European Journal of Medicinal Chemistry 283 (2025) 117126

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: www.elsevier.com/locate/ejmech

Research paper

Triterpenoid phthalimides as selective anti-cancer agents targeting mitochondrial apoptosis

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ARTICLE INFO

Keywords:
Triterpenoids
Betulin
Betulinic acid
Wittig reaction
Diels-Alder reaction
Phthalates
Apoptosis
Cancer
Mitochondria
Cell cycle regulation

ABSTRACT

Starting from benzyl 30-oxobetulinate and 30-oxobetulin diacetate, substituted dienes were synthesized and subjected to Diels-Alder reaction, yielding a variety of triterpenoid phthalates, phthalimides, and related derivatives. A total of 55 new compounds were prepared and tested for *in vitro* cytotoxic activity against eight cancer cell lines and two non-cancerous cell lines. Four compounds with IC_{50} values of 5 μM or lower were selected for further investigation. These compounds induced apoptosis in CCRF-CEM cells in a concentration-dependent manner, accompanied by mitochondrial depolarization and altered expression of key proteins involved in mitochondrial apoptosis. The compounds also disrupted DNA replication and transcriptional activity. Modulation of key proliferation pathways, including PI3K/Akt and STAT3, further supported the anti-proliferative potential of these derivatives. Considering their high cytotoxicity and antiproliferative activity in CCRF-CEM cells, compounds **19**, **26**, **28**, and **30** have been identified as promising candidates for further development.

1. Introduction

Pentacyclic triterpenoids are natural compounds abundant in plants, many of which exhibit interesting biological activity [1–3]. Our research predominantly focuses on lupane derivatives, such as betulinic acid (BA) and betulin, which show significant cytotoxicity, selectively targeting cancer cells [4–12]. The significance of BA and its derivatives as potential drug candidates may be demonstrated by following literature overview. In 2018, we published a review article summarizing all current data about the synthesis and biological activities of semisynthetic derivatives of BA modified at the A-ring [13]. Despite such a narrow focus, our review contained over 40 citations of original articles dealing with numerous ways to optimize the structures to improve a variety of biological activities. As more contemporary literature demonstrates, the interest has not passed yet. In 2021, a minireview was published that summarizes the literature about progress of BA derivatives in the development of potential anti-tumor agents [14]. This minireview is focused on a variety of positions in the betulinic acid structure to be accessible for chemical modifications in order to find the optimum cytostatics. Another review was more focused on the mechanism of action of BA derivatives [15]. Based on the research content within about 100 primary articles, it reveals that the majority of them is in agreement that BA derivatives mostly induce oxidative stress in mitochondria and/or interact with various molecular targets within significant signalling pathways which usually triggers cell death in cancer cells [15]. The authors also highlight the advantage of the use of BA derivatives co-treatment with other anti-tumor drugs and discuss enhancement of bioavailability by using nanoliposomes as delivery system [15]. More information about chemical modifications, biological activities, formulations, and mechanism of action of a number of new BA derivatives may be found in other recent literature [16–23]. Among all of the BA derivatives, the compounds containing heterocyclic moieties are

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https://doi.org/10.1016/j.ejmech.2024.117126
Received 1 November 2024; Received in revised form 29 November 2024; Accepted 29 November 2024
Available online 10 December 2024

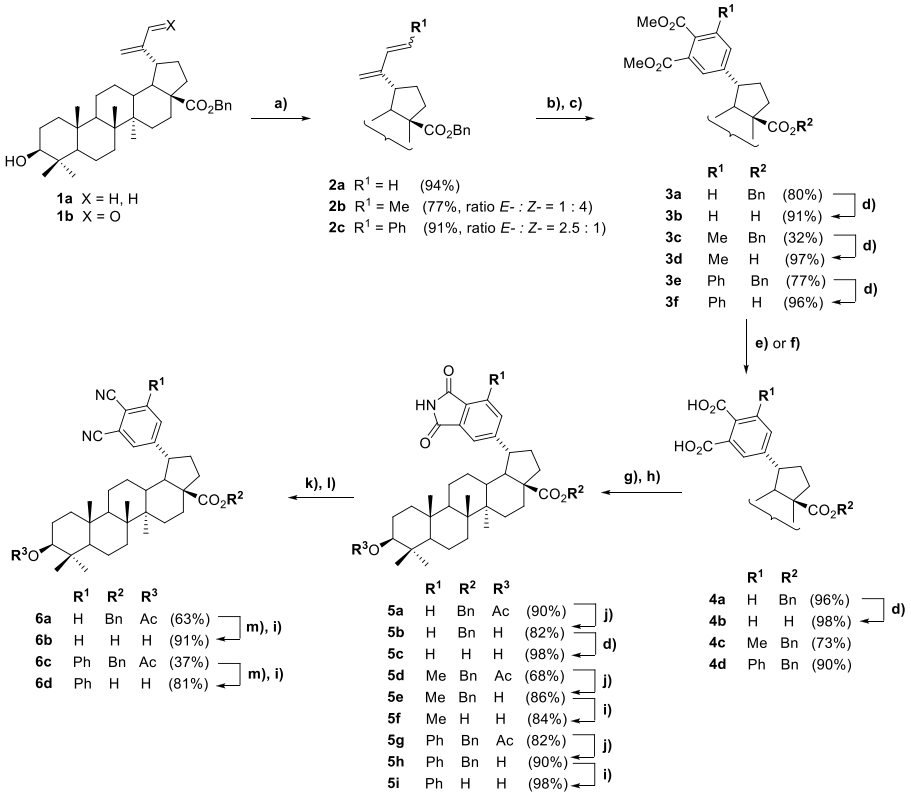
particularly promising, many of them with IC_{50} values around or below 1 μM and some of them effectively targeting resistant cancer cell lines [24–27]. Betulinic acid is also interesting from the chemical point of view. Unlike the majority of other triterpenoids, it contains isopropenyl moiety connected to the E-ring which may undergo a variety of chemical modifications. This may open many new options for the optimization of its biological activities. The allylic position (C-30) of BA may be easily oxidized into an aldehyde to obtain 30-oxobetulinic acid, which is a non-selective cytotoxic compound [28]. In our recent work, we used the Wittig reaction to prepare conjugated dienes from 30-oxobetulinic acid, reducing toxicity in healthy cell lines while retaining strong cytotoxicity against cancer cells [29]. These dienes, however, are not only interesting for their selective cytotoxicity in cancer cells but also as reagents in the Diels-Alder reaction. The primary aim of this work was to explore the possibilities of the use of Diels-Alder reaction between lupane-based dienes and dimethyl acetylenedicarboxylate, with the possibility for further derivatization of the resulting products. This approach yielded a library of substituted phthalates, phthalimides, substituted phthalimides and aromatic dinitriles, which were subsequently tested for biological activity. Such modifications have not been described in the chemistry of lupane triterpenoids to date. There are several reasons that motivated us to perform the following work. First, we were curious about the reactivity of the earlier prepared dienes [29] under the conditions for Diels-Alder reaction. Second, this approach would allow for the preparation of a new type of aromatic and/or heterocyclic derivatives of BA, where the new moiety would be connected to the E-ring by C–C bond and not by ester, ether and similarly labile bonds. In general, lupane derivatives containing a phthalate substituent are well known from the literature[30–36], they often show significant anti-cancer activity and in some cases even hepatoprotective activity. In all these cases, the phthalate moiety is connected to the terpenoid through one of its carboxyl/hydroxyl groups. Our project is new because of the new arrangement at the E-ring substituent, where the phthalate becomes the part of the triterpenoid skeleton replacing the original isopropenyl moiety. When the phthalate part was successfully added to the triterpenoid, more options for chemical modifications have opened. The synthesis of phthalimides was chosen because it was a simple chemical reaction and because phthalimide-substituted triterpenoids are far less common in the literature than triterpenoids substituted with phthtalates.

In the literature, there are only seven records mentioning lupane or oleanane derivatives with a phthalimide ring, including two patents [37–43]. In most cases, these compounds are merely intermediates for introducing an amino group into the molecule, and the resulting derivatives have not been evaluated for any pharmacological activities. In the article [39], only one derivative is reported to have been tested as a TGR45 agonist, but it showed no activity. In our recent work, we prepared several triterpenoids with a phthalimide ring condensed to their A-ring and among them some compounds were selective cytotoxic against cancer cells at low micromolar concentration with good selectivity [43]. This fact deepened our interest in the synthesis and evaluation of new triterpenic molecules with a phthalimide ring connected to the E-ring of lupane skeleton. Last not least, the stability of the phthalimide moiety within various pH is high but there are studies that these compounds may metabolize by splitting the imide ring yielding phthalamic acid [44,45]. Triterpenoid nitriles are more common in the literature and often show interesting biological activities [46–49]. Bardoxolone methyl is probably the most important triterpenoid nitrile [50]. The compound is nephroprotective at low micromolar concentrations and also anti-tumor [51]. Its mechanism of action combines the anti-inflammatory effects through suppression of NF-kB and activation of transcription of antioxidant and anti-inflammatory genes such as Nrf2 [51,52]. Despite that, some later studies pointed at its potential chronic liver toxicity, even questioning its positive effect on kidneys [52]. The toxicity of Bardoxolone methyl is likely associated with the presence of Michael acceptor C═C in the neighborhood of the nitrile group. In our previous study, derivatives containing a CN group demonstrated comparable IC_{50} values on cancer cell lines to the same molecule without the CN group, while showing no toxicity to healthy cells [53]. Despite that, in case of high activity and potential of further development of triterpenic nitriles as anti-cancer drugs, potential toxicity and side-effect must be taken into account.

2. Results and discussion

2.1. Chemistry

During the first step, benzyl ester of betulinic acid (**1a**) was transformed to its 30-oxoderivative **1b**, then Wittig reaction was applied according to our previous work to synthesize dienes **2a**–**2c** (Scheme 1) [29]. Dienes **2a**–**2c** were found to be excellent substrates for the Diels-Alder reaction with dimethyl acetylenedicarboxylate, which yielded isomers of substituted cyclohexadienes (not isolated) that were immediately oxidized with $KMnO_4$ to corresponding phthalates **3a**, **3c**, and **3e** according to the procedure in lit [54]. Treatment of these compounds with LiOH or KOH gave the free phthalic acids **4a**, **4c**, and **4d**. Subsequent reaction of **4a**, **4c**, and **4d** with acetic anhydride followed by the treatment with urea at 170 °C according to Refs. [55,56] yielded phthalimides **5a**, **5d**, and **5g**. To this point of Scheme 1, the reaction pathway went smoothly and only modest optimizations gave high yields of desired target compounds. The last step towards dinitriles **6a** and **6c** according to Ref. [57] was the crux of this pathway and only gave moderate yields of 63 % or 37 % despite many attempts for the optimization. The reason was that in all cases many side-product formed during the reaction with oxalyl chloride even when choosing various combination of solvents and low temperature. Optimized reaction that produced the highest yield is shown. For this reason, the last step was only performed with **5a** and **5g** (Scheme 1). Since benzyl ester and acetate were used as protecting groups, further attention was paid to the synthesis of the free acids **3b**, **3d**, **3f**, **4b**, **5c**, **5f**, **5i**, **6b**, and **6d**. Compounds **3b**, **3d**, **3f**, **4b**, and **5c** were obtained by catalytic debenzylation of parent benzyl esters **3a**, **3c**, **3e**, **4a**, and **5b** using H_2 and Pd/C. However, this simple method failed during the deprotection of other benzyl esters **5e**, **5h**, **6a**, and **6c**, probably due to the low solubility of the product in the solvent mixture used, which inactivated the catalyst. Therefore as an alternative method, catalytic debenzylation using 1, 3-cyclohexadiene as the source of hydrogen was used and with temperature elevated to 50 °C [58], the products **5f**, **5i**, **6b**, and **6d** were obtained in reasonable yields of 81–91 %. The acetates from compounds **5a**, **5d**, and **5g** were removed under acidic conditions using HCl in MeOH/THF, however, for compounds **6a** and **6c**, milder conditions using TsOH in MeOH had to be used, since HCl caused the decomposition of the starting material (Scheme 1) [59,60]. Since the phthalimide moiety offers another functionality for possible modification, a small set of compounds was prepared from **5a** and **5g** by their reaction with acetic anhydride and appropriate amine at elevated temperature (Scheme 2) [56,61]. As the result, compounds **7**, **9**, **11**, **13**, **15**, **17**, **18**, **20**, **22**, **24**, **27**, and **29**, modified at their nitrogen were obtained. Again, the following step was the deprotection of the benzyl- and acetyl-protecting group and based on the substrate, HCl in MeOH/THF or TsOH in MeOH/THF was used for the removal of acetate, while 1,3-cyclohexadiene with Pd/C was used for the deprotection of benzyls (Scheme 2). A small set of similar compounds was prepared from betulin **31** using the same procedures (Scheme 3) to obtain molecules **32**–**40**. This was motivated by our curiosity about how the cytotoxic activity would compare between the derivatives of betulinic acid **3b**–**30** and betulin derivatives **34a**–**40** but since these compounds were cytotoxic in higher concentration levels, their count has not been expanded to a larger library.



Scheme 1. Reagents and conditions: a) $R^1P^+Ph_3Hal^-$, *t*-BuOK, THF, 0–20 °C, 4h; **b**) dimethyl acetylenedicarboxylate, PhMe, 80–90 °C, 12 h; **c)** $KMnO_4/Al_2O_3$, acetone, −10 to −20 °C, 1–2 h; **d)** H_2, Pd/C, THF/MeOH, 20 °C, 13–24 h; **e)** LiOH, MeOH/THF/H_2O, 20 °C, 24 h; **f)** KOH, MeOH, reflux, 5 h; **g)** Ac_2O, 160 °C, 5 h; **h)** $(NH_2)_2CO$, 170 °C, 2 h; **i)** Pd/C, 1,3-cyclohexadiene, EtOH/THF, 50 °C, 14 h; **j)** 3 N HCl in MeOH, THF, 20 °C, 24 h; **k)** 7 N NH_3 in MeOH, 20 °C, 48–96 h; **l)** $(ClCO)_2$, DMF, MeCN, Pyr, 0 °C, 1.5 h; **m)** 0.5 eq *p*-TsOH·H_2O, MeOH, 90 °C, 10 h.

2.2. Biology

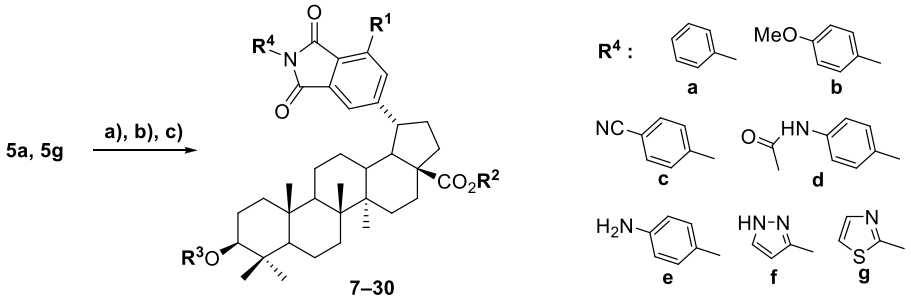
2.2.1. Cytotoxicity assay

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 assay (Table 1) [62]. The rationale for selecting these cancer cell lines is detailed in Borková et al. [4] The cell lines included T-lymphoblastic leukemia (CCRF-CEM), myeloid leukemia (K562) and their multidrug-resistant analogs (CEM-DNR, K562-TAX), as well as solid tumors such as lung (A549) and colon (HCT116, HCT116p53−/−) carcinomas and osteosarcoma (U2OS). For comparison, two non-cancer human fibroblast lines (BJ, MRC-5) were also tested. The majority of compounds exhibited moderate cytotoxic activity in the CCRF-CEM cell line, with IC_{50} values ranging from 5 to 20 μM. Among these, compounds **19**, **26**, **28**, and **30** showed the highest cytotoxic activity in this cell line, with IC_{50} values of 5.18, 4.09, 5.13, and 2.52 μM, respectively. The high selectivity of these compounds toward cancer cells was also demonstrated by their Selectivity Index (SI, Table 2), with compound **30** showing an impressive SI of 11.82, indicating strong selectivity for cancer cells over non-cancerous fibroblasts. Compounds **19**, **26** and **28** also demonstrated favorable selectivity with SI values of >9.70, 7.33 and 5.30, respectively, making them candidates for further evaluation of their mechanism of action. All these compounds **19**, **26**, **28**, and **30** have a free 28-COOH group and a substituted phthalimide attached to the E-ring (in place of naturally occurring isopropenyl moiety). It seems important that the phenyl substituent at position 7 of the aromatic phthalimide ring is crucial for activity. On the other hand, the presence or absence of acetate at position 3 of the terpene, or various substituents at the phthalimide nitrogen, did not significantly affect activity.

Compound **3f** also exhibited notable cytotoxic activity, with IC_{50} values around or below 5 μM in several cancer cell lines, including CEM-DNR (4.85 μM), K562-TAX (4.36 μM), and A549 (4.44 μM), suggesting its potential for further investigation, particularly in resistant cancer types. Although compound **3f** showed activity in resistant cell lines, its SI was lower compared to other leading compounds, indicating a lower degree of selectivity. Additionally, compound **26** demonstrated significant activity in the K562-TAX cell line with an IC_{50} of 4.34 μM.

2.2.2. Pharmacological parameters

The most promising compounds **19**, **26**, **28**, and **30** were selected to evaluate fundamental pharmacokinetic parameters such as absorption, distribution, metabolism, and excretion (ADME, Table 3). Knowledge of the ADME properties of new chemical entities is crucial in their evaluation as potential leading candidates for drug development. Our tested compounds were incubated with human plasma *in vitro* to assess their stability and half-life, both critical for maintaining therapeutic drug levels. All compounds demonstrated high stability, retaining 85 %–100 % of the original substance after 2 h of incubation. This suggests that these compounds may have favorable pharmacological profiles for maintaining therapeutic plasma concentrations. Plasma protein binding is a key factor affecting a drugs bioavailability, distribution, and clearance. The tested compounds exhibited a moderate to high level of plasma protein binding, with values ranging from 57 % to 88 %. Specifically, compounds **19** and **28** displayed the highest binding affinity (77 %, 88 %, respectively), which could influence their distribution and efficacy. In contrast, compound **30** showed the lowest binding (57 %), which might suggest faster systemic clearance but possibly greater bioavailability. Drug metabolism, particularly by liver enzymes, is another critical parameter for drug development. Liver microsomes stability assays, which assess metabolism by the cytochrome P450 system (phase I enzymes), revealed that all compounds had low intrinsic



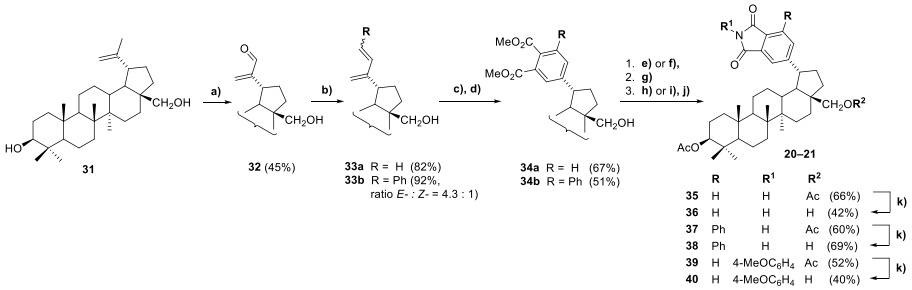
7–17 R^1 = H

	R^1	R^2	R^3	R^4		
7	H	Bn	Ac	a	(61%)	d), f)
8	H	H	H	a	(59%)	
9	H	Bn	Ac	b	(50%)	d), f)
10	H	H	H	b	(63%)	
11	H	Bn	Ac	c	(62%)	e), f)
12	H	H	H	c	(37%)	
13	H	Bn	Ac	d	(47%)	f)
14	H	H	Ac	d	(84%)	
15	H	Bn	Ac	f	(89%)	f)
16	H	H	Ac	f	(76%)	
17	H	Bn	Ac	g	(63%)	

18–30 R^1 = Ph

	R^1	R^2	R^3	R^4		
18	Ph	Bn	Ac	a	(60%)	d), f)
19	Ph	H	H	a	(61%)	
20	Ph	Bn	Ac	b	(60%)	e), f)
21	Ph	H	H	b	(72%)	
22	Ph	Bn	Ac	c	(57%)	e), f)
23	Ph	H	H	c	(49%)	
24	Ph	Bn	Ac	d	(57%)	f), d)
25	Ph	H	Ac	d	(89%)	
26	Ph	H	H	e	(56%)	
27	Ph	Bn	Ac	f	(82%)	f)
28	Ph	H	Ac	f	(79%)	
29	Ph	Bn	Ac	g	(76%)	f)
30	Ph	H	Ac	g	(39%)	

Scheme 2. Reagents and conditions: **a)** Ac_2O, 160 °C, 5 h; **b)** R^4NH_2, THF, 80 °C, 2–4 h; **c)** 170 °C, 2 h; **d)** 3 N HCl in MeOH, THF, 20 °C, 2 h; **e)** *p*-TsOH·H_2O, MeOH, THF, 20 °C, 192 h; **f)** Pd/C, 1,3-cyclohexadiene, EtOH/THF, 50 °C, 13 h.



Scheme 3. Reagents and conditions: **a)** SeO_2, 2-methoxyethanol, 140 °C, 4 h; **b)** $Ph_3P^+CH_2RBr^-$, *t*-BuOK, THF, 20 °C, 4 h; **c)** dimethyl acetylenedicarboxylate, PhMe, THF, 90 °C, 14 h; **d)** $KMnO_4/Al_2O_3$, acetone, 10 °C, 1–2 h; **e)** LiOH·H_2O, THF/MeOH/H_2O, 24 h, 20 °C; **f)** KOH, MeOH, reflux, 4 h; **g)** Ac_2O, 160 °C, 5 h; **h)** $(NH_2)_2CO$, 170 °C, 2 h; **i)** 4-$MeOC_6H_4NH_2$, THF, 50 °C, 3 h; **j)** 170 °C, 2 h; **k)** 6 N HCl in *i*-PrOH, THF, 20 °C, 2–4 d.

clearance. This indicates, that the compounds are metabolicaly stable and not rapidly degraded by hepatic enzymes, a favorable property for maintaining effective drug concentrations over time. Permeability studies using the Parallel Artificial Membrane Permeability Assay (PAMPA) indicated that all tested derivatives had low passive permeability ($-\log P_{app} > 6$ cm/s). This result suggests that these compounds likely use alternative transport mechanisms, such as active transport, to enter cells. This finding is significant as it shows potential for specific targeting or reduced off-target effects.

2.2.3. *Compound derivatives **19**, **26**, **28**, and **30** induce apoptotic cell death in CCRF-CEM cells*

Based on the IC_{50} values below 5 μM observed in the highly sensitive CCRF-CEM cells, we predicted strong anti-tumor activity mediated by compounds **19**, **26**, **28**, and **30**. To further characterize mechanism of cytotoxic action, we used flow cytometry to analyze cell death by using Annexin V and propidium iodide double staining. The main advantage of Annexin V-FITC staining over the other methods commonly used for cell death analysis lies in its ability to recognize early apoptotic cells *via* detection of phosphatidylserine externalization on the cytoplasmic surface of the cell membrane. On the other hand, propidium iodide staining determines cell membrane integrity for identifying late apoptotic or necrotic cells. According to the results (Fig. 1), a high percentage of viable cells was observed in the control (untreated) sample, while a shift from viable to early apoptotic or late apoptotic cells was observed in CCRF-CEM cells treated with compounds **19**, **26**, **28**, and **30** for 24 h (Fig. 1). A significant increment in the population of early and late apoptotic/necrotic cells was primarily induced by the derivatives at 5 × IC_{50} concentrations (Fig. 1). These results show that all four derivatives effectively induce apoptosis in CCRF-CEM cells in a concentration-dependent manner.

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

Comp.	IC_{50} (μmol/L)[a]									
	CCRF-CEM	CEM-DNR	HCT116	HCT116p53-	K562	K562-TAX	A549	U2OS	BJ	MRC-5
3b	14,13 ± 2,73	11,48 ± 1,63	16,58 ± 1,55	16,21 ± 1,52	13,77 ± 3,04	11,46 ± 0,86	11,05 ± 1,57	19,60 ± 0,91	31,09 ± 2,37	25,3 ± 1,56
3d	14,01 ± 1,74	6,78 ± 1,50	16,33 ± 3,54	16,12 ± 2,90	12,07 ± 1,78	7,21 ± 1,33	10,38 ± 1,26	18,98 ± 2,38	32,99 ± 0,93	32,42 ± 1,01
3f	6,30 ± 0,96	4,85 ± 0,88	5,46 ± 0,37	6,17 ± 1,07	6,12 ± 0,75	4,36 ± 1,25	4,44 ± 0,80	9,94 ± 0,78	36,27 ± 1,34	30,15 ± 6,00
4b	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
5c	26,28 ± 3,78	20,7 ± 5,07	31,09 ± 4,52	34,94 ± 6,35	35,34 ± 4,15	17,28 ± 4,92	26,59 ± 2,99	38,52 ± 6,13	>50	>50
5f	7,97 ± 2,19	8,76 ± 1,62	25,87 ± 2,62	27,17 ± 6,60	>50	7,20 ± 1,29	>50	24,84 ± 6,31	>50	34,32 ± 1,52
5i	7,18 ± 0,85	9,65 ± 0,72	7,22 ± 1,11	7,43 ± 1,18	7,58 ± 1,45	10,40 ± 1,74	6,39 ± 1,57	9,82 ± 1,50	34,51 ± 3,09	32,40 ± 2,84
6b	5,90 ± 0,55	5,23 ± 1,21	23,73 ± 2,97	31,78 ± 4,81	30,03 ± 1,67	5,08 ± 1,49	19,95 ± 3,43	20,03 ± 2,84	35,45 ± 1,94	25,32 ± 2,93
6d	5,99 ± 0,78	13,74 ± 3,08	26,03 ± 2,84	31,57 ± 2,36	27,83 ± 6,77	7,00 ± 1,94	20,73 ± 1,73	19,17 ± 2,41	36,22 ± 3,65	25,69 ± 2,55
8	7,93 ± 1,41	19,89 ± 4,27	31,11 ± 3,04	37,83 ± 5,58	>50	14,26 ± 3,29	34,22 ± 6,70	24,88 ± 0,79	>50	30,99 ± 0,63
10	11,10±+2,22	9,85 ± 2,62	>50	>50	>50	5,35 ± 0,92	28,1 ± 7,38	27,51±,3,82	>50	>50
12	19,79 ± 2,14	27,27 ± 6,11	>50	>50	>50	12,67 ± 3,42	>50	>50	>50	>50
14	16,48 ± 2,84	20,76 ± 4,38	24,41 ± 1,59	29,56 ± 4,69	27,09 ± 6,20	18,18 ± 2,18	27,08 ± 3,28	24,29 ± 1,70	31,17 ± 1,42	19,82 ± 1,44
16	18,71 ± 2,09	25,42 ± 3,17	24,01 ± 3,19	30,06 ± 1,72	29,35 ± 5,54	21,06 ± 3,41	27,09 ± 4,54	26,18 ± 1,88	29,97 ± 3,62	28,29 ± 1,93
19	**5,18 ± 0,79**	19,96 ± 4,37	29,36 ± 4,23	>50	>50	23,06 ± 1,82	>50	25,95 ± 6,23	>50	>50
21	13,56 ± 2,41	>50	>50	>50	>50	27,85 ± 4,86	>50	>50	>50	>50
23	14,18 ± 2,14	>50	>50	>50	>50	>50	>50	>50	>50	>50
25	8,08 ± 1,24	>50	16,78 ± 1,79	28,44 ± 6,87	27,91 ± 2,68	>50	34,63 ± 7,26	18,81 ± 4,54	>50	33,7 ± 6,68
26	**4,09 ± 0,47**	6,29 ± 1,57	12,00 ± 1,98	17,14 ± 1,71	20,86 ± 2,74	4,34 ± 0,39	21,02 ± 4,16	15,56 ± 1,19	40,58 ± 2,40	19,36 ± 0,63
28	**5,13 ± 0,52**	11,49 ± 2,83	9,54 ± 0,53	13,69 + 1,62	14,5 ± 3,42	13,28 ± 4,08	18,87 ± 2,63	13,8 ± 3,16	31,8 ± 0,74	22,62 ± 1,83
30	**2,52 ± 0,42**	30,51 ± 5,36	13,11 ± 1,37	18,57 ± 3,63	21,35 ± 3,63	24,46 ± 3,34	20,80 ± 3,52	16,81 ± 3,87	31,74 ± 1,01	27,81 ± 3,78
34a	>50	19,72 ± 4,67	>50	>50	>50	10,33 ± 2,23	>50	>50	>50	>50
34b	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
35	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
36	13,97 ± 2,78	9,21 ± 2,41	19,68 ± 5,32	21,76 ± 6,98	36,04 ± 7,72	7,43 ± 1,77	>50	34,45 ± 5,10	>50	28,48 ± 3,51
37	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
38	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
39	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
40	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

[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. Benzyl ester intermediates did not fully dissolve under the experimental conditions, and thus, their cytotoxicity was not measured.

