from parent compounds betulonic acid **1b**, dihydrobetulonic acid **2b**, and ursonic acid **3b**, significant activity was observed against CCRF-CEM cell line, with $IC_{50}$ = 3.5–6.2 μM for trifluoromethyl pyrones **1f**, **2f**, and **3f**, trifluoromethyldihydrophthalimido derivatives **1h**, **2h**, **3h**, and dimethyl triphluoromethyl phthalate derivatives **1l**, **2l**, and **3l**. Interestingly, the cytotoxicity appears to be minimally influenced by the triterpenoid scaffold, with the heterocyclic or aromatic substituent playing a more significant role. Notably, dihydrophthalimido derivatives showed activity with $IC_{50}$ values of 4.2–4.5 μM, but this activity was completely lost upon full aromatization of the benzene ring. The most active compounds, with $IC_{50}$ below 5 μM, were selected for further pharmacological studies and mechanism of action investigations.

### 2.2.2. Pharmacological parameters

Pharmacological parameters are important indicators to assess whether a compound is suitable for further drug development. The rationale for the selection and measurement of these parameters has been reported earlier in Hodoň et al. [28] The most active compounds **1f**, **1h**, **1l**, **2h**, **2f**, and **3h** were selected for the measurement of *in vitro* pharmacological parameters (Table 2). These six candidates were selected to evaluate fundamental pharmacokinetic parameters such as absorption, distribution, metabolism, and excretion (ADME). Understanding the ADME properties of a new chemical entity is critical during its evaluation to become a leading candidate in a drug discovery programs. The compounds were incubated with human plasma *in vitro* to quickly determine their susceptibility to plasma degradation and to what extent. All compounds demonstrated high stability in human plasma after 2 h incubation (85–100 % of the original quantity of the substance remaining). Plasma protein binding was measured using Equilibrium Dialysis, studied compounds **1f**, **2f**, **1h**, **2h**, **3h** and **1l** were bound by 86 %, 94 %, 73 %, 60 %, 71% and 82 %, respectively. For the microsomal stability assay, human liver microsomes and NADPH cofactor were used to assess phase I oxidation by cytochrome P450 and flavin monooxygenases. The intrinsic clearance calculated from the microsomal stability assay indicated low or medium category. This means that studied compounds were not rapidly metabolized by liver microsome enzymes.

The derivatives exhibited low ability (- log Papp >6 cm/s) to diffuse passively through an artificial cellular membrane in the Parallel Artificial Membrane Permeability Assay (PAMPA), suggesting an alternative intracellular transport mechanism might be involved. Compound **1l**

**Table 1**
Cytotoxic activities of tested compounds against eight tumor (including multidrug resistant variants) and two normal fibroblast cell lines.

| Comp. | $IC_{50}$ (μM)[a] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | CCRF-CEM | CEM-DNR | K562 | K562- TAX | A549 | HCT116 | HCT116 | U2OS | BJ | MRC-5 | Ti[b] |
| **1d** | 7.3 | n.d. | 13 | n.d. | 12 | 24 | 30 | 30 | >50 | >50 | >6.8 |
| **1f** | **4.0** | 7.8 | 22 | 10 | 16 | 13 | 15 | 18 | >50 | 28 | >9.8 |
| **1h** | **4.2** | 20 | 25 | 9.7 | 9.7 | 7.8 | 12 | 7.6 | >50 | 28 | >9.3 |
| **1j** | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | 1 |
| **1l** | **3.8** | >50 | 18 | >50 | 18 | 23 | 13 | 17 | >50 | >50 | >13 |
| **2d** | 8.6 | n.d. | 29 | n.d. | 11 | 23 | 26 | 25 | 43 | 46 | 5 |
| **2f** | **3.5** | **4.9** | 23 | 17 | 11 | 10 | 18 | 19 | >50 | 27 | >11 |
| **2h** | **4.5** | >50 | 30 | >50 | 18 | 29 | 28 | 4.8 | 25 | 28 | >5.9 |
| **2j** | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | 1 |
| **2l** | 5.3 | >50 | >50 | >50 | >50 | >50 | >50 | 11 | >50 | >50 | >9.4 |
| **3d** | 8.3 | n.d. | 23 | n.d. | 23 | 35 | 43 | >50 | >50 | >50 | >6.0 |
| **3f** | 6.3 | 19.5 | 23 | 21 | 17 | 25 | 27 | 23 | >50 | >50 | >7.9 |
| **3h** | **4.3** | >50 | 21 | 23 | 18 | 13 | 12 | 19 | 10 | >50 | >7.0 |
| **3j** | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | >50 | 1 |
| **3l** | 5.1 | >50 | 28 | >50 | 17 | 38 | 26 | 24 | >50 | >50 | >9.8 |

[a] The $IC_{50}$ represents the concentration of the drug required to inhibit cell growth by 50%. The standard deviation in cytotoxicity assays typically reaches up to 15% of the mean value.

[b] The therapeutic index is calculated based on the $IC_{50}$ for the CCRF-CEM line versus the average $IC_{50}$ for both fibroblast lines. Benzyl ester intermediates **1c**, **1e**, **1g**, **1i**, **1k**, **2c**, **2e**, **2g**, **2i**, **2k**, **3c**, **3e**, **3g**, **3i**, **3k**, and allobetulon derivatives **5**–**9** did not fully dissolve under the experimental conditions, and thus, their cytotoxicity was not measured.

**Table 2**
Pharmacological parameters of compounds **1f**, **1h**, **1l**, **2h**, **2f**, and **3h**.

| Compound | Plasma stability | | | | Plasma protein binding | PAMPA | |
|---|---|---|---|---|---|---|---|
| | % Compound remaining | | | | | log Pe | Category[b] |
| | 15min | 30 | 60 | 120 | % Fraction bound | | |
| **1f** | 99.86 | 99.18 | 101.89 | 93.61 | 86.48 | −8.24 | Low |
| **2f** | 98.37 | 97.72 | 97.50 | 94.20 | 94.4 | −8.19 | Low |
| **1h** | 100.04 | 92.72 | 95.84 | 101.00 | 72.73 | −8.46 | Low |
| **2h** | 99.16 | 101.93 | 96.66 | 89.00 | 59.66 | −8.76 | Low |
| **3h** | 101.58 | 99.64 | 101.88 | 94.24 | 70.76 | −8.27 | Low |
| **1l** | 102.94 | 96.67 | 96.18 | 93.78 | 81.56 | −6.91 | Low |

| Compound | MDCK-MDR1 Permeability Assay | | | | | Microsomal stability | | |
|---|---|---|---|---|---|---|---|---|
| | Papp (x10e-6) | Category | Efflux ratio | active efflux | % recovery | % Compound remaining | | |
| | | | | | | 15 min | 30 | 60 |
| **1f** | 0.14 | negative | 26 | Yes | 70.71 | 100 | 86 | 70 |
| **2f** | 0.27 | negative | 6.06 | Yes | 64.85 | 106 | 98 | 82 |
| **1h** | 0.10 | negative | 17.5 | Yes | 94.06 | 99 | 93 | 79 |
| **2h** | 0.05 | negative | 71.81 | Yes | 85.97 | 92 | 92 | 56 |
| **3h** | 0.33 | negative | 8.17 | Yes | 60.49 | 82 | 64 | 45 |
| **1l** | 4.46 | negative | 6.95 | Yes | 71.17 | 91 | 102 | 76 |

| Compound | Caco-2 Permeability Assay | | | | | Microsomal stability |
|---|---|---|---|---|---|---|
| | Papp (x10e-6) | Category | Efflux ratio | active efflux | % recovery | Category of Intrinsic clearence[a] |
| **1f** | 0.04 | Low | 28.45 | Yes | 104.47 | Medium |
| **2f** | 0.15 | Low | 16.1 | Yes | 105.10 | Low |
| **1h** | 0.04 | Low | 36.4 | Yes | 85.99 | Low |
| **2h** | 0.09 | Low | 63.54 | Yes | 84.17 | Medium |
| **3h** | 0.12 | Low | 41.84 | Yes | 69.16 | Medium |
| **1l** | 1.73 | Low | 5.62 | Yes | 75.81 | Low |

[a,b] References, [35,36] error deviations are within a range of values less than 10% (all experiments were done in triplicates, except for cell-based permeability assays, which were performed in duplicates).

showed a minor improvement in cellular permeability (without category change) compared to the other studied compounds in our PAMPA model as well as permeability data from cell permeability assay: Caco-2 and MDCK-MDR1 cell lines. The Caco-2 and MDCK-MDR1 permeability assays are established models of intestinal [33] and blood-brain barriers, respectively [34]. It can be concluded that the studied molecules exhibited low (PappAB $<5 \times 10$-6 cm/s) probability of intestinal absorption and crossing the blood-brain barrier (PappAB $<10 \times 10$-6 cm/s). We assessed rates of transport across Caco-2 and MDCK-MDR1 monolayers in both directions (apical to basolateral (A-B) and basolateral to apical (B-A)) across the cell monolayer, which enabled us to determine the efflux ratio and assess whether the compound undergoes active efflux. The studied derivatives were actively exported from the cells in both barrier models, as indicated by efflux ratios ER $> 2$. Results from all *in vitro* pharmacology testing are summarized in Table 2.

### 2.2.3. Cell death detection by annexin V and propidium iodide staining

Based on the $IC_{50}$ measured in the highly sensitive CCRF-CEM cancer cell line, we have selected the most active compounds **1f**, **2f**, **1h**, **2h**, **3h**, and **1l** for detailed biological testing to gain further insight into their mechanisms of action. It is well documented, that the induction of apoptosis is a primary mechanism of action for anticancer therapies. Therefore, we initially assessed the ability of the selected compounds to induce apoptosis using Annexin V and propidium iodide double staining, followed by flow cytometry analysis. This method enabled us to quantify apoptosis and, additionally, identify necrosis as another potential mode of cell death. Annexin V measures early apoptosis by detecting phosphatidylserine externalized on the cytoplasmic surface of the cell membrane, while propidium iodide assesses membrane integrity. As a result of Annexin V/propidium iodide combined staining, we can easily discriminate healthy cells from cells in early or late stages of apoptosis or cells dying by necrosis. CCRF-CEM cells were treated with **1f**, **2f**, **1h**, **2h**, **3h**, and **1l** at $1 \times IC_{50}$ or $5 \times IC_{50}$ concentrations for 24 h, following the protocol for Annexin V/propidium iodide staining. Our results indicate that cells treated with our compounds underwent apoptosis in a manner corresponding to the concentration used. This was evident from the increase in the proportion of cells stained with Annexin V alone (early apoptotic) or double-stained with Annexin V and PI (late apoptotic; Fig. 1). Among the tested compounds, **1f** and **2f** induced the strongest cytotoxic response, manifested by a significant increase in the apoptotic cell population compared to control cells (Fig. 1).

### 2.2.4. Analysis of mitochondrial membrane potential

Mitochondria play an essential role in the commitment of cells to apoptosis *via* a decreased mitochondrial membrane potential (MMP), resulting in increased permeability of the outer mitochondrial membrane. This event leads to the release of cytochrome c and other apoptosis inducing factors from the mitochondria intermembrane space into the cytosol, ultimately leading to the activation of apoptotic caspase proteases [37]. Therefore, a decrease or loss of mitochondria transmembrane potential is one of the most significant hallmarks associated with apoptotic cell death. We assessed the ability of compounds **1f**, **2f**, **1h**, **2h**, **3h**, and **1l** to promote apoptosis of CCRF-CEM cells by evaluating changes in MMP using JC-1(5,5,6,6-Tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide) probe. JC-1 represents a cationic fluorochrome accumulating in mitochondria of healthy cells (indicating normal MMP), leading to the formation of J-aggregates with emission in the red spectrum (maximum at ~590 nm). On the other hand, the loss of MMP in apoptotic cells leads to J-aggregates dissociation to monomeric form, generating green fluorescence (emission maximum at ~530 nm). Measuring the dye uptake by mitochondria effectively distinguishes between apoptotic and healthy cells. Following 24 h of treatment with the compounds at concentrations corresponding to $1 \times IC_{50}$ or $5 \times IC_{50}$, a significant reduction in the mitochondrial membrane potential was observed in CCRF-CEM cells in a dose-dependent manner compared to control (untreated) cells (Fig. 2). The most pronounced mitochondria depolarization was observed following treatment by **1f**, **2f** and **1l** at $5 \times IC_{50}$ concentration, inducing

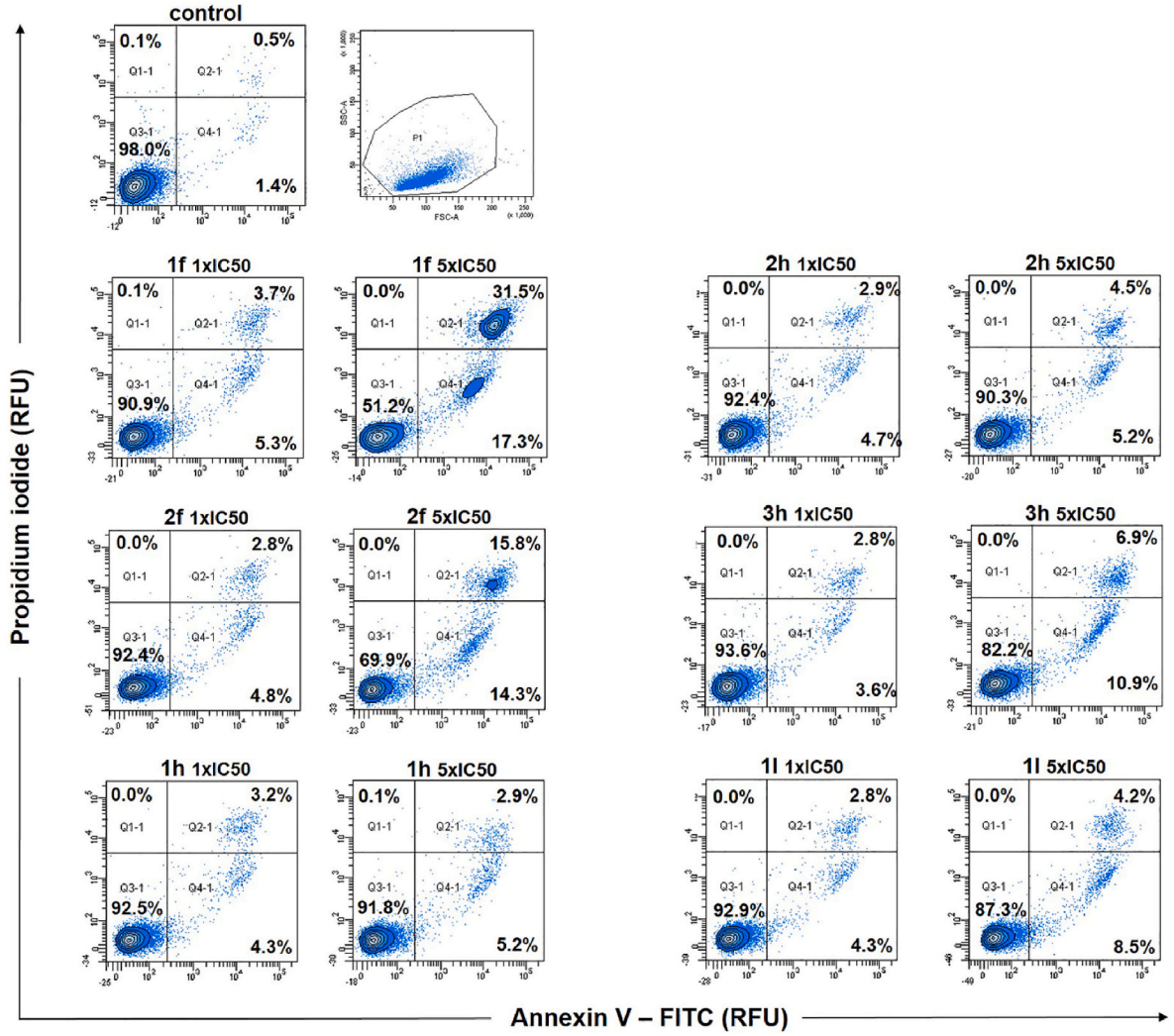



**Fig. 1.** Representative contour plots of dual Annexin V/Propidium iodide flow cytometry analysis. Initially, dead cells and debris were excluded from the analysis. Cells from the P1 region were projected in contour plots of propidium iodide versus Annexin V-FITC, segmented into four quadrants based on appropriate coordinates setting. The Q3-1 region includes cells unstained by both Annexin V and propidium iodide, that are considered as viable; Q4-1 includes cells stained with Annexin V but negative for propidium iodide, classified as early apoptotic; the Q2-1 region includes cells stained with both Annexin V and propidium iodide, considered as late apoptotic or necrotic; Q1-1 includes cells stained only with propidium iodide, which are considered as necrotic. Samples were measured and analyzed using a FACSAria II flow cytometer, with at least 10,000 cells acquired for each sample.

a more than 15-fold increase of low MMP cells compared to the control cells. These results nicely correspond with the cell death analysis result (Fig. 1).

