European Journal of Medicinal Chemistry 302 (2026) 118268

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Synthesis and biological evaluation of novel C-30 modified lupane triterpenoids selectively cytotoxic against cancer cells

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A B S T R A C T

In this work, we describe a new synthetic pathway to 30-methylidyne derivatives of lupane-triterpenoids that were consequently used as excellent substrates for the preparation of new compounds via Sonogashira coupling and via Cu^{I} catalyzed Azide-Alkyne 1,3-dipolar cycloadditions. Reactions had moderate to excellent yields. All prepared compounds were tested for their *in vitro* cytotoxicity on six cancer and two non-cancer cell lines. The most active compounds were triazoles with free 3-OH and 28-OH groups (betulin analogues) and among them, conjugate with triazole substituted by furan **16j** was the best with IC_{50} of 2.68 μM against CCRF-CEM and therapeutic index of 18.66. Mechanistic studies revealed that both **16g** and **16j** induce apoptosis in CCRF-CEM cells, as confirmed by annexin V/PI staining. Cell cycle analysis showed that **16j** causes pronounced S phase arrest, while **16g** modulates G1/S transition in a concentration-dependent manner. Moreover, **16g** strongly suppressed DNA and RNA synthesis, whereas **16j** paradoxically increased RNA synthesis despite replication inhibition. Both compounds triggered mitochondrial hyperpolarization, suggesting early mitochondrial involvement in their apoptotic mechanism. Western blot analysis supported these findings, revealing γH2AX induction and PARP cleavage, alongside distinct modulation of key regulators: **16j** upregulated p21 and phospho-Chk1 (Ser345), while **16g** downregulated both at high dose, consistent with checkpoint activation versus checkpoint bypass. These findings support **16g** and **16j** as promising candidates for further anticancer drug development.

1. Introduction

Triterpenes and triterpenoids are natural compounds that occur throughout nature, especially in plants. Many of them have interesting biological activities, such as antiviral [1] (e.g. anti-HIV [2], anti-herpes [1]), anti-inflammatory [3,4], neuroprotective [5,6], and probably most importantly selective cytotoxic activity against cancer cells [7,8]. The selective cytotoxic activity is the most studied and promising one since it may lead towards new anticancer therapeutics with new mechanism of action. A number of articles were focused on cytotoxic activity of lupane triterpenoids [9,10]. Our recent work was focused on the modification of the position C-30 in betulin and betulinic acid [11–13]. It was found, that the modification of this position with aromatic and/or heterocyclic substituent provides selective cytotoxicity against CCRF-CEM cells. To further explore this position and to open more options for new types of modifications, in this work we focused on the development of a new synthetic pathway leading to new triterpenoid alkynes, highly promising intermediates for further modifications. Alkyne moiety was chosen as it is an electron-rich functional group, that can undergo a wide variety of reactions, such as Sonogashira coupling [14], both electrophilic and nucleophilic additions and most importantly, it may serve as a dipolarophile in various cycloadditions [15]. There are only several published precedents, where lupane-type triterpenoids were furnished with alkyne moiety. Among them, Sonogashira coupling [16,17]. Williamson synthesis [18], esterification [19–23], α-alkylation [24], nucleophilic addition [25] in the position 2 and 3, ester [20–22,25,26] and amide [27] conjugates in the position 28 as well as the position 30, were used. [5]. Especially around the position 30, there is a significant lack of known synthetic procedures leading to triple bond derivatives with exclusive connection of the alkyne moiety to the terpene skeleton by C–C bond. One synthetic pathway was published to obtain such derivative [28,29], however the product was reported to be unstable [30]. Our work, on the other hand, aimed to extend the synthetic portfolio of lupane triterpenoids using the Corey-Fuchs synthesis [31] and to explore

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https://doi.org/10.1016/j.ejmech.2025.118268
Received 29 August 2025; Received in revised form 9 October 2025; Accepted 11 October 2025
Available online 16 October 2025

the synthetic scope of the alkyne substrate in Sonogashira coupling reaction and cycloadditions. All prepared compounds were later tested in a routine evaluation of cytotoxicity in 6 cancer cell lines and 2 non-cancer cell lines. In addition to the cytotoxicity, deeper biological evaluation of selected active derivatives was performed. It is well known that many triterpenoids exert their anticancer effects through induction of apoptosis [32,33], cell cycle arrest [34] and mitochondrial pathway activation [35]. Therefore, mechanistic studies on cell death pathways, cell cycle modulation, mitochondrial membrane potential, and nuclear signaling were conducted to better understand the mode of action of the most promising compounds. In this context, expression and phosphorylation status of key regulators involved in apoptosis, DNA damage response, and cell cycle checkpoints were analyzed by western blotting.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of 30-methylidyne-diethylbetulin 8

First of all, an appropriate protection group for both hydroxyl groups of betulin **1** had to be found in order to prevent cross reactivity while performing reactions at the position C30. For the first experiments and reaction optimization, diethylbetulin **2** was used, that was prepared by the standard Williamson synthesis with 83 % yield. Diethylbetulin **2** was then oxidized with SeO_2 at its allylic position C30 to yield 30-oxo-diethylbetulin **4** in 75 % yield. The procedure has been developed earlier for analogous compounds [7]. The 30-(dibromomethylidene)-diethylbetulin **6** intermediate of the Corey-Fuchs synthesis was obtained by the reaction of the aldehyde **4** with CBr_4 and PPh_3. Although optimized, this step was repeatedly giving only moderate yields around 50 %, which was partly due to the thermolability of the intermediate **6** and partly due to the nature of the Corey-Fuchs reaction [36]. More advanced synthetic approaches were examined, e. g. one-pot reaction with Bestmann Ohira reagent [37], however the outcome was always an inseparable mixture of products. Last step of this pathway towards the desired alkyne **8** was the reaction of the Corey-Fuchs intermediate **6** with *n*-BuLi that provided 30-methylidyne-diethylbetulin **8** in 76 % yield (Scheme 1).

The total yield of this pathway was 24 % over 4 steps, which produced a sufficient amount of compound **8** for subsequent reactions. Even though 30-methylidyne-diethylbetulin **8** could not be deprotected easily, we used the derivative **8** as a perfect model compound for the testing of the synthetic scope and limits of both the Sonogashira coupling and copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC). Due to the harsh conditions that would be necessary for the deprotection of the etheric groups in the final products, better protection had to be found later for the preparation of the final compounds containing the free 3 and 28 hydroxyls.

2.1.2. Sonogashira coupling conjugates with 30-methylidyne-diethylbetulin 8

Several sets of reaction conditions with alkyne **8** as a starting compound were tried. Common reaction conditions [38,39] gave little to no conversion of **8** into desired product. Surprisingly, we found that only the presence of Pd^0 and Cu^I at the same time [40] yields products **11a–11g** (Scheme 2). If Pd^{II} catalysts (e. g. $PdCl_2$ or $Pd(OAc)_2$) were used instead of Pd^0, no reaction was observed.

We found, that this reaction has following limits: 1) Only aryliodides provided desired products, although they can consist of heterocyclic moiety. If arylbromides were used instead, no reaction was observed. 2) If the inert atmosphere was disrupted or the reaction temperature increased over 75 °C, oxidative homocoupling of the terminal alkyne **8** was observed instead of Sonogashira coupling. 3) Aryliodides with acidic protons also spoiled the reaction, e. g. reaction with 4-iodobenzoic acid did not provide any product. Given the limitations above, general limitations of Sonogashira coupling and no previous SAR references, we have designed and prepared a short set of final compounds, which demonstrates scope of the reaction. Non-activated arenes (**11a**, **11c**), arenes with EDG (**11b**), arenes with EWG (**11d**, **11e**) were synthesized as well as two examples of heteroarenes (**11f** and **11g**). Some of the yields (**11b** and **11f**) are rather lower, however they are in the same range as in the lit [40].