Table 2
Selectivity index calculated for each compound and each cancer cell line.

Comp.	SI							
	CCRF-CEM	CEM-DNR	HCT116	HCT116p53-	K562	K562-TAX	A549	U2OS
3b	2.00	2.46	1.70	1.74	2.05	2.46	2.55	1.44
3d	2.33	4.82	2.00	2.03	2.71	4.54	3.15	1.72
3f	5.27	6.85	6.08	5.38	5.43	7.62	7.48	3.34
4b	>1	>1	>1	>1	>1	>1	>1	>1
5c	>1,9	>2,42	>1,61	>1,43	>1,41	>2,89	>1,88	>1,3
5f	>5,29	>4,81	>1,63	>1,55	<1	>5,86	<1	>1,7
5i	4.66	3.47	4.63	4.50	4.41	3.22	5.24	3.41
6b	5.15	5.81	1.28	0.96	1.01	5.98	1.52	1.52
6d	5.17	2.25	1.19	0.98	1.11	4.42	1.49	1.61
8	>5,11	>2,04	>1,3	>1,07	<1	>2,84	>1,18	>1,63
10	>4,50	>5,08	>1	>1	>1	>9,35	>1,78	>1,82
12	>2,53	>1,83	>1	>1	>1	>3,95	>1	>1
14	1.55	1.23	1.04	0.86	0.94	1.40	0.94	1.05
16	1.56	1.15	1.21	0.97	0.99	1.38	1.08	1.11
19	>9,65	>2,51	>1,7	>1	>1	>2,17	>1	>1,93
21	>3,69	>1	>1	>1	>1	>1,8	>1	>1
23	>3,53	>1	>1	>1	>1	>1	>1	>1
25	>5,18	<1	>2,49	>1,47	>1,5	<1	>1,21	>2,22
26	7.33	4.76	2.50	1.75	1.44	6.91	1.43	1.93
28	5.30	2.37	2.85	1.99	1.88	2.05	1.44	1.97
30	11.82	0.98	2.27	1.60	1.39	1.22	1.43	1.77
34a	>1	>2,54	>1	>1	>1	>4,84	>1	>1
34b	>1	>1	>1	>1	>1	>1	>1	>1
35	>1	>1	>1	>1	>1	>1	>1	>1
36	>2,81	>4,26	>1,99	>1,8	>1,09	>5,28	<1	>1,14
37	>1	>1	>1	>1	>1	>1	>1	>1
38	>1	>1	>1	>1	>1	>1	>1	>1
39	>1	>1	>1	>1	>1	>1	>1	>1
40	>1	>1	>1	>1	>1	>1	>1	>1

[b] The therapeutic index is calculated based on the IC_{50} for each cancer line versus the average IC_{50} for both fibroblast lines.

Table 3
Pharmacological parameters of compounds **19**, **26**, **28**, and **30**.

Compound	Plasma stability				Plasma protein binding	PAMPA	
	% Compound remaining						
	15min	30	60	120	% Fraction bound	log Pe	Category[b]
19	96	95	100	89	76.85	−8.22	Low
26	106	92	93	89	62.96	−8.94	Low
28	97	91	92	86	87.88	−8.82	Low
30	98	95	96	85	57.49	−8.47	Low

Compound	Microsomal stability			Microsomal stability
	% Compound remaining			Category of Intrinsic clearence[a]
	15 min	30	60	
19	92	87	79	Low
26	100	102	97	Low
28	99	99	81	Low
30	103	104	98	Low

[a,b] References, [63,64] the error deviations for all experiments are within a range of less than 10 %. All experiments were performed in triplicate, except for cell-based permeability assays, which were performed in duplicate.

2.2.4. *Compound derivatives **19, 26, 28,** and **30** alter mitochondrial membrane potential ($\Delta\Psi_m$) in CCRF-CEM cells*

It is well documented, that apoptotic signaling is partially mediated by the mitochondria in response to various stress stimuli, such as DNA damage, the lack of growth factors, oxidative stress or chemical agents [65,66]. At the mitochondrial level, these stimuli can lead to dysfunction manifested by impaired membrane potential, reduced oxidative phosphorylation (OXPHOS), and reduced ATP production, which initiates mitochondrial apoptotic signaling [67]. As the changes of mitochondrial transmembrane potential are a significant hallmark of apoptosis, we studied the effect of **19**, **26**, **28**, and **30** on this parameter. Mitochondrial membrane potential was analyzed by staining the cells with JC-1 (5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide) dye, which allows detection of alterations in mitochondrial membrane potential. The dye emits different intensities of green and red fluorescence depending on the mitochondria depolarization status. In healthy cells, JC-1 accumulates in the mitochondria in the form of J-aggregates, emitting red fluorescence. However, in apoptotic cells with low $\Delta\Psi_m$, the dye remains in its monomeric form, emitting green fluorescence. To investigate whether derivatives **19**, **26**, **28**, and **30** affect $\Delta\Psi_m$, CCRF-CEM cells were treated with concentrations corresponding to 1 × IC_{50} and 5 × IC_{50} for 24 h, stained with JC-1, and analyzed by flow cytometry. As shown in Fig. 2, a substantial, concentration-dependent increase of low $\Delta\Psi_m$ cells population was detected following derivatives treatment. We observed the most pronounced mitochondria depolarization following treatment with **26** and **28** at 5 × IC_{50}, inducing more than 20-fold increase in the fraction of cells with low $\Delta\Psi_m$ compared to the untreated controls. These results

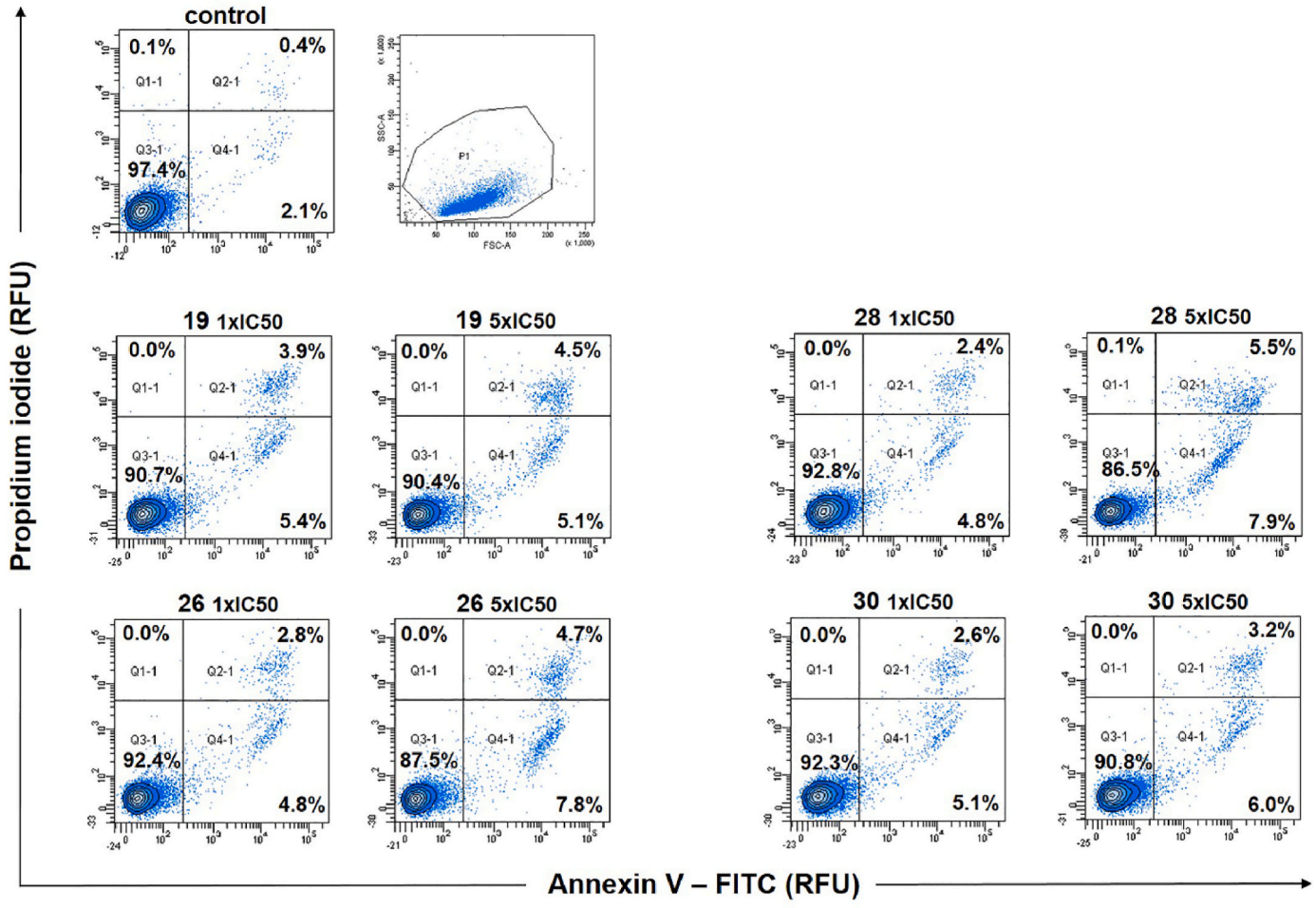


Fig. 1. Flow cytometric analysis of Annexin V-FITC and Propidium iodide staining. Dead cells and debris were excluded from the analysis, and cells from P1 region (upper right picture) were projected by contour plots of propidium iodide versus Annexin V-FITC. Then, contour plots were divided into four quadrants: viable cells (Q3-1), early apoptotic cells (Q4-1), late apoptotic/necrotic (Q2-1) and necrotic cells (Q1-1), respectively. Samples were measured on FACSAria II flow cytometer using a 488 nm laser. At least 10,000 cells were acquired per sample. Picture shows representative contour plots of three independent experiments.

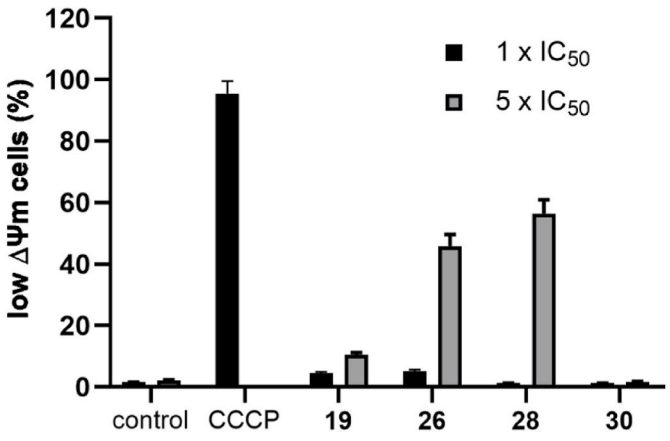


Fig. 2. Quantitative analysis of mitochondrial membrane potential depolarization in CCRF-CEM cells treated with **19**, **26**, **28**, and **30** at 1 × IC_{50} or 5 × IC_{50} concentration for 24 h.

clearly demonstrate the involvement of mitochondria-mediated apoptosis in CCRF-CEM cells treated by **19**, **26**, **28**, and **30**.

Mitochondrial membrane potential was analyzed using a FACSAria II flow cytometer with the JC-1 fluorescent probe. Untreated cells were served as a negative control, while cells treated with 100 μM CCCP were used as a positive control. The bar graph shows the percentage of cells with low $\Delta\Psi_m$. Error bars represent ± SEM (standard error of the mean) of three independent experiments.

*2.2.5. Effect of **19**, **26**, **28**, and **30** on cell cycle modulation and DNA/RNA synthesis in CCRF-CEM cells*

The cytostatic effect of compounds **19**, **26**, **28**, and **30** on CCRF-CEM cells was examined by analyzing the cell cycle profile. As shown in Fig. 3A, a slight increase in the percentage of cells in the G0/G1 phase was observed following treatment by **28** and **30** at 1 × IC_{50} concentration. A more pronounced G0/G1 phase block was detected with compound **26** at 5 × IC_{50} concentration of **26**. Nevertheless, no other significant effects on the cell cycle profile or cell cycle arrest were observed. To further characterize the anti-proliferative potential of these compounds, we have studied their effect on 5-bromo-2-deoxyuridine (BrdU) incorporation into replicating DNA of CCRF-CEM cells. Treatment for 24 h with compounds **26** and **28** at 1 × IC_{50} and 5 × IC_{50} concentrations, and comopound **30** at 5 × IC_{50} concentration, significantly reduced the population of BrdU positive CCRF-CEM cells (Fig. 3B). The most pronounced effect, with almost complete inhibition of DNA synthesis, was observed following treatment with derivative **26** at 5 × IC_{50} concentration (Fig. 3B). These results clearly indicate a reduced rate of cell division in CCRF-CEM cells following treatment with these compounds. Next, we investigated RNA synthesis in CCRF-CEM cells by analyzing 5-bromouridine (BrU) incorporation using flow cytometry. We found that derivatives **26**, **28**, and **30** at 5 × IC_{50} concentration considerably reduced the fraction of BrU incorporating cells, indicating inhibtion of transcription activity (Fig. 3C).

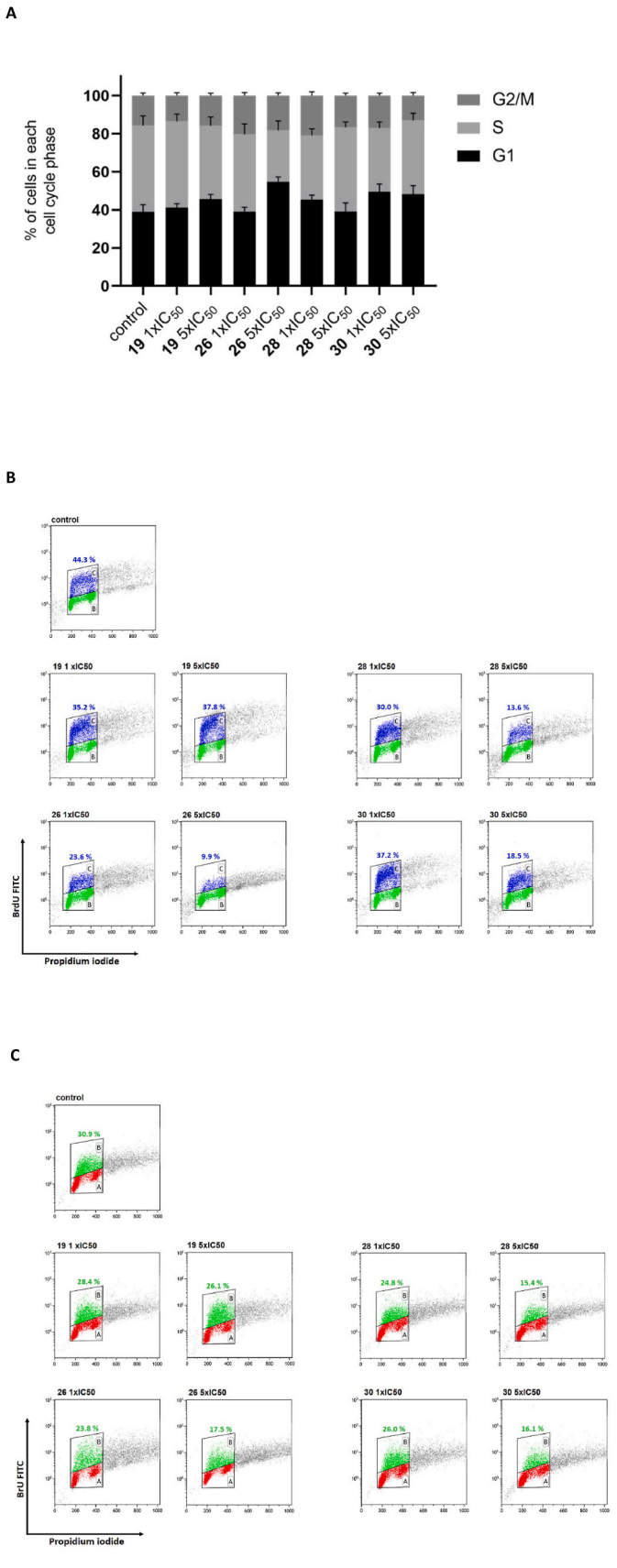
*2.2.6. Effect of **19**, **26**, **28**, and **30** on the expression of apoptosis-, proliferation- and cell cycle-related proteins*

Finally, to better understand the mechanism of action and the involvement of specific signaling pathways triggered by derivatives **19**, **26**, **28**, and **30**, we performed Western blot analysis. As our derivatives modulate the cell cycle and affect DNA synthesis (Fig. 3A and B), we initially examined the expression of proteins related to proliferation pathways. The PI3K/Akt pathway is crucial for both proliferation and apoptosis in cancer cells [68]. It is well known, that initiator kinase PI3K drives cancer initiation and progression and its aberrant activity is associated with several processes contributing to tumor development [69]. An equally important component of this pathway represent Akt kinase which regulates multiple signaling molecules, including those associated with apoptosis, like Bcl-2 or Bax [70,71]. We have examined the impact of **19**, **26**, **28**, and **30** on total Akt protein levels and the phosphorylated active form, P-Akt (Ser473). The results revealed a dramatic reduction in P-Akt expression following treatment with **26** at 5 × IC_{50}, and with **28** and **30** at 5 × and even at 1 × IC_{50} concentrations (Fig. 4). On the other hand, total Akt protein levels remained unaffected or only slightly reduced by **26** and **28** at 5 × IC_{50} concentration (Fig. 4). These results indicate that the PI3K/Akt signaling pathway contributes to the anti-proliferative effects of these compounds in CCRF-CEM cells. STAT3 is another important signaling pathway frequently activated in many cancer cell types. This transcription factor regulates expression of numerous genes involved in cell cycle regulation and proliferation, e.g. Cyclin D and Bcl-2 [72]. We found that treatment with **26** and **28** at 5 × IC_{50} concentration and **30** at 1 × and 5 × IC_{50} concentrations for 24 h reduced the expression of both total STAT3 and also its transcription target Cyclin D (Fig. 4). These results nicely correlate with the observed effects of the compounds on cell cycle modulation and DNA synthesis (Fig. 3A and B). On top of that, we observed Cyclin A expression was reduced by all tested derivatives at 5 × IC_{50} concentration, further promoting previously detected impact on cell cycle progression of CCRF-CEM cells (Fig. 3A). Based on the cell death analysis (Fig. 1), we also investigated the activation fo the Caspase-3, the main executioner protease in apoptosis. Our results showed reduced expression of Caspase-3 precursor form following treatment by **26**, **28**, and **30** at 5 × IC_{50} concentration, indicating Caspase-3 processing and activation (Fig. 4). Ongoing apoptosis was further confirmed by the cleavage of poly (ADP-ribose) polymerase 1 (PARP-1) (Fig. 4), a key substrate of Caspase-3 [73]. Since one of the highlighted mechanisms of triterpenes' anti-cancer effect is induction of apoptosis through regulation of BCL-2 and BH3 family proteins, we studied effect of **19**, **26**, **28**, and **30** on selected proteins expression in more detail. We analyzed the expression of Bid and Bim proteins belonging to the BH3-only subfamily of BCL-2 proteins that promote apoptosis either by directly activating Bax and Bak or by inhibiting anti-apoptotic proteins such as Bcl-2, Bcl-xL, or Mcl-1 [74–76]. Our results clearly indicate that **19**, **26**, **28**, and **30** induce apoptosis through Bid and Bim activation, with increased expression levels observed in treated samples. Interestingly, the derivatives had a more pronounced effect at 1 × IC_{50} than at 5 × IC_{50} (Fig. 4). A similar pattern was observed for the proapoptotic protein Bak. In addition, we investigated the expression of Bcl-xL, a representative anti-apoptotic protein in the BCL-2 family. Exposure of CCRF-CEM cells to derivatives **19**, **26**, and **28** at 5 × IC_{50} for 24 h led to a significant decrease in Bcl-xL expression (Fig. 4), indicating enhanced sensitivity to apoptosis.

3. Conclusion

The main aim of this study was to explore the possibility to expand the structural diversity of lupane derivatives by modifying their isopropenyl moiety, leading to the synthesis of various phthalimides and relative compounds. This was achieved through a two-step process: first, the application of the Wittig reaction as developed in our previous work [29], followed by an unprecedented Diels-Alder reaction. This approach yielded to a broad range of novel compounds, demonstrating the potential for extensive derivatizatization of lupane triterpenoids. As a result of this chemical-exploratory part, 55 new compounds were synthesized, including triterpenoids fused with phthalate, phthalimide and phthalonitrile moieties.

In the second phase of the study, the *in vitro* cytotoxic activity of these compounds was tested using a standard panel of 8 cancer and 2 non-cancer cell lines. Among the compounds tested, four (**19**, **26**, **28**, and **30**) exhibited significant and selective cytotoxic activity in the T-lymphoblastic CCRF-CEM cell line, with favorable selectivity toward cancer cell lines. These compounds were further investigated for their



(caption on next column)

Fig. 3. (A) Cell cycle analysis by flow cytometry. CCRF-CEM cells were treated with **19**, **26**, **28**, and **30** at 1 × IC_{50} and 5 × IC_{50} concentrations for 24 h. Propidium iodide staining was used to examine cell cycle profile and samples were analyzed on a FACSCalibur (Becton Coulter) flow cytometer using a 488 nm laser. At least 10,000 intact cells were acquired per sample, with untreated cells serving as the control. The bar graph shows proportions of cells in each phase of the cell cycle. Error bars represent the SEM (standard error of the mean) from three independent experiments. **(B)** Influence on DNA synthesis in CCRF-CEM cells treated with compounds **19**, **26**, **28**, and **30** at 1 × IC_{50} and 5 × IC_{50} concentrations for 24 h. The numbers represent percentage of proliferating BrdU positive cells (region C). **(C)** Effect on RNA synthesis in CCRF-CEM cells treated with compounds **19**, **26**, **28**, and **30** at 1 × IC_{50} and 5 × IC_{50} concentrations for 24 h. The percentages represent the proportion of BrU positive cells (region B).

pharmacological properties and detailed mechanism of action.

It was found, that **19**, **26**, **28**, and **30** induce apoptosis in CCRF-CEM cells *via* the mitochondrial pathway, primarily by disrupting mitochondrial transmembrane potential. This disruption impacted key proliferation pathways, specifically PI3K/Akt and STAT-3. Mitochondrial dysfuntion led to the activation of the key executioner enzyme caspase-3 and subsequent cleavage of its substrate, such as PARP. Effective anticancer activity of tested derivatives was further demonstrated by inhibiting DNA and RNA synthesis. In summary, the novel triterpenoid phthalimides **19**, **26**, **28**, and **30** showed strong *in vitro* anti-tumor efficacy and represent promising candidates for further *in vivo* studies and the design of more potent derivatives.

4. Experimental procedures

4.1. Chemistry

4.1.1. General information

All reagents were of reagent grade and were used without further purification. Benzyl betulinate (**1a**) and betulin (**31**) in purity 98 %+ were purchased from the company Betulinines (www.betulinines.com). All other chemicals and solvents were purchased from Sigma-Aldrich (www.sigmaaldrich.com), Acros Organics (www.acros.com) or Fluorochem (www.fluorochem.co.uk). Dry solvents were dried over 4 Å molecular sieves or stored as received from commercial suppliers. The course of the reactions was monitored by TLC on Kieselgel 60 F_{254} plates (Merck, Germany) and detected by UV light (254 nm) followed by spraying with 10 % aqueous H_2SO_4 and heating to 150–200 °C. Purification was performed using column chromatography on Silica gel 60 Merck 7734 (Merck, Germany). Melting points (MP) were determined only for crystalline compounds using the STUART SMP30 apparatus and are uncorrected. IR spectra were recorded on a Thermo Nicolet AVATAR 370 FTIR. DRIFT stands for Diffuse Reflectance Infrared Fourier Transform. All ^{1}H and ^{13}C NMR experiments were recorded at 500 MHz (Jeol JNM-ECX-500) or 400 MHz (Jeol JNM-ECA400II) for ^{1}H NMR, and 126 MHz or 100 MHz for ^{13}C NMR, respectively, at 25 °C in $CDCl_3$ or DMSO-d_6. Chemical shifts δ are reported relative to the residual solvent peak (for $CDCl_3$ $\delta_H = 7.26$ ppm, $\delta_C = 77.16$ ppm; for CD_3OD $\delta_H = 3.31$ ppm, $\delta_C = 49.00$ ppm; for DMSO-d_6 $\delta_H = 2.50$ ppm, $\delta_C = 39.52$ ppm). Chemical shifts δ are reported in parts per million (ppm) and coupling constants J are reported in Hertz (Hz). 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) operating in positive and negative full scan mode in the range of $m/z = 400–700$. The settings for electrospray ionization were as follows: oven temperature of 150 °C and source voltage of 3.6 kV. The acquired data were internally calibrated with phthalate as a contaminant in MeOH ($m/z = 297.15909$). The samples were diluted to a final concentration of 0.1 mg/mL in MeOH and injected to the mass spectrometer over an autosampler after HPLC separation (Phenomenex Gemini column, C18, 50 × 2.00 mm, 2.6 μm particles) using the mobile

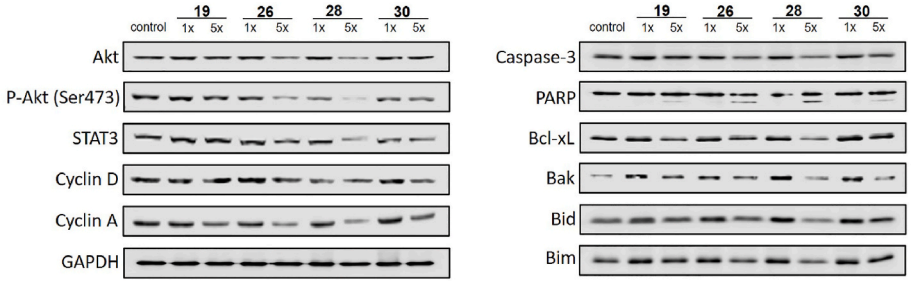


Fig. 4. Western blot analysis of CCRF-CEM cells treated by **19**, **26**, **28**, and **30** at 1 × IC_{50} and 5 × IC_{50} concentrations for 24 h. The left panel depicts expression of proteins associated with the cell cycle and proliferation regulation, while the right panel displays the expression of proteins essential for cell survival and/or apoptotic cell death.

phase isocrat MeOH/ammonium acetate 0.01 mol L^{-1}/HCOOH 95 : 5: 0.1 and a flow rate of 0.3 mL/min.