### 2.2.5. *Effect of **1f, 2f, 1h, 2h, 3h,** and **1l** on cell cycle modulation and DNA/RNA synthesis in CCRF-CEM cells*

To further reveal the mechanism of action and characterize the antitumor properties, we studied cytostatic effect by analyzing the cell cycle profile and another proliferation markers. We used the highly sensitive CCRF-CEM cells, treating them with the studied derivatives at 1 × $IC_{50}$ or 5 × $IC_{50}$ concentration for 24 h (Fig. 3; Table 2). While the 1 × $IC_{50}$ concentration exhibited no significant modulatory effect on cell cycle progression, the 5 × $IC_{50}$ concentration revealed more pronounced effects. Treatment with 5 × $IC_{50}$ concentration of derivatives **1f**, **2f** **1h**, **3h** and **1l** led to a considerable increase in cell accumulation in the G0/G1 phase compared to control cells. This blockage or slowdown in cell cycle progression through G0/G1 was accompanied by a decreased percentage of S-phase cells. To assess the impact on proliferation potential, we focused on proliferation marker BrdU and mitotic marker $pH3^{Ser10}$ after 24 h of incubation with the compounds. Analysis of the mitotic marker indicated a lower rate of cell division in cells treated with 5 × $IC_{50}$ concentration of derivatives **1f**, **2f** and **1l**. Interestingly, derivative **2h** at 5 × $IC_{50}$ concentration induced a significant increase in the fraction of mitotic cells. This effect is not related to tubulin polymerization inhibition (data not shown), suggesting that further studies are needed to clarify the exact mechanism of action. Derivatives **1f**, **2h**, **3h** and **1l** significantly reduced the population of CCRF-CEM cells actively incorporating BrdU into newly synthesized DNA strands. Almost complete DNA synthesis inhibition was observed after treatment with the **2h** derivative even at 1 × $IC_{50}$ concentration, indicating a strong antiproliferative potential. Remarkable effects on BrdU incorporation were seen following treatment with the **2f** derivative. While the 1 × $IC_{50}$ concentration increased the percentage of BrdU positive cells, an opposite effect was observed after treatment with 5 × $IC_{50}$ concentration. Furthermore, we investigated transcription rates by exposing cells to a short pulse of BrU after a 24 h pre-incubation with the selected derivatives. We observed almost complete RNA synthesis inhibition in response to the treatment with **1f**, **2f** and **2h** at 5 × $IC_{50}$ concentration. Interestingly, derivative **3h** at 1 × $IC_{50}$ concentration increased the fraction of BrU positive cells by approximately 50 % compared to

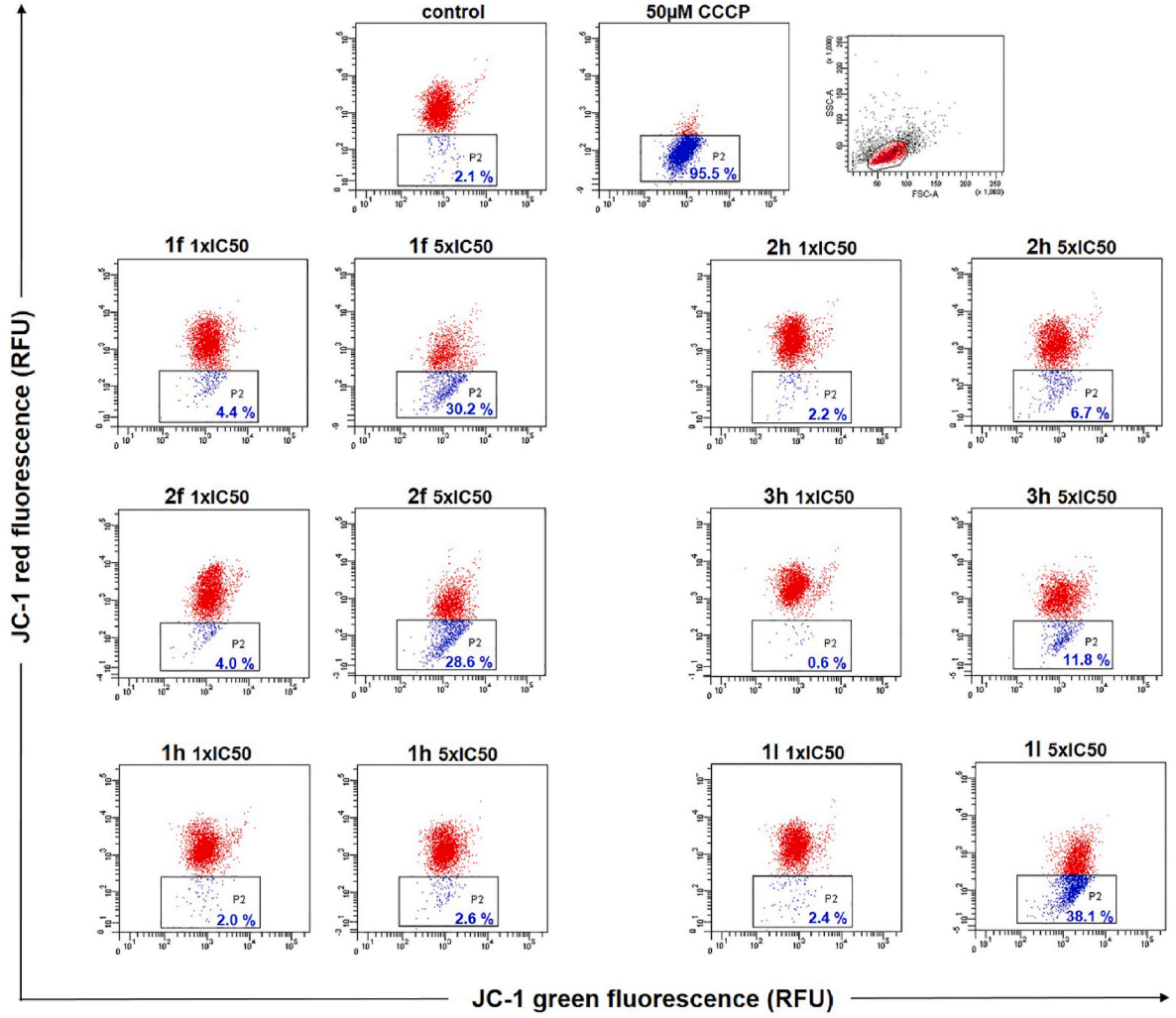



**Fig. 2.** Assessment of mitochondrial membrane potential in CCRF-CEM cells treated with compounds **1f**, **2f**, **1h**, **2h**, **3h**, and **1l** for 24 h at 1 × $IC_{50}$ or 5 × $IC_{50}$ concentration. MMP was analyzed using the FACSAria II flow cytometer and the JC-1 fluorescent probe. Dead cells and debris were excluded from the analysis, as shown in the FCS/SSC dot plot diagram. Cells from the P1 region were displayed in dot plots of propidium iodide (JC-1 red fluorescence; emission maxima at 590 nm) versus fluorescein isothiocyanate (JC-1 green fluorescence; emission maxima at 530 nm). The percentage of cells with depolarized mitochondria (P2 gate) was determined based on the appropriate P2 gate setting in samples treated by CCCP (carbonyl cyanide 3-chlorophenylhydrazone), an uncoupler that completely depolarizes mitochondria. Cells not subjected to treatment served as the control.

untreated cells, suggesting an enhancement of transcription activity.

### *2.2.6. Effect of **1f, 2f, 1h, 2h, 3h,** and **1l** on the expression of apoptosis-related proteins*

In the final part of our study, we investigated the impact of selected derivatives on the cell death machinery through the induction of apoptosis. Caspases, a family of conserved cysteine proteases that typically cleave after an aspartate residue in their substrates, are well-recognized as the pivotal executioners of apoptosis [38]. Once activated, caspases cleave a variety of intracellular polypeptides, including major structural proteins of the cytoplasm and nucleus, components participating in DNA repair machinery [e.g., poly (ADP-ribose) polymerase 1; PARP-1] and several protein kinases [39]. It is generally assumed in apoptosis studies that caspases, existing as precursors in the cytoplasm, are activated by cleavage of their precursor form. Our examination of the effect of derivatives on the activation of the main executioner caspase-3 revealed that compounds **1f**, **2f**, **1h** and **3h** at 5 × $IC_{50}$ significantly reduced the expression of the caspase-3 precursor form, indicating its cleavage into the active form (Fig. 4). The activation of caspase-3, and thus ongoing apoptosis, was further confirmed by the detection of the PARP cleavage fragment (Fig. 4). These results are in line with results from the Annexin V analysis (Fig. 1). As we know from our previous research, triterpenoid derivatives predominantly induce the intrinsic (mitochondrial) apoptosis pathway [40,41]. A crucial component of the mitochondrial pathway includes members of the Bcl-2 protein family, which regulate the integrity of the outer mitochondrial membrane. Our analysis focused on the expression of Bcl-2 and Bcl-XL proteins, critical anti-apoptotic members of the Bcl-2 protein family. Both proteins are often deregulated in cancer and represent promising targets for anticancer therapy. Treating CCRF-CEM cells with all examined derivatives at 5 × $IC_{50}$ for 24 h resulted in reduced expression of both Bcl-2 and Bcl-XL (Fig. 4). These results align with the observed mitochondria depolarization, suggesting disrupted protection against apoptosis (Fig. 2).

## 3. Conclusion

A series of pyrones, dihydrophthalimides, phthalimides, and phthalates were synthesized from four parent triterpenoids – betulinic acid (**1b**), dihydrobetulinic acid (**2b**), ursonic acid (**3b**), and allobetulon (**4**). Although it was initially anticipated that allobetulon (**4**) derivatives might not present significant biological interest due to their low

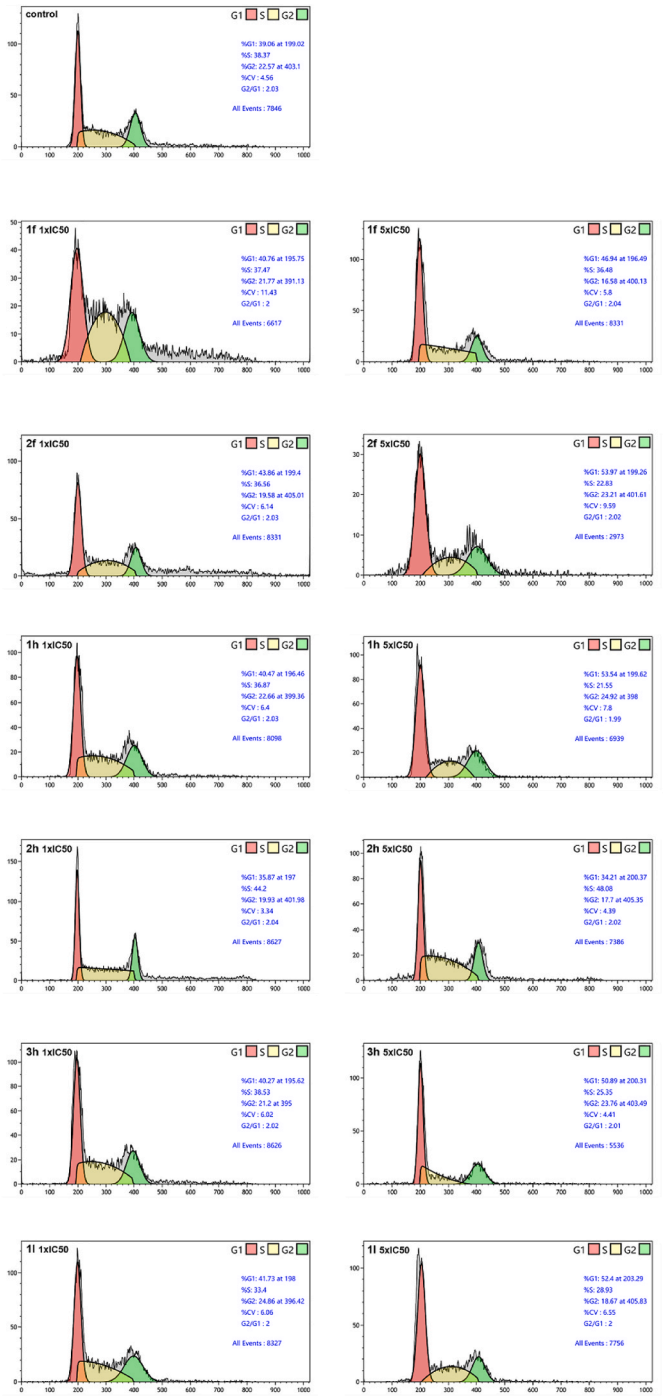



**Fig. 3.** Representative histograms of cell cycle analysis by flow cytometry. CCRF-CEM cells were treated with compounds **1f**, **2f**, **1h**, **2h**, **3h**, and **1l** at 1 × $IC_{50}$ and 5 × $IC_{50}$ concentrations for 24 h prior to analysis. Propidium iodide staining was used to access the cell cycle state. Only live cells were included in the analysis. Untreated cells were taken as a control. The numbers represent the percentage of cells in the G1, S and G2/M phases, respectively.

bioavailability, they were included in this study as a stable triterpenoid benchmark for testing under harsh reaction conditions that were previously unexplored in triterpenoid chemistry. After optimization, these conditions were applied to more sensitive triterpenoid acids or their benzyl esters.

From the basic $IC_{50}$ screening of the target compounds, six derivatives (**1f**, **2f**, **1h**, **2h**, **3h**, and **1l**) with $IC_{50} < 5$ μM were selected for further examination of their pharmacological parameters and mechanism of action. The annexin V and propidium iodide staining experiments showed that all compounds induced selective apoptosis in CCRF-CEM cells, especially at higher concentrations. Further experiments revealed that trifluoromethyl pyrones **1f** and **2f** had a significant impact on mitochondrial potential, causing membrane depolarization. Cell cycle and DNA/RNA synthesis analysis showed that **1f**, **1h**, **3h** and **1l** significantly increased the accumulation of cells in the G0/G1 phase, which was accompanied by a decreased percentage of S-phase cells. Derivatives **1f**, **2h**, **3h** and **1l** significantly reduced the synthesis of new DNA, with almost complete DNA synthesis inhibition observed after treatment with the **2h** derivative even at 1 × $IC_{50}$ concentration, indicating a strong antiproliferative potential. Moreover, compounds **1f**, **2f**, **1h** and **3h** at 5 × $IC_{50}$ reduced the expression of the caspase-3 precursor form, indicating its cleavage into the active form. This activation of caspase-3 and subsequent apoptosis was further confirmed by the detection of the PARP cleavage fragment. Exposure of CCRF-CEM cells to all examined derivatives at 5 × $IC_{50}$ for 24 h led to a reduced expression of Bcl-2 and Bcl-XL, correlating with the results from mitochondrial depolarization and suggesting disrupted protection against apoptosis.

These findings unequivocally demonstrate that all six derivatives (**1f**, **2f**, **1h**, **2h**, **3h**, and **1l**) induce selective apoptosis in CCRF-CEM cells *via* the mitochondrial pathway. Pyrones **1f**, **2f**, and dihydrophthalimide **2h** emerged as the most promising compounds due to their efficacy at lower concentrations, making them excellent candidates for further development. They will be incorporated into a broader set of compounds selected for *in vivo* testing in murine models.

## 4. Experimental procedures

### 4.1. Chemistry

Melting points were determined using either the Büchi B-545 apparatus or the STUART SMP30 apparatus and are uncorrected. Optical rotations were measured on an Autopol III (Rudolph Research, Flanders, USA) polarimeter in MeOH at 25 °C and are in [$10^{-1}$ deg $cm^2$ $g^{-1}$]. Infrared spectra were recorded on a Nicolet Avatar 370 FTIR and processed in the OMNIC 9.8.372. DRIFT stands for Diffuse Reflectance Infrared Fourier Transform. $^1H$ and $^{13}C$ experiments were performed on Jeol ECX-500SS (500 MHz for $^1H$), and Varian$^{UNITY}$ Inova 400 (400 MHz for $^1H$) instruments, using $CDCl_3$, DMSO-$d_6$, $CD_3OD$ or THF-$d_8$ as solvents (25 °C). Chemical shifts ($\delta$) were referenced to the residual signal of the solvent ($CDCl_3$, DMSO-$d_6$, $CD_3OD$ or THF-$d_8$) and are reported in parts per million (ppm). Coupling constants ($J$) are reported in Hertz (Hz). NMR spectra were processed in the ACD/NMR Processor Academic Edition 12.01, MestReNova 6.0.2–5475 or JEOL Delta v5.0.5.1. EI-MS spectra were recorded on an INCOS 50 (Finnigan MAT) spectrometer at 70 eV and an ion source temperature of 150 °C. The samples were introduced from a direct exposure probe at a heating rate of 10 mA/s. Relative abundances stated are related to the most abundant ion in the region of $m/z > 180$. HRMS analysis was performed using an LC-MS Orbitrap Elite high-resolution mass spectrometer with electrospray ionization (Dionex Ultimate 3000, Thermo Exactive plus, MA, USA). Spectra were taken at the positive and negative mode in the range of 400–700 $m/z$. The samples were dissolved in MeOH and injected to the mass spectrometer over autosampler after HPLC separation: precolumn Phenomenex Gemini (C18, 50 × 2 mm, 2.6 μm), mobile phase isocratic MeOH/water/HCOOH 95:5:0.1. The course of the reactions was monitored by TLC on Kieselgel 60 $F_{254}$ plates (Merck) detected first by UV light (254 nm) and then by spraying with 10% aqueous $H_2SO_4$ and heating to 150 °C-200 °C. Purification was performed using column chromatography on Silica gel 60 (Merck 7734).