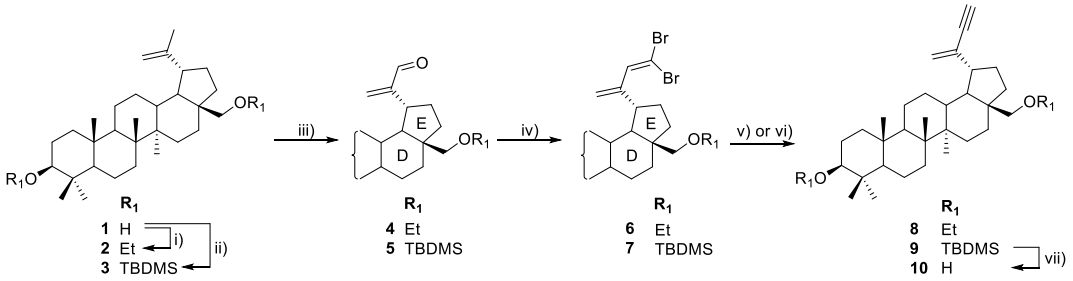
2.1.3. Copper(I)-catalyzed azide-alkyne cycloaddition with 30-methylidyne-diethylbetulin 8

In the second series, we focused on CuAAC. Several routinely used reaction conditions from the literature have been used [41], however, none of them worked generally and the results changed by using various azides. Using copper(II) sulfate pentahydrate and sodium L-ascorbate, triazoles **15a** and **15c** were isolated (Scheme 3). Moreover, triazole **15b** could be derived from **15a** by treatment with *in situ* generated sodium ethoxide.

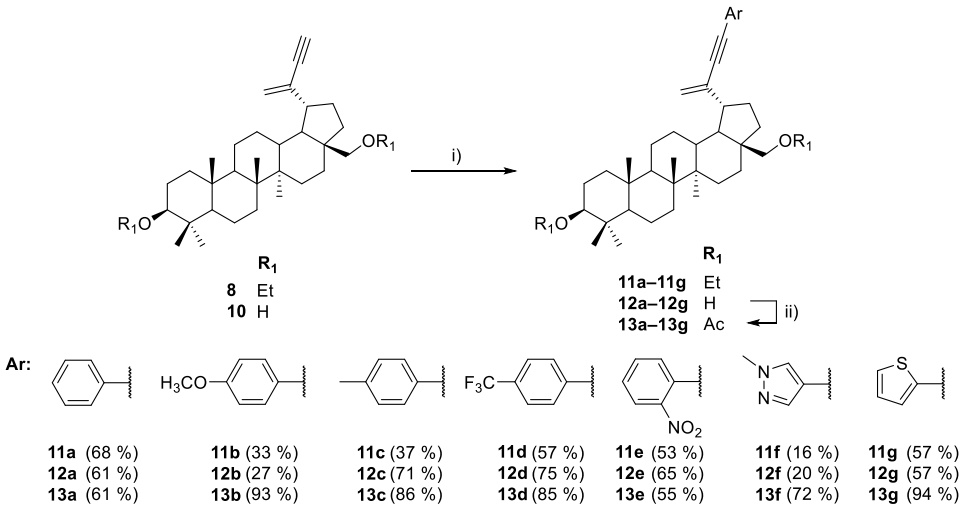
Low reactivity of the alkyne **8** in this reaction could have been caused by its low solubility in polar solvents used for the reaction, therefore we used unprotected alkyne **10** that had to be prepared from TBDMS protected betulin **3** since attempts to directly deprotect compound **8** in strongly acidic conditions led to its decomposition. Design of triazole series is discussed in *2.1.6*.

2.1.4. Synthesis of 30-methylidynebetulin 10

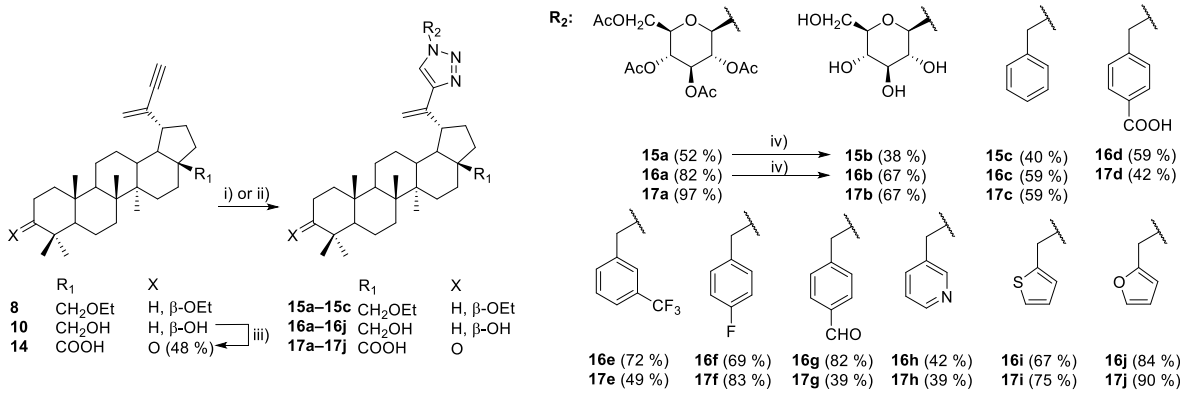
Since ethyl-ether protection was extremely difficult to remove, leading to a decomposition of the protected compound most of the times, *tert*-butyldimethylsilyl (TBDMS) protective group was used (Scheme 1). Protection of betulin **1** with TBDMSCl was successful only in the case of standard Corey protocol [42], which provided excellent yield of 3,28-bis(TBDMS)betulin **3** (97 %). Curiously, the reaction was far less efficient using modern protocols with 4-(dimethylamino)pyridine, giving yields only about 50 % [43]. Allylic oxidation of **3** was held in the analogous manner as in the previous series, yielding 96 % of 30-oxo-3, 28-bis(TBDMS)betulin **5**. During the first step of the Corey-Fuchs synthesis, which was done alike in the first series, low reactivity and decomposition of the product was observed and the yields ranged from 8 to 12 %. After the optimization of the reaction conditions (CBr_4 equivalents increased from 1.2 to 2 and PPh_3 equivalents increased from 2.1 to 4) including the change of the order of adding the reactants (solution



Scheme 1. Synthesis of 30-methylidyne-diethylbetulin and 30-methylidynebetulin. Reagents and conditions: i) NaH, EtI, 1,4-dioxan, reflux; ii) *tert*-butyldimethylsilyl chloride, imidazole, dimethylformamide, r.t.; iii) SeO_2, 2-methoxyethanol, reflux; iv) CBr_4, PPh_3, dichloromethane, 0 °C; v) *n*-BuLi, toluene, −78 °C for compound **8**; vi) *n*-BuLi, *n*-hexane, −78 °C for compound **9**; vii) tetra-*n*-butylammonium fluoride, tetrahydrofuran, 45 °C.



Scheme 2. Synthesis of Sonogashira coupling derivatives. Reagents and conditions: i) aryliodide, Pd/C, PPh_3, CuI, *N*,*N*-diisopropylethylamine, dimethylacetamide, H_2O. 75 °C; ii) Ac_2O, 4-(dimethylamino)pyridine, dichlormethane, r.t.



Scheme 3. CuAAC with 30-methylidyne-lupanoids. Reagents and conditions: i) R_2N_3, $CuSO_4{\cdot}5H_2O$, sodium L-ascorbate, *t*-BuOH, H_2O for compounds **15a–15c**; ii) Copper(I) 3-methylsalicylate, dry tetrahydrofuran, 60 °C for compounds **16a–16j** and **17a–17j**; iii) CrO_3, H_2SO_4, acetone, water, 0 °C; iv) NaH, EtOH, r.t.

of triterpene **5** was added to the mixture of CBr_4 and PPh_3 instead of adding PPh_3 to the mixture of triterpene **5** and CBr_4), the yield of 30-(dibromomethylidene)-3,28-bis(TBDMS)betulin **7** raised to acceptable 49 %. The elimination step of **7** provided excellent yield of 30-methylidyne-3,28-bis(TBDMS)betulin **9** (96 %). Finally, the deprotection step was carried out by tetra-*n*-butylammonium fluoride again yielding 30-methylidynebetulin **10** exceptionally well with the yield of 92 % (Scheme 1). The total yield over 5 steps reached 40 %. Alkyne **10** represents deprotected analogue of 30-methylidyne-diethylbetulin **8**.