4.1.2. *Synthesis of compounds **1a–2c***

Compounds **1a–2c** were prepared earlier in our lab [29].

4.1.3. *General procedure for the synthesis of compounds **3a, 3c, 3e, 34a, 34b***

A solution of diene **2** or **33** (1.25 mmol) and dimethyl acetylenedicarboxylate (3.75 mmol, 3 eq.) in dry toluene (9.8 mL) was stirred at 80–100 °C under N_2 atmosphere for 13 h. Then the solvents were evaporated in vacuo and the mixture of 4,5-di(methoxycarbonyl)-1.4-cyclohexadiene and dimethylphthalate (~5–10 %) was separated from dimethyl acetylenedicarboxylate by flash chromatography on SiO_2 eluting with hexane/ethyl acetate (2 : 1).

The oxidation of 1,4-cyclohexadiene was achieved by modification of known procedure [54] as follows: solution of 4,5-di(methoxycarbonyl)-1.4-cyclohexadiene and dimethylphthalate (0.83 mmol) in acetone (10 mL) were vigorously stirred, then $KMnO_4/Al_2O_3$ (815 mg, 1.66 mmol, 2 eq.) were added in portions over 10 min at 10 to −20 °C. Stirring was continued for another 50 min at 10 to −20 °C. For substituted 4,5-di(methoxycarbonyl)-1.4-cyclohexadiene, this procedure of adding $KMnO_4/Al_2O_3$ (815 mg, 1.66 mmol, 2 eq.) was repeated one more time. Then 1.5 M solution of $N_2H_4{\cdot}H_2O$ (3–6 mL) was added to the reaction mixture at −10 or −20 °C. At the end, the final suspension was filtered through Celite, the precipitate was washed with DCM (3 × 10 mL), combined filtrate was washed with brine (10 mL), and the organic phase was dried over Na_2SO_4. The solvent was evaporated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate. Note: $KMnO_4/Al_2O_3$ was prepared by grinding alumina together with potassium permanganate (3 mmol $KMnO_4$/1 g Al_2O_3) in mortar and pestle.

4.1.4. *Benzyl 3β-hydroxy-19-[3,4-di(methoxycarbonyl)phenyl]-20,29,30-trinorlupan-28-oate **(3a)***

Compound **3a** was prepared according to the general procedure in 2 stages: 1) From diene **2a** (700 mg; 1.25 mmol) and dimethyl acetylenedicarboxylate (533 mg, 3.75 mmol) in toluene (9.8 mL) for 13 h at 80 °C, after purification (mobile phase hexane/EtOAc 2 : 1) the mixture of intermediates (746 mg; 85 %) was obtained as a white solid; R_f 0.29 (silica gel, hexane/EtOAc, 3 : 2); 2). Then the mixture of 4,5-di(methoxycarbonyl)-1.4-cyclohexadiene and dimethylphthalate **3a** (583 mg; 0.83 mmol) reacted with $KMnO_4/Al_2O_3$ (815 mg, 1.66 mmol) in acetone (10 mL) for 1 h at −20 °C. After purification (mobile phase hexane/EtOAc 2 : 1) compound **3a** (543 mg; 94 %, 80 % overall) was obtained as a white solid; **mp** 110–112 °C (hexane/EtOAc); R_f 0.27 (silica gel, hexane/EtOAc, 3 : 2). **IR** (DRIFT): 3500 (O–H), 2943, 2866, 1724 (C═O), 1606, 1286 (C–O), 1124, 697. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ, ppm: 0.73 s (3H, Me), 0.74 s (3H, Me), 0.75 s (3H, Me), 0.87 s (3H, Me), 0.93 s (3H, Me), 1.70 dt (1H, $J_1 = 12.9$ Hz, $J_2 = 10.3$ Hz), 1.85 t (1H, $J =$ 11.3 Hz, H-18), 1.98–2.04 m (1H), 2.14–2.27 m (2H), 2.36 dt (1H, $J_1 =$ 12.7 Hz, $J_2 = 3.2$ Hz), 3.13 dd (1H, $J_1 = 11.2$ Hz, $J_2 = 4.5$ Hz, H-3), 3.55 td (1H, $J_1 = 11.0$ Hz, $J_2 = 5.1$ Hz, H-19), 3.89 s (3H, MeO), 3.91 s (3H, MeO), 5.13 d (1H, $J = 12.2$ Hz, CH_aPh), 5.18 d (1H, $J = 12.2$ Hz, CH_bPh), 7.32–7.39 m (6H, $5H_{Ph}$, H-6′), 7.50 d (1H, $J = 1.7$ Hz, H-3′), 7.64 d (1H, $J = 8.0$ Hz, H-6′). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.7, 15.5, 15.9, 16.2, 18.4, 20.9, 27.49, 27.52, 28.1, 29.6, 32.2, 34.4, 34.8, 37.22, 37.23, 38.3, 38.8, 38.9, 40.7, 42.4, 45.9, 50.3, 52.6, 52.8, 55.40, 55.42, 56.9, 66.1, 79.0, 127.7, 128.3, 128.4, 128.7, 128.8, 129.4, 130.0, 132.7, 136.5, 153.7, 168.0, 168.7, 175.7. **HRMS** (ESI): $C_{44}H_{59}O_7$ found 699.4255 $[M+H]^+$; calcd. 699.4255.

4.1.5. *Benzyl 3β-hydroxy-19-[3,4-di(methoxycarbonyl)-5-methylphenyl]-20,29,30-trinorlupan-28-oate **(3c)***

Compound **3c** was prepared according to the general procedure in 2 stages: 1) From diene **2b** (1.28 g; 2.24 mmol) and dimethyl acetylenedicarboxylate (954 mg, 6.72 mmol) in toluene (16.7 mL) for 13 h at 100 °C, after purification (mobile phase hexane/EtOAc 2 : 1) the mixture of intermediates (659 mg; 41 %) was obtained as a white solid; R_f 0.37 (silica gel, hexane/EtOAc, 3 : 2); 2). The mixture of 4,5-di(methoxycarbonyl)-1.4-cyclohexadiene and dimethylphthalate **3c** (590 mg; 0.83 mmol) reacted with $KMnO_4/Al_2O_3$ (1.63 g (2 × 815 mg), 3.32 mmol) in acetone (10 mL) for 2 h at −10 °C. After purification (mobile phase hexane/EtOAc 2 : 1) compound **3c** (452 mg; 77 %, 32 % overall) was obtained as a white solid; **mp** 120–123 °C (hexane/EtOAc); R_f 0.33 (silica gel, hexane/EtOAc, 3 : 2). **IR** (DRIFT): 3510 (O–H), 2946, 2871, 1725 (C═O), 1605, 1274 (C–O), 698. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.73 s (3H, Me), 0.75 s (3H, Me), 0.76 s (3H, Me), 0.88 s (3H, Me), 0.94 s (3H, Me), 1.70 dt (1H, $J_1 = 12.8$ Hz, $J_2 = 10.3$ Hz), 1.85 t (1H, $J =$ 11.3 Hz, H-18), 1.99–2.04 m (1H), 2.13–2.26 m (2H), 2.32 s (3H, Me), 2.36 dt (1H, $J_1 = 12.9$ Hz, $J_2 = 3.3$ Hz), 3.14 dd (1H, $J_1 = 11.4$ Hz, $J_2 =$ 4.4 Hz, H-3), 3.50 td (1H, $J_1 = 11.0$ Hz, $J_2 = 5.2$ Hz, H-19), 3.88 s (3H, MeO), 3.92 s (3H, MeO), 5.13 d (1H, $J = 12.3$ Hz, CH_aPh), 5.18 d (1H, J = 12.3 Hz, CH_bPh), 7.21 d ($1H_{Ar}$, $J = 1.4$ Hz), 7.33–7.40 m ($5H_{Ph}$), 7.64 d ($1H_{Ar}$, $J = 1.4$ Hz). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.8, 15.5, 15.9, 16.2, 18.4, 19.4, 20.9, 27.5, 27.6, 28.1, 29.6, 32.2, 34.4, 34.8, 37.18, 37.24, 38.4, 38.8, 39.0, 40.7, 42.4, 45.6, 50.3, 52.6 (2C), 55.1, 55.4, 56.8, 66.0, 79.0, 126.4, 128.2, 128.3, 128.4, 128.7, 132.5, 133.7, 135.7, 136.5, 151.0, 166.8, 170.2, 175.8. **HRMS** (ESI): $C_{45}H_{61}O_7$ found 713.4418 $[M+H]^+$; calcd. 713.4412.

4.1.7. *28,3β-dihydroxy-19-[3,4-di(methoxycarbonyl)phenyl]-20,29,30-trinorlupan **(34a)***

Compound **34a** was prepared according to the general procedure in 2 stages: 1) from diene **33a** (500 mg; 1.10 mmol) and dimethyl acetylenedicarboxylate (467 mg, 3.30 mmol) in toluene (5 mL) and THF (1.7 mL) for 17 h at 90 °C, after purification (mobile phase hexane/EtOAc 1 : 1) the mixture of intermediates (587 mg; 89 %) was obtained as a white solid; R_f 0.34 (silica gel, hexane/EtOAc 1 : 1); 2). The mixture of 4,5-di (methoxycarbonyl)-1.4-cyclohexadiene and dimethylphthalate **34a** (550 mg; 0.92 mmol) then reacted with $KMnO_4/Al_2O_3$ (904 mg, 1.84 mmol) in acetone (10 mL) for 1 h at 10 °C. After purification (mobile

phase hexane/EtOAc 1 : 1) compound **34a** (411 mg; 75 %, 67 % overall) was obtained as a white solid; **mp** 174–176 °C (hexane/EtOAc); R_f 0.18 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3524 (O–H), 2948, 1723 (C═O), 1605, 1435, 1290, 1128, 730. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ, ppm: 0.73 s (3H, Me), 0.77 s (3H, Me), 0.92 s (3H, Me), 0.95 s (3H, Me), 1.00 s (3H, Me), 1.90 t (1H, J = 11.8 Hz, H-18), 1.97–2.02 m (2H), 2.32 dq (1H, J_1 = 15.2 Hz, J_2 = 9.7 Hz), 2.85 td (1H, J_1 = 11.1 Hz, J_2 = 6.0 Hz, H-19), 3.14 dd (1H, J_1 = 11.2 Hz, J_2 = 4.7 Hz, H-3), 3.41 d (1H, J = 10.7 Hz, H-28a), 3.86 dd (1H, J_1 = 10.7 Hz, J_2 = 1.8 Hz, H-28b), 3.89 s (3H, MeO), 3.91 s (3H, MeO), 7.35 dd (1$H_{arom.}$, J_1 = 8.0 Hz, J_2 = 1.7 Hz, H-5′), 7.49 d (1$H_{arom.}$, J = 1.7 Hz, H-3′), 7.65 d (1$H_{arom.}$, J = 8.0 Hz, H-6′). **^{13}C NMR** ($CDCl_3$+ CD_3OD, 100 MHz) δ, ppm: 14.6, 15.4, 15.9, 16.0, 18.3, 20.7, 26.9, 27.1, 27.6, 27.9, 29.3, 34.2, 34.4, 37.1, 37.2, 38.6, 38.8, 40.8, 42.6, 46.5, 48.1, 50.0, 52.6, 52.7, 54.5, 55.3, 60.2, 78.8, 127.7, 128.6, 129.3, 129.8, 132.5, 153.6, 168.2, 168.9. **HRMS** (ESI): $C_{37}H_{55}O_6$ found 595.3994 $[M+H]^+$; calcd. 595.3993.

4.1.6. Benzyl 3β-hydroxy-19-[3,4-di(methoxycarbonyl)-5-phenylphenyl]-20,29,30-trinorlupan-28-oate (3e)

Compound **3e** hwas prepared according to the general procedure in 2 stages: 1) From diene **2c** (1.50 g; 2.37 mmol) and dimethyl acetylenedicarboxylate (1.01 g, 7.11 mmol) in toluene (18 mL) for 13 h at 90 °C, after purification (mobile phase hexane/EtOAc 3 : 1 to 2 : 1) the mixture of intermediates (1.54 g; 84 %) was obtained as a white solid; R_f 0.38 (silica gel, hexane/EtOAc, 3 : 2); 2). The mixture of 4,5-di(methoxycarbonyl)-1.4-cyclohexadiene and dimethylphthalate (**3e**) (1.27 g; 1.64 mmol) then reacted with $KMnO_4/Al_2O_3$ (3.24 g (2 × 1.62 g), 6.56 mmol) in acetone (19.5 mL) for 2 h at −10 °C. After purification (mobile phase hexane/EtOAc 2 : 1) compound **3e** (1.17 g; 92 %, 77 % overall) was obtained as a white solid; **mp** 188–190 °C (hexane/EtOAc); R_f 0.30 (silica gel, hexane/EtOAc, 3 : 2). **IR** (DRIFT): 3510 (O–H), 2946, 2868, 1725 (C═O), 1600, 1270 (C–O), 1122, 700. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.74 s (3H, Me), 0.75 s (3H, Me), 0.77 s (3H, Me), 0.89 s (3H, Me), 0.94 s (3H, Me), 1.70 dt (1H, J_1 = 12.6 Hz, J_2 = 10.4 Hz), 1.89 t (1H, J = 11.3 Hz, H-18), 1.99–2.03 m (1H), 2.18–2.28 m (2H), 2.36 dt (1H, J_1 = 12.8 Hz, J_2 = 3.1 Hz), 3.15 dd (1H, J_1 = 11.4 Hz, J_2 = 4.6 Hz, H-3), 3.58 td (1H, J_1 = 11.1 Hz, J_2 = 5.2 Hz, H-19), 3.66 s (3H, MeO), 3.91 s (3H, MeO), 5.13 d (1H, J = 12.2 Hz, CH_aPh), 5.18 d (1H, J = 12.2 Hz, CH_bPh), 7.32–7.41 m (11H_{Ar}), 7.81 d (1H_{Ar}, J = 1.7 Hz). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.8, 15.5, 15.9, 16.2, 18.4, 20.9, 27.5, 27.7, 28.1, 29.6, 32.2, 34.5, 34.9, 37.2, 37.3, 38.4, 38.8, 39.0, 40.7, 42.4, 45.8, 50.3, 52.4, 52.7, 55.35, 55.43, 56.8, 66.1, 79.0, 127.9, 128.0, 128.3, 128.4 (2C), 128.5, 128.68, 128.70, 132.1, 133.3, 136.5, 139.8, 140.7, 151.2, 166.6, 169.7, 175.7. **HRMS** (ESI): $C_{50}H_{63}O_7$ found 775.4570 $[M+H]^+$; calcd. 775.4568.

4.1.8. 28,3β-dihydroxy-19-[3,4-di(methoxycarbonyl)-5-phenylphenyl]-20,29,30-trinorlupan (34b)

Compound **34b** was prepared according to the general procedure in 2 stages: 1) from diene **33b** (600 mg; 1.13 mmol) and dimethyl acetylenedicarboxylate (481 mg, 3.39 mmol) in toluene (6 mL) and THF (2 mL) for 18 h at 90 °C, after purification (mobile phase hexane/EtOAc 1 : 1) the mixture of intermediates (489 mg; yield 74 %, conversion 87 %) was obtained as a white solid; R_f 0.18 (silica gel, hexane/EtOAc 3 : 2); 2). The mixture of 4,5-di(methoxycarbonyl)-1.4-cyclohexadiene and dimethylphthalate **34b** (450 mg; 0.67 mmol) then reacted with $KMnO_4/Al_2O_3$ (1.32 g (2 × 659 mg), 2.68 mmol) in acetone (10 mL) for 2 h at 10 °C. After purification (mobile phase hexane/EtOAc 3 : 2 to 1 : 1) compound **34b** (312 mg; 69 %, 51 % overall) was obtained as a white solid; **mp** 196–198 °C (hexane/EtOAc); R_f 0.20 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3480 (O–H), 2945, 1737 (C═O), 1440, 1357, 1217, 1028, 701. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ, ppm: 0.75 s (3H, Me), 0.79 s (3H, Me), 0.94 s (3H, Me), 0.96 s (3H, Me), 1.02 s (3H, Me), 1.74 qd (1H, J_1 = 12.9 Hz, J_2 = 3.9 Hz), 1.93 t (1H, J = 11.8 Hz, H-18), 1.96–2.02 m (2H), 2.35 dq (1H, J_1 = 15.1 Hz, J_2 = 9.7 Hz), 2.89 td (1H, J_1 = 11.2 Hz, J_2 = 6.0 Hz, H-19), 3.16 dd (1H, J_1 = 11.3 Hz, J_2 = 4.5 Hz, H-3), 3.42 d (1H, J = 10.7 Hz, H-28a), 3.66 s (3H, MeO), 3.87 dd (1H, J_1 = 10.7 Hz, J_2 = 0.9 Hz, H-28b), 3.91 s (3H, MeO), 7.33–7.41 m (6$H_{arom.}$), 7.79 d (1$H_{arom.}$, J = 1.7 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.8, 15.5, 16.06, 16.14, 18.4, 20.9, 34.3, 34.4, 34.6, 37.2, 37.4, 38.7, 38.9, 40.8, 41.0, 42.4, 42.7, 46.4, 48.3, 50.1, 52.4, 52.7, 54.5, 55.4, 56.5, 60.7, 79.0, 125.8, 128.0, 128.4, 128.7, 132.1, 133.3, 139.7, 140.72, 140.74, 150.9, 166.6, 169.7. **HRMS** (ESI): $C_{43}H_{59}O_6$ found 671.4305 $[M+H]^+$; calcd. 671.4306.

4.1.9. General procedure for the synthesis of compounds 3b, 3d, 3f

The cleavage of benzyl group was achieved by modification of the known procedure [39]. To a stirred solution of benzyl ester **3a**, **3c**, or **3e** (0.09 mmol) in a mixture of THF/MeOH (2 mL/0.4 mL), 10 % Pd/C (0.009 mmol, 10 mol. %) was added at room temperature under nitrogen atmosphere. Then the nitrogen atmosphere was replaced by hydrogen gas from a balloon and the reaction mixture was stirred for 13 h. The mixture was filtered through a Celite, washed with $CHCl_3$ and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate.

4.1.10. 19-[3,4-Bis(methoxycarbonyl)phenyl]-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (3b)

Compound **3b** was prepared according to the general procedure from compound **3a** (65 mg; 0.09 mmol) and 10 % Pd/C (10 mg, 0.009 mmol) in mixture THF/MeOH (2 mL/0.4 mL) for 13 h at 20 °C. After purification (mobile phase hexane/EtOAc 3 : 2 to 1 : 1) compound **3b** (50 mg; 91 %) was obtained as a white solid; **mp** 168–170 °C (hexane/EtOAc); R_f 0.18 (silica gel, hexane/EtOAc, 3 : 2). **IR** (DRIFT): 3510 (O–H), 2945, 2845, 1725 (C═O), 1606, 1436, 1289 (C–O), 771. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.73 s (3H, Me), 0.78 s (3H, Me), 0.915 s (3H, Me), 0.922 s (3H, Me), 0.95 s (3H, Me), 1.76 dt (1H, J_1 = 12.9 Hz, J_2 = 10.3 Hz), 1.90 t (1H, J = 11.4 Hz, H-18), 2.10 ddd (1H, J_1 = 12.6 Hz, J_2 = 8.0 Hz, J_3 = 0.8 Hz), 2.25 td (1H, J_1 = 12.7 Hz, J_2 = 3.4 Hz), 2.28–2.38 m (2H), 3.15 dd (1H, J_1 = 11.4 Hz, J_2 = 4.4 Hz, H-3), 3.55 td (1H, J_1 = 11.1 Hz, J_2 = 5.3 Hz, H-19), 3.89 s (3H, MeO), 3.91 s (3H, MeO), 7.39 dd (1H, J_1 = 8.0 Hz, J_2 = 1.7 Hz, H-6′), 7.53 d (1H, J = 1.7 Hz, H-2′), 7.66 d (1H, J = 8.0 Hz, H-5′). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.8, 15.5, 16.1, 16.2, 18.4, 20.9, 27.4, 27.5, 28.1, 29.7, 32.2, 34.4, 34.8, 37.26, 37.33, 38.6, 38.7, 39.0, 40.7, 42.4, 45.9, 50.2, 52.7, 52.8, 55.2, 55.4, 56.7, 79.1, 127.9, 128.9, 129.4, 130.0, 132.6, 153.4, 168.1, 168.7, 181.7. **HRMS** (ESI): $C_{37}H_{53}O_7$ found 609.3790 $[M+H]^+$; calcd. 609.3786.

4.1.11. 19-[3,4-Bis(methoxycarbonyl)-5-methylphenyl]-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (3d)

Compound **3d** was prepared according to the general procedure from compound **3c** (65 mg; 0.09 mmol) and 10 % Pd/C (10 mg, 0.009 mmol) in mixture THF/MeOH (2 mL/0.4 mL) for 13 h at 20 °C. After purification (mobile phase hexane/EtOAc 3 : 2) compound **3d** (54 mg; 97 %) was obtained as a white solid; **mp** 175–177 °C (hexane/EtOAc); R_f 0.23 (silica gel, hexane/EtOAc, 3 : 2). **IR** (DRIFT): 3500 (O–H), 2944, 2869, 1727 (C═O), 1685, 1605, 1272 (C–O), 791. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.73 s (3H, Me), 0.79 s (3H, Me), 0.925 s (3H, Me), 0.928 s (3H, Me), 0.95 s (3H, Me), 1.77 dt (1H, J_1 = 12.9 Hz, J_2 = 10.3 Hz), 1.89 t (1H, J = 11.4 Hz, H-18), 2.08 ddd (1H, J_1 = 12.6 Hz, J_2 = 8.1 Hz, J_3 = 0.8 Hz), 2.22–2.38 m (3H), 2.33 s (3H, Me), 3.16 dd (1H, J_1 = 11.4 Hz, J_2 = 4.5 Hz, H-3), 3.50 td (1H, J_1 = 11.1 Hz, J_2 = 5.4 Hz, H-19), 3.89 s (3H, MeO), 3.93 s (3H, MeO), 7.24 d (1H_{Ar}, J = 1.5 Hz), 7.66 d (1H_{Ar}, J = 1.5 Hz). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.8, 15.5, 16.1, 16.2, 18.4, 19.4, 20.9, 27.4, 27.5, 28.1, 29.8, 32.2, 34.4, 34.8, 37.27, 37.30, 38.6, 38.7, 39.0, 40.7, 42.4, 45.7, 50.3, 52.59, 52.60, 54.8, 55.4, 56.7, 79.1, 126.5, 128.2, 132.6, 133.6, 135.7, 150.8, 166.8, 170.2, 181.9. **HRMS** (ESI): $C_{38}H_{53}O_7$ found 621.3782 $[M-H]^+$; calcd. 621.3786.

4.1.12. 19-[5,6-Bis(methoxycarbonyl)-biphenyl-3-yl]-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (3f)

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

compound **3e** (93 mg; 0.12 mmol) and 10 % Pd/C (13 mg, 0.012 mmol) in mixture THF/MeOH (2.6 mL/0.5 mL) for 13 h at 20 °C. After purification (mobile phase hexane/EtOAc 3 : 2) compound **3f** (79 mg; 96 %) was obtained as a white solid; **mp** 190–192 °C (hexane/EtOAc); R_f 0.2 (silica gel, hexane/EtOAc, 3 : 2). **IR** (DRIFT): 3500 (O–H), 2944, 2869, 1730 (C–O), 1696, 1599, 1272 (C–O), 701. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.74 s (3H, Me), 0.79 s (3H, Me), 0.93 s (3H, Me), 0.94 s (3H, Me), 0.95 s (3H, Me), 1.77 dt (1H, $J_1 = 13.0$ Hz, $J_2 = 10.4$ Hz), 1.92 t (1H, $J =$ 11.4 Hz, H-18), 2.07–2.11 m (1H), 2.24–2.37 m (3H), 3.17 dd (1H, $J_1 =$ 11.5 Hz, $J_2 = 4.6$ Hz, H-3), 3.59 td (1H, $J_1 = 11.2$ Hz, $J_2 = 5.3$ Hz, H-19), 3.67 s (3H, MeO), 3.91 s (3H, MeO), 7.34–7.41 m (6H_{Ar}), 7.84 d (1H_{Ar}, J = 1.8 Hz). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.8, 15.5, 16.1, 16.2, 18.4, 20.9, 27.4, 27.7, 28.1, 29.7, 32.2, 34.4, 34.9, 37.27, 37.31, 38.7, 38.8, 38.9, 40.7, 42.4, 45.8, 50.3, 52.4, 52.7, 55.1, 55.4, 56.7, 79.1, 128.0, 128.1, 128.4 (2C), 128.7, 132.1, 133.3, 139.7, 140.7, 150.9, 166.6, 169.7, 181.9. **HRMS** (ESI): $C_{43}H_{55}O_7$ found 683.3944 $[M - H]^+$; calcd. 683.3942.

4.1.13. Synthesis of phthalic acids *4a–4d*

Phthalic acids were synthesized by modification of the known procedure [77].