Betulonic acid **(1b)**, dihydrobetulonic acid **(2b)**, ursonic acid **(3b)**, and allobetulon **(4)** were purchased from company Betulinines (www.betulinines.com) as well as benzyl esters **1a, 2a, 3a**. All other chemicals and solvents were obtained from Sigma-Aldrich, Lachner or Across

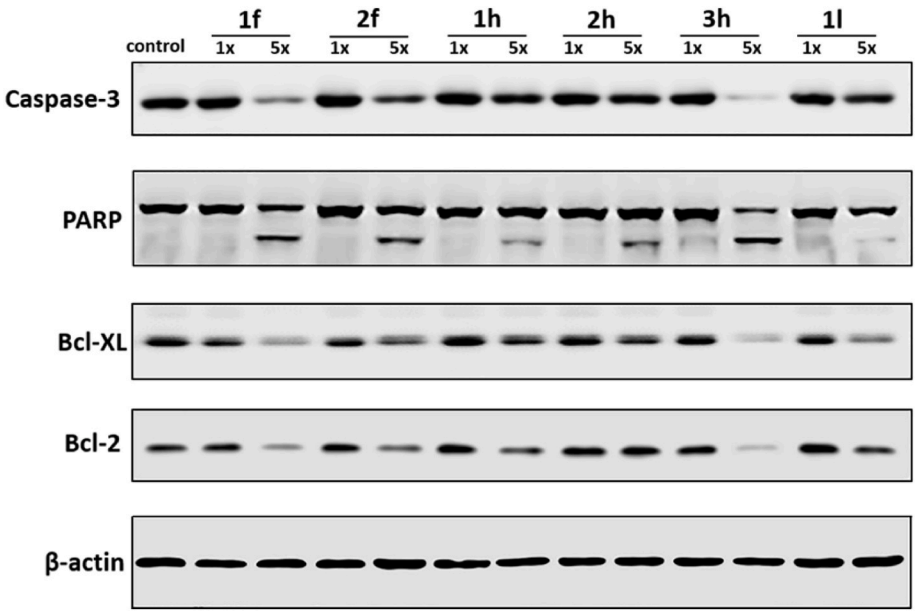



**Fig. 4.** Western blot analysis of CCRF-CEM cells treated with compounds **1f**, **2f**, **1h**, **2h**, **3h** and **1l** at 1 × $IC_{50}$ and 5 × $IC_{50}$ concentrations for 24 h.

Chemicals.

### *4.2. General procedure for the preparation of 2-trifluoroacetylated compounds*

Suspension of NaH in mineral oil (60%, 5.7 mmol, 5 eq.) was added to a mixture of the starting ketone (1.14 mmol) in dry THF (4 mL). After stirring at 20 °C for 5 min, ethyltrifluoroacetate (1.37 mmol, 1.2 eq.) was added dropwise. The reaction mixture was stirred for 12 h at 20 °C and then 10% solution of HCl was added to acidify the mixture to pH 4. Crude product was extracted with $Et_2O$. The organic phase was washed with water, dried over $Na_2SO_4$ and concentrated under vacuum. The crude diketone was used in next step without purification due its low stability.

Note, according to the spectral data, the compounds are in their enol forms.

#### *4.2.1. Benzyl-2-trifluoroacetyl-3-oxolup-20(29)-en-28-oate **1c***

Compound **1c** was prepared according to the general procedure from ketone **1a** (575 mg; 1.06 mmol), 60% suspension of NaH in mineral oil (212 mg, 5.3 mmol) and ethyltrifluoroacetate (151 μL, 180 mg, 1.27 mmol) in THF (6 mL). Compound **1c** (638 mg; 94 %) was obtained as a pale yellow oil. IR (DRIFT): 2928 (C–H), 1725 (C═O), 1570, 1456, 1196, 1144, 732. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.80 s (6H, 2 × Me), 0.96 s (3H, Me), 1.15 s (3H, Me), 1.21 s (3H, Me), 1.69 s (3H, Me), 1.83 d (1H, $J$ = 14.8 Hz, H-1a), 1.85–1.95 m (2H), 2.24 td (1H, $J_1$ = 12.4 Hz, $J_2$ = 3.6 Hz), 2.29 dt (1H, $J_1$ = 6.0 Hz, $J_2$ = 3.2 Hz), 2.58 dq (1H, $J_1$ = 14.8 Hz, $J_2$ = 1.7 Hz, H-1b), 3.03 td (1H, $J_1$ = 11.0 Hz, $J_2$ = 4.5 Hz, H-19β), 4.62 dd (1H, $J_1$ = 1.7 Hz, $J_2$ = 0.6 Hz, H-29a), 4.74 d (1H, $J$ = 1.7 Hz, H-29b), 5.10 d (1H, $J$ = 12.3 Hz, $CH_aPh$), 5.16 d (1H, $J$ = 12.3 Hz, $CH_bPh$), 7.32–7.37 m (5 × HPh), 15.70 s (1H, OH). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.7, 15.1, 15.7, 19.5, 19.7, 21.3, 21.5, 25.7, 29.0, 29.7, 30.7, 32.2, 33.2, 35.9, 37.1, 37.7 q ($J_{C\text{-}F}$ = 2.5 Hz), 38.4, 40.7, 41.0, 42.6, 47.1, 48.7, 49.5, 51.9, 56.7, 65.9, 102.3, 109.8, 117.7 q ($J_{C\text{-}F}$ = 286.9 Hz, $CF_3$), 128.2, 128.4, 128.6, 136.6, 150.6, 175.9, 179.8 q ($JC\text{-}F$ = 33.9 Hz), 196.3. $_{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 72.12 s ($CF_3$). HRMS (ESI): $C_{39}H_{50}F_3O_4$ found 639.3662 $[M - H]^+$; calcd. 639.3656.

#### *4.2.2. 2 Trifluoroacetyl-3-oxolup-20(29)-en-28-oic acid **1d***

Compound **1d** was prepared according to the general procedure from ketone **1b** (1 g; 2.20 mmol), 60% suspension of NaH in mineral oil (368 mg, 9.2 mmol) and ethyltrifluoroacetate (263 μL, 314 mg, 2.21 mmol) in THF (8 mL). Compound **1d** (1.17 g; 99 %) was obtained as a white solid; mp 220–222 °C (ether). IR (DRIFT): 2927 (C–H), 2600 (O–H), 1686 (C═O), 1559, 1456, 1200, 1139, 887, 712. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.82 s (3H, Me), 0.99 s (3H, Me), 1.00 s (3H, Me), 1.15 s (3H, Me), 1.23 s (3H, Me), 1.66 t (1H, $J$ = 11.4 Hz, H-18), 1.71 s (3H, Me), 1.77 dq (1H, $J_1$ = 13.2 Hz, $J_2$ = 3.4 Hz), 1.85 d (1H, $J$ = 15.0 Hz, H-1a), 1.96–2.04 m (2H), 2.23–2.31 m (2H), 2.59 dq (1H, $J_1$ = 15.0 Hz, $J_2$ = 1.8 Hz, H-1b), 3.02 td (1H, $J_1$ = 10.7 Hz, $J_2$ = 4.8 Hz, H-19β), 4.63–4.61 m (1H, H-29a), 4.76 d (1H, $J$ = 1.9 Hz, H-29b), 15.69 s (1H, OH). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.8, 15.2, 15.9, 19.5, 19.6, 21.2, 21.5, 25.6, 29.0, 29.8, 30.7, 32.2, 33.2, 36.0, 37.2, 37.7 q ($J_{C\text{-}F}$ = 2.5 Hz), 38.6, 40.7, 41.0, 42.7, 47.1, 48.6, 49.3, 51.9, 56.6, 102.3, 110.0, 117.7 q ($J_{C\text{-}F}$ = 286.9 Hz, $CF_3$), 150.4, 179.9 q ($J_{C\text{-}F}$ = 33.9 Hz), 182.5, 196.2. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 72.12 d ($J_{H\text{-}F}$ = 1.4 Hz, $CF_3$). HRMS (ESI): $C_{32}H_{44}F_3O_4$ found 549.3190 $[M - H]^+$; calcd. 549.3186.

#### *4.2.3. Benzyl-2-trifluoroacetyl-3-oxolupan-28-oate **2c***

Compound **2c** was prepared according to the general procedure from ketone **2a** (750 mg; 1.37 mmol), 60% suspension of NaH in mineral oil (273 mg, 6.85 mmol) and ethyltrifluoroacetate (195 μL, 233 mg, 1.64 mmol) in THF (8 mL). Compound **2c** (871 mg; 99 %) was obtained as a pale yellow solid; mp 100–103 °C (ether). IR (DRIFT): 2925 (C–H), 1728 (C═O), 1453, 1143, 1121, 696. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.75 d (3H, $J$ = 6.8 Hz, Me), 0.78 s (3H, Me), 0.81 s (3H, Me), 0.85 d (3H, $J$ = 6.8 Hz, Me), 0.95 s (3H, Me), 1.15 s (3H, Me), 1.22 s (3H, Me), 1.70–1.73 m (1H), 1.78–1.86 m (3H), 2.22–2.30 m (3H), 2.60 dq (1H, $J_1$ = 14.9 Hz, $J_2$ = 2.0 Hz, H-1b), 5.09 d (1H, $J$ = 12.3 Hz, CHaPh), 5.13 d (1H, $J$ = 12.3 Hz, CHbPh), 7.30–7.38 m (5 × H-Ph), 15.70 s (1H, OH). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.7, 14.8, 15.1, 15.7, 19.7, 21.3, 21.5, 22.9, 23.1, 27.1, 29.0, 29.7, 29.9, 32.1, 33.3, 35.9, 37.4, 37.7 q ($J_{C\text{-}F}$ = 2.5 Hz), 38.2, 40.7, 41.0, 42.8, 44.3, 48.5, 49.0, 51.9, 57.1, 65.8, 102.3, 117.7 q ($J_{C\text{-}F}$ = 287.1 Hz, $CF_3$), 128.2, 128.4, 128.6, 136.7, 176.1, 179.8 q ($J_{C\text{-}F}$ = 33.8 Hz), 196.3. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 72.12 s ($CF_3$). HRMS (ESI): $C_{39}H_{52}F_3O_4$ found 641.3820 $[M - H]^+$; calcd. 641.3812.

#### *4.2.4. 2-Trifluoroacetyl-3-oxolupan-28-oic acid **2d***

Compound **2d** was prepared according to the general procedure from ketone **2b** (1 g; 2.19 mmol), 60% suspension of NaH in mineral oil (367 mg, 9.15 mmol) and ethyltrifluoroacetate (261 μL, 312 mg, 2.20 mmol)

in THF (8 mL). Compound **2d** (1.24 g; 99 %) was obtained as a white solid; mp 180–185 °C (ether). IR (DRIFT): 3000 (O–H), 2952 (C–H), 1748 (C═O), 1695, 1557, 1456, 1269, 1173, 1140, 870, 715. $^{1}$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.77 d (3H, $J = 6.8$ Hz, Me), 0.83 s (3H, Me), 0.87 d (3H, $J = 6.8$ Hz, Me), 0.98 s (6H, 2Me), 1.15 s (3H, Me), 1.23 s (3H, Me), 1.72–1.76 m (1H), 1.80–1.92 m (4H), 2.24–2.29 m (3H), 2.61 dq (1H, $J_1 = 14.9$ Hz, $J_2 = 1.8$ Hz, H-1b), 15.69 s (1H, OH). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.7, 14.8, 15.1, 15.9, 19.6, 21.2, 21.5, 22.9, 23.1, 27.0, 29.0, 29.8, 29.9, 32.1, 33.3, 35.9, 37.6, 37.7 q ($J_{C\text{-}F} = 2.5$ Hz), 38.5, 40.7, 41.0, 42.8, 44.3, 48.4, 48.8, 51.8, 57.0, 102.3, 117.7 q ($J_{C\text{-}F} = 286.8$ Hz, $CF_3$), 179.8 q ($J_{C\text{-}F} = 33.8$ Hz), 182.7, 196.2. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 72.13 s ($CF_3$). HRMS (ESI): $C_{32}H_{46}F_3O_4$ found 551.3348 $[M - H]^+$; calcd. 551.3343.

#### 4.2.5. Benzyl-2-trifluoroacetyl-3-oxo-ursa-12-en-28-oate **3c**

Compound **3c** was prepared according to the general procedure from ketone **3a** (926 mg; 1.70 mmol), 60% suspension of NaH in mineral oil (340 mg, 8.50 mmol) and ethyltrifluoroacetate (243 μL, 290 mg, 2.04 mmol) in THF (10 mL). Compound **3c** (1.02 g; 94 %) was obtained as a pale yellow oil. IR (DRIFT): 2924 (C–H), 1722 (C═O), 1455, 1199, 1141, 907, 730. $^{1}$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.68 s (3H, Me), 0.88 d (3H, $J = 6.4$ Hz, Me), 0.89 s (3H, Me), 0.95 d (3H, $J = 6.4$ Hz, Me), 1.09 s (3H, Me), 1.18 s (3H, Me), 1.23 s (3H, Me), 1.92–1.96 m (3H), 2.02 td (1H, $J_1 = 13.1$ Hz, $J_2 = 4.5$ Hz), 2.30 dd (1H, $J_1 = 11.5$ Hz, $J_2 = 1.1$ Hz), 2.55 dq (1H, $J_1 = 14.9$ Hz, $J_2 = 2.1$ Hz, H-1b), 4.99 d (1H, $J = 12.5$ Hz, CHaPh), 5.11 d (1H, $J = 12.5$ Hz, CHbPh), 5.29 t (1H, $J = 3.7$ Hz, H-12), 7.31–7.36 m (5HPh), 15.76 s (1H, OH). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.7, 17.0, 17.2, 19.7, 21.3, 21.5, 23.4, 23.5, 24.4, 28.1, 29.2, 29.8, 30.8, 32.3, 35.7, 36.8, 37.5 q ($J_{C\text{-}F} = 2.2$ Hz), 39.0, 39.3, 39.6, 40.9, 42.4, 45.5, 48.3, 52.0, 53.2, 66.2, 102.3, 117.7 q ($J_{C\text{-}F} = 286.7$ Hz, $CF_3$), 125.5, 128.1, 128.3, 128.6, 136.5, 138.3, 177.4, 179.4 q ($J_{C\text{-}F} = 33.9$ Hz), 196.7. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 71.99 d ($J_{H\text{-}F} = 1.6$ Hz, $CF_3$). HRMS (ESI): $C_{39}H_{50}F_3O_4$ found 639.3662 $[M - H]^+$; calcd. 639.3656.

#### 4.2.6. 2-Trifluoroacetyl-3-oxo-ursa-12-en-28-oic acid **3d**

Compound **3d** was prepared according to the general procedure from ketone **3b** (300 mg; 0.54 mmol), 60% suspension of NaH in mineral oil (132 mg, 2.70 mmol) and ethyltrifluoroacetate (94 μL, 112 mg, 0.64 mmol) in THF (3.3 mL). Compound **3d** (265 g; 72 %) was obtained as a pale yellow oil. IR (DRIFT): 2924 (C–H), 1725 (C═O), 1451, 1210, 1150, 903, 734. $^{1}$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.83 s (3H, Me), 0.89 d (3H, $J = 6.4$ Hz, Me), 0.91 s (3H, Me), 0.96 d (3H, $J = 6.4$ Hz, Me), 1.10 s (3H, Me), 1.15 s (3H, Me), 1.23 s (3H, Me), 2.23 d (1H, $J = 11.3$ Hz), 2.56 dd (1H, $J_1 = 14.9$ Hz, $J_2 = 1.8$ Hz), 5.30 t (1H, $J = 3.6$ Hz, H-12), 15.73 s (1H, OH). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.7, 17.1, 17.2, 19.7, 21.3, 21.5, 23.4, 23.6, 24.2, 28.1, 29.2, 30.8, 32.2, 35.7, 36.9, 37.6, 39.0, 39.3, 39.5, 40.9, 42.3, 45.5, 48.2, 52.0, 52.8, 102.3, 117.7 q ($J_{C\text{-}F} = 286.8$ Hz, $CF_3$), 125.6, 138.1, 179.5 q ($J_{C\text{-}F} = 34.1$ Hz),183.7, 196.6. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 77.02 bs. HRMS (ESI): $C_{32}H_{44}F_3O_4$ found 549.3192 $[M - H]^+$; calcd. 549.3179.