*2.1.5. Sonogashira coupling conjugates with 30-methylidynebetulin **10***

Unsurprisingly, we observed similar reactivity of alkyne **10** in the Sonogashira coupling reactions as previously for its diethyl-derivative **8**. Under analogous conditions that were used for the preparation of compounds **11a–11g**, we were able to synthesize analogous series of derivatives **12a–12g**. Since esters of betulin derivatives, especially acetates, are often being used as a prodrugs for several reasons (e. g. better membrane permeability) and often surpass the activity of compounds with free OH groups [41], diacetylderivatives **13a–13g** were prepared by the standard acetylation of **12a–12g** (Scheme 2).

2.1.6. Copper(I)-catalyzed azide-alkyne cycloaddition with 30-methylidynebetulin

The reactivity of 30-methylidynebetulin **10** in CuAAC was as insufficient as using reagent **8**, therefore other reactivity factors were investigated. At first, we explored several sources of catalytic Cu^I ion. Copper(I) iodide [44], $[Cu(\mu\text{-}OH)(TMEDA)]_2Cl_2$ [28] as well as $CuSO_4{\cdot}5H_2O$ in combination with sodium L-ascorbate [41] provided only irreproducible reactions with traces of products or low yields. However, experiments with copper(I) 3-methylsalicylate [45] were reproducible, the reaction times were shortened from week to several tens of hours and yields of the triazole products **16a** and **16c–16j** ranged from 42 to 84 % (Scheme 3). Triazole **16b** was again derived from **16a** by a treatment with sodium ethanolate, which was generated *in situ*. From our previous projects [12,41] studying similar compounds modified in the same position it is clear that aliphatic substituents usually do not improve cytotoxic activity. These compounds were therefore not prepared within this study. On the other hand, 4-formylbenzyl substituent increased the activity the most [41], which motivated us to synthesize benzaldehyde derivative **16g** along with 4-fluorobenzyl derivative **16f** [12]. Triterpenoids are known for their hydrophobicity, which often hampers their solubility and bioavailability. In order to broaden the polarity range of the tested compounds, we have prepared tetra-*O*-acetylglucosyl and glucosyl conjugates **16a** and **16b** containing additional polar functionalities. Based on the fact that interesting biological activites were earlier discovered or significantly improved by adding a heteroaryl ring to active triterpenoid molecules [11,46], during this study we decided to synthesize pyridine **16h**, furan **16j** and thiophene **16i** as common representatives of heteroaryl substituents.

2.1.7. Synthesis of 30-methylidynebetulonic acid

Having optimized the synthesis of alkyne intermediates and following Sonogashira couplings as well as copper-catalyzed cycloadditions, we aimed for compounds that may be potentially selectively cytotoxic in cancer cells. From the previous research [47] we know, that the presence of a carboxyl group in the position 28 is usually essential for higher selective cytotoxic effects in cancer cells. Therefore, the earlier prepared 30-methylidynebetuline **10** was oxidized by Jones reagent to 30-methylidynebetulonic acid **14** (Scheme 3). The reaction provided sufficient yield of 48 % of **14**, which corresponds with lit [48].

2.1.8. Copper(I)-catalyzed azide-alkyne cycloaddition with 30-methylidynebetulonic acid

In the last series, triazoles **17a–17j** were prepared from 30-methylidynebetulonic acid **14** in the analogous manner with copper(I) 3-methylsalicylate as in the previous series. Triazole **17b** was also prepared directly from alkyne **14** by CuAAC. Yields ranged wildly from 18 to 97 % (Scheme 3).

2.2. Biology

2.2.1. Cytotoxicity assay and SAR assumptions

Cytotoxic activity of prepared compounds **10**, **12a–12g**, **13a–13d, 13f**, **14**, **15b**, **16a–16j** and **17a–17j** was tested on six cancer cell lines and two non-cancer cell lines (Table 1). Betulin **1** and betulonic acid **18** were added to the study as standards of the starting natural products. Cytotoxic activity of the compounds **2**–**9**, **11a–11g, 13e, 13g, 15a** and **15c** was not measured due to low solubility of the compounds. If the IC_{50} value exceeds 50 μM, the compound is considered inactive.

Cytotoxicity of alkynes **10** and **14** was moderate and non-selective. Sonogashira derivatives **12a–12g** with free hydroxyls 3 and 28 showed moderate, yet selective cytotoxicity on CCRF-CEM, with IC_{50} = 11.71–22.76 μM. The IC_{50} values remain in the similar range for all substituents used, only slightly improving the activity in comparison to the starting natural compounds. This indicates that the activity is not driven by any specific interaction of the (hetero)aromatic substituent but by the terpenoid structure. On the other hand, none of these substituents shows any negative effects. Diacetylester analogues **13a–13d** and **13f** performed worse than anticipated and showed no cytotoxicity at all. Triazole **15b** was the only derivative of diethylbetulin **2** that was soluble enough for the cytotoxicity measurement, showing low and non-selective cytotoxicity (IC_{50} = 27 μM). Its′ reasonable solubility was obviously the result of the presence of four hydroxyls at the glucosyl moiety. The most active compounds of this study were found among the triazoles derived from betulin **1** that are highly active against CCRF-CEM. Triazole **16g** has IC_{50} 3.99 ± 1.01 μM (TI = 12.53) and derivative **16j** IC_{50} 2.68 ± 0.54 μM (TI = 18.66) while maintaining selectivity. Other compounds containing similar aromatic substituents (e.g. **16c** or halogenated **16e** and **16f**) had IC_{50} above our threshold. Based on these findings it seems that the benzaldehyde moiety might be an important part of the pharmacophore. Comparing the cytotoxicity of benzyl-derivative **16c** and cytotoxicity of heteroarenes **16h–16j** (IC_{50} 2.7–11.7 μM) we can observe dramatic increase in activity in heterocycle-containing compounds. The most promising compound of this study **16j** deserves special attention as it is non-toxic and, so far, the most active triterpenoid triazole prepared at the position C30 of lupane skeleton, compared to our previous studies where similar derivatives reach moderate or low activity [41,49]. To our surprise, analogical compounds derived from betulonic acid (**17g** and **17j**) are far less active on CCRF-CEM cells, which is in contrast with our initial assumption, that derivatives containing 28-carboxylic acid should be more cytotoxic than derivatives with 28-CH_2OH which is commonly stated in the literature [9,50]. In this study, the IC_{50} value of **17j** is significantly higher than the value of analogical betulin derivative **16j**, which further points to a specific mechanism of action of **16j** not barely dependent on the parent triterpenoid. In summary, betulin **1** derivatives with free C-3 and C-28

Table 1
Cytotoxicity assay betulin **1**, betulonic acid **18**, intermediates **10** and **14,** Sonogashira derivatives **12a–12d** and **12f–12g** and triazoles **15b, 16d, 16g–16j, 17a, 17c,** and **17e–17j** against 6 cancer and 2 non-cancer cell lines.