4.1.14. Benzyl 19-[3,4-bis(carboxy)phenyl]-3β-hydroxy-20,29,30-trinorlupan-28-oate (*4a*)

To solution of dimethyl phthalate (650 mg, 0.93 mmol) in 16 mL (2 : 1: 1) of MeOH, THF, H_2O under vigorous stirring was added LiOH monohydrate (344 mg, 8.18 mmol) at room temperature. The reaction was stirred 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 residue was purified by column chromatography on SiO_2 eluting with chloroform/methanol (98 : 2, 95 : 5, 5 : 1), giving the phthalic acid **4a** as a white solid (598 mg, 96 %); **mp** 200–202 °C (hexane/EtOAc); R_f 0.18 ($CHCl_3$/MeOH/AcOH 4 : 1: 0.002). **IR** (DRIFT): 3400 (O–H), 2942, 1716 (C═O), 1455, 1375, 1128, 696. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ, ppm: 0.64 s (3H, Me), 0.67 s (3H, Me), 0.69 s (3H, Me), 0.83 s (3H, Me), 0.86 s (3H, Me), 1.56 td (1H, $J_1 = 13.1$ Hz, $J_2 = 3.2$ Hz), 1.79–1.89 m (2H), 1.92 t (1H, $J = 11.3$ Hz, H-18), 2.06–2.23 m (3H), 2.90 dd (1H, $J_1 =$ 10.7 Hz, $J_2 = 5.0$ Hz, H-3), 3.43 td (1H, $J_1 = 11.0$ Hz, $J_2 = 5.2$ Hz, H-19), 4.21 br. s (1H, OH), 5.13 d (1H, $J = 12.2$ Hz, CH_aPh), 5.17 d (1H, $J =$ 12.2 Hz, CH_bPh), 7.32–7.42 m (6H_{Ar}), 7.82–7.89 m (2H_{Ar}). **^{13}C NMR** (DMSO-d_6, 100 MHz) δ, ppm: 14.3, 15.5, 15.77, 15.83, 17.9, 20.4, 26.8, 27.1, 28.1, 29.0, 31.3, 33.8, 34.4, 36.2, 36.6, 37.7, 38.1, 38.4, 40.0, 41.9, 45.3, 49.5, 54.1, 54.8, 56.1, 65.3, 76.7, 128.0, 128.2 (2C), 128.4, 129.0, 131.3, 131.5, 134.5, 136.5, 151.8, 168.2, 168.6, 175.0. **HRMS** (ESI): $C_{42}H_{53}O_7$ found 669.3778 $[M - H]^+$; calcd. 699.3786.

4.1.15. 19-[3,4-Bis(carboxy)phenyl]-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (*4b*)

The cleavage of benzyl group was achieved by modification of the known procedure [39]. To a stirred solution of benzyl ester **4a** (0.11 mmol) in a mixture of THF/MeOH (2.4 mL/0.5 mL), 10 % Pd/C (0.011 mmol, 10 mol.%) was added at room temperature under nitrogen atmosphere. After nitrogen atmosphere was replaced by hydrogen gas from a balloon and the reaction mixture was stirred for 13 h. The mixture was filtered through a Celite, washed with EtOAc and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate.

The compound **4b** was prepared according to the general procedure from compound **4a** (76 mg; 0.11 mmol) and 10 % Pd/C (12 mg, 0.011 mmol) in mixture THF/MeOH (2.4 mL/0.5 mL) for 13 h at 20 °C. After purification (mobile phase $CHCl_3$/MeOH/AcOH 5 : 1: 0.001 to 3 : 1: 0.002) compound **4b** (44 mg; 98 %) was obtained as a white solid; **mp** 190–192 °C ($CHCl_3$/MeOH/AcOH); R_f 0.20 (silica gel, $CHCl_3$/MeOH/AcOH 3 : 1: 0.002). **IR** (DRIFT): 3300 (O–H), 2970, 1739 (C═O), 1548, 1366, 1217, 667. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ, ppm: 0.62 s (3H, Me), 0.71 s (3H, Me), 0.83 s (3H, Me), 0.865 s (3H, Me), 0.87 s (3H, Me), 1.68–1.74 m (1H), 1.85 t (1H, $J = 11.2$ Hz, H-18), 1.88–1.93 m (1H), 2.12–2.20 m (2H), 2.29–2.33 m (1H), 2.91 dd (1H, $J_1 = 10.7$ Hz, $J_2 =$ 5.1 Hz, H-3), 3.42–3.45 m (1H, H-19), 4.22 br. s (1H, OH), 7.38 d (1H_{Ar}, $J = 7.9$ Hz), 8.01 s (1H_{Ar}), 8.05 d (1H_{Ar}, $J = 7.9$ Hz). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.76, 15.84, 17.9, 20.4, 26.9, 27.1, 28.1, 29.2, 31.8, 33.9, 34.6, 36.5, 36.6, 37.6, 38.1, 38.4, 40.2, 41.9, 45.2, 49.6, 54.0, 54.8, 55.6, 76.7, 79.2, 128.9, 131.4, 132.1, 132.8, 135.0, 151.8, 168.1, 168.3, 177.6. **HRMS** (ESI): $C_{35}H_{47}O_7$ found 579.3315 $[M - H]^+$; calcd. 579.3316.

4.1.16. General procedure for the synthesis of substituted phthalic acids *4c, 4d*

A mixture of dimethyl phthalate (0.56 mmol) and KOH (25.2 mmol, 45 eq.) in MeOH (9 mL) was heated to reflux for 4 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 residue was purified by column chromatography on SiO_2 eluting with chloroform/methanol (98 : 2, 95 : 5, 5 : 1).

4.1.17. Benzyl 3β-hydroxy-19-[3,4-bis(carboxy)-5-methylphenyl]-20,29,30-trinorlupan-28-oate (*4c*)

Compound **4c** was prepared according to the general procedure from dimethyl phthalate **3c** (400 mg; 0.56 mmol) and KOH (1.41 g, 25.6 mmol) in MeOH (9 mL). After purification (mobile phase chloroform/methanol 98 : 2, 95 : 5, 5 : 1) compound **4c** (280 mg; 73 %) was obtained as a white solid; **mp** 155–157 °C (chloroform/methanol); R_f 0.3 ($CHCl_3$/MeOH/AcOH 4 : 1: 0.002). **IR** (DRIFT): 3480 (O–H), 2970, 1739 (C═O), 1441, 1366, 1217, 895. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ, ppm: 0.62 s (3H, Me), 0.67 s (3H, Me), 0.69 s (3H, Me), 0.84 s (3H, Me), 0.87 s (3H, Me), 1.56 td (1H, $J_1 = 12.9$ Hz, $J_2 = 2.7$ Hz), 1.76–7.93 m (3H), 2.03–2.23 m (3H), 2.28 s (3H, Me), 2.90 dd (1H, $J_1 = 10.5$ Hz, $J_2 = 5.5$ Hz, H-3), 3.37 td (1H, $J_1 = 11.1$ Hz, $J_2 = 5.3$ Hz, H-19), 5.13 d (1H, $J =$ 12.3 Hz, CH_aPh), 5.17 d (1H, $J = 12.3$ Hz, CH_bPh), 7.28 s (1H_{Ar}), 7.31–7.42 m (5H_{Ph}), 7.52 s (1H_{Ar}). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.7, 15.5, 15.7, 15.8, 17.9, 19.6, 20.4, 26.9, 27.1, 28.0, 29.0, 31.2, 33.8, 34.5, 36.1, 36.6, 37.7, 38.1, 38.4, 40.0, 41.8, 45.0, 49.5, 53.7, 54.8, 56.1, 65.2, 76.7, 125.8, 128.0, 128.1, 128.4, 130.3, 132.2, 134.3, 134.7, 136.5, 148.9, 168.5, 170.5, 174.9. **HRMS** (ESI): $C_{43}H_{55}O_7$ found 683.3937 $[M - H]^+$; calcd. 683.3942.

4.1.18. Benzyl 3β-hydroxy-19-[3,4-bis(carboxy)-5-phenylphenyl]-20,29,30-trinorlupan-28-oate (*4d*)

Compound **4d** was prepared according to the general procedure from dimethyl phthalate **3e** (810 mg; 1.05 mmol) and KOH (2.65 g, 47.25 mmol) in MeOH (11 mL). After purification (mobile phase chloroform/methanol 98 : 2, 95 : 5, 5 : 1) compound **4d** (710 mg; 90 %) was obtained as a white solid; **mp** 188–190 °C (chloroform/methanol); R_f 0.41 ($CHCl_3$/MeOH/AcOH 4 : 1: 0.002). **IR** (DRIFT): 2939, 2867, 1723, 1701 (C═O), 1598, 698. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ, ppm: 0.62 s (3H, Me), 0.68 s (3H, Me), 0.70 s (3H, Me), 0.84 s (3H, Me), 0.89 s (3H, Me), 1.57 td (1H, $J_1 = 13.1$ Hz, $J_2 = 3.5$ Hz), 1.79–1.89 m (2H), 1.96 t (1H, J = 11.3 Hz, H-18), 2.10–2.21 m (3H), 2.91 dd (1H, $J_1 = 10.7$ Hz, $J_2 = 5.2$ Hz, H-3), 3.46 td (1H, $J_1 = 10.6$ Hz, $J_2 = 5.1$ Hz, H-19), 5.13 d (1H, $J =$ 12.3 Hz, CH_aPh), 5.16 d (1H, $J = 12.3$ Hz, CH_bPh), 7.29–7.42 m (11H_{Ar}), 7.67 br. s (1H_{Ar}). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.5, 15.7, 15.8, 17.9, 20.4, 27.0, 27.1, 28.1, 29.0, 31.2, 33.9, 34.5, 36.1, 36.6, 37.8, 38.1, 38.4, 40.1, 41.9, 45.2, 49.6, 53.8, 54.8, 56.1, 65.2, 76.7, 127.1 (2C), 128.0 (2C), 128.1, 128.4, 128.5 (2C), 131.6, 133.8, 136.5, 139.3, 140.9, 148.7, 168.8, 170.2, 175.0. **HRMS** (ESI): $C_{48}H_{57}O_7$ found 745.4089 $[M - H]^+$; calcd. 745.4099.

*4.1.19. General procedure for the synthesis of phtalimides **5a, 5d, 5g***

Phthalimides were synthesized in 2 steps by modification of the known procedures [55,56]. The phthalic acid (**4**, 0.30 mmol) was added to acetic anhydride (1.5 mL) and the solution was refluxed for 5 h under nitrogen atmosphere. The acetic anhydride was removed in vacuo and the crude phthalic anhydride was used in next step without purification. The phthalic anhydride and urea (360 mg, 6.0 mmol, 20 eq.) were heated to 170 °C for 2 h. The crude product was cooled to room temperature and was diluted with water, extracted with DCM (100 mL), dried over Na_2SO_4, concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate (3 : 1).

*4.1.20. Benzyl 3β-acetoxy-19-(1,3-dioxoisoindolin-5-yl)-20,29,30-trinorlupan-28-oate (**5a**)*

Compound **5a** was prepared according to the general procedure in 2 stages from phthalic acid **4a** (460 mg; 0.69 mmol) and acetic anhydride (3 mL), then urea (828 mg; 13.8 mmol). After purification (mobile phase hexane/EtOAc 3 : 1) compound **5a** (436 mg; 90 %) was obtained as a white solid; **mp** 155–157 °C (hexane/EtOAc); R_f 0.44 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3263 (N–H), 2943, 2870, 1773, 1720 (C═O), 1618, 747, 695. **^{1}H NMR** ($CDCl_3$, 400 MHz) *δ*, ppm: 0.74 s (3H, Me), 0.78 s (3H, Me), 0.80 s (3H, Me), 0.81 s (3H, Me), 0.87 s (3H, Me), 1.75 dt (1H, J_1 = 12.6 Hz, J_2 = 10.4 Hz), 1.88 t (1H, J = 11.2 Hz, H-18), 2.01 s (3H, MeCO), 2.02–2.08 m (1H), 2.20–2.31 m (2H), 2.38 dt (1H, J_1 = 12.7 Hz, J_2 = 3.0 Hz), 3.64 td (1H, J_1 = 11.0 Hz, J_2 = 5.0 Hz, H-19), 4.41 dd (1H, J_1 = 8.0 Hz, J_2 = 6.0 Hz, H-3), 5.13 d (1H, J = 12.2 Hz, CH_aPh), 5.19 d (1H, J = 12.2 Hz, CH_bPh), 7.31–7.40 m ($5H_{Ph}$), 7.57 dd (1H, J_1 = 7.8 Hz, J_2 = 1.5 Hz, H-6′), 7.67 br. s (1H, NH), 7.70 d (1H, J = 1.5 Hz, H-4′), 7.73 d (1H, J = 7.8 Hz, H-7′). **^{13}C NMR** ($CDCl_3$, 100 MHz) *δ*, ppm: 14.7, 15.9, 16.2, 16.6, 18.2, 20.9, 21.4, 23.7, 27.6, 28.0, 29.5, 32.1, 34.3, 35.1, 37.1, 37.2, 37.9, 38.3, 38.4, 40.7, 42.4, 46.5, 50.2, 55.4, 56.2, 57.0, 66.2, 80.9, 122.2, 123.8, 128.36, 128.4, 128.7, 130.2, 133.4, 133.9, 136.4, 157.8, 168.2, 168.6, 171.1, 175.7. **HRMS** (ESI): $C_{44}H_{54}NO_6$ found 692.3941 $[M-H]^+$; calcd. 692.3946.

*4.1.21. Benzyl 3β-acetoxy-19-(7-methyl-1,3-dioxoisoindolin-5-yl)-20,29,30-trinorlupan-28-oate (**5d**)*

Compound **5d** was prepared according to the general procedure in 2 stages from phthalic acid **4c** (170 mg; 0.25 mmol) and acetic anhydride (1.5 mL), then urea (300 mg; 5.0 mmol). After purification (mobile phase hexane/EtOAc 3 : 1) compound **5d** (121 mg; 68 %) was obtained as a white solid; **mp** 170–172 °C (hexane/EtOAc); R_f 0.52 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3278 (N–H), 2943, 2870, 1766, 1720 (C═O), 1619, 753, 696. **^{1}H NMR** ($CDCl_3$, 500 MHz) *δ*, ppm: 0.75 s (3H, Me), 0.78 s (3H, Me), 0.80 s (3H, Me), 0.81 s (3H, Me), 0.87 s (3H, Me), 1.73 dt (1H, J_1 = 12.4 Hz, J_2 = 10.4 Hz), 1.87 t (1H, J = 11.3 Hz, H-18), 2.02 s (3H, MeCO), 2.02–2.06 m (1H), 2.17–2.29 m (2H), 2.37 dt (1H, J_1 = 12.9 Hz, J_2 = 3.3 Hz), 2.65 s (3H, Me), 3.58 td (1H, J_1 = 11.1 Hz, J_2 = 5.1 Hz, H-19), 4.41 dd (1H, J_1 = 9.2 Hz, J_2 = 6.7 Hz, H-3), 5.13 d (1H, J = 12.2 Hz, CH_aPh), 5.19 d (1H, J = 12.2 Hz, CH_bPh), 7.29 s ($1H_{Ar}$), 7.31–7.40 m ($5H_{Ph}$), 7.49 s (1H, NH), 7.53 s ($1H_{Ar}$). **^{13}C NMR** ($CDCl_3$, 100 MHz) *δ*, ppm: 14.7, 15.8, 16.2, 16.6, 17.9, 18.2, 20.9, 21.4, 23.7, 27.5, 28.0, 29.5, 32.0, 34.2, 34.9, 37.1, 37.2, 37.9, 38.3, 38.4, 40.6, 42.3, 46.3, 50.1, 55.4, 55.7, 56.9, 66.1, 80.9, 119.7, 127.0, 128.3, 128.4, 128.7, 133.8, 136.2, 136.4, 138.4, 157.2, 168.7, 169.1, 171.1, 175.7. **HRMS** (ESI): $C_{45}H_{56}NO_6$ found 706.4116 $[M-H]^+$; calcd. 706.4102.

*4.1.22. Benzyl 3β-acetoxy-19-(1,3-dioxo-7-phenylisoindolin-5-yl)-20,29,30-trinorlupan-28-oate (**5g**)*

Compound **5g** was prepared according to the general procedure in 2 stages from phthalic acid **4d** (224 mg; 0.30 mmol) and acetic anhydride (2 mL), then urea (396 mg; 6.6 mmol). After purification (mobile phase hexane/EtOAc 4 : 1 to 3 : 1) compound **5g** (189 mg; 82 %) was obtained as a white solid; **mp** 170–172 °C (hexane/EtOAc); R_f 0.45 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3270 (N–H), 2943, 2870, 1769, 1719 (C═O), 1615, 769, 696. **^{1}H NMR** ($CDCl_3$, 500 MHz) *δ*, ppm: 0.75 s (3H, Me), 0.79 s (3H, Me), 0.81 s (3H, Me), 0.82 s (3H, Me), 0.89 s (3H, Me), 1.75 dt (1H, J_1 = 12.7 Hz, J_2 = 10.3 Hz), 1.92 t (1H, J = 11.3 Hz, H-18), 2.02 s (3H, MeCO), 2.03–2.07 m (1H), 2.20–2.32 m (2H), 2.38 dt (1H, J_1 = 12.9 Hz, J_2 = 3.2 Hz), 3.67 td (1H, J_1 = 11.1 Hz, J_2 = 5.1 Hz, H-19), 4.42 dd (1H, J_1 = 10.2 Hz, J_2 = 5.8 Hz, H-3), 5.14 d (1H, J = 12.2 Hz, CH_aPh), 5.19 d (1H, J = 12.2 Hz, CH_bPh), 7.33–7.40 m ($5H_{Ar}$), 7.46–7.50 m (5H, $4H_{Ar}$, NH), 7.53–7.56 m ($2H_{Ar}$), 7.71 d ($1H_{Ar}$, J = 1.5 Hz). **^{13}C NMR** ($CDCl_3$, 126 MHz) *δ*, ppm: 14.7, 15.9, 16.2, 16.6, 18.3, 20.9, 21.4, 23.8, 27.7, 28.1, 29.5, 32.1, 34.3, 35.0, 37.16, 37.22, 37.9, 38.4, 38.5, 40.7, 42.4, 46.5, 50.2, 55.4, 55.9, 57.0, 66.2, 80.9, 121.0, 125.6, 128.3, 128.4, 128.5, 128.7, 128.8, 129.5, 134.6, 136.0, 136.39, 136.43, 141.4, 157.5, 167.6, 168.1, 171.1, 175.6. **HRMS** (ESI): $C_{50}H_{60}NO_6$ found 770.4418 $[M+H]^+$; calcd. 770.4415.

*4.1.23. General procedure for the synthesis of phtalimides **5b, 5e, 5h***

To a stirred solution of acetate **5a**, **5d**, **5g** (0.13 mmol) in THF (1 mL) at room temperature was added 3 *N* HCl in MeOH or 6 *N* HCl in PrOH (3 mL) and the reaction mixture was stirred for 24 h or 4 d. The resulting mixture was diluted with water, extracted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$, water, dried over Na_2SO_4, concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate.

*4.1.24. Benzyl 19-(1,3-dioxoisoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oate (**5b**)*

Compound **5b** was prepared according to the general procedure from acetate **5a** (77 mg; 0.11 mmol) and 3 *N* HCl in MeOH (2.5 mL) for 24 h. After purification (mobile phase hexane/EtOAc 2 : 1) compound **5b** (59 mg; 82 %) was obtained as a white solid; **mp** 165–167 °C (hexane/EtOAc); R_f 0.32 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3500 (O–H), 3250 (N–H), 2940, 2867, 1769, 1718 (C═O), 1618, 748, 695. **^{1}H NMR** ($CDCl_3$, 500 MHz) *δ*, ppm: 0.72 s (3H, Me), 0.746 s (3H, Me), 0.754 s (3H, Me), 0.87 s (3H, Me), 0.93 s (3H, Me), 1.74 dt (1H, J_1 = 13.3 Hz, J_2 = 10.2 Hz), 1.88 t (1H, J = 11.3 Hz, H-18), 2.02–2.07 m (1H), 2.19–2.30 m (2H), 2.38 dt (1H, J_1 = 13.0 Hz, J_2 = 3.3 Hz), 3.12 dd (1H, J_1 = 11.2 Hz, J_2 = 4.7 Hz, H-3), 3.65 td (1H, J_1 = 11.0 Hz, J_2 = 5.0 Hz, H-19), 5.14 d (1H, J = 12.2 Hz, CH_aPh), 5.20 d (1H, J = 12.2 Hz, CH_bPh), 7.33–7.40 m ($5H_{Ar}$), 7.57 dd ($1H_{Ar}$, J_1 = 7.7 Hz, J_2 = 1.5 Hz, H-6′), 7.63 br. s (1H, NH), 7.71 d ($1H_{Ar}$, J = 1.5 Hz, H-4′), 7.72 d ($1H_{Ar}$, J = 7.7 Hz, H-7′). **^{13}C NMR** ($CDCl_3$, 126 MHz) *δ*, ppm: 14.7, 15.5, 15.9, 16.1, 18.4, 20.9, 27.4, 27.7, 28.1, 29.5, 32.1, 34.4, 35.0, 37.2, 37.3, 38.4, 38.8, 38.9, 40.7, 42.4, 46.5, 50.3, 55.4, 56.1, 57.0, 66.2, 79.0, 122.1, 123.7, 128.4, 128.5, 128.7, 130.2, 133.4, 134.0, 136.4, 157.9, 168.3, 168.7, 175.7. **HRMS** (ESI): $C_{42}H_{52}NO_5$ found 650.3845 $[M-H]^+$; calcd. 650.3840.

*4.1.25. Benzyl 19-(7-methyl-1,3-dioxoisoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oate (**5e**)*

Compound **5e** was prepared according to the general procedure from acetate **5d** (110 mg; 0.16 mmol) and 3 *N* HCl in MeOH (3.8 mL) for 24 h. After purification (mobile phase hexane/EtOAc 2 : 1) compound **5e** (91 mg; 86 %) was obtained as a white solid; **mp** 172–174 °C (hexane/EtOAc); R_f 0.27 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3500 (O–H), 3250 (N–H), 2941, 2866, 1764, 1719 (C═O), 1619, 754, 695. **^{1}H NMR** ($CDCl_3$, 500 MHz) *δ*, ppm: 0.73 s (3H, Me), 0.75 s (3H, Me), 0.76 s (3H, Me), 0.88 s (3H, Me), 0.93 s (3H, Me), 1.73 dt (1H, J_1 = 12.7 Hz, J_2 = 10.3 Hz), 1.87 t (1H, J = 11.3 Hz, H-18), 2.03 ddd (1H, J_1 = 12.6 Hz, J_2 = 8.0 Hz, J_2 = 0.9 Hz), 2.16–2.29 m (2H), 2.37 dt (1H, J_1 = 13.0 Hz, J_2 = 3.4 Hz), 2.64 s (3H, Me), 3.13 dd (1H, J_1 = 11.2 Hz, J_2 = 4.7 Hz, H-3), 3.58 td (1H, J_1 = 11.0 Hz, J_2 = 5.1 Hz, H-19), 5.13 d (1H, J = 12.2 Hz, CH_aPh), 5.19 d (1H, J = 12.2 Hz, CH_bPh), 7.29 s ($1H_{Ar}$), 7.32–7.40 m ($5H_{Ph}$), 7.53 d ($1H_{Ar}$, J = 1.1 Hz), 7.55 br. s (1H, NH). **^{13}C NMR** ($CDCl_3$, 126 MHz) *δ*, ppm: 14.8, 15.5, 15.9, 16.1, 17.8, 18.4, 20.9, 27.4, 27.6, 28.1, 29.6, 32.1, 34.4, 34.9, 37.20, 37.23, 38.3, 38.8, 38.9, 40.7, 42.4, 46.3, 50.3, 55.4, 55.7, 56.9, 66.1, 79.0, 119.6, 127.0, 128.3, 128.4,

128.7, 133.8, 136.3, 136.4, 138.3, 157.2, 168.8, 169.1, 175.7. **HRMS** (ESI): $C_{43}H_{56}NO_5$ found 666.4149 $[M+H]^+$; calcd. 666.4153.

4.1.26. Benzyl 19-(1,3-dioxo-7-phenylisoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oate (5h)

Compound **5h** was prepared according to the general procedure from acetate **5g** (100 mg; 0.13 mmol) and 3 *N* HCl in MeOH (3 mL) for 24 h. After purification (mobile phase hexane/EtOAc 3 : 1 to 2 : 1) compound **5h** (85 mg; 90 %) was obtained as a white solid; **mp** 167–169 °C (hexane/EtOAc); R_f 0.27 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3500 (O–H), 3250 (N–H), 2941, 2867, 1767, 1716 (C═O), 1614, 752, 696. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.73 s (3H, Me), 0.75 s (3H, Me), 0.76 s (3H, Me), 0.90 s (3H, Me), 0.94 s (3H, Me), 1.75 dt (1H, J_1 = 12.0 Hz, J_2 = 10.4 Hz), 1.92 t (1H, J = 11.3 Hz, H-18), 2.03–2.07 m (1H), 2.21–2.32 m (2H), 2.38 dt (1H, J_1 = 13.0 Hz, J_2 = 3.2 Hz), 3.13 dd (1H, J_1 = 11.0 Hz, J_2 = 4.7 Hz, H-3), 3.68 td (1H, J_1 = 11.0 Hz, J_2 = 5.1 Hz, H-19), 5.14 d (1H, J = 12.2 Hz, CH_aPh), 5.19 d (1H, J = 12.2 Hz, CH_bPh), 7.31–7.41 m ($5H_{Ar}$), 7.46–7.50 m ($3H_{Ar}$), 7.50 d ($1H_{Ar}$, J = 1.5 Hz), 7.53–7.56 m (3H, $2H_{Ar}$, NH), 7.71 d ($1H_{Ar}$, J = 1.5 Hz). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.8, 15.5, 15.9, 16.1, 18.4, 20.9, 27.4, 27.8, 28.1, 29.5, 32.1, 34.4, 34.9, 37.22, 37.23, 38.4, 38.8, 38.9, 40.7, 42.4, 46.4, 50.3, 55.4, 55.9, 56.9, 66.2, 79.0, 120.9, 125.6, 128.3, 128.4, 128.5, 128.7, 128.8, 129.5, 134.6, 136.1, 136.38, 136.42, 141.4, 157.6, 167.7, 168.3, 175.7. **HRMS** (ESI): $C_{48}H_{56}NO_5$ found 726.4165 $[M-H]^+$; calcd. 726.4153.

4.1.27. General procedure for the synthesis of phtalimides 5c, 5f, 5i

The cleavage of benzyl group was achieved by modification of the known procedures [39,58].

General procedure A: To a stirred solution of benzyl ester (0.09 mmol) in a mixture of THF/MeOH (2 mL/0.4 mL), 10 % Pd/C (0.009 mmol, 10 mol. %) was added at room temperature under nitrogen atmosphere. Then the nitrogen atmosphere was replaced by hydrogen gas from a balloon and the reaction mixture was stirred for 13 h. The mixture was filtered through a Celite, washed with EtOAc and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate.

General procedure B: To a stirred solution of benzyl ester (0.10 mmol) in a mixture of THF/EtOH (8 mL, 1 : 1), 10 % Pd/C (0.07 mmol, 0.67 eq.) was added at room temperature under nitrogen atmosphere. After that 1,3-cyclohexadiene (0.76 mmol, 7.6 eq.) was added and the reaction mixture was stirred at 50 °C for 13 h. The resulting mixture was filtered through a Celite, washed with EtOAc and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate.