#### 4.2.7. 19β,28-epoxy-2-trifluoroacetyl-(18α)-oleanan-3-one 5

Compound **5** was prepared according to the general procedure from 3-oxo derivative **4** (500 mg; 1.14 mmol), 60% suspension of NaH in mineral oil (228 mg, 5.7 mmol) and ethyltrifluoroacetate (165 μL, 195 mg, 1.37 mmol) in THF (4 mL). Compound **5** (580 mg; 95 %) was obtained as a pale yellow solid; mp 158–160 °C (ether). IR (DRIFT): 2925, 2863, 1583, 1456, 1197, 1141, 1034, 894, 712. $^{1}$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.80 s (3H, Me), 0.85 s (3H, Me), 0.936 s (3H, Me), 0.943 s (3H, Me), 1.02 s (3H, Me), 1.17 s (3H, Me), 1.24 s (3H, Me), 1.71 dq (1H, $J_1 = 13.3$ Hz, $J_2 = 3.2$ Hz), 1.88 d (1H, $J = 14.9$ Hz, H-1a), 2.63 dq (1H, $J_1 = 14.9$ Hz, $J_2 = 2.0$ Hz, H-1b), 3.46 d (1H, $J = 7.8$ Hz, H-28a), 3.55 s (1H, H-19), 3.79 dd (1H, $J_1 = 7.8$ Hz, $J_2 = 1.4$ Hz, H-28b), 15.70 s (1H, OH). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 13.6, 15.5, 15.6, 19.6, 21.2, 21.6, 24.7, 26.4, 26.5, 26.6, 28.9, 29.0, 29.8, 32.9, 34.4, 36.0, 36.4, 36.9, 37.9 q ($J_{C\text{-}F} = 2.5$ Hz), 40.6, 40.96, 41.0, 41.6, 46.9, 49.2, 52.0, 71.4, 88.1, 102.3, 117.7 q ($J_{C\text{-}F} = 287.0$ Hz, $CF_3$), 179.8 q ($J_{C\text{-}F} = 33.8$ Hz), 196.3. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 72.12 d ($J_{H\text{-}F} = 1.6$ Hz, $CF_3$). HRMS (ESI): $C_{32}H_{46}F_3O_3$ found 535.3398 $[M - H]^+$; calcd. 535.3394.

### 4.3. General procedure for the preparation of unsaturated lactones

A modified procedure from Ref. [29] was used.

To a solution of hydroxy ketone (0.99 mmol) in dry toluene (6 mL) at 90 °C under nitrogen atmosphere was added in one portion of (triphenylphosphoranylidene)ethenone (1.19–1.88 mmol, 1.2–1.9 eq) and the resulting mixture was stirred at 90 °C for 3–12 h. Then the resulting residue was purified by column chromatography on $SiO_2$ eluting with hexane/ethyl acetate.

Procedure from Ref. [42] was used for the synthesis of (triphenylphosphoranylidene)ethenone.

#### 4.3.1. Benzyl-4′-trifluoromethyl lupa-2,20(29)-dieno [3,2-b]-pyran-6′-one-28-oate **1e**

Compound **1e** was prepared according to the general procedure from **1c** (600 mg; 0.94 mmol) and (triphenylphosphoranylidene)ethenone (377 mg, 1.13 mmol) in toluene (5 mL) at 90 °C for 3 h. After purification (mobile phase hexane/EtOAc 6:1) compound **1e** (389 mg; 62%) was obtained as a white solid; mp 105–108 °C (hexane/EtOAc); $R_f$ 0.56 (silica gel, hexane/EtOAc, 3:1). IR (DRIFT): 2944 (C–H), 1743, 1726 (C═O), 1644, 1552, 1455, 1371, 1269, 1141 (C–F), 870, 730, 697. $^{1}$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.81 s (3H, Me), 0.83 s (3H, Me), 0.97 s (3H, Me), 1.19 s (3H, Me), 1.26 s (3H, Me), 1.62 t (1H, $J = 11.4$ Hz, H-18), 1.69 s (3H, Me), 1.76 dq (1H, $J_1 = 12.7$ Hz, $J_2 = 3.6$ Hz), 1.86–1.95 m (3H), 2.22–2.32 m (2H), 2.57 d (1H, $J = 15.7$ Hz, H-1b), 3.03 td (1H, $J_1 = 11.0$ Hz, $J_2 = 4.6$ Hz, H-19β), 4.62 dd (1H, $J_1 = 2.1$ Hz, $J_2 = 1.4$ Hz, H-29a), 4.74 d (1H, $J = 2.1$ Hz, H-29b), 5.10 d (1H, $J = 12.3$ Hz, CHaPh), 5.16 d (1H, $J = 12.3$ Hz, CHbPh), 6.50 s (1H, H-5′), 7.32–7.37 m (5HPh). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.7, 15.6, 15.8, 19.4, 19.5, 20.9, 21.5, 25.6, 29.0, 29.7, 30.7, 32.2, 33.2, 36.0, 37.0, 38.3, 38.5, 38.7 q ($J_{C\text{-}F} = 1.7$ Hz), 40.7, 42.6, 47.1, 48.9, 49.5, 52.2, 56.7, 65.9, 106.0, 109.8, 112.7 q ($J_{C\text{-}F} = 6.4$ Hz), 121.8 q ($J_{C\text{-}F} = 275.5$ Hz, $CF_3$), 128.2, 128.4, 128.6, 136.6, 145.4 q ($J_{C\text{-}F} = 31.0$ Hz), 150.6, 161.1, 167.7, 175.9. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 65.04 d ($J_{H\text{-}F} = 1.2$ Hz, $CF_3$). HRMS (ESI): $C_{41}H_{52}F_3O_4$ found 665.3815 $[M+H]^+$; calcd. 665.3812.

#### 4.3.2. 4′-trifluoromethyl lupa-2,20(29)-dieno [3,2-b]-pyran-6′-one-28-oic acid **1f**

Compound **1f** was prepared according to the general procedure from **1d** (500 mg; 0.93 mmol) and (triphenylphosphoranylidene)ethenone (591 mg, 1.77 mmol) in toluene (6 mL) at 90 °C for 12 h. After purification (mobile phase hexane/EtOAc 4:1 → 3:1) compound **1f** (195 mg; 37%) was obtained as a white solid; mp 176–180 °C (hexane/EtOAc); $R_f$ 0.25 (silica gel, hexane/EtOAc, 3:1). IR (DRIFT): 2927 (C–H), 2700 (O–H), 1686 (C═O), 1559, 1456, 1200, 1139 (C–F), 887, 712. $^{1}$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.86 s (3H, Me), 1.00 s (3H, Me), 1.01 s (3H, Me), 1.19 s (3H, Me), 1.27 s (3H, Me), 1.66 t (1H, $J = 11.4$ Hz, H-18), 1.71f d (3H, $J = 0.5$ Hz, Me), 1.78 dq (1H, $J_1 = 12.9$ Hz, $J_2 = 3.6$ Hz), 1.94 (1H, $J = 15.9$ Hz, H-1a), 1.98–2.04 m (2H), 2.24–2.32 m (2H), 2.58 d (1H, $J = 15.9$ Hz, H-1b), 3.02 td (1H, $J_1 = 10.8$ Hz, $J_2 = 4.9$ Hz, H-19β), 4.64 dd (1H, $J_1 = 2.2$ Hz, $J_2 = 1.4$ Hz, H-29a), 4.76 d (1H, $J = 2.2$ Hz, H-29b), 6.50 s (1H, H-5′). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.8, 15.77, 15.82, 19.4, 19.5, 20.9, 21.5, 25.6, 29.0, 29.8, 30.7, 32.2, 33.2, 36.1, 37.2, 38.5, 38.57, 38.64 q (JC-F = 1.5 Hz), 40.8, 42.7, 47.0, 48.9, 49.3, 52.1, 56.6, 106.0, 110.0, 112.8 q ($J_{C\text{-}F} = 6.4$ Hz), 121.8 q ($J_{C\text{-}F} = 276.0$ Hz, $CF_3$), 145.3 q ($J_{C\text{-}F} = 31.3$ Hz), 150.4, 161.2, 167.7, 182.4. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 65.03 s ($CF_3$). HRMS (ESI): $C_{34}H_{46}F3O_4$ found 575.3345 $[M+H]^+$; calcd. 575.3343.

*4.3.3. Benzyl-4′-trifluoromethyl-20(29)-dihydrolup-2-en [3,2-b]-pyran-6′-one-28-oate **2e***

Compound **2e** was prepared according to the general procedure from **2c** (830 mg; 1.29 mmol) and (triphenylphosphoranylidene)ethenone (518 mg, 1.55 mmol) in toluene (6 mL) at 90 °C for 3 h. After purification (mobile phase hexane/EtOAc 6:1) compound **2e** (556 mg; 65%) was obtained as a white solid; mp 102–108 °C (hexane/EtOAc); $R_f$ 0.59 (silica gel, hexane/EtOAc, 3:1). IR (DRIFT): 2951 (C–H), 1743, 1726 (C–O), 1552, 1455, 1268, 1144 (C–F), 908, 730, 696. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.75 d (3H, $J$ = 6.8 Hz, Me), 0.79 s (3H, Me), 0.84 s (3H, Me), 0.86 d (3H, $J$ = 6.8 Hz, Me), 0.95 s (3H, Me), 1.19 s (3H, Me), 1.26 s (3H, Me), 1.71–1.75 m (1H), 1.78–1.86 m (2H), 1.93 d (1H, $J$ = 15.9 Hz, H-1a), 2.23–2.31 m (3H), 2.58 d (1H, $J$ = 15.9 Hz, H-1b), 5.09 d (1H, $J$ = 12.3 Hz, CHaPh), 5.14 d (1H, $J$ = 12.3 Hz, CHbPh), 6.50 s (1H, H-5′), 7.31–7.37 m (5HPh). $^{13}$C NMR ($CDCl^3$, 126 MHz) $\delta$, ppm: 14.7, 14.8, 15.67, 15.73, 19.4, 20.9, 21.6, 22.9, 23.1, 27.0, 29.0, 29.7, 29.9, 32.1, 33.3, 36.0, 37.4, 38.2, 38.5, 38.6 q ($J_{C\text{-}F}$ = 2.1 Hz), 40.7, 42.8, 44.3, 48.7, 49.0, 52.1, 57.1, 65.8, 106.0, 112.7 q ($J_{C\text{-}F}$ = 6.2 Hz), 121.9 q ($J_{C\text{-}F}$ = 275.8 Hz, $CF_3$), 128.2, 128.4, 128.6, 136.7, 145.3 q ($J_{C\text{-}F}$ = 31.0 Hz), 161.1, 167.8, 176.1. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 65.05 s ($CF_3$). HRMS (ESI): $C_{41}H_{54}F_3O_4$ found 667.3969 $[M+H]^+$; calcd. 667.3969.

*4.3.4. 4′-trifluoromethyl-20(29)-dihydrolup-2-en [3,2-b]-pyran-6′-one-28-oic acid **2f***

Compound **2f** was prepared according to the general procedure from **2d** (500 mg; 0.93 mmol) and (triphenylphosphoranylidene)ethenone (591 mg, 1.77 mmol) in toluene (6 mL) at 90 °C for 12 h. After purification (mobile phase hexane/EtOAc 4:1 → 3:1) compound **2f** (293 mg; 56%) was obtained as a white solid; mp 180–185 °C (hexane/EtOAc); $R_f$ 0.25 (silica gel, hexane/EtOAc, 3:1). IR (DRIFT): 2925 (C–H), 2700 (O–H), 1683 (C═O), 1569, 1456, 1202, 1134 (C–F), 958, 713. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.77 d (3H, $J$ = 6.8 Hz, Me), 0.86 s (3H, Me), 0.87 d (3H, $J$ = 6.8 Hz, Me), 0.99 s (6H, 2Me), 1.19 s (3H, Me), 1.27 s (3H, Me), 1.75 dq (1H, $J_1$ = 12.6 Hz, $J_2$ = 2.9 Hz), 1.83 dtd (1H, $J_1$ = 13.6 Hz, $J_2$ = 6.8 Hz, $J_3$ = 2.4 Hz), 1.91 dd (1H, $J_1$ = 12.5 Hz, $J_2$ = 7.4 Hz), 1.95 d (1H, $J$ = 15.9 Hz, H-1a), 2.24–2.31 m (3H), 2.60 d (1H, $J$ = 15.9 Hz, H-1b), 6.51 s (1H, H-5′). $_{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.6, 14.7, 15.7, 15.8, 19.3, 20.8, 21.4, 22.8, 23.0, 26.9, 28.9, 29.75, 29.83, 32.0, 33.3, 36.0, 37.5, 38.36, 38.44, 38.6 q ($J_{C\text{-}F}$ = 1.8 Hz), 40.7, 42.8, 44.2, 48.6, 48.7, 52.0, 56.9, 105.9, 112.7 q ($J_{C\text{-}F}$ = 6.2 Hz), 121.8 q ($J_{C\text{-}F}$ = 275.6 Hz, $CF_3$), 145.3 q ($J_{C\text{-}F}$ = 31.3 Hz), 161.1, 167.6, 182.6. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 65.04 s ($CF_3$). HRMS (ESI): $C_{34}H_{48}F_3O_4$ found 577.3500 $[M+H]^+$; calcd. 577.3499.

*4.3.5. Benzyl-4′-trifluoromethyl-ursa-2,12-dieno [3,2-b]-pyran-6′-one-28-oate **3e***

Compound **3e** was prepared according to the general procedure from **3c** (970 mg; 1.52 mmol) and (triphenylphosphoranylidene)ethenone (608 mg, 1.82 mmol) in toluene (7 mL) at 90 °C for 3 h. After purification (mobile phase hexane/EtOAc 6:1) compound **3e** (700 mg; 70%) was obtained as a white solid; mp 107–110 °C (hexane/EtOAc); $R_f$ 0.61 (silica gel, hexane/EtOAc, 3:1). IR (DRIFT): 2925 (C–H), 1739 (C═O), 1551, 1455, 1269, 1137 (C–F), 908, 730. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.69 s (3H, Me), 0.89 d (3H, $J$ = 6.4 Hz, Me), 0.92 s (3H, Me), 0.95 d (3H, $J$ = 6.4 Hz, Me), 1.10 s (3H, Me), 1.21 s (3H, Me), 1.28 s (3H, Me), 1.95–2.05 m (4H), 2.31 d (1H, $J$ = 11.3 Hz), 2.54 d (1H, $J$ = 15.8 Hz, H-1b), 4.99 d (1H, $J$ = 12.4 Hz, CHaPh), 5.12 d (1H, $J$ = 12.4 Hz, CHbPh), 5.29 t (1H, $J$ = 3.6 Hz, H-12), 6.51 s (1H, H-5′), 7.31–7.36 m (5HPh). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 15.2, 17.0, 17.2, 19.5, 21.1, 21.3, 23.3, 23.6, 24.4, 28.1, 29.2, 30.8, 32.3, 35.8, 36.7, 38.38, 38.43 q ($J_{C\text{-}F}$ = 1.8 Hz), 39.0, 39.3, 39.6, 42.4, 45.8, 48.3, 52.2, 53.1, 66.2, 105.9, 112.7 q ($J_{C\text{-}F}$ = 6.5 Hz), 121.9 q ($J_{C\text{-}F}$ = 275.8 Hz, $CF_3$), 125.3, 128.1, 128.3, 128.6, 136.5, 138.4, 145.3 q ($J_{C\text{-}F}$ = 31.2 Hz), 161.1, 167.8, 177.3. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 65.00 s ($CF_3$). HRMS (ESI): $C_{41}H_{52}F_3O_4$ found 665.3815 [M+H]+; calcd. 665.3812.

*4.3.6. 4′-trifluoromethyl-ursa-2,12-dieno [3,2-b]-pyran-6′-one-28-oic acid **3f***

Compound **3f** was prepared according to the general procedure from benzyl ester **3d** (50 mg; 0.08 mmol), 10% Pd/C (53 mg, 0.05 mmol) and 1,3-cyclohexadiene (54 μL, 46 mg, 0.57 mmol) in mixture THF/EtOAc (6 mL, 1:1). After purification (mobile phase hexane/EtOAc 3:1) compound **3f** (31 mg; 72 %) was obtained as a white solid; mp 180–184 °C (hexane/EtOAc); $R_f$ 0.52 (silica gel, hexane/EtOAc, 3:2). IR (DRIFT): 2924 (C–H), 2600 (O–H), 1749 (C═O), 1696, 1552, 1269, 1139 (C–F), 870, 708. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.84 s (3H, Me), 0.89 d (3H, $J$ = 6.4 Hz, Me), 0.946 s (3H, Me), 0.95 d (3H, $J$ = 6.4 Hz, Me), 1.11 s (3H, Me), 1.19 s (3H, Me), 1.28 s (3H, Me), 1.88 td (1H, $J_1$ = 13.7 Hz, $J_2$ = 4.3 Hz), 1.95–2.09 m (4H), 2.23 d (1H, $J$ = 11.5 Hz), 2.55 d (1H, $J$ = 15.6 Hz, H-1b), 5.31 t (1H, $J$ = 3.6 Hz, H-12), 6.51 s (1H, H-5′). $_{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 15.2, 17.0, 17.1, 19.4, 21.1, 21.3, 23.4, 23.6, 24.2, 28.1, 29.2, 30.8, 32.2, 35.8, 36.8, 38.36, 38.40, 39.0, 39.3, 39.6, 42.3, 45.8, 48.2, 52.2, 52.8, 105.9, 112.8 q ($J_{C\text{-}F}$ = 6.0 Hz), 121.8 q ($J_{C\text{-}F}$ = 276.1 Hz, $CF_3$), 125.4, 138.2, 145.2 q ($J_{C\text{-}F}$ = 31.2 Hz), 161.1, 167.7, 183.9. $^{19}$F NMR ($CDCl^3$, 500 MHz) $\delta$, ppm: 65.02 s ($CF_3$). HRMS (ESI): $C_{34}H_{46}F_3O_4$ found 575.3345 $[M+H]^+$; calcd. 575.3343.