Comp.	IC50 (μM)[a]								
	CCRF-CEM	K562	HCT116	HCT116p53$^{-/-}$	A549	U2OS	BJ	MRC-5	TI[b]
cisplatin[c]	5.82	10 ± 2	20.79	**NA**	9.79 ± 0.63	54.16	**NA**	21.22 ± 1.24	3.65
1	13.80 ± 3.01	≥50	40.30 ± 7.14	36.47 ± 2.72	29.11 ± 5.07	25.64 ± 6.72	12.74 ± 2.48	14.75 ± 4.64	1.00
18	15.61 ± 0.87	≥50	35.96 ± 0.88	41.00 ± 2.92	37.24 ± 4.80	40.39 ± 6.52	≥50	31.66 ± 2.72	2.62
10	24.62 ± 2.70	16.95 ± 1.24	12.09 ± 1.49	8.14 ± 0.60	11.81 ± 2.47	7.16 ± 0.72	16.36 ± 3.17	10.74 ± 0.62	0.55
14	11.69 ± 1.03	≥50	45.02 ± 2.12	≥50	41.12 ± 0.88	≥50	27.80 ± 3.61	25.54 ± 3.64	2.28
12a	11.71 ± 2.05	≥50	≥50	≥50	≥50	≥50	≥50	≥50	4.27
12b	12.02 ± 2.38	≥50	≥50	≥50	≥50	≥50	≥50	≥50	4.16
12c	22.76 ± 6.61	≥50	≥50	≥50	≥50	≥50	≥50	≥50	2.20
12d	14.50 ± 3.32	≥50	≥50	≥50	≥50	≥50	≥50	≥50	3.45
12f	12.06 ± 2.19	≥50	≥50	≥50	≥50	≥50	≥50	≥50	4.15
12g	15.43 ± 3.49	≥50	≥50	≥50	≥50	≥50	≥50	≥50	3.24
15b	26.80 ± 1.44	26.98 ± 3.12	29.12 ± 0.43	29.36 ± 0.84	34.60 ± 3.89	26.54 ± 0.65	29.82 ± 2.44	29.53 ± 0.97	1.11
16d	40.92 ± 2.80	≥50	≥50	≥50	≥50	≥50	≥50	≥50	1.22
16g	**3.99 ± 1.01**	≥50	≥50	≥50	≥50	≥50	≥50	≥50	12.53
16h	11.74 ± 2.88	≥50	≥50	≥50	≥50	≥50	≥50	≥50	4.26
16i	7.26 ± 1.77	≥50	≥50	≥50	≥50	≥50	≥50	≥50	6.89
16j	**2.68 ± 0.54**	≥50	≥50	≥50	≥50	≥50	≥50	≥50	18.66
17a	17.22 ± 2.48	28.53 ± 3.99	30.02 ± 0.50	28.96 ± 2.17	28.55 ± 3.46	25.35 ± 2.24	30.32 ± 0.46	27.39 ± 2.68	1.68
17c	33.13 ± 7.30	≥50	≥50	≥50	≥50	≥50	≥50	≥50	1.51
17e	18.88 ± 3.95	≥50	33.30 ± 3.85	41.40 ± 5.58	≥50	≥50	≥50	≥50	2.65
17f	29.25 ± 3.88	≥50	≥50	≥50	≥50	≥50	≥50	≥50	1.71
17g	7.72 ± 1.92	16.62 ± 2.95	13.10 ± 1.64	10.75 ± 0.87	32.98 ± 2.36	14.10 ± 3.12	>50	41.05 ± 1.97	5.90
17h	32.78 ± 6.40	≥50	≥50	≥50	≥50	≥50	≥50	≥50	1.53
17i	27.39 ± 4.84	≥50	36.80 ± 1.36	43.67 ± 3.04	≥50	≥50	≥50	≥50	1.83
17j	21.00 ± 3.83	≥50	34.39 ± 2.13	43.43 ± 1.93	≥50	≥50	≥50	≥50	2,38

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

[b] The therapeutic index is calculated based on the IC_{50} for the CCRF-CEM line versus the average IC_{50} for both fibroblast lines. All compounds were tested in at least 3 biological replicates, those with IC_{50} > 50 μM in all tested cell lines are not shown since they are considered inactive.

[c] The IC_{50} values for cisplatin were retrieved from peer-reviewed literature [51–56].

hydroxyls were more cytotoxic in CCRF-CEM cells than derivatives containing ethyl- or acetyl-substituents. They were also better than derivatives made from betulonic acid **18**. It is also clear that the triazole substituent plays the key role as a part of the pharmacophore increasing the selective cytotoxicity of the parent compound and it may be further tuned by its substitution by benzaldehyde or another heteroaromatic ring such as furan. Majority of the studied final derivatives were found non-toxic, which makes them suitable for further derivatization and investigation.

2.2.2. Induction of cell death by compounds **16g** and **16j** in CCRF-CEM cells analyzed via annexin V/propidium iodide staining

To investigate whether compounds **16g** and **16j** induce apoptotic cell death in CCRF-CEM cells, we employed annexin V-FITC and propidium iodide (PI) double staining followed by flow cytometric analysis (Fig. 1). Cells were treated with compounds **16g** and **16j** at concentrations corresponding to 1 × and 5 × IC_{50} for 24 h. The extent and nature of cell death were assessed by distinguishing between early apoptotic (annexin V^+/PI^-), late apoptotic or necrotic (annexin V^+/PI^+), and viable (annexin V^-/PI^-) cell populations. The results clearly demonstrated a marked increase in apoptotic cells following treatment with both **16g** and **16j**, as compared to the untreated control, which maintained over 97 % viability with negligible annexin V or PI staining. At both concentration levels, the two compounds induced a comparable shift from viable to apoptotic populations, with a dose-dependent increase in late apoptotic (annexin V^+/PI^+) cells, suggesting progression of apoptosis over time. Notably, the proportion of early apoptotic cells was already elevated at 1 × IC_{50}, and further accumulation in the late apoptotic quadrant was observed at 5 × IC_{50}, highlighting the time- and concentration-sensitive nature of the apoptotic response. Importantly, PI-only positive cells indicating necrosis remained negligible across all treated samples, reinforcing the conclusion that the observed cytotoxicity is driven predominantly by programmed cell death rather than by membrane-compromising necrotic mechanisms. Together, these data provide strong evidence that both compounds trigger apoptosis as the main mode of cytotoxicity in CCRF-CEM cells. This controlled and selective mechanism of action is particularly desirable in the context of anticancer therapy, as it may be associated with reduced off-target toxicity and improved therapeutic indices. The comparable pro-apoptotic activity of both compounds also suggests a potential structural or mechanistic convergence, warranting further investigation into their molecular targets and signalling pathways involved in apoptosis induction.