4.1.28. 19-(1,3-Dioxoisoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (5c)

Compound **5c** was prepared according to the general procedure A from benzyl ester **5b** (55 mg; 0.08 mmol), 10 % Pd/C (8 mg, 0.008 mmol) in mixture THF/MeOH (1.7 mL/0.3 mL). After purification (mobile phase $CHCl_3$/MeOH 96 : 4) compound **5c** (44 mg; 98 %) was obtained as a white solid; **mp** 227–229 °C ($CHCl_3$/MeOH); R_f 0.49 (silica gel, $CHCl_3$/MeOH, 9 : 2). **IR** (DRIFT): 3480 (O–H), 3211 (N–H), 2937, 2869, 1771, 1715 (C═O), 1686 (C═O), 1619, 748, 692. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ, ppm: 0.62 s (3H, Me), 0.71 s (3H, Me), 0.84 s (3H, Me), 0.85 s (3H, Me), 0.88 s (3H, Me), 1.56 td (1H, J_1 = 13.0 Hz, J_2 = 3.0 Hz), 1.86–1.90 m (2H), 1.93 t (1H, J = 11.5 Hz, H-18), 2.15–2.33 m (3H), 2.91 dt (1H, J_1 = 10.3 Hz, J_2 = 5.0 Hz, H-3), 3.57 td (1H, J_1 = 10.7 Hz, J_2 = 5.0 Hz, H-19), 4.22 d (1H, J = 5.0 Hz, OH), 7.67 d ($1H_{Ar}$, J = 8.1 Hz), 7.72 d ($1H_{Ar}$, J = 8.1 Hz), 7.73 s ($1H_{Ar}$), 11.2 s (1H, NH), 12.2 br. s (1H, COOH). **^{13}C NMR** (DMSO-d_6, 100 MHz) δ, ppm: 14.3, 15.6, 15.7, 15.8, 17.9, 20.4, 27.0, 27.1, 28.0, 29.1, 31.3, 33.9, 34.8, 36.2, 36.6, 37.6, 38.1, 38.4, 40.1, 41.9, 45.8, 49.5, 54.5, 54.8, 55.8, 76.7, 121.4, 122.9, 130.0, 133.1, 133.6, 157.2, 169.1, 169.4, 177.2. **HRMS** (ESI): $C_{35}H_{46}NO_5$ found 560.3368 $[M-H]^+$; calcd. 560.3370.

4.1.29. 19-(7-Methyl-1,3-dioxoisoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (5f)

Compound **5f** was prepared according to the general procedure B from benzyl ester **5e** (94 mg; 0.14 mmol), 10 % Pd/C (95 mg, 0.09 mmol) and 1,3-cyclohexadiene (100 μL, 85 mg, 1.06 mmol) in mixture THF/EtOH (10 mL, 1 : 1). After purification (mobile phase hexane/EtOAc 3 : 2) compound **5f** (68 mg; 84 %) was obtained as a white solid; **mp** 224–226 °C (hexane/EtOAc); R_f 0.16 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3460 (O–H), 3210 (N–H), 2937, 2868, 1765, 1712 (C═O), 1619, 755. **^{1}H NMR** (DMSO-d_6 500 MHz) δ, ppm: 0.62 s (3H, Me), 0.71 s (3H, Me), 0.84 s (3H, Me), 0.85 s (3H, Me), 0.89 s (3H, Me), 1.56 td (1H, J_1 = 13.0 Hz, J_2 = 3.0 Hz), 1.85–1.88 m (2H), 1.93 t (1H, J = 11.3 Hz, H-18), 2.12–2.20 m (2H), 2.29 td (1H, J_1 = 12.3 Hz, J_2 = 3.0 Hz), 2.55 (3H, Me), 2.90 dt (1H, J_1 = 10.7 Hz, J_2 = 5.1 Hz, H-3), 3.51 td (1H, J_1 = 10.9 Hz, J_2 = 5.0 Hz, H-19), 4.21 d (1H, J = 5.1 Hz, OH), 7.49 s ($1H_{Ar}$), 7.53 s ($1H_{Ar}$), 11.07 s (1H, NH), 12.18 s (1H, COOH). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.6, 15.7, 15.8, 17.0, 17.9, 20.4, 27.0, 27.1, 28.0, 29.1, 31.3, 33.9, 34.7, 36.1, 36.6, 37.6, 38.2, 38.4, 40.1, 41.9, 45.7, 49.6, 54.1, 54.8, 55.8, 76.8, 118.9, 126.7, 133.6, 135.6, 137.0, 156.6, 169.2, 169.9, 177.2. **HRMS** (ESI): $C_{36}H_{48}NO_5$ found 574.3501 $[M-H]^+$; calcd. 574.3527.

4.1.30. 19-(1,3-Dioxo-7-phenylisoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (5i)

Compound **5i** was prepared according to the general procedure B from benzyl ester **5h** (75 mg; 0.10 mmol), 10 % Pd/C (74 mg, 0.07 mmol) and 1,3-cyclohexadiene (72 μL, 61 mg, 0.76 mmol) in mixture THF/EtOH (8 mL, 1 : 1). After purification (mobile phase hexane/EtOAc 3 : 2) compound **5i** (63 mg; 98 %) was obtained as a white solid; **mp** 230–232 °C (hexane/EtOAc); R_f 0.18 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3460 (O–H), 3213 (N–H), 2940, 2867, 1767, 1715 (C═O), 1615, 754, 697. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.73 s (3H, Me), 0.78 s (3H, Me), 0.935 s (3H, Me), 0.942 s (3H, Me), 0.95 s (3H, Me), 1.81 dt (1H, J_1 = 12.9 Hz, J_2 = 10.4 Hz), 1.97 t (1H, J = 11.3 Hz, H-18), 2.11–2.16 m (1H), 2.28–2.40 m (3H), 3.15 dd (1H, J_1 = 11.1 Hz, J_2 = 4.7 Hz, H-3), 3.68 td (1H, J_1 = 11.0 Hz, J_2 = 5.3 Hz, H-19), 7.45–7.50 m ($3H_{Ar}$), 7.53 d ($1H_{Ar}$, J = 1.4 Hz), 7.54–7.56 m ($2H_{Ar}$), 7.74 d ($1H_{Ar}$, J = 1.4 Hz), 7.83 br. s (1H, NH). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.6, 15.7, 15.8, 17.9, 20.4, 27.1 (2C), 28.0, 29.1, 31.2, 33.9, 34.7, 36.1, 36.6, 37.7, 38.3, 38.4, 40.1, 41.9, 45.6, 49.6, 54.2, 54.8, 55.8, 76.8, 120.1, 125.4, 127.9, 128.2, 129.6, 134.5, 134.9, 136.2, 139.8, 157.0, 168.6, 168.8, 177.3. **HRMS** (ESI): $C_{41}H_{50}NO_5$ found 636.3686 $[M-H]^+$; calcd. 636.3684.

4.1.31. General procedure for the synthesis of phtalonitriles 6a, 6c

3,4-Dicyanophenyl- derivatives of betulinic acid (phthalonitrile) were synthesized in 2 steps by modification of the known procedures [57]. Solution of the phthalimide (0.17 mmol) and 7 *N* ammonia solution in MeOH (3 mL) was stirred at room temperature for 48–96 h. The reaction mixture was diluted with water, extracted with EtOAc (40 mL). Organic layer was washed with water (10 mL × 2), dried over Na_2SO_4, concentrated in vacuo. The crude phthalamide was used in next step without purification. Anhydrous DMF (64 μL, 0.82 mmol, 4.8 eq.) was added to acetonitrile (3 mL) cooled to 0 °C and then oxalyl chloride (64 μL, 0.75 mmol, 4.4 eq.) was slowly added with formation of a white precipitate and gas evolution. When the evolution of gas ceased, the crude phthalamide was added as a suspension in acetonitrile (5 mL). Pyridine (121 μl, 1.5 mmol, 8.8 eq.) was then added, and the reaction mixture was stirred for an additional 45 min at 0 °C. The reaction was then quenched by the addition of Et_2O (2 mL) and 10 % HCl (2 mL), extracted with Et_2O (10 mL × 3). Organic layer was washed with sat. NaCl, dried over Na_2SO_4, concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate (4 : 1).

*4.1.32. Benzyl 3β-acetoxy-19-(3,4-dicyanophenyl)-20,29,30-trinorlupan-28-oate (**6a**)*

Compound **6a** was prepared according to the general procedure in 2 stages from phthalimide **5a** (121 mg; 0.17 mmol) and 7 *N* ammonia in MeOH (3 mL), then oxalyl chloride (64 μL, 0.75 mmol), DMF (64 μL, 0.82 mmol) and pyridine (121 μL, 1.5 mmol). After purification (mobile phase hexane/EtOAc 4 : 1) compound **6a** (72 mg; 63 %) was obtained as a white solid; **mp** 138–140 °C (hexane/EtOAc); R_f 0.57 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 2943, 2233 (C≡ N), 1723, 1598, 1455, 1366, 1243, 1130, 1028, 978, 733, 697. **^{1}H NMR** ($CDCl_3$, 500 MHz) *δ*, ppm: 0.74 s (3H, Me), 0.79 s (3H, Me), 0.81 s (3H, Me), 0.82 s (3H, Me), 0.87 s (3H, Me), 1.71 dt (1H, J_1 = 12.9 Hz, J_2 = 10.3 Hz), 1.78 t (1H, J = 11.3 Hz, H-18), 2.02 s (3H, MeCO), 2.06 ddd (1H, J_1 = 9.8 Hz, J_2 = 8.2 Hz, J_3 = 1.0 Hz), 2.18–2.29 m (2H), 2.38 dt (1H, J_1 = 13.1 Hz, J_2 = 3.4 Hz), 3.59 td (1H, J_1 = 11.1 Hz, J_2 = 5.2 Hz, H-19), 4.43 dd (1H, J_1 = 10.0 Hz, J_2 = 6.1 Hz, H-3), 5.12 d (1H, J = 12.2 Hz, CH_aPh), 5.19 d (1H, J = 12.2 Hz, CH_bPh), 7.32–7.38 m ($5H_{Ph}$), 7.55 dd (1H, J_1 = 8.1 Hz, J_2 = 1.7 Hz, H-6′), 7.62 d (1H, J = 1.7 Hz, H-2′), 7.69 d (1H, J = 8.1 Hz, H-5’). **^{13}C NMR** ($CDCl_3$, 126 MHz) *δ*, ppm: 14.6, 15.8, 16.2, 16.6, 18.2, 20.8, 21.4, 23.7, 27.9, 28.0, 29.4, 32.0, 34.3, 34.9, 37.14, 37.15, 37.9, 38.2, 38.4, 40.7, 42.4, 46.1, 50.1, 55.4, 56.1, 56.9, 66.3, 80.9, 113.0, 115.7, 115.8, 116.0, 128.4, 128.5, 128.7, 132.3, 132.7, 133.7, 136.2, 156.5, 171.1, 175.4. **HRMS** (ESI): $C_{44}H_{54}NO_6$ found 692.4428 $[M + NH_4]^+$; calcd. 692.4422.

*4.1.33. Benzyl 3β-acetoxy-19-(5,6-dicyanobiphenyl-3-yl)-20,29,30-trinorlupan-28-oate (**6c**)*

Compound **6c** was prepared according to the general procedure in 2 stages from phthalimide **5g** (232 mg; 0.30 mmol) and 7 *N* ammonia in MeOH (9 mL), then oxalyl chloride (114 μL, 1.32 mmol), DMF (111 μL, 1.44 mmol) and pyridine (213 μL, 2.64 mmol). After purification (mobile phase hexane/EtOAc 4 : 1 to 3 : 1) compound **6c** (84 mg; 37 %) was obtained as a white solid; **mp** 156–158 °C (hexane/EtOAc); R_f 0.55 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 2943, 2870, 2228 (C≡ N), 1727 (C═O), 1592, 1244 (C–O), 698. **^{1}H NMR** ($CDCl_3$, 400 MHz) *δ*, ppm: 0.74 s (3H, Me), 0.79 s (3H, Me), 0.81 s (3H, Me), 0.82 s (3H, Me), 0.89 s (3H, Me), 1.71 dt (1H, J_1 = 12.5 Hz, J_2 = 10.5 Hz), 1.82 t (1H, J = 11.3 Hz, H-18), 2.02 s (3H, MeCO), 2.03–2.08 m (1H), 2.22–2.40 m (3H), 3.63 td (1H, J_1 = 10.9 Hz, J_2 = 5.0 Hz, H-19), 4.43 dd (1H, J_1 = 9.8 Hz, J_2 = 6.0 Hz, H-3), 5.12 d (1H, J = 12.2 Hz, CH_aPh), 5.19 d (1H, J = 12.2 Hz, CH_bPh), 7.33–7.38 m ($5H_{Ph}$), 7.50–7.54 m ($5H_{Ph}$), 7.56 s ($1H_{Ar}$), 7.61 s ($1H_{Ar}$). **^{13}C NMR** ($CDCl_3$, 100 MHz) *δ*, ppm: 14.7, 15.8, 16.2, 16.6, 18.2, 20.9, 21.4, 23.7, 28.0 (2C), 29.4, 31.9, 34.3, 34.9, 37.1 (2C), 37.9, 38.2, 38.4, 40.7, 42.4, 46.1, 50.1, 55.4, 56.0, 56.9, 66.2, 80.8, 111.6, 115.6, 116.2, 117.4, 128.4, 128.5, 128.7, 128.8, 129.1, 129.7, 131.2, 133.4, 136.2, 136.9, 147.2, 156.2, 171.0, 175.4. **HRMS** (ESI): $C_{50}H_{62}N_3O_4$ found 768.4739 $[M + NH_4]^+$; calcd. 768.4735.

*4.1.34. General procedure for the synthesis of phtalonitriles **6b, 6d***

The cleavage of acetyl group was achieved by modification of the known procedure [59,60]. To a solution of acetate (0.12 mmol) in methanol (4 mL) was added *p*-TsOH·H_2O (0.06 mmol, 50 mol. %). The mixture was stirred under reflux for 7 h and monitored by TLC until starting material was consumed. The solvent was evaporated in vacuo and the residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate.

The cleavage of benzyl group was achieved by modification of the known procedure [58]. To a stirred solution of benzyl ester **6a**, **6c** (0.10 mmol) in a mixture of THF/EtOH (8 mL, 1 : 1), 10 % Pd/C (0.07 mmol, 0.67 eq.) was added at room temperature under nitrogen atmosphere. After that 1,3-cyclohexadiene (0.76 mmol, 7.6 eq.) was added and the reaction mixture was stirred at 50 °C for 13 h. The resulting mixture was filtered through a Celite, washed with EtOAc and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate.

*4.1.35. 19-(3,4-Dicyanophenyl)-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (**6b**)*

Compound **6b** was prepared according to the general procedures above from benzyl ester **6a** (62 mg; 0.10 mmol), 10 % Pd/C (71 mg, 0.07 mmol) and 1,3-cyclohexadiene (72 μL, 61 mg, 0.76 mmol) in mixture THF/EtOH (8 mL, 1 : 1). After purification (mobile phase hexane/EtOAc 2 : 1) compound **6b** (53 mg; 98 %, 91 % afterall) was obtained as a white solid; **mp** 198–200 °C (hexane/EtOAc); R_f 0.16 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3402 (O–H), 2937, 2871, 2233 (C≡ N), 1697 (C═O), 1597, 1190, 734. **^{1}H NMR** (DMSO-d_6, 500 MHz) *δ*, ppm: 0.63 s (3H, Me), 0.72 s (3H, Me), 0.85 s (6H, 2Me), 0.88 s (3H, Me), 1.54 td (1H, J_1 = 13.1 Hz, J_2 = 3.2 Hz), 1.83–1.94 m (3H), 2.12–2.19 m (2H), 2.27 td (1H, J_1 = 12.4 Hz, J_2 = 3.4 Hz), 2.92 dt (1H, J_1 = 10.5 Hz, J_2 = 5.1 Hz, H-3), 3.53 td (1H, J_1 = 10.9 Hz, J_2 = 4.9 Hz, H-19), 4.22 d (1H, J = 5.1 Hz, OH), 7.88 dd (1H, J_1 = 8.1 Hz, J_2 = 1.4 Hz, H-6′), 7.99 d (1H, J = 8.1 Hz, H-5′), 8.19 d (1H, J = 1.4 Hz, H-2’), 12.22 s (1H, COOH). **^{13}C NMR** (DMSO-d_6, 126 MHz) *δ*, ppm: 14.3, 15.6, 15.7, 15.8, 17.9, 20.4, 22.07, 22.11, 28.0, 29.1, 31.1, 33.8, 34.6, 36.0, 36.6, 37.6, 38.2, 38.4, 40.1, 42.0, 45.3, 49.5, 54.6, 54.8, 55.8, 76.7, 111.4, 114.5, 116.10, 116.14, 133.0, 133.2, 133.8, 157.0, 177.2. **HRMS** (ESI): $C_{35}H_{45}N_2O_3$ found 541.3434 $[M - H]^+$; calcd. 541.3425.

*4.1.36. 19-(5,6-Dicyanobiphenyl-3-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (**6d**)*

Compound **6d** was prepared according to the general procedures above from benzyl ester **6c** (62 mg; 0.09 mmol), 10 % Pd/C (64 mg, 0.06 mmol) and 1,3-cyclohexadiene (64 μL, 54 mg, 0.68 mmol) in mixture THF/EtOH (7 mL, 1 : 1). After purification (mobile phase hexane/EtOAc 2 : 1) compound **6d** (46 mg; 82 %, 81 % afterall) was obtained as a white solid; **mp** 200–203 °C (hexane/EtOAc); R_f 0.18 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3500 (O–H), 2939, 2868, 2229 (C≡ N), 1695 (C═O), 1592, 771, 699. **^{1}H NMR** (DMSO-d_6, 500 MHz) *δ*, ppm: 0.63 s (3H, Me), 0.72 s (3H, Me), 0.85 s (3H, Me), 0.86 s (3H, Me), 0.91 s (3H, Me), 1.57 td (1H, J_1 = 13.0 Hz, J_2 = 3.1 Hz), 1.85 dd (1H, J_1 = 11.7 Hz, J_2 = 8.6 Hz), 1.92–1.98 m (1H), 1.98 t (1H, J = 11.2 Hz), 2.14–2.24 m (2H), 2.29 td (1H, J_1 = 12.7 Hz, J_2 = 3.1 Hz), 2.92 dt (1H, J_1 = 10.7 Hz, J_2 = 5.2 Hz, H-3), 3.60 td (1H, J_1 = 10.9 Hz, J_2 = 5.0 Hz, H-19), 4.22 d (1H, J = 5.2 Hz, OH), 7.54–7.63 m ($5H_{Ph}$), 7.88 s ($1H_{Ar}$), 8.21 s ($1H_{Ar}$), 12.21 s (1H, COOH). **^{13}C NMR** (DMSO-d_6, 126 MHz) *δ*, ppm: 14.3, 15.6, 15.7, 15.8, 17.9, 20.5, 27.1, 27.2, 28.1, 29.1, 31.0, 33.9, 34.5, 35.9, 36.7, 37.7, 38.3, 38.4, 40.1, 42.0, 45.4, 49.5, 54.4, 54.8, 55.9, 76.8, 110.1, 116.0, 116.4, 118.9, 128.8, 128.9, 129.4, 131.9, 133.7, 136.5, 145.9, 157.1, 177.2. **HRMS** (ESI): $C_{41}H_{49}N_2O_3$ found 617.3747 $[M - H]^+$; calcd. 617.3738.

*4.1.37. General procedure for the synthesis of protected N-substituted phthalimides **7, 9, 11, 13, 15, 17, 18, 20, 22, 24, 27, 29***

N-substituted phthalimides were synthesized in 3 steps by modification of the known procedures [56,61]. To the phthalic acid **5a** or **5g** (0.30 mmol) was added acetic anhydride (1.5 mL) and the solution was refluxed for 5 h under nitrogen atmosphere. The acetic anhydride was removed in vacuo and the crude phthalic anhydride was used in next step without purification. A mixture of phthalic anhydride and primary amine (0.30 mmol) in THF (1.5 mL) was stirred for 2–4 h at 40 °C or refluxed under nitrogen atmosphere. Removal of the solvent in vacuo afforded the corresponding carboxylic acid amide. The carboxylic acid amide was then heated at 170 °C for 2 h under nitrogen atmosphere. The crude product was cooled to room temperature and purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate (4 : 1 to 1 : 1).

*4.1.38. Benzyl 3β-acetoxy-19-(1,3-dioxo-2-phenylisoindolin-5-yl)-20,29,30-trinorlupan-28-oate (**7**)*

Compound **7** was prepared according to the general procedure in 3 stages from phthalic acid **5a** (200 mg; 0.30 mmol) and acetic anhydride (1.5 mL), then aniline (28 mg; 0.30 mmol) in THF (1.5 mL) for 2 h at

40 °C. After purification (mobile phase hexane/EtOAc 4 : 1) compound **7** (140 mg; 61 %) was obtained as a white solid; **mp** 125–127 °C (hexane/EtOAc); R_f 0.59 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 2942, 2860, 1775, 1723 (C═O), 1617, 751, 690. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.76 s (3H, Me), 0.79 s (3H, Me), 0.80 s (3H, Me), 0.81 s (3H, Me), 0.89 s (3H, Me), 1.78 dt (1H, $J_1 = 12.7$ Hz, $J_2 = 10.4$ Hz), 1.91 t (1H, $J = 11.3$ Hz, H-18), 2.02 s (3H, MeCO), 2.04–2.09 m (1H), 2.21–2.32 m (2H), 2.39 dt (1H, $J_1 = 13.1$ Hz, $J_2 = 3.2$ Hz), 3.67 td (1H, $J_1 = 11.0$ Hz, $J_2 = 5.1$ Hz, H-19), 4.41 dd (1H, $J_1 = 8.7$ Hz, $J_2 = 7.3$ Hz, H-3), 5.15 d (1H, $J = 12.2$ Hz, CH_aPh), 5.20 d (1H, $J = 12.2$ Hz, CH_bPh), 7.34–7.44 m ($8H_{Ph}$), 7.49–7.52 m ($2H_{Ph}$), 7.61 dd (1H, $J_1 = 7.7$ Hz, $J_2 = 1.4$ Hz, H-6′), 7.80 d (1H, $J = 1.4$ Hz, H-4′), 7.82 d (1H, $J_1 = 7.7$ Hz, H-7′). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.7, 15.9, 16.2, 16.6, 18.3, 20.9, 21.4, 23.8, 27.7, 28.0, 29.5, 32.1, 34.3, 35.1, 37.2, 37.3, 37.9, 38.3, 38.5, 40.7, 42.4, 46.6, 50.2, 55.4, 56.2, 57.0, 66.2, 80.9, 122.3, 123.9, 126.7, 128.1, 128.4, 128.5, 128.7, 129.2, 129.3, 132.0, 132.5, 134.0, 136.4, 158.0, 167.4, 167.7, 171.0, 175.6. **HRMS** (ESI): $C_{50}H_{60}NO_6$ found 770.4421 $[M+H]^+$; calcd. 770.4415.

4.1.39. Benzyl 3β-acetoxy-19-(1,3-dioxo-2-(4-methoxyphenyl)isoindolin-5-yl)-20,29,30-trinorlupan-28-oate (9)

Compound **9** was prepared according to the general procedure in 3 stages from phthalic acid **5a** (206 mg; 0.31 mmol) and acetic anhydride (1.5 mL), then 4-methoxyaniline (38 mg; 0.31 mmol) in THF (1.5 mL) for 2 h at 40 °C. After purification (mobile phase hexane/EtOAc 3 : 1) compound **9** (122 mg; 50 %) was obtained as a pale yellow solid; **mp** 140–142 °C (hexane/EtOAc); R_f 0.57 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 2943, 2860, 1774, 1717 (C═O), 1613, 1512, 1377, 1245 (C–O), 746, 696. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.76 s (3H, Me), 0.79 s (3H, Me), 0.80 s (3H, Me), 0.81 s (3H, Me), 0.88 s (3H, Me), 1.77 dt (1H, $J_1 = 12.7$ Hz, $J_2 = 10.4$ Hz), 1.91 t (1H, $J = 11.3$ Hz, H-18), 2.02 s (3H, MeCO), 2.04–2.08 m (1H), 2.22–2.32 m (2H), 2.39 dt (1H, $J_1 = 12.9$ Hz, $J_2 = 3.2$ Hz), 3.67 td (1H, $J_1 = 11.0$ Hz, $J_2 = 5.0$ Hz, H-19), 3.85 s (3H, MeO), 4.41 dd (1H, $J_1 = 8.7$ Hz, $J_2 = 7.3$ Hz, H-3), 5.15 d (1H, $J = 12.2$ Hz, CH_aPh), 5.20 d (1H, $J = 12.2$ Hz, CH_bPh), 7.02 d ($2H_{Ar}$, $J = 9.0$ Hz), 7.33 d ($2H_{Ar}$, $J = 9.0$ Hz), 7.33–7.39 m ($5H_{Ph}$), 7.59 dd (1H, $J_1 = 7.7$ Hz, $J_2 = 1.4$ Hz, H-6′), 7.79 d (1H, $J = 1.4$ Hz, H-4′), 7.80 d (1H, $J_1 = 7.7$ Hz, H-7′). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.7, 15.9, 16.2, 16.6, 18.3, 20.9, 21.4, 23.8, 27.7, 28.0, 29.5, 32.1, 34.3, 35.1, 37.2, 37.3, 37.9, 38.4, 38.5, 40.7, 42.4, 46.9, 50.2, 55.5, 55.6, 56.2, 57.0, 66.2, 80.9, 114.6, 122.2, 123.8, 124.6, 128.1, 128.4, 128.5, 128.7, 129.4, 132.6, 134.0, 136.4, 157.8, 159.3, 167.7, 168.0, 171.1, 175.7. **HRMS** (ESI): $C_{51}H_{62}NO_7$ found 800.4525 $[M+H]^+$; calcd. 800.4521.

4.1.40. Benzyl 3β-acetoxy-19-(1,3-dioxo-2-(4-cyanophenyl)isoindolin-5-yl)-20,29,30-trinorlupan-28-oate (11)

Compound **11** was prepared according to the general procedure in 3 stages from phthalic acid **5a** (206 mg; 0.31 mmol) and acetic anhydride (1.5 mL), then 4-aminobenzonitrile (37 mg; 0.31 mmol) in THF (1.5 mL) for 4 h at reflux. After purification (mobile phase hexane/EtOAc 4 : 1) compound **11** (152 mg; 62 %) was obtained as a white solid; **mp** 163–164 °C (hexane/EtOAc); R_f 0.55 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 2943, 2869, 2229 (C≡ N), 1779, 1721 (C═O), 1605, 1363, 1243, 745, 695. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.75 s (3H, Me), 0.79 s (3H, Me), 0.80 s (3H, Me), 0.81 s (3H, Me), 0.88 s (3H, Me), 1.77 dt (1H, $J_1 = 12.7$ Hz, $J_2 = 10.3$ Hz), 1.90 t (1H, $J = 11.3$ Hz, H-18), 2.01 s (3H, MeCO), 2.07 ddd (1H, $J_1 = 13.0$ Hz, $J_2 = 8.1$ Hz, $J_3 = 0.9$ Hz), 2.22–2.33 m (2H), 2.40 dt (1H, $J_1 = 13.1$ Hz, $J_2 = 3.3$ Hz), 3.69 td (1H, $J_1 = 11.0$ Hz, $J_2 = 5.0$ Hz, H-19), 4.40 dd (1H, $J_1 = 8.1$ Hz, $J_2 = 7.9$ Hz, H-3), 5.14 d (1H, $J = 12.2$ Hz, CH_aPh), 5.20 d (1H, $J = 12.2$ Hz, CH_bPh), 7.34–7.41 m ($5H_{Ph}$), 7.65 dd (1H, $J_1 = 7.8$ Hz, $J_2 = 1.4$ Hz, H-6′), 7.68 d ($2H_{Ar}$, $J = 8.9$ Hz), 7.79 d ($2H_{Ar}$, $J = 8.9$ Hz), 7.82 d (1H, $J = 1.4$ Hz, H-4′), 7.84 d (1H, $J_1 = 7.8$ Hz, H-7′). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.7, 15.9, 16.2, 16.6, 18.3, 20.9, 21.4, 23.7, 27.8, 28.0, 29.5, 32.1, 34.3, 35.1, 37.2, 37.3, 37.9, 38.3, 38.5, 40.7, 42.4, 46.6, 50.2, 55.5, 56.3, 57.0, 66.2, 80.9, 111.3, 118.4, 122.7, 124.3, 126.6, 128.4, 128.5, 128.7, 128.9, 132.1, 133.0, 134.5, 136.2, 136.4, 158.7, 166.5, 166.9, 171.1, 175.6. **HRMS** (ESI): $C_{51}H_{62}N_3O_6$ found 812.4639 $[M + NH_4]^+$; calcd. 812.4633.