*4.3.7. 19β,28-epoxy-4′-trifluoromethyl-18a-oleanan-2-eno [3,2-b]-pyran-6′-one **6***

Compound **6** was prepared according to the general procedure from **5** (2.1 g; 3.92 mmol) and (triphenylphosphoranylidene)ethenone (1.44 g, 4.31 mmol) in toluene (15 mL) at 90 °C for 3 h. After purification (mobile phase hexane/EtOAc 5:1) compound **6** (1.41 g; 67%) was obtained as a white solid; mp 212–215 °C (hexane/EtOAc); $R_f$ 0.52 (silica gel, hexane/EtOAc, 3:1). IR (DRIFT): 2921 (C–H), 1739 (C═O), 1556, 1268, 1174, 1138, 874. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.81 s (3H, Me), 0.89 s (3H, Me), 0.94 s (3H, Me), 0.95 s (3H, Me), 1.03 s (3H, Me), 1.20 s (3H, Me), 1.28 s (3H, Me), 1.70–1.74 m (1H), 1.97 d (1H, $J$ = 16.0 Hz, H-1a), 2.62 d (1H, $J$ = 16.0 Hz, H-1b), 3.46 d (1H, $J$ = 7.8 Hz, H-28a), 3.55 s (1H, H-19β), 3.79 dd (1H, $J_1$ = 7.8 Hz, $J_2$ = 1.4 Hz, H-28b), 6.51 d (1H, $J$ = 0.6 Hz, H-5′). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 13.6, 15.6, 16.1, 19.4, 20.9, 21.6, 24.7, 26.3, 26.5, 26.6, 28.9, 29.0, 32.83, 32.84, 34.4, 36.1, 36.4, 36.9, 38.5, 38.8 q ($J_{C\text{-}F}$ = 2.1 Hz), 40.7, 41.0, 41.6, 46.9, 49.4, 52.3, 71.4, 88.1, 105.9, 112.8 q ($J_{C\text{-}F}$ = 6.4 Hz, C-5′), 121.9 q ($J_{C\text{-}F}$ = 275.9 Hz, $CF_3$), 145.3 q ($J_{C\text{-}F}$ = 31.2 Hz), 161.1, 167.8. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 65.02 s ($CF_3$). HRMS (ESI): $C_{34}H_{48}F_3O_3$ found 561.3552 $[M+H]^+$; calcd. 561.3550.

*4.4. General procedure for the preparation of dihydrophenyl phthalimides*

A solution of each pyrone (0.78 mmol) and *N*-phenylmaleimide (2.34 mmol, 3 eq.) in dry diphenyl ether (2 mL) was stirred at 180–200 °C under nitrogen for 3 h. Then the resulting residue was purified by column chromatography on $SiO_2$ eluting with hexane/ethyl acetate.

*4.4.1. Benzyl-1′,3′-Dioxo-2′-phenyl-5′-trifluoromethyl lupa-2,20(29)-dieno [2,3-g]-2′,3′,3a′,7a′-tetrahydro-1H-isoindole-28-oate **1g***

Compound **1g** was prepared according to the general procedure from pyrone **1e** (350 mg; 0.53 mmol) and *N*-phenylmaleimide (275 mg, 1.59 mmol) in diphenyl ether (1.5 mL) at 180 °C for 3 h. After purification (mobile phase hexane/EtOAc 5:1) compound **1g** (220 mg; 53 %) was obtained as a white solid; mp 146–150 °C (hexane/EtOAc); $R_f$ 0.43 (silica gel, hexane/EtOAc, 3:1). IR (DRIFT): 2942 (C–H), 1722 (C═O), 1499, 1370, 1122 (C–F), 738, 692. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.815 s (3H, Me), 0.824 s (3H, Me), 0.97 s (3H, Me), 1.02 s (3H, Me), 1.25 s (3H, Me), 1.63 t (1H, $J$ = 11.4 Hz, H-18), 1.70 s (3H, Me), 1.74 dq (1H, $J_1$ = 12.8 Hz, $J_2$ = 2.6 Hz), 1.83 d (1H, $J$ = 16.5 Hz, H-1a), 1.84–1.94 m (2H), 2.22–2.32 m (2H), 2.34 d (1H, $J$ = 16.5 Hz, H-1b), 3.04 td (1H, $J_1$ = 10.9 Hz, $J_2$ = 4.7 Hz, H-19), 3.75 dqu (1H, $J_1$ = 8.5 Hz, $J_2$ = 2.9 Hz, H-7′a), 3.85 d (1H, $J$ = 8.5 Hz, H-3′a), 4.62–4.63 m (1H, H-29a), 4.74 d (1H, $J$ = 2.1 Hz, H-29b), 5.10 d (1H, $J$ = 12.3 Hz, CHaPh),

5.16 d (1H, $J = 12.3$ Hz, CHbPh), 6.23 s (1H, H-4′), 7.26–7.28 m (2HPh), 7.30–7.40 m (6HPh), 7.44–7.48 m (2HPh). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.8, 15.6, 16.8, 19.5, 19.8, 21.3, 22.5, 25.7, 28.5, 29.7, 30.7, 32.2, 33.6, 36.0, 37.1, 38.4 (2C), 40.7 q ($J_{C\text{-}F} = 2.0$ Hz), 40.8, 41.9, 42.6, 44.9, 47.1, 49.3, 49.5, 52.0, 56.7, 65.9, 109.8, 123.0 q ($J_{C\text{-}F} = 274.3$ Hz, CF3), 123.77 q ($J_{C\text{-}F} = 7.1$ Hz), 123.84, 126.3, 128.2, 128.4, 128.6, 128.8, 129.3, 131.9, 133.7 q ($J_{C\text{-}F} = 29.0$ Hz), 135.6, 136.6, 150.7, 175.5, 175.9, 176.0. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 61.04 d ($J_{H\text{-}F} = 1.2$ Hz, $CF_3$). HRMS (ESI): $C_{50}H_{57}F_3NO_4$ found 792.4233 $[M - H]^+$; calcd. 792.4234.

#### 4.4.2. 1′,3′-Dioxo-2′-phenyl-5′-trifluoromethyl lupa-2,20(29)-dieno[2,3-g]-2′,3′,3a′,7a′-tetrahydro-1H-isoindole-28-oic acid **1h**

Compound **1h** was prepared according to the general procedure from pyrone **1f** (264 mg; 0.47 mmol) and *N*-phenylmaleimide (244 mg, 1.41 mmol) in diphenyl ether (1.5 mL) at 200 °C for 3 h. After purification (mobile phase hexane/EtOAc 3:1) compound **1h** (130 mg; 40 %) was obtained as a white solid; mp 227–229 °C (hexane/EtOAc); $R_f$ 0.30 (silica gel, hexane/EtOAc, 3:2). IR (DRIFT): 2945 (C–H), 2750 (O–H), 1728 (C═O), 1684, 1499, 1456, 1370, 1272, 1125 (C–F), 876, 738, 690. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.85 s (3H, Me), 1.00 s (3H, Me), 1.01 s (3H, Me), 1.02 s (3H, Me), 1.26 s (3H, Me), 1.66 t (1H, $J = 11.4$ Hz, H-18), 1.71 s (3H, Me), 1.74–1.78 m (1H), 1.85 d (1H, $J = 16.5$ Hz, H-1a), 1.96–2.05 m (2H), 2.23–2.31 m (2H), 2.35 d (1H, $J = 16.5$ Hz, H-1b), 3.02 td (1H, $J_1 = 10.8$ Hz, $J_2 = 4.9$ Hz, H-19β), 3.75 d qu (1H, $J_1 = 8.4$ Hz, $J_2 = 2.7$ Hz, H-7′a), 3.85 d (1H, $J = 8.4$ Hz, H-3′a), 4.64 dd (1H, $J_1 = 2.1$ Hz, $J_2 = 1.4$ Hz, H-29a), 4.76 d (1H, $J = 2.1$ Hz, H-29b), 6.24 s (1H, H-4′), 7.26–7.28 m (2HPh), 7.37–7.40 m (1HPh), 7.44–7.48 m (2HPh). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.8, 15.9, 16.8, 19.5, 19.8, 21.3, 22.5, 25.6, 28.5, 29.8, 30.7, 32.2, 33.6, 36.0, 37.2, 38.4, 38.6, 40.7, 40.8, 41.9, 42.6, 44.9, 47.1, 49.29, 49.34, 52.0, 56.6, 110.0, 123.0 q ($J_{C\text{-}F} = 274.6$ Hz, $CF_3$), 123.8 q ($J_{C\text{-}F} = 6.5$ Hz), 123.9, 126.3, 128.8, 129.3, 131.9, 133.7 q ($J_{C\text{-}F} = 29.2$ Hz), 135.6, 150.5, 175.6, 175.9, 181.8. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 61.03 s ($CF_3$). HRMS (ESI): $C_{43}H_{51}F_3NO_4$ found 702.3768 $[M - H]^+$; calcd. 702.3765.

#### 4.4.3. 1 benzyl-1′,3′-Dioxo-2′-phenyl-5′-trifluoromethyl-20(29)-dihydrolup-2-en[2,3-g]-2′,3′,3a′,7a′-tetrahydro-1H-isoindole-28-oate **2g**

Compound **2g** was prepared according to the general procedure from pyrone **2e** (520 mg; 0.78 mmol) and *N*-phenylmaleimide (405 mg, 2.34 mmol) in diphenyl ether (2 mL) at 180 °C for 3 h. After purification (mobile phase hexane/EtOAc 5:1) compound **2g** (401 mg; 65 %) was obtained as a white solid; mp 155–157 °C (hexane/EtOAc); $R_f$ 0.43 (silica gel, hexane/EtOAc, 3:1). IR (DRIFT): 2970 (C–H), 1736, 1726 (C═O), 1455, 1366, 1217, 1121 (C–F), 737, 692. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.75 d (3H, $J = 6.8$ Hz, Me), 0.80 s (3H, Me), 0.83 s (3H, Me), 0.85 d (3H, $J = 6.8$ Hz, Me), 0.95 s (3H, Me), 1.02 s (3H, Me), 1.25 s (3H, Me), 1.71 dq (1H, $J_1 = 12.6$ Hz, $J_2 = 2.6$ Hz), 1.79–1.86 m (3H), 2.22–2.30 m (3H), 2.35 d (1H, $J = 16.5$ Hz, H-1b), 3.76 dqu (1H, $J_1 = 8.5$ Hz, $J_2 = 2.9$ Hz, H-7′a), 3.85 d (1H, $J = 8.5$ Hz, H-3′a), 5.09 d (1H, $J = 12.3$ Hz, CHaPh), 5.14 d (1H, $J = 12.3$ Hz, CHbPh), 6.24 s (1H, H-4′), 7.26–7.28 m (2HPh), 7.30–7.40 m (6HPh), 7.44–7.48 m (2HPh). $_{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.7, 14.8, 15.7, 16.8, 19.8, 21.4, 22.5, 22.9, 23.1, 27.1, 28.5, 29.7, 29.9, 32.1, 33.7, 36.0, 37.4, 38.3, 38.4, 40.7 q ($J_{C\text{-}F} = 2.0$ Hz), 40.8, 41.9, 42.8, 44.3, 44.9, 49.0, 49.1, 52.0, 57.2, 65.8, 123.0 q ($J_{C\text{-}F} = 274.5$ Hz, $CF_3$), 123.78 q ($J_{C\text{-}F} = 6.7$ Hz), 123.83, 126.3, 128.1, 128.4, 128.6, 128.8, 129.3, 132.0, 133.7 q ($J_{C\text{-}F} = 29.0$ Hz), 135.6, 136.8, 175.5, 175.9, 176.2. 19F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 61.05 s ($CF_3$). HRMS (ESI): $C_{50}H_{59}F_3NO_4$ found 794.4393 $[M - H]^+$; calcd. 794.4391.

#### 4.4.4. 1′,3′-Dioxo-2′-phenyl-5′-trifluoromethyl-20(29)-dihydrolup-2-en [2,3-g]-2′,3′,3a′,7a′-tetrahydro-1H-isoindole-28-oic acid **2h**

Compound **2h** was prepared according to the general procedure from pyrone **2f** (510 mg; 0.90 mmol) and *N*-phenylmaleimide (467 mg, 2.70 mmol) in diphenyl ether (1.5 mL) at 200 °C for 3 h. After purification (mobile phase hexane/EtOAc 3:1) compound **2h** (363 mg; 58 %) was obtained as a white solid; mp 225–227 °C (hexane/EtOAc); $R_f$ 0.39 (silica gel, hexane/EtOAc, 3:2). IR (DRIFT): 2950 (C–H), 2700 (O–H), 1724 (C═O), 1685, 1499, 1456, 1367, 1121 (C–F), 871, 731, 691. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.77 d (3H, $J = 6.8$ Hz, Me), 0.86 s (3H, Me), 0.87 d (3H, $J = 6.8$ Hz, Me), 0.99 s (6H, 2Me), 1.02 s (3H, Me), 1.26 s (3H, Me), 1.71–1.74 m (1H), 1.81–1.92 m (3H), 2.23–2.30 m (3H), 2.37 d (1H, $J = 16.5$ Hz, H-1b), 3.76 d qu (1H, $J_1 = 8.5$ Hz, $J_2 = 2.8$ Hz, H-7′a), 3.85 d (1H, $J = 8.5$ Hz, H-3′a), 6.24 s (1H, H-4′), 7.26–7.28 m (2HPh), 7.37–7.40 m (1HPh), 7.44–7.48 m (2HPh). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.7, 14.8, 15.9, 16.8, 19.8, 21.3, 22.5, 22.9, 23.1, 27.0, 28.5, 29.8, 29.9, 32.1, 33.7, 36.0, 37.6, 38.4, 38.5, 40.7, 40.8, 41.9, 42.8, 44.3, 44.9, 48.8, 49.1, 52.0, 57.0, 123.0 q ($J_{C\text{-}F} = 274.7$ Hz, $CF_3$), 123.76 q ($J_{C\text{-}F} = 6.7$ Hz), 123.8, 126.3, 128.8, 129.3, 131.9, 133.7 q ($J_{C\text{-}F} = 29.4$ Hz), 135.6, 175.6, 175.9, 182.5. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 61.05 s ($CF_3$). HRMS (ESI): $C_{43}H_{53}F_3NO_4$ found 704.3925 $[M - H]^+$; calcd. 704.3921.

#### 4.4.5. Benzyl-1′,3′-dioxo-2′-phenyl-5′-trifluoromethyl-ursa-2,12-dieno[2,3-g]-2′,3′,3a′,7a′-tetrahydro-1H-isoindole-28-oate **3g**

Compound **3g** was prepared according to the general procedure from pyrone **3e** (600 mg; 0.90 mmol) and *N*-phenylmaleimide (467 mg, 2.70 mmol) in diphenyl ether (2.3 mL) at 180 °C for 3 h. After purification (mobile phase hexane/EtOAc 5:1) compound **3g** (482 mg; 68 %) was obtained as a white solid; mp 155–157 °C (hexane/EtOAc); $R_f$ 0.41 (silica gel, hexane/EtOAc, 3:1). IR (DRIFT): 2925 (C–H), 1722 (C═O), 1499, 1367, 1124 (C–F), 740, 692. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.70 s (3H, Me), 0.89 d (3H, $J = 6.4$ Hz, Me), 0.91 s (3H, Me), 0.95 d (3H, $J = 6.4$ Hz, Me), 1.04 s (3H, Me), 1.10 s (3H, Me), 1.27 s (3H, Me), 1.90–1.95 m (3H), 2.03 td (1H, $J_1 = 13.2$ Hz, $J_2 = 4.4$ Hz), 2.29–2.33 m (2H), 3.76 dqu (1H, $J_1 = 8.5$ Hz, $J_2 = 2.8$ Hz, H-7′a), 3.87 d (1H, $J = 8.5$ Hz, H-3′a), 4.99 d (1H, $J = 12.5$ Hz, CHaPh), 5.12 d (1H, $J = 12.5$ Hz, CHbPh), 5.29 t (1H, $J = 3.6$ Hz, H-12), 6.25 s (1H, H-4′), 7.27–7.40 m (8HPh), 7.44–7.48 m (2HPh). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 16.1, 17.0, 17.2, 19.9, 21.3, 22.7, 23.2, 23.6, 24.4, 28.0, 28.7, 30.8, 32.6, 35.8, 36.8, 38.3, 39.0, 39.3, 39.7, 40.4, 41.9, 42.3, 44.9, 46.2, 48.4, 52.0, 53.2, 66.1, 123.0 q ($J_{C\text{-}F} = 274.4$ Hz, $CF_3$), 123.7, 123.8 q ($J_{C\text{-}F} = 6.9$ Hz), 125.6, 126.3, 128.1, 128.3, 128.6, 128.8, 129.3, 131.9, 133.5 q ($J_{C\text{-}F} = 28.8$ Hz), 135.6, 136.5, 138.3, 175.5, 175.9, 177.4. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 61.05 s ($CF_3$). HRMS (ESI): $C_{50}H_{57}F_3NO_4$ found 792.4236 $[M - H]^+$; calcd. 792.4234.