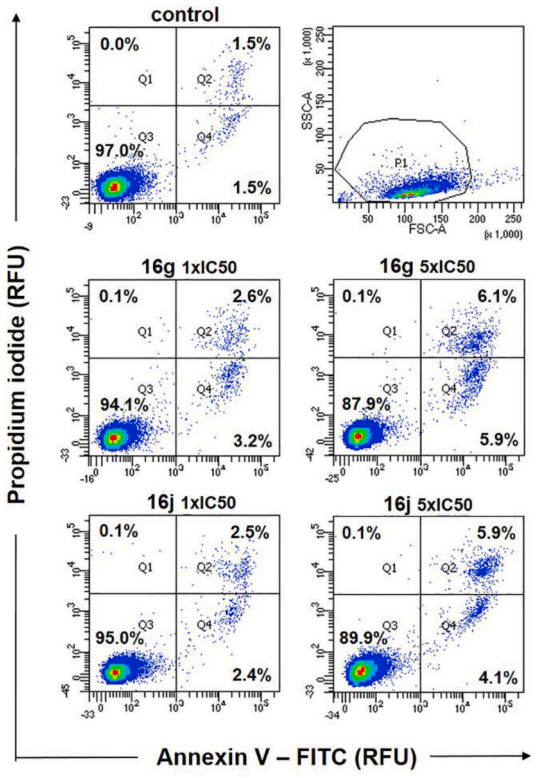
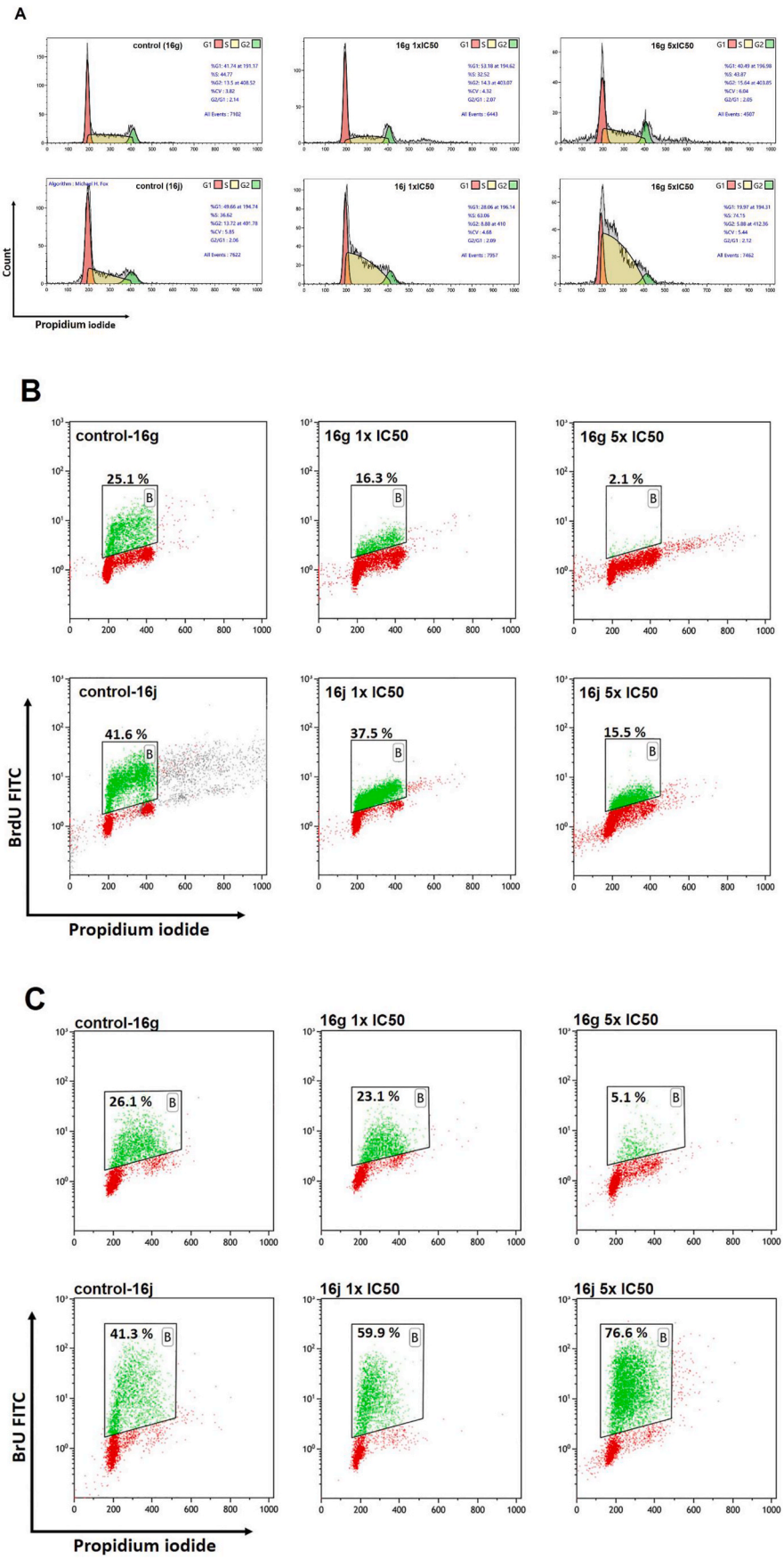


Fig. 1. Flow cytometric analysis of apoptosis in CCRF-CEM cells treated with compounds 16g and 16j. Cells were incubated with the tested compounds at 1 × IC_{50} and 5 × IC_{50} concentrations for 24 h and subsequently stained with annexin V-FITC and propidium iodide (PI). Dot plots represent the distribution of viable (Annexin V^-/PI^-), early apoptotic (Annexin V^+/PI^-), and late apoptotic or necrotic (Annexin V^+/PI^+) cells. Representative results from one of three independent experiments are shown.

2.2.3. Modulation of cell cycle and DNA/RNA synthesis by **16g** and **16j** in CCRF-CEM cells

To explore the cytostatic activity of compounds **16g** and **16j**, we evaluated their influence on cell cycle progression in CCRF-CEM cells treated for 24 h with concentrations corresponding to 1 × IC_{50} and 5 × IC_{50} (see Figure 2). The results were compared to the cell cycle distribution of untreated control cells. Treatment with compound **16g** at a lower concentration (1 × IC_{50}) led to a moderate accumulation of cells in the G1 phase, accompanied by a corresponding decrease in the S phase population, suggesting a G1 phase delay or block. However, at the higher concentration (5 × IC_{50}), this trend was reversed (Fig. 2a). In contrast, compound **16j** produced a distinctly different cell cycle profile. Even at the lower concentration 1 × IC_{50}, it caused a marked enrichment of cells in the S phase and a pronounced reduction in both G1 and G2/M populations, which became even more pronounced at the higher concentration 5 × IC_{50} (Fig. 2a). This suggests that **16j** strongly impairs progression through the S phase, potentially by inhibiting key enzymes involved in DNA synthesis or by inducing replication fork collapse. The substantial depletion of cells in G2/M further supports the idea that cells are unable to complete DNA replication and progress to mitosis. Overall, the comparison of these two compounds highlights divergent mechanisms of cell cycle interference: **16g** exhibits a biphasic response depending on concentration, indicative of partial cell cycle modulation, whereas **16j** acts as a potent S phase blocker. This distinct behaviour of **16j** may reflect a more direct interaction with DNA or replication-associated targets, and warrants further mechanistic studies, such as investigation of replication checkpoint activation or DNA damage response pathways. To complement the cell cycle analysis and further elucidate the cytostatic mechanisms of compounds **16g** and **16j**, we evaluated their impact on nucleic acid synthesis by measuring the proportion of CCRF-CEM cells actively synthesizing DNA and RNA after 24-h treatment at 1 × IC_{50} and 5 × IC_{50} concentrations. Compound **16g** induced a progressive and marked suppression of both DNA and RNA synthesis, with a stronger effect at the higher concentration (Fig. 2a and b). At the 5 × IC_{50} concentration, DNA replication was nearly abolished, suggesting that cells either fail to initiate proper replication or stall early in S phase. The simultaneous inhibition of transcriptional activity further supports the hypothesis that compound **16g** triggers a global suppression of nuclear functions, potentially through interference with key components of replication or transcription machinery. This broad inhibition is consistent with a cytostatic phenotype marked by cellular quiescence or arrest prior to S phase progression. In contrast, compound **16j** displayed a distinct and somewhat paradoxical effect on nucleic acid synthesis. Despite inducing a strong S phase accumulation, particularly at 5 × IC_{50}, RNA synthesis was substantially increased, even exceeding levels observed in untreated cells (Fig. 2c). Meanwhile, DNA synthesis showed only a moderate reduction at the lower dose but was markedly suppressed at the higher concentration (Fig. 2b). This profile suggests that **16j** promotes or maintains transcriptional activity within S phase-arrested cells, potentially due to persistent activation of transcriptional programs or compensatory RNA production under replication



(caption on next page)

Fig. 2. Flow cytometry analysis of the effect of compounds 16g and 16j on cell cycle progression, DNA synthesis, and RNA synthesis in CCRF-CEM cells. (A) Representative histograms of cell cycle profiles after 24 h treatment with compounds 16g and 16j at 1 × IC_{50} and 5 × IC_{50} concentrations. Propidium iodide staining was used to assess the distribution of cells in G1, S, and G2/M phases. Only live cells were included in the analysis. Untreated cells served as control. (B) BrdU incorporation assay to evaluate DNA synthesis after 24 h treatment. CCRF-CEM cells were pulse-labeled with BrdU and analyzed by flow cytometry. The percentages represent the proportion of BrdU-positive cells (region B). (C) BrU incorporation assay for RNA synthesis under the same treatment conditions. The numbers indicate the percentage of BrU-positive cells (region B). All data are representative of at least three independent experiments.

stress. The pronounced increase in RNA synthesis, despite DNA replication inhibition, may reflect a disrupted coordination between replication and transcription, possibly resulting in increased transcription–replication conflicts or altered chromatin dynamics. In summary, the observed inhibition of DNA synthesis by both compounds aligns with their effects on cell cycle progression, however, the divergent patterns of RNA synthesis point to distinct underlying mechanisms. Compound **16g** appears to act through a broad nuclear shutdown, likely halting both DNA and RNA synthesis upstream of S phase. On the other hand, compound **16j** induces replication stress with paradoxically increased transcriptional output, raising interesting questions about its effect on chromatin structure, transcription regulation, or checkpoint signalling.