4.1.41. Benzyl 3β-acetoxy-19-(1,3-dioxo-2-(4-acetamidophenyl) isoindolin-5-yl)-20,29,30-trinorlupan-28-oate (13)

Compound **13** was prepared according to the general procedure in 3 stages from phthalic acid **5a** (206 mg; 0.31 mmol) and acetic anhydride (1.5 mL), then *N*-(4-aminophenyl)acetamide (47 mg; 0.31 mmol) in THF (1.5 mL) for 4 h at reflux. After purification (mobile phase hexane/EtOAc 1 : 1) compound **13** (120 mg; 47 %) was obtained as a pale yellow solid; **mp** 172–174 °C (hexane/EtOAc); R_f 0.14 (silica gel, hexane/EtOAc, 1 : 1). **IR** (DRIFT): 3400 (N–H), 2943, 2820, 1775, 1716 (C═O), 1605, 1514, 1367, 1245 (C–O), 746, 695. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.75 s (3H, Me), 0.79 s (3H, Me), 0.80 s (3H, Me), 0.81 s (3H, Me), 0.88 s (3H, Me), 1.77 dt (1H, $J_1 = 11.9$ Hz, $J_2 = 10.3$ Hz), 1.90 t (1H, $J = 11.3$ Hz, H-18), 2.01 s (3H, MeCO), 2.04–2.08 m (1H), 2.20 s (3H, MeCO), 2.22–2.32 m (2H), 2.39 dt (1H, $J_1 = 13.1$ Hz, $J_2 = 3.3$ Hz), 3.67 td (1H, $J_1 = 11.0$ Hz, $J_2 = 5.0$ Hz, H-19), 4.44 dd (1H, $J_1 = 8.8$ Hz, $J_2 = 7.2$ Hz, H-3), 5.14 d (1H, $J = 12.2$ Hz, CH_aPh), 5.20 d (1H, $J = 12.2$ Hz, CH_bPh), 7.31 br. s (1H, NH), 7.34–7.41 m ($7H_{Ar}$), 7.60 dd (1H, $J_1 = 7.7$ Hz, $J_2 = 1.4$ Hz, H-6′), 7.64 d ($2H_{Ar}$, $J = 8.7$ Hz), 7.79 d (1H, $J = 1.4$ Hz, H-4′), 7.80 d (1H, $J_1 = 7.7$ Hz, H-7′). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.7, 15.8, 16.2, 16.6, 18.2, 20.8, 21.4, 23.7, 24.7, 27.6, 28.0, 29.5, 32.0, 34.2, 35.1, 37.1, 37.2, 37.9, 38.3, 38.4, 40.6, 42.4, 46.5, 50.1, 55.4, 56.2, 57.0, 66.1, 80.9, 120.2, 122.3, 123.9, 127.3, 127.5, 128.35, 128.43, 128.7, 129.2, 132.4, 134.1, 136.3, 138.0, 158.0, 167.5, 167.8, 168.6, 171.1, 175.6. **HRMS** (ESI): $C_{52}H_{61}N_2O_7$ found 825.4487 $[M - H]^+$; calcd. 825.4473.

4.1.42. Benzyl 3β-acetoxy-19-(1,3-dioxo-2-(1h-pyrazol-3-yl)isoindolin-5-yl)-20,29,30-trinorlupan-28-oate (15)

Compound **15** was prepared according to the general procedure in 3 stages from phthalic acid **5a** (206 mg; 0.31 mmol) and acetic anhydride (1.5 mL), then *1H*-pyrazol-3-amine (26 mg; 0.31 mmol) in THF (1.5 mL) for 4 h at reflux. After purification (mobile phase hexane/EtOAc 1 : 1) compound **15** (208 mg; 89 %) was obtained as a white solid; **mp** 165–167 °C (hexane/EtOAc); R_f 0.11 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3200 (N–H), 2942, 2869, 1780, 1721 (C═O), 1244 (C–O), 745, 694. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.75 s (3H, Me), 0.78 s (3H, Me), 0.80 s (3H, Me), 0.81 s (3H, Me), 0.88 s (3H, Me), 1.77 dt (1H, $J_1 = 12.7$ Hz, $J_2 = 10.4$ Hz), 1.90 t (1H, $J = 11.3$ Hz, H-18), 2.01 s (3H, MeCO), 2.05–2.09 m (1H), 2.23–2.31 m (2H), 2.39 dt (1H, $J_1 = 12.9$ Hz, $J_2 = 3.1$ Hz), 3.67 td (1H, $J_1 = 11.0$ Hz, $J_2 = 5.1$ Hz, H-19), 4.41 dd (1H, $J_1 = 8.8$ Hz, $J_2 = 7.3$ Hz, H-3), 5.14 d (1H, $J = 12.2$ Hz, CH_aPh), 5.20 d (1H, $J = 12.2$ Hz, CH_bPh), 6.63 d (1H, $J = 2.3$ Hz), 7.34–7.39 m ($5H_{Ph}$), 7.61 dd (1H, $J_1 = 7.7$ Hz, $J_2 = 1.4$ Hz, H-6′), 7.69 d (1H, $J = 2.3$ Hz), 7.81 d (1H, $J = 1.4$ Hz, H-4′), 7.82 d (1H, $J_1 = 7.7$ Hz, H-7′). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.7, 15.8, 16.2, 16.6, 18.2, 20.8, 21.4, 23.7, 27.6, 28.0, 29.5, 32.0, 34.2, 35.0, 37.1, 37.2, 37.9, 38.3, 38.4, 40.6, 42.4, 46.6, 50.1, 55.4, 56.2, 56.9, 66.1, 80.9, 100.6, 122.5, 124.1, 128.3, 128.4, 128.7, 129.3, 131.4, 132.4, 134.2, 136.4, 140.3, 158.2, 166.4, 166.8, 171.1, 175.7. **HRMS** (ESI): $C_{47}H_{58}N_3O_6$ found 760.4324 $[M+H]^+$; calcd. 760.4320.

4.1.43. Benzyl 3β-acetoxy-19-(1,3-dioxo-2-(thiazol-2-yl)isoindolin-5-yl)-20,29,30-trinorlupan-28-oate (17)

Compound **17** was prepared according to the general procedure in 3 stages from phthalic acid **5a** (206 mg; 0.31 mmol) and acetic anhydride (1.5 mL), then thiazol-2-amine (31 mg; 0.31 mmol) in THF (1.5 mL) for 4 h at reflux. After purification (mobile phase hexane/EtOAc 2 : 1) compound **17** (151 mg; 63 %) was obtained as a white solid; **mp** 155–157 °C (hexane/EtOAc); R_f 0.32 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 2943, 2867, 1787, 1727 (C═O), 1333, 1243 (C–O), 741, 696. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.75 s (3H, Me), 0.78 s (3H, Me), 0.80 s (3H, Me), 0.81 s (3H, Me), 0.87 s (3H, Me), 1.78 dt (1H, $J_1 = 12.9$

Hz, J_2 = 10.4 Hz), 1.90 t (1H, J = 11.3 Hz, H-18), 2.01 s (3H, MeCO), 2.05–2.10 m (1H), 2.23–2.31 m (2H), 2.39 dt (1H, J_1 = 13.0 Hz, J_2 = 3.2 Hz), 3.69 td (1H, J_1 = 11.0 Hz, J_2 = 5.2 Hz, H-19), 4.41 dd (1H, J_1 = 8.8 Hz, J_2 = 7.3 Hz, H-3), 5.14 d (1H, J = 12.2 Hz, CH_aPh), 5.20 d (1H, J = 12.2 Hz, CH_bPh), 7.31 d (1H, J = 3.5 Hz), 7.34–7.39 m ($5H_{Ph}$), 7.65 dd (1H, J_1 = 7.8 Hz, J_2 = 1.4 Hz, H-6'), 7.81 d (1H, J = 3.5 Hz), 7.85 d (1H, J = 1.4 Hz, H-4'), 7.88 d (1H, J_1 = 7.8 Hz, H-7'). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.7, 15.9, 16.2, 16.6, 18.2, 20.9, 21.4, 23.7, 27.8, 28.0, 29.5, 32.1, 34.3, 35.0, 37.1, 37.2, 37.9, 38.3, 38.4, 40.7, 42.4, 46.6, 50.2, 55.4, 56.3, 57.0, 66.2, 80.9, 117.3, 123.1, 124.6, 128.4, 128.5, 128.7, 131.8, 134.8, 136.4, 140.1, 152.6, 158.9, 164.7, 165.1, 171.0, 175.6. **HRMS** (ESI): $C_{47}H_{57}N_2O_6S$ found 777.3920 $[M+H]^+$; calcd. 777.3932.

*4.1.44. Benzyl 3β-acetoxy-19-(1,3-dioxo-2,7-diphenylisoindolin-5-yl)-20,29,30-trinorlupan-28-oate (**18**)*

Compound **18** was prepared according to the general procedure in 3 stages from phthalic acid **5g** (230 mg; 0.31 mmol) and acetic anhydride (1.7 mL), then aniline (29 mg; 0.31 mmol) in THF (1.5 mL) for 2 h at 40 °C. After purification (mobile phase hexane/EtOAc 4 : 1) compound **18** (158 mg; 60 %) was obtained as a white solid; **mp** 170–172 °C (hexane/EtOAc); R_f 0.57 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 2942, 2867, 1775, 1719 (C═O), 1376, 1243 (C–O), 751, 695. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.76 s (3H, Me), 0.80 s (3H, Me), 0.81 s (3H, Me), 0.82 s (3H, Me), 0.91 s (3H, Me), 1.78 dt (1H, J_1 = 12.4 Hz, J_2 = 10.5 Hz), 1.96 t (1H, J = 11.4 Hz, H-18), 2.02 s (3H, MeCO), 2.05–2.09 m (1H), 2.25–2.34 m (2H), 2.39 dt (1H, J_1 = 13.0 Hz, J_2 = 3.0 Hz), 3.71 td (1H, J_1 = 11.0 Hz, J_2 = 4.9 Hz, H-19), 4.42 dd (1H, J_1 = 9.5 Hz, J_2 = 6.4 Hz, H-3), 5.15 d (1H, J = 12.2 Hz, CH_aPh), 5.20 d (1H, J = 12.2 Hz, CH_bPh), 7.33–7.48 m ($13H_{Ph}$), 7.55 d ($1H_{Ar}$, J = 1.4 Hz), 7.57–7.60 m ($2H_{Ph}$), 7.82 d ($1H_{Ar}$, J_1 = 1.4 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.7, 15.9, 16.2, 16.6, 18.2, 20.9, 21.4, 23.7, 27.7, 28.0, 29.5, 32.0, 34.3, 35.0, 37.1, 37.2, 37.9, 38.3, 38.4, 40.7, 42.4, 46.5, 50.2, 55.4, 56.0, 57.0, 66.1, 80.9, 121.2, 124.6, 126.8, 128.0, 128.2, 128.3, 128.5, 128.7, 128.8, 129.1, 129.6, 131.9, 133.7, 136.1, 136.37, 136.41, 141.5, 157.7, 166.9, 167.4, 171.1, 175.6. **HRMS** (ESI): $C_{56}H_{64}NO_6$ found 846.4735 $[M+H]^+$; calcd. 846.4728.

*4.1.45. Benzyl 3β-acetoxy-19-(2-(4-methoxyphenyl)-1,3-dioxo-7-phenylisoindolin-5-yl)-20,29,30-trinorlupan-28-oate (**20**)*

Compound **20** was prepared according to the general procedure in 3 stages from phthalic acid **5g** (260 mg; 0.35 mmol) and acetic anhydride (2 mL), then 4-methoxyaniline (43 mg; 0.35 mmol) in THF (1.7 mL) for 2 h at reflux. After purification (mobile phase hexane/EtOAc 3 : 1) compound **20** (185 mg; 60 %) was obtained as a pale yellow solid; **mp** 180–182 °C (hexane/EtOAc); R_f 0.52 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 2942, 2869, 1773, 1717 (C═O), 1612, 1512, 1380, 1246 (C–O), 749, 696. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ, ppm: 0.76 s (3H, Me), 0.79 s (3H, Me), 0.81 s (3H, Me), 0.82 s (3H, Me), 0.90 s (3H, Me), 1.78 dt (1H, J_1 = 12.6 Hz, J_2 = 10.2 Hz), 1.95 t (1H, J = 11.2 Hz, H-18), 2.02 s (3H, MeCO), 2.04–2.09 m (1H), 2.21–2.34 m (2H), 2.39 dt (1H, J_1 = 13.1 Hz, J_2 = 3.1 Hz), 3.70 td (1H, J_1 = 11.0 Hz, J_2 = 5.0 Hz, H-19), 3.83 s (3H, MeO), 4.42 dd (1H, J_1 = 9.6 Hz, J_2 = 6.3 Hz, H-3), 5.14 d (1H, J = 12.2 Hz, CH_aPh), 5.20 d (1H, J = 12.2 Hz, CH_bPh), 6.98 d ($2H_{Ar}$, J = 9.0 Hz), 7.31 d ($2H_{Ar}$, J = 9.0 Hz), 7.32–7.49 m ($8H_{Ph}$), 7.53 d ($1H_{Ar}$, J = 1.4 Hz), 7.57–7.60 m ($2H_{Ph}$), 7.80 d ($1H_{Ar}$, J_1 = 1.4 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.7, 15.8, 16.2, 16.6, 18.2, 20.9, 21.4, 23.7, 27.7, 28.0, 29.5, 32.0, 34.2, 35.0, 37.1, 37.2, 37.9, 38.3, 38.4, 40.7, 42.4, 46.5, 50.2, 55.4, 55.6, 56.0, 57.0, 66.1, 80.9, 114.4, 121.1, 124.5, 124.7, 128.1, 128.2, 128.3, 128.5, 128.7, 128.8, 129.6, 133.7, 136.0, 136.37, 136.43, 141.4, 157.5, 159.2, 167.2, 167.7, 171.1, 175.6. **HRMS** (ESI): $C_{57}H_{66}NO_7$ found 876.4844 $[M+H]^+$; calcd. 876.4834.

*4.1.46. Benzyl 3β-acetoxy-19-(2-(4-cyanophenyl)-1,3-dioxo-7-phenylisoindolin-5-yl)-20,29,30-trinorlupan-28-oate (**22**)*

Compound **22** was prepared according to the general procedure in 3 stages from phthalic acid **5g** (260 mg; 0.35 mmol) and acetic anhydride (2 mL), then 4-aminobenzonitrile (41 mg; 0.35 mmol) in THF (1.7 mL) for 4 h at reflux. After purification (mobile phase hexane/EtOAc 4 : 1) compound **22** (174 mg; 57 %) was obtained as a pale yellow solid; **mp** 180–183 °C (hexane/EtOAc); R_f 0.55 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 2943, 2870, 2228 (C≡ N), 1777, 1722 (C═O), 1605, 1367, 1244 (C–O), 749, 695. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ, ppm: 0.76 s (3H, Me), 0.79 s (3H, Me), 0.80 s (3H, Me), 0.81 s (3H, Me), 0.90 s (3H, Me), 1.78 dt (1H, J_1 = 12.8 Hz, J_2 = 10.3 Hz), 1.94 t (1H, J = 11.3 Hz, H-18), 2.02 s (3H, MeCO), 2.05–2.10 m (1H), 2.25–2.34 m (2H), 2.40 dt (1H, J_1 = 13.0 Hz, J_2 = 2.7 Hz), 3.72 td (1H, J_1 = 10.9 Hz, J_2 = 5.1 Hz, H-19), 4.39–4.43 m (1H, H-3), 5.14 d (1H, J = 12.2 Hz, CH_aPh), 5.20 d (1H, J = 12.2 Hz, CH_bPh), 7.33–7.36 m ($5H_{Ph}$), 7.47–7.52 m ($3H_{Ph}$), 7.55–7.57 m ($2H_{Ph}$), 7.57 d ($1H_{Ar}$, J = 1.0 Hz), 7.65 d ($2H_{Ar}$, J = 8.8 Hz), 7.76 d ($2H_{Ar}$, J = 8.8 Hz), 7.83 d ($1H_{Ar}$, J_1 = 1.1 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.7, 15.8, 16.2, 16.6, 18.2, 20.9, 21.4, 23.7, 27.8, 28.0, 29.5, 32.0, 34.2, 35.0, 37.1, 37.2, 37.9, 38.3, 38.5, 40.7, 42.4, 46.5, 50.2, 55.4, 56.1, 57.0, 66.2, 80.9, 111.2, 118.4, 121.2, 124.3, 126.7, 128.3, 128.4, 128.6, 128.7, 129.1, 129.5, 132.9, 133.2, 136.16, 136.19, 136.3, 136.5, 142.0, 158.3, 166.1, 166.6, 171.1, 175.6. **HRMS** (ESI): $C_{57}H_{66}N_3O_6$ found 888.4957 $[M + NH_4]^+$; calcd. 888.4946.

*4.1.47. Benzyl 3β-acetoxy-19-(2-(4-acetamidophenyl)-1,3-dioxo-7-phenylisoindolin-5-yl)-20,29,30-trinorlupan-28-oate (**24**)*

Compound **24** was prepared according to the general procedure in 3 stages from phthalic acid **5g** (260 mg; 0.35 mmol) and acetic anhydride (2 mL), then *N*-(4-aminophenyl)acetamide (53 mg; 0.35 mmol) in THF (1.7 mL) for 4 h at reflux. After purification (mobile phase hexane/EtOAc 1 : 1) compound **24** (174 mg; 57 %) was obtained as a yellow solid; **mp** 210–212 °C (hexane/EtOAc); R_f 0.07 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3400 (N–H), 2943, 2870, 1713 (C═O), 1604, 1514, 1377, 1245, 750, 696. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ, ppm: 0.74 s (3H, Me), 0.78 s (3H, Me), 0.79 s (3H, Me), 0.80 s (3H, Me), 0.89 s (3H, Me), 1.76 dt (1H, J_1 = 12.2 Hz, J_2 = 10.6 Hz), 1.93 t (1H, J = 11.1 Hz, H-18), 2.00 s (3H, MeCO), 2.02–2.08 m (1H), 2.15 s(3H, MeCO), 2.22–2.39 m (3H), 3.68 td (1H, J_1 = 10.8 Hz, J_2 = 4.8 Hz, H-19), 4.39–4.43 m (1H, H-3), 5.13 d (1H, J = 12.2 Hz, CH_aPh), 5.18 d (1H, J = 12.2 Hz, CH_bPh), 7.32–7.39 m (8H, $7H_{Ph}$, NH), 7.43–7.48 m ($3H_{Ph}$), 7.52 s ($1H_{Ar}$), 7.52–7.59 m ($4H_{Ph}$), 7.79 s ($1H_{Ar}$). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.7, 15.8, 16.2, 16.6, 18.2, 20.9, 21.4, 23.7, 24.6, 27.7, 28.0, 29.5, 32.0, 34.2, 35.0, 37.1, 37.2, 37.9, 38.3, 38.4, 40.7, 42.4, 46.5, 50.2, 55.4, 56.0, 57.0, 66.1, 80.9, 120.1, 121.2, 124.6, 127.37, 127.44, 128.2, 128.4, 128.5, 128.66, 128.69, 128.7, 129.6, 133.6, 136.36, 136.43, 137.8, 141.5, 157.7, 167.1, 167.5, 168.5, 171.1, 175.6. **HRMS** (ESI): $C_{58}H_{67}N_2O_7$ found 903.4952 $[M+H]^+$; calcd. 903.4943.

*4.1.48. Benzyl 3β-acetoxy-19-(1,3-dioxo-7-phenyl-2-(1h-pyrazol-3-yl) isoindolin-5-yl)-20,29,30-trinorlupan-28-oate (**27**)*

Compound **27** was prepared according to the general procedure in 3 stages from phthalic acid **5g** (260 mg; 0.35 mmol) and acetic anhydride (2 mL), then 1*H*-pyrazol-3-amine (29 mg; 0.35 mmol) in THF (1.7 mL) for 2 h at reflux. After purification (mobile phase hexane/EtOAc 3 : 2) compound **27** (239 mg; 82 %) was obtained as a white solid; **mp** 175–177 °C (hexane/EtOAc); R_f 0.14 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3200 (N–H), 2944, 2870, 1778, 1724 (C═O), 1244 (C–O), 747, 695. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ, ppm: 0.75 s (3H, Me), 0.79 s (3H, Me), 0.80 s (3H, Me), 0.81 s (3H, Me), 0.90 s (3H, Me), 1.78 dt (1H, J_1 = 12.6 Hz, J_2 = 10.3 Hz), 1.94 t (1H, J = 11.3 Hz, H-18), 2.01 s (3H, MeCO), 2.04–2.09 m (1H), 2.24–2.33 m (2H), 2.39 dt (1H, J_1 = 13.0 Hz, J_2 = 3.1 Hz), 3.70 td (1H, J_1 = 10.9 Hz, J_2 = 5.0 Hz, H-19), 4.42 dd (1H, J_1 = 9.2 Hz, J_2 = 6.8 Hz, H-3), 5.14 d (1H, J = 12.2 Hz, CH_aPh), 5.20 d (1H, J = 12.2 Hz, CH_bPh), 6.59 d (1H, J = 2.3 Hz), 7.33–7.41 m ($8H_{Ar}$), 7.46–7.51 m ($3H_{Ar}$), 7.54 d ($1H_{Ar}$, J = 1.4 Hz), 7.55–7.59 m ($2H_{Ar}$), 7.62 d (1H, J = 2.3 Hz), 7.82 d ($1H_{Ar}$, J = 1.4 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.7, 15.8, 16.2, 16.6, 18.2, 20.9, 21.4, 23.7, 27.8, 28.0, 29.5, 32.0, 34.3, 34.9, 37.1, 37.2, 37.9, 38.3, 38.4, 40.7, 42.4, 46.5,

50.1, 55.4, 56.0, 56.9, 66.1, 80.9, 100.3, 121.4, 124.7, 128.2, 128.3, 128.5, 128.7, 128.9, 129.6, 131.7, 133.6, 136.3, 136.4, 140.1, 141.1, 146.3, 157.8, 165.9, 166.4, 171.1, 175.6. **HRMS** (ESI): $C_{53}H_{60}N_3O_6$ found 834.4475 $[M-H]^+$; calcd. 834.4477.

4.1.49. Benzyl 3β-acetoxy-19-(1,3-dioxo-7-phenyl-2-(thiazol-2-yl) isoindolin-5-yl)-20,29,30-trinorlupan-28-oate (29)

Compound **29** was prepared according to the general procedure in 3 stages from phthalic acid **5g** (119 mg; 0.16 mmol) and acetic anhydride (1 mL), then thiazol-2-amine (16 mg; 0.16 mmol) in THF (1.5 mL) for 3 h at reflux. After purification (mobile phase hexane/EtOAc 3 : 1) compound **29** (104 mg; 76 %) was obtained as a white solid; **mp** 170–172 °C (hexane/EtOAc); R_f 0.41 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 2941, 2867, 1784, 1729 (C═O), 1697, 1617, 1333, 1244 (C–O), 746, 695. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.76 s (3H, Me), 0.79 s (3H, Me), 0.80 s (3H, Me), 0.81 s (3H, Me), 0.90 s (3H, Me), 1.79 dt (1H, J_1 = 12.7 Hz, J_2 = 10.5 Hz), 1.94 t (1H, J = 11.3 Hz, H-18), 2.01 s (3H, MeCO), 2.08 ddd (1H, J_1 = 13.0 Hz, J_2 = 7.5 Hz, J_2 = 0.9 Hz), 2.23–2.33 m (2H), 2.39 dt (1H, J_1 = 12.7 Hz, J_2 = 3.0 Hz), 3.72 td (1H, J_1 = 11.0 Hz, J_2 = 5.3 Hz, H-19), 4.42 dd (1H, J_1 = 9.4 Hz, J_2 = 6.5 Hz, H-3), 5.15 d (1H, J = 12.2 Hz, CH_aPh), 5.20 d (1H, J = 12.2 Hz, CH_bPh), 7.28 d (1H, J = 3.5 Hz), 7.33–7.41 m ($5H_{Ar}$), 7.47–7.51 m ($3H_{Ar}$), 7.56–7.59 m ($3H_{Ar}$), 7.79 d (1H, J = 3.5 Hz), 7.86 d ($1H_{Ar}$, J = 1.3 Hz). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.7, 15.9, 16.2, 16.6, 18.3, 20.9, 21.4, 23.7, 27.9, 28.0, 29.5, 32.1, 34.3, 34.9, 37.1, 37.2, 37.9, 38.35, 38.44, 40.7, 42.4, 46.6, 50.2, 55.4, 56.1, 57.0, 66.2, 80.9, 117.3, 121.9, 124.0, 128.36, 128.37, 128.5, 128.7, 129.1, 129.5, 133.1, 136.0, 136.4, 140.0, 142.3, 152.5, 158.6, 164.5, 164.6, 171.0, 175.6. **HRMS** (ESI): $C_{53}H_{61}N_2O_6S$ found 853.4243 $[M+H]^+$; calcd. 853.4245.

4.1.50. General procedure for the acetyl deprotection of derivatives 8, 10, 12, 19, 21, 23, 26

General procedure A: To a stirred solution of acetate (0.13 mmol) in THF (1 mL) at room temperature was added 3 *N* HCl in MeOH (3 mL) and the reaction mixture was stirred for 24 h. The resulting mixture was diluted with water, extracted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$, water, dried over Na_2SO_4, concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate.

General procedure B: The cleavage of acetyl group was achieved by modification of the known procedure [59,60]. To a solution of acetate (0.20 mmol) in methanol (7 mL) and THF (3.5 mL) was added *p*-TsOH·H_2O (0.24–0.48 mmol, 1.2–2.4 eq.). The mixture was stirred at room temperature for 7–8 days and monitored by TLC until starting material was consumed. The solvents were evaporated in vacuo and the residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate.