#### 4.4.6. 1′,3′-Dioxo-2′-phenyl-5′-trifluoromethyl-ursa-2,12-dieno[2,3-g]-2′,3′,3a′,7a′-tetrahydro-1H-isoindole-28-oic acid **3h**

Compound **3h** was prepared from benzyl ester **3g** (45 mg; 0.06 mmol) by catalytic hydrogenation using 10% Pd/C (42 mg, 0.04 mmol) and 1,3-cyclohexadiene (44 μL, 37 mg, 0.46 mmol) in a mixture THF/EtOAc (6 mL, 1:1). After purification on silica gel (mobile phase hexane/EtOAc 4:1 → 3:1) compound **3h** (27 mg; 68 %) was obtained as a white solid; mp 227–230 °C (hexane/EtOAc); $R_f$ 0.32 (silica gel, hexane/EtOAc, 3:2). IR (DRIFT): 2923 (C–H), 2750 (O–H), 1722 (C═O), 1500, 1456, 1367, 1290, 1125 (C–F), 738, 690. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.84 s (3H, Me), 0.89 d (3H, $J = 6.5$ Hz, Me), 0.95 s (3H, Me), 0.96 d (3H, $J = 6.5$ Hz, Me), 1.03 s (3H, Me), 1.11 s (3H, Me), 1.27 s (3H, Me), 2.23 d (1H, $J = 11.0$ Hz), 2.32 d (1H, $J = 16.5$ Hz, H-1b), 3.76 dqu (1H, $J_1 = 8.5$ Hz, $J_2 = 2.8$ Hz, H-7′a), 3.86 d (1H, $J = 8.5$ Hz, H-3′a), 5.31 t (1H, $J = 3.6$ Hz, H-12), 6.25 s (1H, H-4′), 7.26–7.28 m (2HPh), 7.37–7.40 m (1HPh), 7.44–7.48 m (2HPh). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 16.2, 17.0, 17.2, 19.9, 21.3, 22.7, 23.2, 23.6, 24.2, 28.1, 28.7, 30.8, 32.5, 35.9, 36.9, 38.3, 39.0, 39.3, 39.7, 40.4, 41.9, 42.3, 44.9, 46.2, 48.2, 52.0, 52.8, 123.0 q ($J_{C\text{-}F} = 274.2$ Hz, $CF_3$), 123.76, 123.8 q ($J_{C\text{-}F} = 7.2$ Hz), 125.8, 126.3, 128.8, 129.3, 131.9, 133.5 q ($J_{C\text{-}F} = 28.8$ Hz), 135.6, 138.1, 175.5, 175.8, 183.8. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 61.07 s ($CF_3$). HRMS (ESI): $C_{43}H_{51}F_3NO_4$ found 702.3770 $[M - H]^+$; calcd. 702.3765.

#### 4.4.7. 1′,3′-Dioxo-2′-phenyl-5'-trifluoromethyl-19β,28-epoxy[2,3-g]-2′,3′,3a′,7a′-tetrahydro-1H-isoindole-18a-oleanan 7

Compound **7** was prepared according to the general procedure from pyrone **6** (1.2g mg; 2.14 mmol) and *N*-phenylmaleimide (1.11 g, 6.42 mmol) in diphenyl ether (4 mL) at 200 °C for 3 h. After purification (mobile phase hexane/EtOAc 5:1) compound **7** (1.11 g; 75 %) was obtained as a white solid; mp 234–236 °C (hexane/EtOAc); $R_f$ 0.34 (silica gel, hexane/EtOAc, 4:1). IR (DRIFT): 2927 (C–H), 1713 (C═O), 1497, 137, 1123 (C-F)740. $^1$H NMR ($CDCl_3$, 500 MHz) δ, ppm: 0.81 s (3H, Me), 0.87 s (3H, Me), 0.94 s (6H, 2Me), 1.03 s (3H, Me), 1.04 s (3H, Me), 1.15 dq (1H, $J_1$ = 12.9 Hz, $J_2$ = 2.1 Hz), 1.27 s (3H, Me), 1.69-1.72 m (1H), 1.89 d (1H, $J$ = 16.6 Hz, H-1a), 2.39 d (1H, $J$ = 16.6 Hz, H-1b), 3.46 d (1H, $J$ = 7.8 Hz, H-28a), 3.55 s (1H, H-19), 3.76 dqu(1H, $J_1$ = 8.6 Hz, $J_2$ = 2.9 Hz, H-7'a), 3.79 dd (1H, $J_1$ = 7.8 Hz, $J_1$ = 0.8 Hz, H-28b), 3.86 d (1H, $J$ = 8.6 Hz, H-3'a), 6.25 s (1H, H-4'), 7.27-7.29 m ($2H_{Ph}$), 7.37-7.40 m ($1H_{Ph}$), 7.44-7.48 m ($2H_{Ph}$).$^{13}$C NMR ($CDCl_3$, 126 MHz) δ, ppm: 13.6, 15.6, 17.1, 19.8, 21.4, 22.5, 24.7, 26.4, 26.57, 26.58, 28.5, 29.0, 32.9, 33.2, 34.4, 36.1, 36.4, 36.9, 38.5, 40.7, 40.89 q ($J_{C-F}$ = 1.9 Hz), 40.9, 41.6, 41.9, 44.9, 46.9, 49.9, 52.2, 71.4, 88.1, 123.0 q ($J_{C-F}$ = 274.6 Hz, $CF_3$), 123.80, 123.84 q ($J_{C-F}$ = 6.7 Hz), 126.3, 128.8, 129.3, 132.0, 133.7 q ($J_{C-F}$ = 29.1 Hz), 135.7, 175.5, 175.9.$^{19}$F NMR ($CDCl_3$, 500 MHz) δ, ppm: - 61.04 s ($CF_3$).HRMS (ESI): $C_{43}H_{53}F_3NO_3$ found 688.3973 $[M-H]^+$; calcd. 688.3972

### 4.5. General procedure for the preparation of phthalimides

A modified procedure from Ref. [30] was used.

A solution of each dihydrophthalimide (1.45 mmol) and elemental sulfur (2.78 mmol, 2 eq.) in dry 1,3-dimethyl-3,4,5,6-tetrahydro-2(*1H*)-pyrimidinone (DMPU, 2 mL) was stirred at 220 °C under an atmosphere of nitrogen for 2 h. Then to the reaction mixture was added water and the formed precipitate was collected by filtration. Washing the filtrate with water, then the filtrate was dissolved in ether. The organic layer was dried over $Na_2SO_4$, concentrated in vacuo. The residue was purified by column chromatography on $SiO_2$ eluting with hexane/ethyl acetate.

#### 4.5.1. Benzyl-1′,3′-dioxo-2′-phenyl-5′-trifluoromethyl lupa-2,20(29)-dieno [2,3-g]-isoindoline-28-oate 1i

Compound **1i** was prepared according to the general procedure from **1g** (165 mg; 0.21 mmol) and elemental sulfur (13 mg, 0.42 mmol) in DMPU (1 mL). After purification (mobile phase hexane/EtOAc 7:1) compound **1i** (119 mg; 72 %) was obtained as a pale yellow; mp 147–150 °C (hexane/EtOAc); $R_f$ 0.55 (silica gel, hexane/EtOAc, 4:1). IR (DRIFT): 2942 (C–H), 1719 (C═O), 1500, 1456, 1376, 1233, 1123 (C–F), 756, 689. $^1$H NMR ($CDCl_3$, 500 MHz) *δ*, ppm: 0.80 s (3H, Me), 0.85 s (3H, Me), 1.00 s (3H, Me), 1.55 s (3H, Me), 1.59 s (3H, Me), 1.65 t (1H, $J$ = 11.4 Hz, H-18), 1.72 s (3H, Me), 1.80 dq (1H, $J_1$ = 13.0 Hz, $J_2$ = 3.7 Hz), 1.86–1.96 m (2H), 2.25–2.33 m (3H), 3.06 td (1H, $J_1$ = 10.9 Hz, $J_2$ = 4.6 Hz, H-19), 3.36 d (1H, $J$ = 17.0 Hz, H-1b), 4.65 dd (1H, $J_1$ = 2.2 Hz, $J_2$ = 1.4 Hz, H-29a), 4.77 d (1H, $J$ = 2.2 Hz, H-29b), 5.11 d (1H, $J$ = 12.3 Hz, CHaPh), 5.17 d (1H, $J$ = 12.3 Hz, CHbPh), 7.33–7.44 m (8HPh), 7.49–7.53 m (2HPh), 8.14 s (1H, H-4′). $^{13}$C NMR ($CDCl_3$, 126 MHz) *δ*, ppm: 14.8, 15.6, 15.8, 19.6, 20.1, 21.6, 22.5, 25.8, 29.5, 29.7, 30.7, 32.2, 33.5, 35.0, 37.1, 38.1, 38.4, 40.8, 42.7, 43.8 q ($J_{C-F}$ = 2.6 Hz), 47.1, 49.1, 49.5, 55.2, 56.7, 65.9, 109.8, 119.6 q ($J_{C-F}$ = 6.2 Hz), 123.8 q ($J_{C-F}$ = 275.3 Hz, $CF_3$), 127.1, 128.2, 128.4, 128.5, 128.6, 129.3, 131.76, 131.77, 132.3, 135.0 q ($J_{C-F}$ = 29.5 Hz), 136.6, 143.6, 150.7, 151.1, 166.0, 167.3, 175.9. $^{19}$F NMR ($CDCl_3$, 500 MHz) *δ*, ppm: 60.86 s ($CF_3$). HRMS (ESI): $C_{50}H_{55}F_3NO_4$ found 790.4082 $[M-H]^+$; calcd. 790.4078.

#### 4.5.2. 1′,3′-Dioxo-2′-phenyl-5′-trifluoromethyl lupa-2,20(29)-dieno[2,3-g] isoindoline-28-oic acid 1j

Compound **1j** was prepared according to the general procedure from **1h** (155 mg; 0.22 mmol) and elemental sulfur (14 mg, 0.44 mmol) in DMPU (1 mL). After purification (mobile phase hexane/EtOAc 3:1) compound **1j** (61 mg; 40 %) was obtained as a pale yellow solid; mp 228–230 °C (hexane/EtOAc); $R_f$ 0.48 (silica gel, hexane/EtOAc, 3:2). IR (DRIFT): 2947 (C–H), 2700 (O–H), 1720 (C═O), 1693, 1500, 1455, 1375, 1238, 1123 (C–F), 756, 688. $^1$H NMR ($CDCl_3$, 500 MHz) *δ*, ppm: 0.82 s (3H, Me), 1.04 s (6H, 2Me), 1.16 qd (1H, $J1$ = 13.1 Hz, $J2$ = 4.6 Hz), 1.54 s (3H, Me), 1.59 s (3H, Me), 1.69 t (1H, $J$ = 11.4 Hz, H-18), 1.73 s (3H, Me), 1.82 dq (1H, $J_1$ = 12.8 Hz, $J_2$ = 2.5 Hz), 1.98–2.07 m (2H), 2.27–2.33 m (3H), 3.05 td (1H, $J_1$ = 10.8 Hz, $J_2$ = 4.8 Hz, H-19), 3.37 d (1H, $J$ = 16.9 Hz, H-1b), 4.67 dd (1H, $J1$ = 2.1 Hz, $J2$ = 1.4 Hz, H-29a), 4.79 d (1H, $J$ = 2.1 Hz, H-29b), 7.39–7.43 m (3HPh), 7.49–7.52 m (2HPh), 8.14 s (1H, H-4′). $^{13}$C NMR ($CDCl_3$, 126 MHz) *δ*, ppm: 14.8, 15.79, 15.81, 19.6, 20.0, 21.5, 22.4, 25.7, 29.5, 29.8, 30.8, 32.2, 33.5, 35.0, 37.2, 38.1, 38.7, 40.8, 42.7, 43.8 q ($J_{C-F}$ = 2.7 Hz), 47.1, 49.0, 49.3, 55.2, 56.6, 110.0, 119.6 q ($J_{C-F}$ = 6.6 Hz), 123.8 q ($J_{C-F}$ = 275.1 Hz, $CF_3$), 127.1, 128.5, 129.3, 131.7, 131.8, 132.3, 135.0 q ($J_{C-F}$ = 29.8 Hz), 143.5, 150.5, 151.1, 166.0, 167.3, 182.5. $^{19}$F NMR ($CDCl_3$, 500 MHz) *δ*, ppm: 60.85 s ($CF_3$). HRMS (ESI): $C_{43}H_{51}F_3NO_4$ found 702.3763 $[M+H]^+$; calcd. 702.3765.

#### 4.5.3. Benzyl-1′,3′-dioxo-2′-phenyl-5′-trifluoromethyl-20(29)-dihydrolup-2-en[2,3-g]isoindoline-28-oate 2i

Compound **2i** was prepared according to the general procedure from **2g** (370 mg; 0.47 mmol) and elemental sulfur (30 mg, 0.94 mmol) in DMPU (1.5 mL). After purification (mobile phase hexane/EtOAc 7:1) compound **2i** (296 mg; 80 %) was obtained as a pale yellow solid; mp 155–158 °C (hexane/EtOAc); $R_f$ 0.55 (silica gel, hexane/EtOAc, 4:1). IR (DRIFT): 2952 (C–H), 1720 (C═O), 1501, 1455, 1377, 1233, 1122 (C–F), 909, 731, 689. $^1$H NMR ($CDCl_3$, 500 MHz) *δ*, ppm: 0.77 d (3H, $J$ = 6.8 Hz, Me), 0.80 s (3H, Me), 0.83 s (3H, Me), 0.87 d (3H, $J$ = 6.8 Hz, Me), 0.99 s (3H, Me), 1.56 s (3H, Me), 1.59 s (3H, Me), 1.77 dq (1H, $J_1$ = 12.3 Hz, $J_2$ = 2.4 Hz), 1.82–1.87 m (2H), 2.26–2.33 m (4H), 3.38 d (1H, $J$ = 16.7 Hz, H-1b), 5.10 d (1H, $J$ = 12.3 Hz, CHaPh), 5.15 d (1H, $J$ = 12.3 Hz, CHbPh), 7.31–7.44 m (8HPh), 7.49–7.54 m (2HPh), 8.14 s (1H, H-4′). $^{13}$C NMR ($CDCl_3$, 126 MHz) *δ*, ppm: 14.7, 14.8, 15.6, 15.8, 20.1, 21.6, 22.5, 22.9, 23.1, 27.2, 29.5, 29.7, 29.9, 32.1, 33.6, 35.0, 37.4, 38.1, 38.3, 40.8, 42.8, 43.8 q ($J_{C-F}$ = 2.5 Hz), 44.3, 48.9, 49.0, 55.2, 57.2, 65.8, 119.6 q ($J_{C-F}$ = 6.3 Hz), 123.8 q ($J_{C-F}$ = 275.3 Hz, $CF_3$), 127.1, 128.2, 128.4, 128.5, 128.6, 129.3, 131.76, 131.79, 132.3, 135.0 q ($J_{C-F}$ = 29.8 Hz), 136.7, 143.6, 151.1, 166.0, 167.3, 176.1. $^{19}$F NMR ($CDCl_3$, 500 MHz) *δ*, ppm: 60.87 s ($CF_3$). HRMS (ESI): $C_{50}H_{57}F_3NO_4$ found 792.4239 $[M-H]^+$; calcd. 792.4234.

#### 4.5.4. 1′,3′-Dioxo-2′-phenyl-5′-trifluoromethyl-20(29)-dihydrolup-2-en [2,3-g]isoindoline-28-oic acid 2j

Compound **2j** was prepared according to the general procedure from tetrahydroisoindole **2h** (260 mg; 0.38 mmol) and elemental sulfur (24 mg, 0.76 mmol) in DMPU (1.5 mL). After purification (mobile phase hexane/EtOAc 3:1) compound **2j** (126 mg; 48 %) was obtained as a pale yellow solid; mp 226–228 °C (hexane/EtOAc); $R_f$ 0.52 (silica gel, hexane/EtOAc, 3:2). IR (DRIFT): 2953 (C–H), 2900 (O–H), 1721 (C═O), 1501, 1378, 1233, 1126 (C–F), 911, 757, 689. $^1$H NMR ($CDCl_3$, 500 MHz) *δ*, ppm: 0.79 d (3H, $J$ = 6.8 Hz, Me), 0.836 s (3H, Me), 0.89 d (3H, $J$ = 6.8 Hz, Me), 1.02 s (3H, Me), 1.04 s (3H, Me), 1.54 s (3H, Me), 1.59 s (3H, Me), 1.77–1.88 m (2H), 1.92 dd (1H, $J_1$ = 12.3 Hz, $J_2$ = 7.4 Hz), 2.26–2.35 m (4H), 3.39 d (1H, $J$ = 17.2 Hz, H-1b), 7.39–7.43 m (3HPh), 7.49–7.52 m (2HPh), 8.14 s (1H, H-4′). $^{13}$C NMR ($CDCl_3$, 126 MHz) *δ*, ppm: 14.7, 14.8, 15.8, 15.9, 20.0, 21.5, 22.4, 22.9, 23.1, 27.1, 29.5, 29.8, 29.9, 32.1, 33.5, 35.0, 37.6, 38.1, 38.6, 40.8, 42.9, 43.8, 44.3, 48.8, 48.9, 55.2, 57.1, 119.6 q ($J_{C-F}$ = 6.4 Hz), 123.8 q ($J_{C-F}$ = 275.3 Hz, $CF_3$), 127.1, 128.5, 129.3, 131.7, 131.8, 132.4, 135.0 q ($J_{C-F}$ = 29.6 Hz), 143.5, 151.1, 166.0, 167.3, 182.7. $^{19}$F NMR ($CDCl_3$, 500 MHz) *δ*, ppm: 60.87 s ($CF_3$). HRMS (ESI): $C_{43}H_{51}F_3NO_4$ found 702.3773 $[M-H]^+$; calcd. 702.3765.