*2.2.4. Compounds **16g** and **16j** modulate mitochondrial membrane potential (ΔΨm) in CCRF-CEM cells*

Mitochondrial membrane potential (ΔΨm) is a key indicator of mitochondrial function and plays a critical role in the intrinsic apoptotic pathway. Its disruption often marks an early step in apoptosis, leading to the release of pro-apoptotic factors and caspase activation. To further investigate the mechanisms underlying the pro-apoptotic activity of the derivatives **16g** and **16j**, changes in mitochondrial transmembrane potential (ΔΨm) were assessed using the JC-1 dye in flow cytometric analysis. JC-1 is a cationic dye that selectively accumulates in mitochondria (Fig. 3). In healthy, non-apoptotic cells with high ΔΨm, JC-1 forms aggregates that emit red fluorescence. In contrast, in apoptotic cells with depolarized mitochondria, the dye exists in its monomeric form, emitting green fluorescence. Interestingly, treatment with compounds **16g** and **16j** did not result in the expected mitochondrial depolarization commonly associated with early apoptosis. Instead, JC-1 staining revealed a prominent shift of the cell population toward the P3 region (high red and green fluorescence) in cells treated with compounds **16g** and **16j**. This effect closely resembled the response observed with the positive control curcumin, which is known to induce early mitochondrial hyperpolarization preceding apoptotic progression. The observed fluorescence pattern indicates an increase in mitochondrial membrane potential (ΔΨm), characteristic of hyperpolarized mitochondria. These findings are intriguing, especially in the context of prior results demonstrating that both **16g** and **16j** induced pronounced apoptotic cell death, as confirmed by annexin V/PI staining. The observed mitochondrial hyperpolarization may represent an early or transient phase preceding depolarization and cytochrome *c* release. It has been suggested that certain compounds can induce a biphasic response in mitochondria, initially leading to increased ΔΨm due to changes in ion fluxes, ROS accumulation, or altered electron transport activity, ultimately triggering mitochondrial outer membrane permeabilization (MOMP) and apoptotic cascade initiation [57–59]. This phenomenon has been reported for other small molecules and natural compounds, where mitochondrial hyperpolarization is followed by the collapse of ΔΨm and subsequent apoptotic cell death [58–60]. Thus, despite the lack of depolarization at the tested time point, the current results do not contradict the pro-apoptotic nature of **16g** and **16j**, but rather suggest that hyperpolarization may be a relevant step in their mechanism of action.

2.2.5. Protein-level analysis of apoptotic, cell cycle and stress response pathways by western blot

To further elucidate the molecular mechanisms underlying the distinct cellular phenotypes induced by compounds **16g** and **16j**, we conducted Western blot analysis focusing on key regulators of cell cycle progression, DNA damage response, and apoptosis (Fig. 4). These experiments aimed to validate and mechanistically contextualize the phenotypic outcomes previously observed at the functional level, including S-phase arrest, transcriptional modulation, and apoptotic

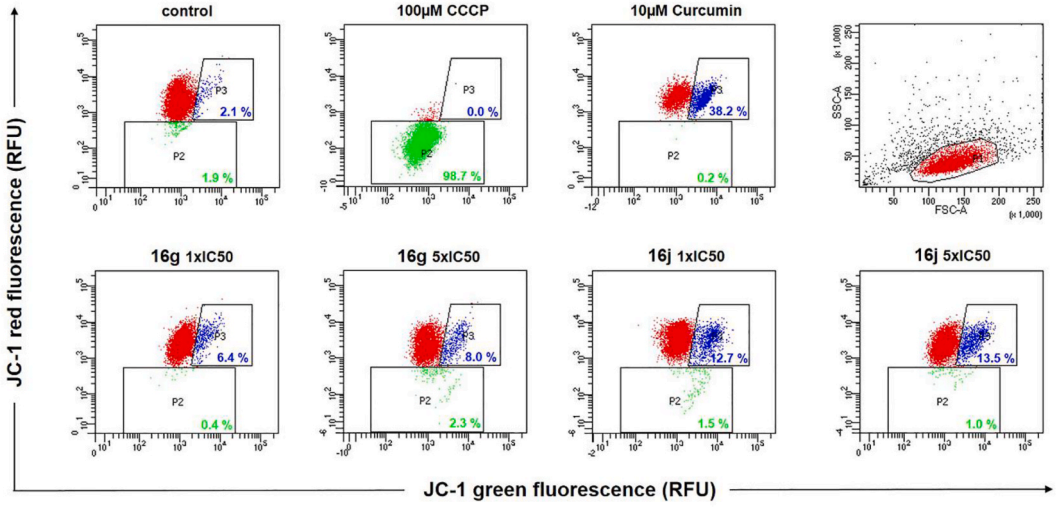


Fig. 3. JC-1-based assessment of mitochondrial membrane potential changes in CCRF-CEM cells treated with compounds 16g and 16j (24 h, 1 × and 5 × IC_{50}). Dead cells and debris were excluded from the analysis based on FSC/SSC parameters (rightmost plot). Cells from the P1 gate were analyzed for red (JC-1 aggregates; emission at 590 nm) versus green (JC-1 monomers; emission at 530 nm) fluorescence to evaluate mitochondrial polarization status. As controls, untreated cells (control) and cells treated with 100 μM CCCP (a mitochondrial uncoupler inducing complete depolarization) or 10 μM curcumin (a reference compound inducing mitochondrial hyperpolarization) were included. CCCP treatment led to a significant shift of the population into the P2 gate, confirming the gate settings for depolarized cells, whereas curcumin treatment increased the proportion of cells in the upper right quadrant (P3), consistent with hyperpolarized mitochondria. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

induction.

Cyclin A, a hallmark of active S-phase progression, exhibited a marked downregulation in response to **16g** at 5 × IC_{50}, which aligns with the nearly complete suppression of DNA synthesis and global transcriptional shutdown observed in earlier assays. This finding reinforces the hypothesis that **16g** disrupts cell cycle progression upstream of DNA replication initiation, rather than by engaging canonical replication checkpoints. In contrast, **16j** caused a moderate increase in Cyclin A expression at both concentrations, consistent with pronounced S-phase accumulation and persistent replication stress, suggesting that cells are arrested within S phase rather than before it. Analysis of Chk1 signaling further delineated these mechanistic differences. Total Chk1 levels remained stable or slightly elevated with **16j** at 1 × IC_{50}, while phospho-Chk1 (Ser345), a canonical marker of ATR-mediated replication checkpoint activation, was robustly induced at both 1 × and 5 × IC_{50}. This pattern clearly reflects active engagement of the replication stress response pathway in cells exposed to **16j**, supporting the interpretation that **16j** disrupts DNA replication fidelity and activates ATR-Chk1-dependent signaling to halt cell cycle progression. Conversely, **16g** at 5 × IC_{50} led to a pronounced downregulation of both total and phospho-Chk1, indicating that checkpoint signaling is bypassed or suppressed, potentially due to the global inhibition of transcriptional and replicative machinery. This observation aligns well with the marked reduction in p21 levels under the same conditions, further supporting the idea that **16g** induces a cytostatic phenotype characterized by nuclear silencing rather than checkpoint-mediated arrest. Interestingly, p21 expression was strongly induced by **16j** at 1 × IC_{50}, consistent with checkpoint activation and S-phase stalling. The dissipation of this response at higher concentration may reflect a transition from regulated arrest to apoptotic commitment. In contrast, **16g** at high dose resulted in a profound loss of p21, reinforcing the notion of transcriptional repression and loss of regulatory control over cell cycle checkpoints. γH2AX, a sensitive marker of DNA double-strand breaks and replication fork collapse, was substantially upregulated by **16g** at 5 × IC_{50}, consistent with the notion that extensive nuclear stress and impaired replication initiation can culminate in DNA damage. **16j** also induced γH2AX at both concentrations, albeit more moderately, in agreement with a sustained but less catastrophic replication stress response. In line with previous Annexin V/PI and JC-1 data indicating apoptosis induction, Western blot analysis confirmed activation of downstream apoptotic signaling. Bcl-2 expression was diminished at 5 × IC_{50} for both compounds, reflecting a shift in the apoptotic balance towards cell death. Although caspase-8 levels remained unchanged, Western blot revealed two distinct bands corresponding to isoforms naturally present in CCRF-CEM cells, suggesting lineage-specific expression rather than treatment-induced cleavage [61]. The pro-form of caspase-3 was clearly diminished at high concentrations of both compounds. This reduction suggests proteolytic activation, given that the antibody used specifically recognizes the uncleaved precursor. Importantly, cleaved PARP, a downstream substrate of executioner caspases, was detected as a faint but distinct fragment in both **16g-** and **16j**-treated cells at 5 × IC_{50}. Despite its relatively weak intensity, the presence of this band provides direct biochemical evidence of executioner caspase activity and apoptotic commitment, in line with the functional data. The absence of detectable caspase-8 cleavage, coupled with Bcl-2 downregulation and PARP fragmentation, supports the conclusion that apoptosis proceeds predominantly via the intrinsic mitochondrial pathway rather than through extrinsic death receptor signaling.