4.1.51. General procedure for the benzyl deprotection of derivatives 8, 10, 12, 14, 16, 19, 21, 23, 25, 26, 28, 30

The cleavage of benzyl group was achieved by modification of the known procedure [58]. To a stirred solution of benzyl ester (0.10 mmol) in a mixture of THF/EtOH (8 mL, 1 : 1), 10 % Pd/C (0.07 mmol, 0.67 eq.) was added at room temperature under nitrogen atmosphere. After that, 1,3-cyclohexadiene (0.76 mmol, 7.6 eq.) was added and the reaction mixture was stirred at 50 °C for 13 h. The resulting mixture was filtered through a Celite, washed with EtOAc and concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate.

4.1.52. 19-(1,3-Dioxo-2-phenylisoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (8)

Compound **8** was prepared from compound **7**, acetyl group was removed using general procedure A. Then, benzyl ester was cleaved according to the general procedure using 10 % Pd/C (71 mg, 0.067 mmol) and 1,3-cyclohexadiene (72 μL, 61 mg, 0.76 mmol) in mixture THF/EtOH (8 mL, 1 : 1). After purification (mobile $CHCl_3$/MeOH 97 : 3 to 96 : 4) compound **8** (57 mg; 90 %, 59 % overall) was obtained as a white solid; **mp** 235–327 °C ($CHCl_3$/MeOH); R_f 0.56 (silica gel, $CHCl_3$/MeOH 9 : 2). **IR** (DRIFT): 3480 (O–H), 2939, 2866, 1774, 1723 (C═O), 1686, 1375, 751, 689. **^{1}H NMR** (DMSO-d_6, 400 MHz) δ, ppm: 0.63 s (3H, Me), 0.72 s (3H, Me), 0.85 s (3H, Me), 0.87 s (3H, Me), 0.91 s (3H, Me), 1.87–1.99 m (2H), 1.99 t (1H, J = 11.3 Hz, H-18), 2.16–2.36 m (3H), 2.92 dt (1H, J_1 = 10.4 Hz, J_2 = 5.2 Hz, H-3), 3.62 td (1H, J_1 = 10.9 Hz, J_2 = 5.9 Hz, H-19), 4.22 d (1H, J = 5.2 Hz, OH), 7.42–7.45 m ($3H_{Ar}$), 7.51–7.54 m ($2H_{Ar}$), 7.79–7.83 m ($2H_{Ar}$), 7.90 s ($1H_{Ar}$), 12.20 s (1H, COOH). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.66, 15.74, 15.8, 17.9, 20.4, 27.1, 27.2, 28.0, 29.1, 31.2, 33.9, 34.9, 36.1, 36.6, 37.7, 38.1, 38.4, 40.1, 42.0, 45.9, 49.6, 54.5, 54.8, 55.8, 76.7, 121.9, 123.4, 127.4, 127.9, 128.8, 128.9, 132.0, 132.1, 134.0, 157.7, 166.9, 167.1, 177.2. **HRMS** (ESI): $C_{41}H_{50}NO_5$ found 636.3682 $[M-H]^+$; calcd. 636.3684.

4.1.53. 19-(1,3-Dioxo-2-(4-methoxyphenyl)isoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (10)

Compound **10** was prepared from compound **9**, acetyl group was removed using general procedure A. Then, benzyl ester was cleaved according to the general procedure using 10 % Pd/C (71 mg, 0.067 mmol) and 1,3-cyclohexadiene (72 μL, 61 mg, 0.76 mmol) in mixture THF/EtOH (8 mL, 1 : 1). After purification (mobile phase hexane/EtOAc 2 : 1 to 1 : 1) compound **10** (57 mg; 85 %, 63 % overall) was obtained as a pale yellow solid; **mp** 220–223 °C (hexane/EtOAc); R_f 0.27 (silica gel, hexane/EtOAc 1 : 1). **IR** (DRIFT): 3460 (O–H), 2936, 2868, 1715 (C═O), 1686, 1513, 1380, 1247 (C–O), 746, 692. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ, ppm: 0.63 s (3H, Me), 0.72 s (3H, Me), 0.85 s (3H, Me), 0.87 s (3H, Me), 0.91 s (3H, Me), 1.59 td (1H, J_1 = 13.0 Hz, J_2 = 3.2 Hz), 1.87–1.95 m (2H), 1.99 t (1H, J = 11.3 Hz, H-18), 2.16–2.35 m (3H), 2.92 dt (1H, J_1 = 10.7 Hz, J_2 = 5.2 Hz, H-3), 3.61 td (1H, J_1 = 11.0 Hz, J_2 = 5.1 Hz, H-19), 3.81 s (3H, MeO), 4.21 d (1H, J = 5.2 Hz, OH), 7.06 d ($2H_{Ar}$, J = 9.1 Hz), 7.34 d ($2H_{Ar}$, J = 9.1 Hz), 7.77–7.81 m (2H, H-6′, H-7′), 7.88 s (1H, H-4'), 12.20 s (1H, COOH). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.65, 15.7, 15.8, 17.9, 20.4, 27.1, 27.2, 28.0, 29.1, 31.2, 33.9, 34.8, 36.1, 36.6, 37.7, 38.1, 38.4, 40.1, 42.0, 45.9, 49.5, 54.5, 54.8, 55.4, 55.8, 76.7, 114.1, 121.7, 123.3, 124.5, 128.8, 129.0, 132.1, 133.9, 157.6, 158.8, 167.1, 167.4, 177.2. **HRMS** (ESI): $C_{42}H_{54}NO_6$ found 668.3947 $[M+H]^+$; calcd. 668.3946.

4.1.54. 19-(1,3-Dioxo-2-(4-cyanophenyl)isoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (12)

Compound **12** was prepared from compound **11**, acetyl group was removed using general procedure A, and also B. Then, benzyl ester was cleaved according to the general procedure using 10 % Pd/C (74 mg, 0.07 mmol) and 1,3-cyclohexadiene (79 μL, 67 mg, 0.84 mmol) in mixture THF/EtOH (8.8 mL, 1 : 1). After purification (mobile phase hexane/EtOAc 2 : 1) compound **12** (57 mg; 78 %, 37 % overall) was obtained as a white solid; **mp** 210–212 °C (hexane/EtOAc); R_f 0.38 (silica gel, hexane/EtOAc 1 : 1). **IR** (DRIFT): 3450 (O–H), 2926, 2868, 2230 (C≡ N), 1776, 1726 (C═O), 1689 (C═O), 1374, 745, 689. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ, ppm: 0.62 s (3H, Me), 0.72 s (3H, Me), 0.84 s (3H, Me), 0.87 s (3H, Me), 0.91 s (3H, Me), 1.59 td (1H, J_1 = 13.1 Hz, J_2 = 3.4 Hz), 1.87–1.96 m (2H), 2.00 t (1H, J = 11.3 Hz, H-18), 2.16–2.35 m (3H), 2.91 dt (1H, J_1 = 10.5 Hz, J_2 = 5.2 Hz, H-3), 3.63 td (1H, J_1 = 10.9 Hz, J_2 = 5.0 Hz, H-19), 4.21 d (1H, J = 5.2 Hz, OH), 7.70 d ($2H_{Ar}$, J = 8.8 Hz), 7.82 dd (1H, J_1 = 7.7 Hz, J_2 = 0.7 Hz, H-6′), 7.85 d (1H, J_1 = 7.7 Hz, H-7′), 7.94 s (1H, H-4'), 8.01 d ($2H_{Ar}$, J = 8.8 Hz), 12.21 s (1H, COOH). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.66, 15.75, 15.8, 17.9, 20.4, 27.1, 27.2, 28.0, 29.1, 31.2, 33.9, 34.9, 36.1, 36.6, 37.7, 38.1, 38.4, 40.1, 42.0, 45.9, 49.6, 54.5, 54.8, 55.8, 76.7, 110.2, 118.5, 122.1, 123.6, 127.8, 128.9, 132.0, 132.9, 134.3, 136.3, 158.0, 166.3, 166.5, 177.3. **HRMS** (ESI): $C_{42}H_{51}N_2O_5$ found 663.3792 $[M+H]^+$; calcd. 663.3792.

*4.1.55. 3β-Acetoxy-19-(1,3-dioxo-2-(4-acetamidophenyl)isoindolin-5-yl)-20,29,30-trinorlupan-28-oic acid **(14)***

Compound **14** was prepared according to the general procedure from benzyl ester **13** (144 mg; 0.17 mmol), 10 % Pd/C (117 mg, 0.11 mmol) and 1,3-cyclohexadiene (122 μL, 103 mg, 1.29 mmol) in mixture THF/EtOH (10 mL, 1 : 1). After purification (mobile phase $CHCl_3$/MeOH 98 : 2 to 96 : 4) compound **14** (105 mg; 84 %) was obtained as a pale yellow solid; **mp** 228–230 °C ($CHCl_3$/MeOH); R_f 0.30 (silica gel, $CHCl_3$/MeOH 9 : 1). **IR** (DRIFT): 3300 (O–H), 2944, 2860, 1709 (C═O), 1604, 1514, 1397, 1245 (C–O), 729, 690. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.807 s (3H, Me), 0.81 s (3H, Me), 0.82 s (3H, Me), 0.93 s (3H, Me), 0.94 s (3H, Me), 1.84 dt (1H, J_1 = 12.8 Hz, J_2 = 10.1 Hz), 1.95 t (1H, J = 11.4 Hz, H-18), 2.02 s (3H, MeCO), 2.15 dd (1H, J_1 = 13.0 Hz, J_2 = 8.6 Hz), 2.21 s (3H, MeCO), 2.29–2.43 m (3H), 3.68 td (1H, J_1 = 11.0 Hz, J_2 = 5.2 Hz, H-19), 4.42 dd (1H, J_1 = 9.3 Hz, J_2 = 6.6 Hz, H-3), 7.36 s (1H, NH), 7.39 d ($2H_{Ar}$, J = 8.4 Hz), 7.63 d ($1H_{Ar}$, J = 7.1 Hz), 7.64 d ($2H_{Ar}$, J = 8.4 Hz), 7.82 s ($1H_{Ar}$), 7.82 d ($1H_{Ar}$, J_1 = 7.1 Hz). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.7, 16.1, 16.2, 16.6, 18.2, 20.9, 21.4, 23.8, 24.7, 27.6, 28.0, 29.7, 32.2, 34.3, 35.2, 37.2, 37.4, 37.9, 38.4, 38.6, 40.7, 42.5, 46.6, 50.1, 55.4, 55.9, 56.8, 81.0, 120.3, 122.4, 124.0, 127.3, 127.6, 129.3, 132.5, 134.0, 137.9, 157.8, 167.5, 167.8, 168.8, 171.2, 181.2. **HRMS** (ESI): $C_{45}H_{57}N_2O_7$ found 737.4158 $[M+H]^+$; calcd. 737.4160.

*4.1.56. 3β-Acetoxy-19-(1,3-dioxo-2-(1H-pyrazol-3-yl)isoindolin-5-yl)-20,29,30-trinorlupan-28-oic acid **(16)***

Compound **16** was prepared according to the general procedure from benzyl ester **15** (150 mg; 0.20 mmol), 10 % Pd/C (138 mg, 0.13 mmol) and 1,3-cyclohexadiene (144 μL, 122 mg, 1.52 mmol) in mixture THF/EtOH (10 mL, 1 : 1). After purification (mobile phase $CHCl_3$/MeOH 98 : 2 to 96 : 4) compound **16** (102 mg; 76 %) was obtained as a white solid; **mp** 189–191 °C ($CHCl_3$/MeOH); R_f 0.36 (silica gel, $CHCl_3$/MeOH 9 : 1). **IR** (DRIFT): 3500, 2970, 1736, 1727, 1366, 1229, 1217, 745, 687. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ, ppm: 0.76 s (9H, 3Me), 0.87 s (3H, Me), 0.92 s (3H, Me), 1.87–1.95 m (2H), 1.97 s (3H, MeCO), 1.98 t (1H, J = 11.3 Hz, H-18), 2.16–2.36 m (3H), 3.62 td (1H, J_1 = 10.9 Hz, J_2 = 5.1 Hz, H-19), 4.31 dd (1H, J_1 = 11.3 Hz, J_2 = 4.5 Hz, H-3), 6.36 d (1H, J = 2.3 Hz), 7.82–7.85 m (3H), 7.90 s (1H), 12.24 s (1H), 13.06 s (1H). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.6, 15.8, 16.4, 17.7, 20.4, 20.9, 23.3, 27.0, 27.6, 29.1, 31.2, 33.7, 34.8, 36.1, 36.6, 37.3, 37.6, 40.1, 42.0, 45.9, 49.3, 54.57, 54.61, 55.8, 79.2, 79.8, 102.6, 122.1, 123.5, 128.8, 130.2, 131.9, 134.3, 139.7, 157.9, 166.5, 166.8, 170.0, 177.3. **HRMS** (ESI): $C_{40}H_{52}N_3O_6$ found 670.3850 $[M+H]^+$; calcd. 670.3851.

*4.1.57. 19-(1,3-Dioxo-2,7-diphenylisoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oic acid **(19)***

Compound **19** was prepared from compound **18**, acetyl group was removed using general procedure A. Then, benzyl ester was cleaved according to the general procedure using 10 % Pd/C (95 mg, 0.09 mmol) and 1,3-cyclohexadiene (100 μL, 85 mg, 1.06 mmol) in mixture THF/EtOH (10 mL, 1 : 1). After purification (mobile phase hexane/EtOAc 2 : 1 to 1 : 1) compound **19** (76 mg; 78 %, 61 % overall) was obtained as a white solid; **mp** 240–242 °C (hexane/EtOAc); R_f 0.30 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3230 (O–H), 2944, 2868, 1716 (C═O), 1698 (C═O), 1389, 752, 738. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ, ppm: 0.63 s (3H, Me), 0.72 s (3H, Me), 0.85 s (3H, Me), 0.88 s (3H, Me), 0.93 s (3H, Me), 1.62 td (1H, J_1 = 13.1 Hz, J_2 = 3.3 Hz), 1.87–1.99 m (2H), 2.05 t (1H, J = 11.3 Hz, H-18), 2.16–2.37 m (3H), 2.92 dt (1H, J_1 = 10.6 Hz, J_2 = 5.1 Hz, H-3), 3.68 td (1H, J_1 = 11.0 Hz, J_2 = 5.2 Hz, H-19), 4.22 d (1H, J = 5.1 Hz, OH), 7.40–7.52 m ($8H_{Ar}$), 7.62–7.65 m ($2H_{Ar}$), 7.67 s ($1H_{Ar}$), 7.89 s ($1H_{Ar}$), 12.20 s (1H, COOH). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.67, 15.73, 15.8, 17.9, 20.5, 27.1, 27.3, 28.1, 29.2, 31.1, 33.9, 34.8, 36.0, 36.6, 37.7, 38.2, 38.4, 40.1, 42.0, 45.7, 49.6, 54.1, 54.8, 55.8, 76.8, 120.8, 124.4, 124.9, 127.5, 127.9, 128.4, 128.7, 129.6, 132.1, 133.4, 135.5, 136.1, 140.1, 157.4, 166.3, 166.7, 177.3. **HRMS** (ESI): $C_{47}H_{56}NO_5$ found 714.4150 $[M+H]^+$; calcd. 714.4153.

*4.1.58. 19-(2-(4-Methoxyphenyl)-1,3-dioxo-7-phenylisoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oic acid **(21)***

Compound **21** was prepared from compound **20**, acetyl group was removed using general procedure B. Then, benzyl ester was cleaved according to the general procedure using 10 % Pd/C (117 mg, 0.11 mmol) and 1,3-cyclohexadiene (116 μL, 98 mg, 1.22 mmol) in mixture THF/EtOH (12 mL, 1 : 1). After purification (mobile phase hexane/EtOAc 2 : 1 to 1 : 1) compound **21** (97 mg; 82 %, 72 % overall) was obtained as a white solid; **mp** 231–233 °C (hexane/EtOAc); R_f 0.30 (silica gel, hexane/EtOAc 1 : 1). **IR** (DRIFT): 3480 (O–H), 2938, 2868, 1709 (C═O), 1612, 1512, 1383, 1248 (C–O), 747, 696. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ, ppm: 0.63 s (3H, Me), 0.72 s (3H, Me), 0.85 s (3H, Me), 0.88 s (3H, Me), 0.93 s (3H, Me), 1.61 td (1H, J_1 = 13.0 Hz, J_2 = 3.1 Hz), 1.87–1.99 m (2H), 2.04 t (1H, J = 11.3 Hz, H-18), 2.15–2.37 m (3H), 2.92 dt (1H, J_1 = 10.7 Hz, J_2 = 5.2 Hz, H-3), 3.67 td (1H, J_1 = 11.0 Hz, J_2 = 5.6 Hz, H-19), 3.80 s (3H, MeO), 4.22 d (1H, J = 5.2 Hz, OH), 7.04 d ($2H_{Ar}$, J = 9.0 Hz), 7.33 d ($2H_{Ar}$, J = 9.0 Hz), 7.44–7.49 m ($3H_{Ph}$), 7.61–7.64 m ($2H_{Ph}$), 7.65 s ($1H_{Ar}$), 7.86 s ($1H_{Ar}$), 12.20 s (1H, COOH). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.67, 15.73, 15.8, 17.9, 20.5, 27.1, 27.3, 28.1, 29.2, 31.1, 33.9, 34.8, 36.1, 36.6, 37.7, 38.2, 38.4, 40.1, 42.0, 45.7, 49.6, 54.1, 54.8, 55.4, 55.8, 76.8, 114.0, 121.2, 124.5, 124.6, 127.9, 128.4, 128.8, 129.6, 133.4, 134.0, 136.1, 140.0, 157.3, 158.7, 166.5, 166.9, 177.3. **HRMS** (ESI): $C_{48}H_{58}NO_6$ found 744.4256 $[M+H]^+$; calcd. 744.4259.

*4.1.59. 19-(2-(4-Cyanophenyl)-1,3-dioxo-7-phenylisoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oic acid **(23)***

Compound **23** was prepared from compound **22**, acetyl group was removed using general procedure B. Then, benzyl ester was cleaved according to the general procedure using 10 % Pd/C (117 mg, 0.11 mmol) and 1,3-cyclohexadiene (116 μL, 98 mg, 1.22 mmol) in mixture THF/EtOH (12 mL, 1 : 1). After purification (mobile phase hexane/EtOAc 2 : 1 to 1 : 1) compound **23** (80 mg; 68 %, 49 % overall) was obtained as a white solid; **mp** 218–220 °C (hexane/EtOAc); R_f 0.36 (silica gel, hexane/EtOAc 1 : 1). **IR** (DRIFT): 3500 (O–H), 2941, 2867, 2229 (C≡ N), 1775, 1721 (C═O), 1375, 749, 696. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ, ppm: 0.62 s (3H, Me), 0.71 s (3H, Me), 0.84 s (3H, Me), 0.87 s (3H, Me), 0.93 s (3H, Me), 1.62 td (1H, J_1 = 13.2 Hz, J_2 = 3.4 Hz), 1.87–2.00 m (2H), 2.06 t (1H, J = 11.3 Hz, H-18), 2.15–2.36 m (3H), 2.91 dt (1H, J_1 = 10.6 Hz, J_2 = 5.2 Hz, H-3), 3.68 td (1H, J_1 = 11.0 Hz, J_2 = 5.2 Hz, H-19), 4.21 d (1H, J = 5.2 Hz, OH), 7.45–7.49 m ($3H_{Ph}$), 7.63–7.66 m ($2H_{Ph}$), 7.68 d ($2H_{Ar}$, J = 8.8 Hz), 7.70 br. s ($1H_{Ar}$), 7.92 br. s ($1H_{Ar}$), 7.99 d ($2H_{Ar}$, J = 8.8 Hz), 12.20 s (1H, COOH). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.66, 15.73, 15.8, 17.9, 20.5, 27.1, 27.3, 28.1, 29.2, 31.1, 33.9, 34.8, 36.0, 36.6, 37.7, 38.2, 38.4, 40.1, 42.0, 45.8, 49.6, 54.1, 54.8, 55.8, 76.7, 110.2, 118.5, 123.2, 124.4, 127.89, 127.93, 128.5, 129.6, 132.8, 133.3, 135.8, 136.0, 136.3, 140.3, 157.7, 165.7, 166.1, 177.3. **HRMS** (ESI): $C_{48}H_{55}N_2O_5$ found 739.4100 $[M+H]^+$; calcd. 739.4105.

*4.1.60. 3β-Acetoxy-19-(2-(4-acetamidophenyl)-1,3-dioxo-7-phenylisoindolin-5-yl)-20,29,30-trinorlupan-28-oic acid **(25)***

Compound **25** was prepared according to the general procedure from benzyl ester **24** (125 mg; 0.14 mmol), 10 % Pd/C (95 mg, 0.09 mmol) and 1,3-cyclohexadiene (100 μL, 85 mg, 1.06 mmol) in mixture THF/EtOH (10 mL, 1 : 1). After purification (mobile phase $CHCl_3$/MeOH 98 : 2 to 96 : 4) compound **25** (100 mg; 89 %) was obtained as an yellow solid; **mp** 234–236 °C ($CHCl_3$/MeOH); R_f 0.48 (silica gel, $CHCl_3$/MeOH 9 : 1). **IR** (DRIFT): 3300 (O–H), 2941, 2870, 1709 (C═O), 1514, 1375, 1245 (C–O), 749, 696. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.81 s (3H, Me), 0.82 s (3H, Me), 0.83 s (3H, Me), 0.95 s (6H, 2Me), 1.84 dt (1H, J_1 = 12.9 Hz, J_2 = 10.4 Hz), 1.99 t (1H, J = 11.3 Hz, H-18), 2.02 s (3H, MeCO), 2.13–2.17 m (1H), 2.17 s(3H, MeCO), 2.30–2.45 m (3H), 3.71 td (1H, J_1 = 11.2 Hz, J_2 = 5.2 Hz, H-19), 4.43 dd (1H, J_1 = 10.2 Hz, J_2 = 5.7 Hz, H-3), 7.33 s ($1H_{Ar}$), 7.36 d ($2H_{Ar}$, J = 8.6 Hz), 7.43–7.49 m ($3H_{Ar}$), 7.56–7.60 m ($5H_{Ar}$), 7.83 s ($1H_{Ar}$). **^{13}C NMR** ($CDCl_3$, 126 MHz)

δ, ppm: 14.8, 16.1, 16.2, 16.6, 18.3, 20.9, 21.4, 23.8, 26.6, 27.7, 28.1, 29.7, 32.1, 34.3, 35.1, 37.2, 37.3, 37.9, 38.5, 38.6, 40.8, 42.5, 46.6, 50.1, 55.4, 55.7, 56.8, 81.0, 120.2, 124.7, 126.2, 127.4, 127.6, 128.3, 128.9, 129.6, 133.7, 136.1, 136.4, 137.8, 141.6, 157.5, 167.1, 167.5, 168.7, 171.2, 181.3. **HRMS** (ESI): $C_{51}H_{61}N_2O_7$ found 813.4472 $[M+H]^+$; calcd. 813.4473.

4.1.61. 19-(2-(4-aminophenyl)-1,3-dioxo-7-phenylisoindolin-5-yl)-3β-hydroxy-20,29,30-trinorlupan-28-oic acid (26)

Compound **26** was prepared according to the general procedure A using 3*N*–HCl in MeOH (2.6 mL) for 30 h. The reaction mixture was poured in water, added CH_3COONH_4 to neutral pH, extracted with EtOAc. Then, benzyl ester was cleaved according to the general procedure. After purification (mobile phase $CHCl_3$/MeOH 98 : 2 to 96 : 4) compound **26** (63 mg; 79 %, 56 % overall) was obtained as a yellow solid; **mp** 235–237 °C (hexane/EtOAc); R_f 0.36 (silica gel, $CHCl_3$/MeOH 9 : 1). **IR** (DRIFT): 3365, 2936, 1705, 1618, 1516, 1448, 1386, 1105, 750, 695. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ, ppm: 0.62 s (3H, Me), 0.72 s (3H, Me), 0.85 s (3H, Me), 0.87 s (3H, Me), 0.93 s (3H, Me), 1.61 td (1H, $J_1 = 12.6$ Hz, $J_2 = 2.6$ Hz), 1.86–1.97 m (2H), 2.03 t (1H, $J = 11.3$ Hz, H-18), 2.15–2.25 m (2H), 2.31–2.37 m (1H), 2.92 dd (1H, $J_1 = 10.6$ Hz, $J_2 = 5.2$ Hz, H-3), 3.65 td (1H, $J_1 = 10.9$ Hz, $J_2 = 4.9$ Hz, H-19), 4.22 br. s (1H, OH), 5.33 br. s (2H, NH_2), 6.62 d (2H_{Ar}, $J = 8.7$ Hz), 6.99 d (2H_{Ar}, $J = 8.7$ Hz), 7.43–7.48 m (3H_{Ar}), 7.60–7.62 m (3H_{Ar}), 7.83 s (1H_{Ar}), 12.19 s (1H, COOH). **^{13}C NMR** (DMSO-d_6, 100 MHz) δ, ppm: 14.4, 15.7, 15.75, 15.8, 18.0, 20.5, 27.1, 27.3, 28.1, 29.2, 31.2, 33.9, 34.8, 36.1, 36.7, 37.7, 38.3, 38.5, 40.1, 42.0, 45.7, 49.6, 54.1, 54.8, 55.8, 76.8, 112.0, 113.6, 120.0, 124.5, 127.9, 128.3, 128.4, 129.6, 133.5, 136.2, 138.9, 139.9, 148.5, 157.2, 166.9, 167.3, 177.3. **HRMS** (ESI): $C_{47}H_{57}N_2O_5$ found 729.4260 $[M+H]^+$; calcd. 729.4262.

4.1.62. 3β-Acetoxy-19-(1,3-dioxo-7-phenyl-2-(1H-pyrazol-3-yl) isoindolin-5-yl)-20,29,30-trinorlupan-28-oic acid (28)

Compound **28** was prepared according to the general procedure from benzyl ester **27** (117 mg; 0.14 mmol), 10 % Pd/C (95 mg, 0.09 mmol) and 1,3-cyclohexadiene (100 μL, 85 mg, 1.06 mmol) in mixture THF/EtOH (10 mL, 1 : 1). After purification (mobile phase $CHCl_3$/MeOH 98 : 2 to 96 : 4) compound **28** (82 mg; 79 %) was obtained as a white solid; **mp** 229–232 °C ($CHCl_3$/MeOH); R_f 0.43 (silica gel, $CHCl_3$/MeOH 9 : 1). **IR** (DRIFT): 3300 (O–H), 3200 (N–H), 2942, 2871, 1725 (C═O), 1244, 746, 696. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ, ppm: 0.76 s (9H, 3Me), 0.88 s (3H, Me), 0.94 s (3H, Me), 1.87–1.96 m (2H), 1.97 s (3H, MeCO), 2.04 t (1H, $J = 11.2$ Hz, H-18), 2.15–2.37 m (3H), 3.67 td (1H, $J_1 = 11.1$ Hz, $J_2 = 5.7$ Hz, H-19), 4.31 dd (1H, $J_1 = 11.4$ Hz, $J_2 = 4.7$ Hz, H-3), 6.32 d (1H, $J = 2.1$ Hz), 7.44–7.49 m (3H_{Ar}), 7.59–7.61 m (2H_{Ar}), 7.67 s (1H_{Ar}), 7.82 d (1H, $J = 2.1$ Hz), 7.90 s (1H_{Ar}), 12.24 s (1H), 13.04 s (1H). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.6, 15.8, 16.4, 17.7, 20.5, 20.9, 23.3, 27.14, 27.15, 27.6, 29.1, 31.1, 33.7, 34.7, 36.1, 36.6, 37.3, 37.7, 40.1, 42.0, 45.8, 49.3, 54.2, 54.6, 55.8, 79.9, 102.6, 120.9, 124.3, 126.0, 127.9, 128.4, 129.6, 130.1, 133.3, 136.0, 139.8, 140.3, 157.6, 165.9, 166.4, 170.0, 177.3. **HRMS** (ESI): $C_{46}H_{56}N_3O_6$ found 746.4159 $[M+H]^+$; calcd. 746.4164.