#### 4.5.5. Benzyl-1′,3′-dioxo-2′-phenyl-5′-trifluoromethyl-ursa-2,12-dieno[2,3-g]isoindoline-28-oate 3i

Compound **3i** was prepared according to the general procedure from

**3g** (420 mg; 0.53 mmol) and elemental sulfur (34 mg, 1.06 mmol) in DMPU (1.5 mL). After purification (mobile phase hexane/EtOAc 7:1) compound **3i** (385 mg; 90 %) was obtained as a pale yellow solid; mp 157–160 °C (hexane/EtOAc); $R_f$ 0.50 (silica gel, hexane/EtOAc, 4:1). IR (DRIFT): 2925 (C–H), 1720 (C═O), 1501, 1455, 1377, 1231, 1126 (C–F), 756. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.73 s (3H, Me), 0.88 s (3H, Me), 0.91 d (3H, $J$ = 6.4 Hz, Me), 0.96 d (3H, $J$ = 6.4 Hz, Me), 1.13 s (3H, Me), 1.57 s (3H, Me), 1.61 s (3H, Me), 1.97–2.10 m (3H), 2.33 d (1H, $J$ = 11.4 Hz), 2.38 d (1H, $J$ = 16.9 Hz, H-1a), 3.33 d (1H, $J$ = 16.9 Hz, H-1b), 5.00 d (1H, $J$ = 12.5 Hz, CHaPh), 5.12 d (1H, $J$ = 12.5 Hz, CHbPh), 5.34 t (1H, $J$ = 3.6 Hz, H-12), 7.30–7.38 m (5HPh), 7.40–7.44 m (3HPh), 7.50–7.53 m (2HPh), 8.15 s (1H, H-4′). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 15.4, 17.0, 17.2, 20.1, 21.3, 22.7, 23.3, 23.6, 24.4, 28.0, 29.6, 30.9, 32.6, 34.8, 36.8, 38.0, 39.0, 39.4, 39.7, 42.5, 43.7 q ($J_{C\text{-}F}$ = 2.1 Hz), 45.8, 48.4, 53.2, 55.4, 66.2, 119.6 q ($J_{C\text{-}F}$ = 6.5 Hz), 123.8 q ($J_{C\text{-}F}$ = 275.3 Hz, $CF_3$), 125.6, 127.1, 128.1, 128.4, 128.5, 128.6, 129.3, 131.7, 131.9, 132.4, 134.9 q ($J_{C\text{-}F}$ = 29.9 Hz), 136.5, 138.4, 143.4, 151.1, 166.0, 167.2, 177.4. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 60.73 s ($CF_3$). HRMS (ESI): $C_{50}H_{55}F_3NO_4$ found 790.4083 $[M - H]^+$; calcd. 790.4078.

#### 4.5.6. 1′,3′-Dioxo-2′-phenyl-5′-trifluoromethyl-ursa-2,12-dieno[2,3-g] isoindoline-28-oic acid **3j**

Compound **3j** was prepared from benzyl ester **3i** (50 mg; 0.06 mmol) by catalytic hydrogenation using 10% Pd/C (6 mg, 0.006 mmol) in a mixture THF/MeOH (1.3 mL/0.3 mL) for 1 h. After purification in silica gel (mobile phase hexane/EtOAc 4:1) compound **3j** (42 mg; 99 %) was obtained as a white solid; mp 228–230 °C (hexane/EtOAc); $R_f$ 0.34 (silica gel, hexane/EtOAc, 3:1). IR (DRIFT): 2924 (C–H), 2600 (O–H), 1720 (C═O), 1695, 1501, 1456, 1377, 1233, 1126 (C–F), 910, 750, 688. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.89 s (3H, Me), 0.91 s (3H, Me), 0.92 d (3H, $J$ = 6.4 Hz, Me), 0.97 d (3H, $J$ = 6.4 Hz, Me), 1.14 s (3H, Me), 1.52 s (3H, Me), 1.60 s (3H, Me), 1.91 td (1H, $J1$ = 13.6 Hz, $J1$ = 4.5 Hz), 2.03 td (1H, $J_1$ = 13.6 Hz, $J_2$ = 4.3 Hz), 2.08–2.11 m (2H), 2.26 d (1H, $J$ = 11.3 Hz), 2.39 d (1H, $J$ = 16.9 Hz, H-1a), 3.33 d (1H, $J$ = 16.9 Hz, H-1b), 5.36 t (1H, $J$ = 3.6 Hz, H-12), 7.39–7.43 m (3HPh), 7.48–7.51 m (2HPh), 8.15 s (1H, H-4′). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 15.4, 17.0, 17.2, 20.1, 21.3, 22.6, 23.3, 23.6, 24.2, 28.1, 29.6, 30.8, 32.5, 34.8, 36.9, 38.0, 39.0, 39.3, 39.7, 42.4, 43.7 q ($J_{C\text{-}F}$ = 3.1 Hz), 45.9, 48.3, 52.9, 55.3, 119.7 q ($J_{C\text{-}F}$ = 6.6 Hz), 121.8 q ($J_{C\text{-}F}$ = 275.4 Hz, $CF_3$), 125.7, 127.1, 128.5, 129.3, 131.7, 131.9, 132.4, 134.9 q ($J_{C\text{-}F}$ = 30.4 Hz), 138.2, 143.3, 151.0, 166.0, 167.3, 184.1. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 60.76 s ($CF_3$). HRMS (ESI): $C_{43}H_{49}F_3NO_4$ found 700.3610 $[M - H]^+$; calcd. 700.3608.

#### 4.5.7. 1′,3′-Dioxo-2′-phenyl-5′-trifluoromethyl-19β,28-epoxy[2,3-g]-isoindoline-18a-oleanan **8**

Compound **8** was prepared according to the general procedure from **7** (1.0 g; 1.45 mmol) and elemental sulfur (89 mg, 2.78 mmol) in DMPU (2 mL). After purification (mobile phase hexane/EtOAc 8:1) compound **8** (864 mg; 87 %) was obtained as a pale yellow solid; mp 235–237 °C (hexane/EtOAc); $R_f$ 0.36 (silica gel, hexane/EtOAc, 6:1). IR (DRIFT): 2926 (C–H), 1719 (C═O), 1503, 1448, 1233, 1129 (C–F), 758, 704. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.82 s (3H, Me), 0.85 s (3H, Me), 0.96 s (3H, Me), 0.98 s (3H, Me), 1.07 s (3H, Me), 1.57 s (3H, Me), 1.60 s (3H, Me), 2.36 d (1H, $J$ = 16.8 Hz, H-1a), 3.41 d (1H, $J$ = 16.8 Hz, H-1b), 3.48 d (1H, $J$ = 7.9 Hz, H-28a), 3.58 s (1H, H-19β), 3.81 dd (1H, $J_1$ = 7.9 Hz, $J_2$ = 0.8 Hz, H-28b), 7.41–7.44 m (3HPh), 7.50–7.53 m (2HPh), 8.15 s (1H, H-4′). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 13.6, 15.6, 16.1, 20.0, 21.6, 22.4, 24.7, 26.4, 26.6, 26.7, 29.0, 29.6, 32.9, 33.1, 34.5, 35.1, 36.4, 36.9, 38.2, 40.7, 41.0, 41.7, 44.0 q ($J_{C\text{-}F}$ = 2.5 Hz), 46.9, 49.6, 55.4, 71.4, 88.1, 119.6 q ($J_{C\text{-}F}$ = 6.5 Hz), 123.8 q ($J_{C\text{-}F}$ = 275.3 Hz, $CF_3$), 127.1, 128.5, 129.3, 131.75, 131.81, 132.4, 135.1 q ($J_{C\text{-}F}$ = 29.9 Hz), 143.5, 151.1, 166.0, 167.2. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 60.88 s ($CF_3$). HRMS (ESI): $C_{43}H_{51}F_3NO_3$ found 686.3814 $[M - H]^+$; calcd. 686.3816.

### 4.6. General procedure for the preparation of triterpenic methylphthalates

A mixture of each triterpenic phenylphthalimide (0.25 mmol) and KOH (11.25 mmol, 45 eq.) in MeOH/$H_2O$ (4 mL/0.4 mL) was heated to reflux (95 °C) for 24 h, then concentrated in vacuo. Water (250 mL) was added, the solution was acidified to pH 3.0 by 10% HCl and the phthalic acid was salted out with solid NaCl and extracted with EtOAc (3 × 100 mL). Organic layer was dried over $Na_2SO_4$, concentrated in vacuo. The crude phthalic acid used in next step without purification.

A mixture of phthalic acid in acetone (4.7 mL), $K_2CO_3$ (0.50 mmol, 2 eq.) and dimethyl sulfate (0.50 mmol, 2 eq.) was stirred and refluxed (60 °C) for 13 h under nitrogen atmosphere. Then the mixture was cooled and diluted with water, extracted with $CH_2Cl_2$. The organic layer was washed with water, dried over $Na_2SO_4$, concentrated in vacuo. The residue was purified by column chromatography on $SiO_2$ eluting with hexane/ethyl acetate.

#### 4.6.1. Benzyl lupa-2,20(29)-dieno [2,3-d]-1′,2′-dimethyl-4′-trifluoromethylphthalate-28-oate **1k**

Compound **1k** was prepared according to the general procedure in 2 stages: from phenylphthalimide **1i** (110 mg; 0.14 mmol) and KOH (353 mg, 6.30 mmol) in MeOH/$H_2O$ (2 mL/0.2 mL); then $K_2CO_3$ (39 mg, 0.28 mmol), dimethyl sulfate (26 μL, 35 mg, 0.28 mmol) in acetone (2 mL). After purification (mobile phase hexane/EtOAc 8:1) compound **1k** (74 mg; 69 %) was obtained as a white solid; mp 117–120 °C (hexane/EtOAc); $R_f$ 0.43 (silica gel, hexane/EtOAc, 4:1). IR (DRIFT): 2948 (C–H), 1728 (C═O), 1454, 1258, 1193, 1148, 1121 (C–F), 1105, 911, 729, 697. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.74 s (3H, Me), 0.83 s (3H, Me), 0.98 s (3H, Me), 1.32 s (3H, Me), 1.37 s (3H, Me), 1.64 t (1H, $J$ = 11.4 Hz, H-18), 1.71 s (3H, Me), 1.77 dq (1H, $J_1$ = 13.0 Hz, $J_2$ = 3.3 Hz), 1.85–1.95 m (2H), 2.23–2.32 m (3H), 3.04 td (1H, $J_1$ = 11.0 Hz, $J_2$ = 4.8 Hz, H-19β), 3.25 d (1H, $J$ = 17.0 Hz, H-1b), 3.878 s (3H, MeO), 3.879 s (3H, MeO), 4.64 dd (1H, $J_1$ = 2.2 Hz, $J_2$ = 1.4 Hz, H-29a), 4.76 d (1H, $J$ = 2.2 Hz, H-29b), 5.10 d (1H, $J$ = 12.3 Hz, CHaPh), 5.16 d (1H, $J$ = 12.3 Hz, CHbPh), 7.30–7.39 m (5H), 8.16 s (1H, H-3'). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.8, 15.4, 15.6, 19.6, 20.4, 21.7, 23.0, 25.8, 29.7, 30.7, 31.2, 32.2, 33.6, 34.8, 37.1, 38.5, 39.2, 40.6, 42.6, 43.8 q ($J_{C\text{-}F}$ = 2.1 Hz), 47.1, 49.2, 49.5, 52.77, 52.78, 55.6, 56.7, 65.9, 109.8, 124.1 q ($J_{C\text{-}F}$ = 274.8 Hz, $CF_3$), 126.0 q ($J_{C\text{-}F}$ = 6.8 Hz), 127.6, 128.2, 128.4, 128.7, 130.3 q ($J_{C\text{-}F}$ = 29.3 Hz), 136.6, 138.6, 140.8, 146.3, 150.7, 165.7, 170.5, 176.0. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 60.56 s ($CF_3$). HRMS (ESI): $C_{46}H_{58}F_3O_6$ found 763.4175 $[M+H]^+$; calcd. 763.4180.

#### 4.6.2. Lupa-2,20(29)-dieno [2,3-d]-1′,2′-dimethyl-4′-trifluoromethylphthalate-28-oic acid **1l**

Compound **1l** was prepared from benzyl ester **1k** (50 mg; 0.07 mmol) by catalytic hydrogenation using, 10% Pd/C (42 mg, 0.04 mmol) and 1,3-cyclohexadiene (47 μL, 40 mg, 0.50 mmol) in a mixture THF/EtOAc (5 mL, 1:1). After purification (mobile phase hexane/EtOAc 3:1) compound **1l** (35 mg; 80 %) was obtained as a white solid; mp 226–228 °C (hexane/EtOAc); $R_f$ 0.47 (silica gel, hexane/EtOAc, 3:2). IR (DRIFT): 2948 (C–H), 2800 (O–H), 1733 (C═O), 1685 (C═O), 1448, 1320, 1266, 1216, 1117, 1104, 880, 726. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 0.76 s (3H, Me), 1.01 s (3H, Me), 1.02 s (3H, Me), 1.13 qd (1H, $J_1$ = 12.9 Hz, $J_2$ = 4.4 Hz), 1.32 s (3H, Me), 1.38 s (3H, Me), 1.68 t (1H, $J$ = 11.4 Hz, H-18), 1.72 s (3H, Me), 1.79 dq (1H, $J_1$ = 12.9 Hz, $J_2$ = 2.7 Hz), 1.97–2.05 m (2H), 2.25–2.31 m (3H), 3.03 td (1H, $J_1$ = 10.8 Hz, $J_2$ = 4.9 Hz, H-19β), 3.27 d (1H, $J$ = 16.8 Hz, H-1b), 3.876 s (3H, MeO), 3.880 s (3H, MeO), 4.65 dd (1H, $J_1$ = 2.1 Hz, $J_2$ = 1.4 Hz, H-29a), 4.77 d (1H, $J$ = 2.1 Hz, H-29b), 8.16 s (1H, H-3'). $^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$, ppm: 14.8, 15.4, 15.8, 19.6, 20.4, 21.6, 23.0, 25.8, 29.8, 30.7, 31.2, 32.2, 33.5, 34.8, 37.2, 38.7, 39.2, 40.7, 42.6, 42.8, 47.0, 49.2, 49.3, 52.78, 52.79, 55.5, 56.6, 110.0, 124.1 q ($J_{C\text{-}F}$ = 274.7 Hz, $CF_3$), 126.0 q ($J_{C\text{-}F}$ = 6.5 Hz), 127.6, 130.3 q ($J_{C\text{-}F}$ = 29.5 Hz), 138.6, 140.8, 146.2, 150.5, 165.7, 170.5, 182.5. $^{19}$F NMR ($CDCl_3$, 500 MHz) $\delta$, ppm: 60.55 s ($CF_3$). HRMS (ESI): $C_{39}H_{50}F_3O_6$ found 671.3557 $[M+H]^+$; calcd. 671.3554.

#### 4.6.3. Benzyl-lup-2-en [2,3-d]1′,2′-dimethyl-4′-trifluoromethylphthalate-28-oate **2k**

Compound **2k** was prepared according to the general procedure in 2 stages: from phenylphthalimide **2i** (265 mg; 0.33 mmol) and KOH (832 mg, 14.85 mmol) in MeOH/$H_2O$ (4 mL/0.4 mL); then $K_2CO_3$ (91 mg, 0.66 mmol), dimethyl sulfate (62 μL, 83 mg, 0.66 mmol) in acetone (4.5 mL). After purification (mobile phase hexane/EtOAc 8:1) compound **2k** (89 mg; conversion 66%, yield 52%) was obtained as a white solid; mp 115–120 °C (hexane/EtOAc); $R_f$ 0.41 (silica gel, hexane/EtOAc, 4:1). IR (DRIFT): 2951 (C–H), 1728 (C═O), 1454, 1258, 1147, 1121 (C–F), 1104, 908, 729, 696. $^1$H NMR ($CDCl_3$, 500 MHz) δ, ppm: 0.75 s (3H, Me), 0.76 d (3H, $J$ = 6.8 Hz, Me), 0.81 s (3H, Me), 0.86 d (3H, $J$ = 6.8 Hz, Me), 0.97 s (3H, Me), 1.33 s (3H, Me), 1.38 s (3H, Me), 1.75 dq (1H, $J_1$ = 12.7 Hz, $J_2$ = 2.9 Hz), 1.81–1.86 m (2H), 2.25–2.31 m (4H), 3.27 d (1H, $J$ = 16.8 Hz, H-1b), 3.880 s (3H, MeO), 3.881 s (3H, MeO), 5.09 d (1H, $J$ = 12.3 Hz, CHaPh), 5.14 d (1H, $J$ = 12.3 Hz, CHbPh), 7.29–7.38 m (5HPh), 8.16 s (1H, H-3'). $^{13}$C NMR ($CDCl_3$, 126 MHz) δ, ppm: 14.7, 14.8, 15.4, 15.6, 20.4, 21.7, 22.9, 23.0, 23.1, 27.2, 29.7, 29.9, 31.2, 32.1, 33.7, 34.8, 37.4, 38.3, 39.2, 40.7, 42.8, 43.8 q ($J_{C\text{-}F}$ = 2.2 Hz), 44.3, 49.00, 49.01, 52.76, 52.77, 55.6, 57.2, 65.8, 124.1 q ($J_{C\text{-}F}$ = 275.1 Hz, $CF_3$), 126.0 q ($J_{C\text{-}F}$ = 6.5 Hz), 127.6, 128.2, 128.4, 128.6, 130.3 q ($J_{C\text{-}F}$ = 29.3 Hz), 136.7, 138.6, 140.8, 146.3, 165.7, 170.5, 176.2. $^{19}$F NMR ($CDCl_3$, 500 MHz) δ, ppm: 60.57 s ($CF_3$). HRMS (ESI): $C_{46}H_{60}F_3O_6$ found 765.4335 $[M+H]^+$; calcd. 765.4337.