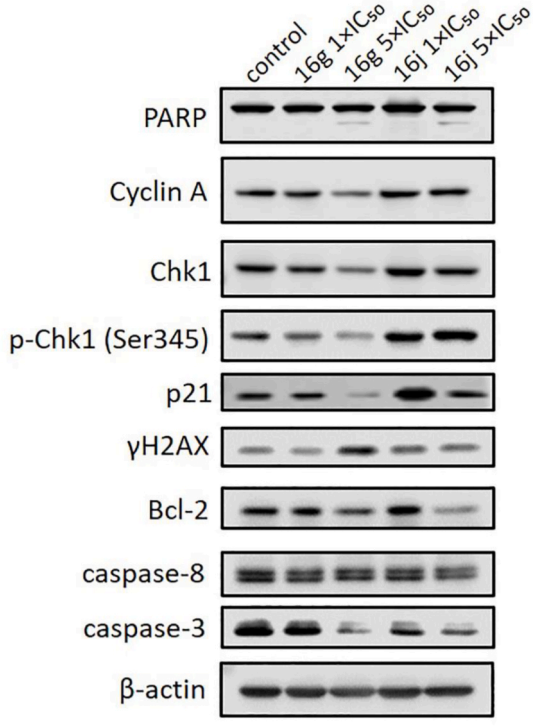


Fig. 4. Western blot analysis of selected cell cycle, DNA damage response, and apoptotic proteins in CCRF-CEM cells treated with compounds 16g and 16j. CCRF-CEM cells were treated with compounds **16g** and **16j** at 1 × and 5 × IC_{50} concentrations for 24 h. Whole-cell lysates were analyzed by Western blot for the expression of Cyclin A, p21, total Chk1, phospho-Chk1 (Ser345), γH2AX, Bcl-2, caspase-8, caspase-3, and PARP. β-actin served as a loading control.

Collectively, these data consolidate the functional divergence between **16g** and **16j**: **16g** promotes a transcriptionally repressive, checkpoint-independent cytostatic state accompanied by apoptotic commitment and DNA damage, while **16j** elicits a checkpoint-active response driven by replication stress, ultimately progressing to apoptosis upon checkpoint exhaustion. These mechanistic differences not only validate the earlier phenotypic observations but also underscore the distinct therapeutic potential of each compound.

2.2.6. *Pharmacokinetic parameters*

Preclinical studies of absorption, distribution, metabolism and excretion (ADME) serve as a crucial link between the laboratory development of candidate compounds and the initiation of human clinical trials. Indeed, the ADME parameters obtained from *in vitro* and *in vivo* models can further refine the selection of potential clinical candidates.

In vitro ADME analyses were conducted on **16g** and **16j**, with a particular focus on plasma stability (Table 2). This is of paramount importance for expeditious determination of the instability of test compounds in plasma, as it can result in rapid *in vivo* clearance and suboptimal pharmacokinetics [62]. Both compounds were found to be stable in human plasma (maintaining over 99 % presence after 120 min).

Data from the plasma protein binding study can provide insights into the distribution of the compounds into bodily tissues and the subsequent reduction in the amount of drug available for metabolic clearance or elimination from the body. The measurement of plasma protein binding was performed using a Rapid Equilibrium Dialysis device, with **16g** and **16j** reporting percent of fraction bound values of approximately 86 % and 97 %, respectively.

In the microsomal stability assay, human liver microsomes and NADPH cofactor were utilized to evaluate phase I oxidation by cytochrome P450 and flavin monooxygenases. The intrinsic clearance calculated from the microsomal stability assay indicated a high category for both derivatives. The compounds demonstrating high intrinsic clearance values are usually immediately removed from the body, which leads to a short duration of their therapeutic effect. On the other hand, there are established pharmaceuticals employed in clinical practice, including diclofenac, midazolam, chlorpromazine and verapamil, which possess higher intrinsic clearance. Consequently, it is imperative to

Table 2
Pharmacokinetic parameters of compounds **16g** and **16j**.

	Plasma stability				Plasma protein binding	PAMPA	
	% Compound remaining						
Compound	15min	30	60	120	% Fraction bound	log Pe	Category[b]
16g	104	104	99	99	85.81	-9.11	Low
16j	102	96	98	103	96.67	-7.5	Low

	Microsomal stability			Microsomal stability
	% Compound remaining			Category of Intrinsic clearence[a]
Compound	15 min	30	60	
16g	78	58	23	High
16j	54	28	11	High

	MDCK-MDR1 Permeability Assay					Caco-2 Permeability Assay				
Compound	Papp (x10e-6)	Category	Efflux ratio	active efflux	% recovery	Papp (x10e-6)	Category	Efflux ratio	active efflux	% recovery
16g	0.272	negative	2.43	Yes	91.67	0.0699	Low	9.39	Yes	52.09
16j	1.28	negative	2.96	Yes	104.72	0.169	Low	9.48	Yes	91.32

[a,b] References [67,68] 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.

ascertain the actual half-life, select the optimal dose and determine the frequency of administration during the day [63,64].

The Parallel Artificial Membrane Permeability Assay (PAMPA) has emerged as a primary screen for determining passive transcellular permeability. The tested derivatives **16g** and **16j** exhibited low passive diffusivity through an artificial cellular membrane (log Papp >6 cm/s), suggesting an alternative transport mechanism. The Caco-2 and MDCK-MDR1 permeability assays are well-established models of intestinal [65] and blood-brain barriers [66], respectively. It can be concluded that molecules **16g** and **16j** exhibited low probability of intestinal absorption and *trans*-blood-brain barrier penetration (PappAB $<5 \times 10^{-6}$ cm/s; PappAB $<10 \times 10^{-6}$ cm/s; CNS+). The assessment of rates of transport across Caco-2 and MDCK-MDR1 monolayers in both directions (apical to basolateral (A-B) and basolateral to apical (B-A)) across the cell monolayer was undertaken, thus enabling the determination of the efflux ratio and demonstrating whether the compound undergoes active efflux. The compounds under scrutiny exhibited efflux ratio values that were higher than the limit of active and passive efflux ($\leq$2), especially in the Caco-2 cell line. This finding indicates that compounds are likely to be substrates of the MDR1 efflux pump, given the presence of the pump in both cell types.

Collectively, these data underscore the importance of integrative ADME interpretation. While microsomal clearance is a critical parameter, it does not singularly define the pharmacokinetic fate of a compound. The favorable plasma stability, high protein binding, and potential for active transport suggest that **16g** and **16j** possess a pharmacokinetic profile amenable to further development. In this context, *in vivo* pharmacokinetic evaluation is planned as a logical next step, contingent upon further structural optimization of the lead compounds. This progression reflects a rational and resource-efficient strategy in early drug discovery, ensuring that only the most promising candidates are advanced to animal studies. The insights gained from the current ADME profiling will directly inform future modifications aimed at improving metabolic stability while preserving the potent and selective anticancer activity of **16g** and **16j**.