4.1.63. 3β-Acetoxy-19-(1,3-dioxo-7-phenyl-2-(thiazol-2-yl)isoindolin-5-yl)-20,29,30-trinorlupan-28-oic acid (30)

Compound **30** was prepared according to the general procedure from benzyl ester **29** (93 mg; 0.11 mmol), 10 % Pd/C (74 mg, 0.07 mmol) and 1,3-cyclohexadiene (79 μL, 67 mg, 0.84 mmol) in mixture THF/EtOH (8 mL, 1 : 1). After purification (mobile phase hexane/EtOAc 2 : 1 to 3 : 2) compound **30** (33 mg; 39 %) was obtained as a white solid; **mp** 227–229 °C (hexane/EtOAc); R_f 0.25 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3180 (O–H), 2942, 2870, 1731 (C═O), 1333, 1243, 746, 696. **^{1}H NMR** (DMSO-d_6, 500 MHz) δ, ppm: 0.76 s (9H, 3Me), 0.88 s (3H, Me), 0.95 s (3H, Me), 1.63 td (1H, $J_1 = 13.2$ Hz, $J_2 = 3.1$ Hz), 1.89 dd (1H, $J_1 = 11.8$ Hz, $J_2 = 8.4$ Hz), 1.93–1.99 m (1H), 1.96 s (3H, MeCO), 2.04 t (1H, $J = 11.3$ Hz, H-18), 2.15–2.37 m (3H), 3.69 td (1H, $J_1 = 10.9$ Hz, $J_2 = 5.0$ Hz, H-19), 4.31 dd (1H, $J_1 = 11.4$ Hz, $J_2 = 4.6$ Hz, H-3), 7.47–7.51 m (3H_{Ar}), 7.63–7.64 m (2H_{Ar}), 7.73 br. s (1H_{Ar}), 7.76 d (1H, $J = 3.5$ Hz), 7.80 d (1H, $J = 3.5$ Hz), 7.95 br. s (1H_{Ar}), 12.21 s (1H, COOH). **^{13}C NMR** (DMSO-d_6, 126 MHz) δ, ppm: 14.3, 15.6, 15.7, 16.4, 17.7, 20.5, 20.9, 23.3, 27.2, 27.6, 29.2, 31.1, 33.7, 34.7, 36.0, 36.6, 37.3, 37.7, 40.1, 42.0, 45.8, 46.4, 49.3, 54.3, 54.6, 55.8, 79.8, 120.1, 124.1, 126.9, 128.0, 128.6, 129.6, 132.9, 135.9, 136.4, 139.8, 140.6, 151.8, 158.1, 164.5, 164.6, 170.0, 177.3. **HRMS** (ESI): $C_{46}H_{55}N_2O_6S$ found 763.3772 $[M+H]^+$; calcd. 763.3775.

4.1.64. Synthesis of compounds 32, 33a, 33b

Compounds **32**, **33a**, and **33b** were prepared earlier in our lab [29].

4.1.65. Synthesis of phtalimides 35, 37, 39

The detailed description of synthesis of compounds 35, 37, 39 is described in the following sections 4.1.66 - 4.1.70.

4.1.66. Preparation of compound 35

To solution of dimethyl phthalate (250 mg, 0.42 mmol) in 8 mL (2 : 1: 1) of MeOH, THF, H_2O under vigorous stirring was added LiOH monohydrate (155 mg, 3.70 mmol, 8.8 eq.) at room temperature. The reaction was stirred for 24 h, then concentrated in vacuo. Water 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. Organic layer was dried over Na_2SO_4, concentrated in vacuo and the crude phthalic acid was used in next step without purification. To the phthalic acid was added acetic anhydride (4 mL) and the solution was refluxed for 5 h under nitrogen atmosphere. The acetic anhydride was removed in vacuo and the crude phthalic anhydride was used in next step without purification. The phthalic anhydride and urea (504 mg, 8.4 mmol, 20 eq.) were heated to 170 °C for 2 h. The crude product was cooled to room temperature and was diluted with water, extracted with DCM (100 mL), dried over Na_2SO_4, concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate (2 : 1).

4.1.67. 3β, 28-Diacetoxy-19-(1,3-dioxoisoindolin-5-yl)-20,29,30-trinorlupan (35)

Compound **35** was prepared according to the general procedure in 3 stages from phthalate (250 mg, 0.42 mmol) and LiOH monohydrate (155 mg, 3.70 mmol) in 8 mL (2 : 1: 1) of MeOH, THF, H_2O; after acetic anhydride (4 mL), then urea (504 mg, 8.4 mmol). After purification (mobile phase hexane/EtOAc 2 : 1) compound **35** (176 mg, 66 %) was obtained as a white solid; **mp** 190–193 °C (hexane/EtOAc); R_f 0.41 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3490 (N–H), 2970, 1738, 1440, 1365, 1229, 1217, 1029. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ, ppm: 0.80 s (3H, Me), 0.81 s (3H, Me), 0.82 s (3H, Me), 0.92 s (3H, Me), 1.02 s (3H, Me), 1.71 td (1H, $J_1 = 13.7$ Hz, $J_2 = 4.0$ Hz), 1.79 td (1H, $J_1 = 12.3$ Hz, $J_2 = 3.6$ Hz), 1.90–1.98 m (3H), 2.02 s (3H, MeCO), 2.10 s (3H, MeCO), 2.37 dq (1H, $J_1 = 13.8$ Hz, $J_2 = 9.9$ Hz), 3.01 td (1H, $J_1 = 11.1$ Hz, $J_2 = 5.7$ Hz, H-19), 3.96 d (1H, $J = 11.0$ Hz, H-28a), 4.31 d (1H, $J = 11.0$ Hz, H-28b), 4.42 dd (1H, $J_1 = 8.8$ Hz, $J_2 = 7.3$ Hz, H-3), 7.55 dd (1H, $J_1 = 7.7$ Hz, $J_2 = 1.3$ Hz, H-6′), 7.69 s (1H, NH), 7.70 s (1H, H-4′), 7.74 d (1H, $J = 7.7$ Hz, H-7′). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.7, 16.05, 16.12, 16.6, 18.2, 20.8, 21.1, 21.4, 23.7, 27.0, 27.7, 28.0, 29.9, 34.2, 34.6, 34.9, 37.1, 37.6, 37.9, 38.4, 40.9, 42.7, 47.0, 47.1, 49.9, 55.2, 55.3, 62.7, 80.9, 122.2, 123.8, 130.3, 133.4, 133.6, 157.1, 168.3, 168.7, 171.1, 171.7. **HRMS** (ESI): $C_{39}H_{52}NO_6$ found 630.3802 $[M-H]^+$; calcd. 630.3789.

4.1.68. Preparation of compound 37

A mixture of dimethyl phthalate (0.40 mmol) and KOH (1.0 g, 18.0 mmol, 45 eq.) in MeOH (5 mL) was heated to reflux for 4 h, then concentrated in vacuo. Water 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 and the crude phthalic acid was used in next step without purification. To the phthalic acid was added acetic anhydride (4 mL) and the solution was refluxed for 5 h under nitrogen atmosphere. The acetic anhydride was removed in vacuo and the crude phthalic anhydride was used in next step without purification. The phthalic anhydride and urea (408 mg, 8.0 mmol, 20 eq.) were heated to 170 °C for 2 h. The crude product was cooled to room temperature and was diluted with water, extracted with DCM (100 mL), dried over Na_2SO_4, concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate (2 : 1), giving the final phthalimide.

*4.1.69. 3β, 28-Diacetoxy-19-(1,3-dioxo-7-phenylisoindolin-5-yl)-20,29,30-trinorlupan **(37)***

Compound **37** was prepared according to the general procedure in 3 stages from phthalate (270 mg, 0.40 mmol) and KOH (1.0 g, 18.0 mmol) in 5 mL of MeOH; after acetic anhydride (4 mL), then urea (480 mg; 8.0 mmol). After purification (mobile phase hexane/EtOAc 2 : 1) compound **37** (131 mg; 60 %) was obtained as a white solid; **mp** 206–208 °C (hexane/EtOAc); R_f 0.45 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3246 (N–H), 2942, 1718 (C═O), 1616, 1449, 1365, 1240, 1028, 753, 697. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ, ppm: 0.81 s (6H, 2Me), 0.83 s (3H, Me), 0.94 s (3H, Me), 1.03 s (3H, Me), 2.02 s (3H, MeCO), 2.10 s (3H, MeCO), 2.39 dq (1H, J_1 = 14.0 Hz, J_2 = 9.4 Hz), 3.03 td (1H, J_1 = 11.5 Hz, J_2 = 5.6 Hz, H-19), 3.96 d (1H, J = 10.9 Hz, H-28a), 4.32 d (1H, J = 10.9 Hz, H-28b), 4.41–4.45 m (1H, H-3), 7.46–7.55 m ($6H_{Ar}$), 7.60 s (1H, NH), 7.71 s ($1H_{Ar}$). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.8, 16.1, 16.2, 16.6, 18.2, 20.9, 21.2 (2C), 21.4, 23.7, 27.0, 27.8, 28.0, 29.9, 34.2, 34.5, 35.0, 37.1, 37.6, 37.9, 38.4, 40.9, 42.8, 47.0, 50.0, 55.1, 55.4, 62.7, 80.9, 123.8, 125.7, 128.3, 128.9, 129.5, 134.4, 134.6, 136.4, 141.4, 156.9, 167.6, 168.1, 171.1, 171.7. **HRMS** (ESI): $C_{45}H_{58}NO_6$ found 708.4254 $[M+H]^+$; calcd. 708.4259.

*4.1.70. 3β, 28-Diacetoxy-19-(1,3-dioxo-2-(4-methoxyphenyl)isoindolin-5-yl)-20,29,30-trinorlupan **(39)***

Compound **39** was prepared according to the general procedure of preparation of N-substituted phtalimides in 3 stages from phthalate **34a** (125 mg, 0.21 mmol) and LiOH monohydrate (78 mg, 1.85 mmol) in 4 mL (2 : 1: 1) of MeOH, THF, H_2O; after acetic anhydride (4 mL), then 4-methoxyaniline (26 mg; 0.21 mmol) in THF (2 mL) for 2 h at 40 °C. After purification (mobile phase hexane/EtOAc 3 : 1) compound **39** (80 mg; 52 %) was obtained as a pale yellow solid; **mp** 176–178 °C (hexane/EtOAc); R_f 0.50 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 2944, 1736 (C═O), 1727, 1513, 1441, 1366, 1230, 1029, 825, 747, 691. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ, ppm: 0.807 s (3H, Me), 0.81 s (3H, Me), 0.82 s (3H, Me), 0.93 s (3H, Me), 1.03 s (3H, Me), 1.72 td (1H, J_1 = 13.9 Hz, J_2 = 4.5 Hz), 1.80 td (1H, J_1 = 12.5 Hz, J_2 = 3.7 Hz), 1.91–1.98 m (3H), 2.02 s (3H, MeCO), 2.11 s (3H, MeCO), 2.38 dq (1H, J_1 = 14.0 Hz, J_2 = 10.0 Hz), 3.03 td (1H, J_1 = 11.0 Hz, J_2 = 5.6 Hz, H-19), 3.85 s (3H, MeO), 3.97 d (1H, J = 11.0 Hz, H-28a), 4.32 d (1H, J = 11.0 Hz, H-28b), 4.42 dd (1H, J_1 = 8.8 Hz, J_2 = 7.3 Hz, H-3), 7.02 d ($2H_{Ar}$, J = 9.1 Hz), 7.33 d ($2H_{Ar}$, J = 9.1 Hz), 7.57 dd (1H, J_1 = 7.7 Hz, J_2 = 1.1 Hz, H-6′), 7.78 s (1H, H-4′), 7.81 d (1H, J_1 = 7.7 Hz, H-7'). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ, ppm: 14.8, 16.1, 16.2, 16.6, 18.3, 20.8, 21.2, 21.4, 23.8, 27.0, 27.8, 28.1, 29.9, 34.2, 34.7, 35.0, 37.1, 37.6, 37.9, 38.4, 40.9, 42.8, 47.1, 47.2, 50.0, 55.3, 55.4, 55.6, 62.7, 80.9, 114.6, 122.2, 123.9, 124.6, 128.1, 129.5, 132.6, 133.8, 157.2, 159.4, 167.6, 168.0, 171.1, 171.7. **HRMS** (ESI): $C_{46}H_{60}NO_7$ found 738.4363 $[M+H]^+$; calcd. 738.4364.

*4.1.71. General procedure for the synthesis of compounds **36, 38, 40***

To a stirred solution of derivative **35**, **37**, or **39** (0.13 mmol) in THF (1 mL) at room temperature was added 3 *N* HCl in MeOH or 6 *N* HCl in PrOH (3 mL) and the reaction mixture was stirred for 24 h or 4 d. The resulting mixture was diluted with water, extracted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$, water, dried over Na_2SO_4, concentrated in vacuo. The residue was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate.

*4.1.72. 3β-Acetoxy-19-(1,3-dioxoisoindolin-5-yl)-20,29,30-trinorlupan-28-ol **(36)***

Compound **36** was prepared according to the general procedure from acetate **35** (120 mg; 0.19 mmol), 6 *N* HCl in PrOH (5 mL) and THF (1 mL) for 4 days. After purification (mobile phase hexane/EtOAc 3 : 2) compound **36** (47 mg, 42 %) was obtained as a white solid; **mp** 193–195 °C (hexane/EtOAc); R_f 0.25 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3500, 2970, 1738, 1440, 1366, 1217, 1027, 900, 749. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.80 s (3H, Me), 0.81 s (3H, Me), 0.82 s (3H, Me), 0.92 s (3H, Me), 1.01 s (3H, Me), 1.69–1.79 m (2H), 1.94 t (1H, J = 11.7 Hz, H-18), 2.00–2.06 m (2H), 2.02 s (3H, MeCO), 2.37 dq (1H, J_1 = 14.0 Hz, J_2 = 10.0 Hz), 2.96 td (1H, J_1 = 11.1 Hz, J_2 = 5.9 Hz, H-19), 3.44 d (1H, J = 10.7 Hz, H-28a), 3.87 d (1H, J = 10.7 Hz, H-28b), 4.42 dd (1H, J_1 = 8.7 Hz, J_2 = 7.5 Hz, H-3), 7.56 dd (1H, J_1 = 7.7 Hz, J_2 = 0.7 Hz, H-6′), 7.63 s (1H, NH), 7.70 s (1H, H-4′), 7.73 d (1H, J = 7.7 Hz, H-7'). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.8, 16.0, 16.2, 16.6, 18.3, 20.8, 21.4, 23.7, 27.0, 27.8, 28.0, 29.4, 34.2, 34.4, 34.9, 37.1, 37.3, 37.9, 38.4, 40.9, 42.8, 47.3, 48.5, 50.0, 55.3, 55.4, 60.7, 80.9, 122.3, 123.8, 130.3, 133.3, 133.6, 157.5, 168.2, 168.6, 171.1. **HRMS** (ESI): $C_{37}H_{50}NO_5$ found 588.3695 $[M-H]^+$; calcd. 588.3684.

*4.1.73. 3β-Acetoxy-19-(1,3-dioxo-7-phenylisoindolin-5-yl)-20,29,30-trinorlupan-28-ol **(38)***

Compound **38** was prepared according to the general procedure from acetate **37** (90 mg; 0.13 mmol), 6 *N* HCl in PrOH (4 mL) and THF (1 mL) for 2 days. After purification (mobile phase hexane/EtOAc 2 : 1 to 3 : 2) compound **38** (58 mg; 69 %) was obtained as a white solid; **mp** 205–207 °C (hexane/EtOAc); R_f 0.23 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3250, 2930, 1716, 1616, 1471, 1368, 1243, 1027, 753, 697. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.81 s (3H, Me), 0.814 s (3H, Me), 0.83 s (3H, Me), 0.95 s (3H, Me), 1.02 s (3H, Me), 1.73 td (1H, J_1 = 13.7 Hz, J_2 = 4.0 Hz), 1.78 td (1H, J_1 = 12.3 Hz, J_2 = 3.6 Hz), 1.98 t (1H, J = 11.7 Hz, H-18), 2.00–2.06 m (2H), 2.02 s (3H, MeCO), 2.39 dq (1H, J_1 = 14.2 Hz, J_2 = 10.0 Hz), 2.98 td (1H, J_1 = 11.0 Hz, J_2 = 5.9 Hz, H-19), 3.44 d (1H, J = 10.7 Hz, H-28a), 3.88 d (1H, J = 10.7 Hz, H-28b), 4.43 dd (1H, J_1 = 9.9 Hz, J_2 = 6.2 Hz, H-3), 7.45–7.50 m ($4H_{Ar}$), 7.53–7.56 m (2H), 7.56 s (1H, NH), 7.71 s ($1H_{Ar}$). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.8, 16.1, 16.2, 16.6, 18.3, 20.9, 21.4, 23.7, 27.0, 27.9, 28.1, 29.4, 34.2, 34.4, 34.8, 37.1, 37.4, 37.9, 38.4, 41.0, 42.8, 47.2, 48.5, 50.0, 55.1, 55.4, 60.8, 80.9, 120.7, 125.6, 128.3, 128.9, 129.5, 134.6, 135.4, 136.4, 141.4, 157.2, 167.6, 168.1, 171.1. **HRMS** (ESI): $C_{43}H_{56}NO_5$ found 666.4153 $[M+H]^+$; calcd. 666.4153.

*4.1.74. 3β,-Acetoxy-19-(1,3-dioxo-2-(4-methoxyphenyl)isoindolin-5-yl)-20,29,30-trinorlupan-28-ol **(40)***

Compound **40** was prepared according to the general procedure from acetate **39** (45 mg; 0.06 mmol), 6 *N* HCl in PrOH (3 mL) and THF (0.5 mL) for 3 days. After purification (mobile phase hexane/EtOAc 2 : 1) compound **40** (17 mg; 40 %) was obtained as a white solid; **mp** 190–193 °C (hexane/EtOAc); R_f 0.25 (silica gel, hexane/EtOAc 3 : 2). **IR** (DRIFT): 3462 (O–H), 2934, 1715 (C═O), 1512, 1379, 1246, 1027, 825, 747, 691. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ, ppm: 0.805 s (3H, Me), 0.81 s (3H, Me), 0.83 s (3H, Me), 0.94 s (3H, Me), 1.02 s (3H, Me), 1.73 td (1H, J_1 = 13.3 Hz, J_2 = 4.5 Hz), 1.77 td (1H, J_1 = 12.1 Hz, J_2 = 3.5 Hz), 1.96 t (1H, J = 11.7 Hz, H-18), 2.01–2.07 m (2H), 2.02 s (3H, MeCO), 2.38 dq (1H, J_1 = 13.9 Hz, J_2 = 10.0 Hz), 2.98 td (1H, J_1 = 11.1 Hz, J_2 = 5.9 Hz, H-19), 3.44 d (1H, J = 10.7 Hz, H-28a), 3.85 s (3H, MeO), 3.88 dd (1H, J_1 = 10.7 Hz, J_2 = 0.7 Hz, H-28b), 4.42 dd (1H, J_1 = 9.4 Hz, J_2 = 6.7 Hz, H-3), 7.02 d ($2H_{Ar}$, J = 9.1 Hz), 7.33 d ($2H_{Ar}$, J = 9.1 Hz), 7.58 d (1H, J = 7.7 Hz, H-6′), 7.78 s (1H, H-4′), 7.80 d (1H, J_1 = 7.7 Hz, H-7'). **^{13}C NMR** ($CDCl_3$, 126 MHz) δ, ppm: 14.8, 16.1, 16.2, 16.6, 18.3, 20.9, 21.4, 23.8, 27.0, 27.8, 28.1, 29.4, 34.2, 34.4, 34.9, 37.1, 37.4, 37.9, 38.4, 41.0, 42.8, 47.3, 48.5, 50.0, 55.3, 55.4, 55.6, 60.8, 80.9, 114.6, 123.8, 124.6, 126.2, 128.1, 129.4, 132.5, 133.7, 157.5, 159.4, 167.7, 168.0,

171.1. **HRMS** (ESI): $C_{44}H_{58}NO_6$ found 696.4258 $[M+H]^+$; calcd. 696.4259.

4.2. Biological evaluation

4.2.1. Cell culture and MTS cytotoxicity assay

The detailed procedure for cell culture and MTS analysis is described in [24,25,62,78,79].

4.2.2. Pharmacological parameters

The detailed procedure for cell culture and MTS analysis is described in [24,26,80].

4.2.3. Annexin V assay

The Annexin V kit (Exbio) was used to analyze cell death in samples treated with **19**, **26**, **28**, and **30** at 1 × IC_{50} and 5 × IC_{50} concentrations for 24 h. The manufacturer's instructions were followed with minor modifications. Briefly, cells diluted to the appropriate concentration were washed with Ca^{2+} containing Annexin V binding buffer prior to staining with Annexin V-FITC conjugate and propidium iodide. Because of the high sensitivity of CCRF-CEM cells to propidium iodide itself, we reduced manufacturer's protocol recommended concentration by half. Cells were stained for 15 min at room temperature in the dark, centrifuged and re-suspended in 100 µl volume of Annexin V binding buffer. Samples were immediately analyzed on FACSAria II flow cytometer (Becton Dickinson). At least 10,000 cells of each sample were acquired.

4.2.4. JC-1 mitochondrial membrane potential assay

Mitochondrial membrane potential ($\Delta\Psi_m$) was measured using the membrane-permeant JC-1 cationic probe. CCRF-CEM cells were treated with **19**, **26**, **28**, and **30** at 1 × IC_{50} and 5 × IC_{50} concentrations for 24 h. A cell suspension at an approximate density of 0.5 × 10^6 cells/mL was labelled with 1 µM JC-1 for 10 min at 37 °C. As a positive control, 100 µM CCCP uncoupler was added to one well 5 min prior to JC-1. Following staining, cells were pelleted by centrifugation (1500 rpm, 5 min, room temperature), re-suspended in 0.5 ml of 1 × PBS and immediately analyzed on FACSAria II flow cytometer (Becton Dickinson) using a 488 nm laser. JC-1 monomers were detected at 529 nm of emission maximum, while J-aggregates were detected at 590 nm of emission maximum. At least 10,000 cells were acquired for each sample.

4.2.5. Cell cycle analysis

The detailed procedure for cell cycle analysis is described elsewhere [78].

4.2.6. BrdU incorporation analysis

Cells were cultivated and treated with derivatives **19**, **26**, **28**, and **30** at 1 × IC_{50} and 5 × IC_{50} concentrations for 24 h. Prior to harvesting, cells were pulse-labelled with 10 µM 5-bromo-2′-deoxyuridine (BrdU) for 30 min. Cells were collected by centrifugation (1500 rpm/5 min/RT), washed with cold 1 × PBS and fixed in ice-cold 70 % ethanol. Following washing with 1 × PBS, cells were incubated in 2 M HCl for 30 min at room temperature to denature their DNA. Then, HCl was neutralized with 0.1 M $Na_2B_4O_7$ (borax) and cells were washed with 0.5 % Tween-20 and 1 % BSA in 1 × PBS. Further, cell pellets were incubated with a primary anti-BrdU antibody (Exbio) for 30 min at room temperature. Following washing with 1 × PBS, cells were stained with secondary anti-mouse-FITC antibody (Sigma) for 30 min at room temperature in the dark.

The samples were then washed with 1 × PBS and incubated with propidium iodide (0.1 mg/mL) and RNase A (0.5 mg/mL) for 1 h at room temperature in the dark and finally analyzed by flow cytometry (FACSCalibur) using a 488 nm single beam laser.

4.2.7. BrU incorporation analysis

Cells were cultured and treated as for BrdU analysis. Prior to harvesting, they were pulse-labelled with 1 mM 5-bromouridine (BrU) for 30 min. Then, the cells were fixed in 1 % buffered paraformaldehyde with 0.05 % NP-40 at room temperature for 15 min, followed by incubation at 4 °C overnight. Pelleted cells were washed with 1 % glycine in 1 × PBS followed by washing with 1 × PBS alone. The cells were stained with primary anti-BrdU antibody (Exbio) cross-reacting to BrU for 45 min at room temperature. Following washing with 1 × PBS, the cells were stained with secondary anti-mouse-FITC antibody (Sigma) for 45 min at room temperature in the dark. The cells were washed with 1 × PBS and fixed with 1 % PBS buffered paraformaldehyde containing 0.05 % NP-40. The samples were then washed with 1 × PBS and incubated with propidium iodide (0.1 mg/mL) and RNase A (0.5 mg/mL) for 1 h at room temperature in the dark. The analysis was performed similarly to the BrdU analysis.

4.2.8. Western blot

CCRF-CEM cells were treated with derivatives **19**, **26**, **28**, and **30** at 1 × IC_{50} and 5 × IC_{50} concentrations for 24 h. Then, the cells were washed with ice cold 1 × PBS and total proteins of CCRF-CEM cells were extracted using RIPA lysis 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). Proteins were quantified using the Pierce™ BCA Protein Assay Kit (Thermo Scientific). Aliquots containing 30 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 further transferred from the gel onto a nitrocellulose membrane using the Trans-Blot® Turbo™ Transfer System (Bio-Rad). Membranes were blocked in 5 % TBS buffered BSA with 0.1 % Tween 20 for 1 h at room temperature and afterwards incubated with primary antibodies against Akt, P-Akt (Ser473), STAT-3, Caspase-3, PARP (all from Cell Signaling Technology), Cyclin D, GAPDH, Bcl-xL, Bak, Bid, Bim (all from Abcam) and Cyclin A (Sigma Aldrich) at 4 °C overnight. Washed (TBS/0.1 % Tween 20) membranes were incubated with an appropriate HRP-conjugated secondary antibody (Sigma Aldrich) for 1 h at room temperature. Finally, the chemiluminescence signal was developed by ECL Prime (Amersham) reagent and detected by Li-cor Odyssey (LI-COR Biotechnology) imaging system. Signals from anti-GAPDG served as loading controls.

CRediT authorship contribution statement

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

Acknowledgment

The chemistry work and basic biological tests were paid by the Czech Science Agency (grant number 21-00902S), advanced biological experiments were supported by the National Institute for Cancer Research (Program EXCELES, ID Project No. LX22NPO5102) - next generation EU and the infrastructure was supported in parts by the infrastructural projects (CZ-OPENSCREEN – LM2023052; EATRIS-CZ – LM2023053). Pharmacology studies were supported by internal Palacky University grants IGA_LF_2024_038 and IGA_PrF_2024_028.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2024.117126.

Data availability

Data will be made available on request.

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