#### 4.6.4. Lup-2-en [2,3-d]1′,2′-dimethyl-4′-trifluoromethylphthalate-28-oic acid **2l**

Compound **2l** was prepared from benzyl ester **2k** (45 mg; 0.06 mmol) by catalytic hydrogenation using 10% Pd/C (6 mg, 0.006 mmol) in mixture THF/MeOH (1.3 mL/0.3 mL) for 2 h. After purification on silica gel (mobile phase hexane/EtOAc 3:1) compound **2l** (36 mg; 90 %) was obtained as a white solid; mp 225–227 °C (hexane/EtOAc); $R_f$ 0.52 (silica gel, hexane/EtOAc, 3:2). IR (DRIFT): 2951 (C–H), 2750 (O–H), 1741 (C═O), 1684 (C═O), 1450, 1321, 1263, 1216, 1200, 1118, 1106, 916, 727. $^1$H NMR ($CDCl_3$, 500 MHz) δ, ppm: 0.77 s (3H, Me), 0.78 d (3H, $J$ = 6.8 Hz, Me), 0.87 d (3H, $J$ = 6.8 Hz, Me), 1.00 s (3H, Me), 1.01 s (3H, Me), 1.32 s (3H, Me), 1.38 s (3H, Me), 1.74–1.78 m (1H), 1.85 dtd (1H, $J_1$ = 13.5 Hz, $J_2$ = 6.8 Hz, $J_3$ = 2.7 Hz), 1.91 dd (1H, $J_1$ = 12.3 Hz, $J_2$ = 7.4 Hz), 2.25–2.32 m (4H), 3.28 d (1H, $J$ = 17.0 Hz, H-1b), 3.878 s (3H, MeO), 3.881 s (3H, MeO), 8.16 s (1H, H-3'). $^{13}$C NMR ($CDCl_3$, 126 MHz) δ, ppm: 14.7, 14.8, 15.4, 15.8, 20.4, 21.6, 22.9, 23.0, 23.1, 27.1, 29.8, 29.9, 31.2, 32.1, 33.6, 34.8, 37.6, 38.6, 39.2, 40.7, 42.8, 43.8, 44.3, 48.8, 48.9, 52.78, 52.79, 55.5, 57.1, 124.1 q ($J_{C\text{-}F}$ = 274.6 Hz, $CF_3$), 126.0 q ($J_{C\text{-}F}$ = 6.4 Hz), 127.6, 130.3 q ($J_{C\text{-}F}$ = 28.8 Hz), 138.6, 140.7, 146.2, 165.7, 170.5, 182.7. $^{19}$F NMR ($CDCl_3$, 500 MHz) δ, ppm: 60.56 s ($CF_3$). HRMS (ESI): $C_{39}H_{52}F_3O_6$ found 673.3711 $[M-H]^+$; calcd. 673.3711.

#### 4.6.5. Benzyl ursa-2,12-dieno [2,3-d]-1′,2′-dimethyl-4′-trifluoromethylphthalate-28-oate **3k**

Compound **3k** was prepared according to the general procedure in 2 stages: from phenylphthalimide **3i** (280 mg; 0.35 mmol) and KOH (882 mg, 15.75 mmol) in MeOH/$H_2O$ (4 mL/0.4 mL); then $K_2CO_3$ (97 mg, 0.70 mmol), dimethyl sulfate (66 μL, 88 mg, 0.70 mmol) in acetone (4.5 mL). After purification (mobile phase hexane/EtOAc 8:1) compound **3k** (93 mg; conversion 52%, yield 66%) was obtained as a white solid; mp 120–123 °C (hexane/EtOAc); $R_f$ 0.41 (silica gel, hexane/EtOAc, 4:1). IR (DRIFT): 2948 (C–H), 1735 (C═O), 1454, 1371, 1261, 1215, 1122, 1104, 908, 729, 696. $^1$H NMR ($CDCl_3$, 500 MHz) δ, ppm: 0.71 s (3H, Me), 0.83 s (3H, Me), 0.90 d (3H, $J$ = 6.4 Hz, Me), 0.95 d (3H, $J$ = 6.4 Hz, Me), 1.11 s (3H, Me), 1.34 s (3H, Me), 1.40 s (3H, Me), 1.94–2.08 m (3H), 2.31–2.35 m (2H), 3.22 d (1H, $J$ = 16.4 Hz, H-1b), 3.88 s (3H, OMe), 3.89 s (3H, OMe), 4.99 d (1H, $J$ = 12.5 Hz, CHaPh), 5.11 d (1H, $J$ = 12.5 Hz, CHbPh), 5.33 t (1H, $J$ = 3.6 Hz, H-12), 7.29–7.37 m (5HPh), 8.17 s (1H, H-3'). $^{13}$C NMR ($CDCl_3$, 126 MHz) δ, ppm: 15.1, 17.0, 17.2, 20.5, 21.3, 23.2, 23.4, 23.5, 24.4, 28.0, 30.9, 31.4, 32.7, 34.6, 36.8, 39.0, 39.1, 39.4, 39.6, 42.4, 43.7, 45.9, 48.4, 52.77, 52.79, 53.3, 55.8, 66.2, 124.1 q ($J_{C\text{-}F}$ = 274.6 Hz, $CF_3$), 125.7, 126.0 q (JC-F = 6.5 Hz), 127.7, 128.1, 128.3, 128.6, 130.2 q ($J_{C\text{-}F}$ = 29.4 Hz), 136.5, 138.3, 138.7, 140.7, 146.2, 165.6, 170.5, 177.4. $^{19}$F NMR ($CDCl_3$, 500 MHz) δ, ppm: 60.43 s ($CF_3$). HRMS (ESI): $C_{46}H_{58}F_3O_6$ found 763.4178 $[M+H]^+$; calcd. 763.4180.

#### 4.6.6. Ursa-2,12-dieno [2,3-d]-1′,2′-dimethyl-4′-trifluoromethylphthalate-28-oic acid **3l**

Compound **3l** was prepared from benzyl ester **3k** (60 mg; 0.08 mmol) by catalytic hydrogenation using 10% Pd/C (53 mg, 0.05 mmol) and 1,3-cyclohexadiene (58 μL, 49 mg, 0.61 mmol) in mixture THF/EtOAc (6 mL, 1:1). After purification (mobile phase hexane/EtOAc 3:1) compound **3l** (43 mg; 81 %) was obtained as a white solid; mp 231–233 °C (hexane/EtOAc); $R_f$ 0.39 (silica gel, hexane/EtOAc, 3:2). IR (DRIFT): 2949 (C–H), 2800 (O–H), 1734 (C═O), 1694 (C═O), 1446, 1314, 1262, 1214, 1146, 1123, 1104, 976, 910, 728. $^1$H NMR ($CDCl_3$, 500 MHz) δ, ppm: 0.86 s (6H, 2Me), 0.91 d (3H, $J$ = 6.4 Hz, Me), 0.96 d (3H, $J$ = 6.4 Hz, Me), 1.12 s (3H, Me), 1.31 s (3H, Me), 1.39 s (3H, Me), 1.88 td (1H, $J_1$ = 13.7 Hz, $J_2$ = 4.5 Hz), 2.00–2.07 m (3H), 2.24 d (1H, $J$ = 11.2 Hz), 2.34 d (1H, $J$ = 16.9 Hz, H-1a), 3.23 d (1H, $J$ = 16.9 Hz, H-1b), 3.877 s (3H, OMe), 3.884 s (3H, OMe), 5.34 t (1H, $J$ = 3.6 Hz, H-12), 8.17 s (1H, H-3'). $^{13}$C NMR ($CDCl_3$, 126 MHz) δ, ppm: 15.1, 17.0, 17.2, 20.5, 21.3, 23.3, 23.4, 23.6, 24.2, 28.1, 30.8, 31.3, 32.6, 34.7, 36.9, 39.0, 39.1, 39.3, 39.6, 42.3, 43.7, 45.9, 48.3, 52.79, 52.80, 52.9, 55.7, 124.1 q ($J_{C\text{-}F}$ = 274.0 Hz, $CF_3$), 125.8, 126.0 q ($J_{C\text{-}F}$ = 7.2 Hz), 127.7, 130.2 q ($J_{C\text{-}F}$ = 29.2 Hz), 138.1, 138.7, 140.6, 146.2, 165.6, 170.4, 183.9. $^{19}$F NMR ($CDCl_3$, 500 MHz) δ, ppm: 60.44 s ($CF_3$). HRMS (ESI): $C_{39}H_{50}F_3O_6$ found 671.3557 $[M-H]^+$; calcd. 671.3554.

#### 4.6.7. 19′β,28′-Epoxy [5,6-b]-18′α-oleanan-4-trifluoromethylphthalic acid dimethyl ester **9**

Compound **9** was prepared according to the general procedure in 2 stages: from phenylphthalimide **8** (55 mg; 0.08 mmol) and KOH (202 mg, 3.6 mmol) in MeOH/$H_2O$ (2 mL/0.2 mL); then $K_2CO_3$ (22 mg, 0.16 mmol), dimethyl sulfate (15 μL, 20 mg, 0.16 mmol) in acetone (1.5 mL). After purification (mobile phase hexane/EtOAc 7:1) compound **9** (31 mg; 59 %) was obtained as a white solid; mp 230–232 °C (hexane/EtOAc); $R_f$ 0.39 (silica gel, hexane/EtOAc, 7:1). IR (DRIFT): 2946 (C–H), 1741 (C═O), 1448, 1316, 1250, 1200, 1144, 1102, 1035, 798. $^1$H NMR ($CDCl_3$, 500 MHz) δ, ppm: 0.79 s (3H, Me), 0.81 s (3H, Me), 0.948 s (3H, Me), 0.95 s (3H, Me), 1.04 s (3H, Me), 1.34 s (3H, Me), 1.40 s (3H, Me), 1.65 dq (1H, $J_1$ = 12.6 Hz, $J_2$ = 2.8 Hz), 1.75 dq (1H, $J_1$ = 12.6 Hz, $J_2$ = 2.8 Hz), 2.31 d (1H, $J$ = 16.8 Hz, H-1a), 3.30 d (1H, $J$ = 16.8 Hz, H-1b), 3.46 d (1H, $J$ = 7.9 Hz, H-28a), 3.57 s (1H, H-19), 3.80 d (1H, $J$ = 7.9 Hz, H-28b), 3.88 s (6H, MeO), 8.17 s (1H, H-3'). $^{13}$C NMR ($CDCl_3$, 126 MHz) δ, ppm: 13.6, 15.5, 15.7, 20.4, 21.7, 22.9, 24.7, 26.4, 26.6, 26.7, 29.0, 31.4, 32.9, 33.1, 34.5, 34.9, 36.4, 36.9, 39.3, 40.6, 40.9, 41.7, 44.0, 46.9, 49.7, 52.77, 52.78, 55.7, 71.5, 88.1, 124.1 q ($J_{C\text{-}F}$ = 274.9 Hz, $CF_3$), 126.0 q ($J_{C\text{-}F}$ = 6.7 Hz), 127.6, 130.3 q ($J_{C\text{-}F}$ = 29.7 Hz), 138.6, 140.8, 146.3, 165.6, 170.5. $^{19}$F NMR ($CDCl_3$, 500 MHz) δ, ppm: 60.59 s ($CF_3$). HRMS (ESI): $C_{39}H_{54}F_3O_5$ found 659.3919 $[M+H]^+$; calcd. 659.3918.

### 4.7. Biological evaluation

#### 4.7.1. Cell culture and MTS cytotoxicity assay

Cytotoxicity screening was done according to the routine protocol developed earlier at our department [28,31,41,43].

#### 4.7.2. Pharmacological parameters

Detailed protocol for the screening of pharmacological parameters was described in our earlier work [28].

#### 4.7.3. Annexin V assay

We utilized the Annexin kit from Exbio, adhering to the

recommended protocol with minor modifications. Briefly, cells were diluted to the appropriate concentration, washed with Annexin V binding buffer, and stained with Annexin V-FITC and propidium iodide. We used only half of the recommended volume of propidium iodide, as the suggested concentration induced acute toxicity in CCRF-CEM cells. Cells were incubated for 15 min at room temperature in the dark, centrifuged and resuspended in 100 μl of Annexin V binding buffer. Samples were immediately analyzed by a FACSAria II flow cytometer (Becton Dickinson), acquiring at least 10,000 cells per sample.

#### 4.7.4. JC-1 mitochondrial membrane potential assay

Mitochondrial membrane potential was assessed using the membrane-permeant JC-1 cationic probe. CCRF-CEM cells were treated with specific compounds at 1 x $IC_{50}$ and 5 x $IC_{50}$ concentration for 24 h. Cell suspension at a density of $0.5 \times 10^6$ cells/mL was labeled with JC-1 at a final concentration of 1 μM for 10 min. For the control tube, CCCP at a final concentration of 50 μM was added 5 min prior to JC-1. Labeled cells were pelleted by centrifugation (1500 rpm, 5 min, room temperature), resuspended in 0.5 ml of PBS, and immediately analyzed by a FACSAria II flow cytometer (Becton Dickinson) with excitation by a 488 nm laser. JC-1 monomers and J-aggregates were detected separately using emission filters appropriate for emission peaks at 529 nm (monomers) and 590 nm (aggregates), with at least 10,000 cells acquired for each sample.

#### 4.7.5. Cell cycle and DNA/RNA synthesis analysis

The detailed protocol for cell cycle analysis as well as DNA/RNA synthesis analysis is described in our previous work [44].

#### 4.7.6. Western blot

CCRF-CEM cells were treated with derivatives **1f**, **2f**, **1h**, **2h**, **3h**, and **1l** at 1 × $IC_{50}$ and 5 × $IC_{50}$ concentrations for 24 h. Cells were washed with ice-cold PBS and lysed in RIPA buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1% NP-40, 0.5 % sodium deoxycholate, 0.1% SDS, 1 mM EDTA) supplemented with cOmplete™ Protease and Phosphatase Inhibitor Cocktails (Roche). Lysis occurred on ice for 30 min with occasional vortexing. Subsequently, cell lysates were clarified by centrifugation (15,000 g, 20 min, 4 °C) and protein concentration was determined using the Pierce™ BCA Protein Assay Kit (Thermo Scientific). Aliquots containing 20 μg of total cellular proteins were denatured in Laemmli buffer (50 mM DTT, 0.06% bromophenol blue, 47% glycerol, 12% SDS, 0.5 M Tris pH 6.8) and separated by SDS-polyacrylamide gel electrophoresis. Proteins were then transferred from the gel onto a 0.2 μM pore-size nitrocellulose membrane using the Trans-Blot® Turbo™ Transfer System (Bio-Rad). Following blocking (5% BSA/TBS/0.1% Tween 20) for 1 h, membranes were incubated with primary antibodies against Caspase-3, STAT3, PARP (all from Cell Signaling Technology), Bcl-2, Bcl-XL (both from Abcam), and β-actin (Sigma Aldrich) overnight at 4 °C. Washed (TBS/0.1% Tween 20) membranes were incubated with an appropriate HRP-conjugated secondary antibody (Sigma Aldrich) for 1 h at room temperature. The chemiluminescence signal was developed using ECL Prime (Amersham) reagent and detected by the Li-cor Odyssey (LI-COR Biotechnology) imaging system.

## CRediT authorship contribution statement

**Anna Kazakova:** Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Ivo Frydrych:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nikola Jakubcová:** Visualization, Validation, Methodology, Data curation. **Jan Pokorný:** Writing – review & editing, Validation, Methodology, Conceptualization. **Barbora Lišková:** Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation. **Soňa Gurská:** Visualization, Methodology, Data curation. **Petr Džubák:** Writing – review & editing, Validation, Methodology, Formal analysis. **Marián Hajdúch:** Supervision, Funding acquisition, Conceptualization. **Milan Urban:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

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

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

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