3. Conclusions

New synthetic pathway to lupane triterpenoids (**8**–**10** and **14**) modified with alkyne moiety at the position C30 was developed. These molecules might be excellent substrates for a number of further modifications, two of them, Sonogashira coupling and copper(I)-catalyzed azide-alkyne cycloaddition, were explored in this work. As a result, three sets of betulin derivatives modified in the position C30 were prepared by Sonogashira coupling. Series **11a–11g** has 3-*O*-ethyl and 28-*O*-ethyl groups, series **12a–12g** has free 3β-OH and 28-OH groups and finally series **13a–13g** has 3β-*O*-acetyl and 28-*O*-acetyl groups. Next, three more sets of triazoles were prepared in the position C30 from intermediates **8**, **10**, and **14** – series **15a–15c** containing 3β-*O*-ethyl and 28-*O*-ethyl groups, series **16a–16j** with free 3β-OH and 28-OH groups and lastly series **17a–17j**, derivatives of betulonic acid (with 3-oxo and 28-carboxyl groups). All compounds were subjected to tests of cytotoxicity levels against 6 cancer cell lines and 2 non-cancer cell lines. Several compounds from series **16a–16j** had IC_{50} in low micromolar ranges of concentrations. The best compounds were triazoles **16g** and **16j**, containing furan and benzaldehyde substituent, both of them also had very high therapeutic index. Compounds **16g** and **16j** were chosen for more detailed study of their mechanism of action.

In-depth biological characterization of selected compounds **16g** and **16j** in CCRF-CEM cells confirmed apoptosis as the primary mode of cytotoxicity. Annexin V/PI staining demonstrated a dose-dependent increase in early and late apoptotic populations with minimal necrosis, indicating a selective and controlled induction of cell death. Investigation of mitochondrial membrane potential (ΔΨm) revealed a shift toward hyperpolarization, rather than the expected depolarization, a phenomenon resembling the action of curcumin. This suggests that compounds **16g** and **16j** may initiate apoptosis through an early hyperpolarization event, consistent with previously reported biphasic mitochondrial responses that precede mitochondrial outer membrane permeabilization (MOMP) and caspase activation. Cell cycle analysis further distinguished the mechanistic profiles of the two compounds. Compound **16g** showed a concentration-dependent biphasic effect, initially causing G1 accumulation and later reversing this trend. In contrast, compound **16j** caused pronounced S phase arrest at both concentrations, likely reflecting direct interference with DNA replication. These observations were supported by DNA/RNA synthesis assays: compound **16g** broadly suppressed both DNA and RNA synthesis, consistent with a cytostatic effect upstream of S phase. Conversely, compound **16j** induced transcriptional upregulation despite impaired DNA replication, suggesting uncoupling of transcription and replication machinery under stress conditions. These findings were further supported by Western blot analysis, which confirmed activation of the DNA

damage response and apoptotic signaling. Phosphorylated Chk1 (Ser345) and γH2AX were induced by **16j**, indicating activation of replication stress and DNA damage checkpoints, whereas **16g** suppressed both markers at high concentration, suggesting checkpoint bypass. Differential regulation of p21 further supported this divergence, with strong upregulation by **16j** and suppression by **16g**, in line with their respective effects on cell cycle arrest. Reduced levels of full-length caspase-3 and cleaved PARP fragments confirmed apoptotic progression induced by both compounds. Downregulation of Bcl-2 was also observed, consistent with mitochondrial involvement in apoptosis induction. Taken together, these data support the conclusion that **16j** and **16g** exert their cytotoxic effects through distinct molecular pathways converging on apoptotic cell death. The integration of transcriptional deregulation, checkpoint modulation, and mitochondrial signaling emphasizes their mechanistic complementarity and therapeutic potential. The combination of high cytotoxic potency, selectivity, and multifaceted biological activity renders compound **16j** and to a lesser extent **16g** particularly promising for further *in vivo* validation and target-oriented development.

4. Experimental procedures

4.1. Chemistry

Melting points were determined using either the STUART SMP30 apparatus or Büchi B-545 apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet Avatar 370 FTIR and processed in OMNIC 9.8.372. DRIFT stands for Diffuse Reflectance Infrared Fourier Transform. ^{1}H and ^{13}C experiments were performed on Jeol ECX-500SS (500 MHz for ^{1}H) instrument, using $CDCl_3$, $(CD_3)_2SO$, CD_3OD or CD_2Cl_2 as solvents (25 °C). Currently used solvent is always specified for each compound separately. Chemical shifts (δ) were referenced to the residual signal of the solvent ($CDCl_3$, $(CD_3)_2SO$, CD_3OD or CD_2Cl_2) and are reported in parts per million (ppm). Coupling constants (J) are reported in Hertz (Hz). NMR spectra were processed in the MestReNova 6.0.2, MestReNova 14.1.0 or JEOL Delta 5.0.5.1. HRMS analysis was performed using LC-MS Orbitrap Elite high-resolution mass spectrometer (Dionex Ultimate 3000, Thermo Exactive plus, MA, USA) with electrospray ionization (ESI) or atmospheric-pressure chemical ionization (APCI). Used ionization method is specified for each compound separately. Spectra were taken at the positive and negative mode in the range of 250–1000 *m/z*. The samples were dissolved in MeCN or MeOH and injected to the mass spectrometer over autosampler after HPLC separation: precolumn Phenomenex Gemini (C18, 50 × 2 mm, 2.6 μm), mobile phase isocratic MeOH/water/HCOOH 95:5:0.1. Some of the nonpolar products could not be measured for HRMS – they were either insoluble or did not ionize well. The course of the reactions was monitored by TLC on Kieselgel 60 F_{254} plates (Merck) detected by UV light (254 nm) first and then 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). The ratios of solvents both in TLC and column chromatography are always volumetric.

Betulin (**1**) was purchased from the company Betulinines (www.betulinines.com). All other chemicals and solvents were obtained from Sigma-Aldrich, Lachner, Across Chemicals or VWR.

4.1.1. Diethylbetulin **2**

NaH (5.42 g; 226 mmol) was added in small portions to a stirred solution of betulin **1** (10 g, 22.6 mmol) in 1,4-dioxane (200 mL) under argon. The reaction mixture was then heated under reflux for 1 h. After cooling the reaction mixture down to the r.t., ethyl iodide (18.2 mL, 226 mmol) was added dropwise. The mixture was then heated under reflux overnight. The completion of the reaction was confirmed by TLC analysis (toluene). The reaction mixture was then diluted with water and extracted with EtOAc 3 times. The collected organic phase was washed with brine 3 times, dried over $MgSO_4$, filtered and solvents were removed under reduced pressure. The residue was purified by column chromatography on silica-gel (mobile phase – toluene). Collected fractions were evaporated, which yielded white crystals of **2**. Yield: 9.35 g (83 %), white solid, m. p. 179–181 °C (toluene); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.76 (s, 3H), 0.83 (s, 3H), 0.94 (s, 3H), 0.97 (s, 3H), 1.03 (s, 3H, 5 × CH_3), 1.17 (t, J = 7.0 Hz, 3H), 1.19 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 1.68 (s, 3H, CH_3–H-30) 2.40 (td, J_1 = 10.6 Hz, J_2 = 5.5 Hz, 1H, H-19β), 2.72 (dd, J_1 = 11.6 Hz, J_2 = 4.1 Hz, 1H, H-3α), 3.09 (d, J = 9.1 Hz, 1H, H-28a), 3.35 (dq, J_1 = 9.5 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.42–3.57 (m, 3H, H-28b and CH_2 – Et), 3.65 (dq, J_1 = 9.5 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 4.53–4.61 (m, 1H, H-29-*pro E*), 4.66–4.72 (m, 1H, H-29-*pro Z*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.91, 15.33, 15.82, 16.15, 16.27, 16.42, 18.46, 19.27, 21.06, 23.56, 25.44, 27.41, 28.21, 30.21, 34.43, 35.00, 37.31, 37.64, 38.90, 38.95, 41.15, 42.82, 47.32, 48.17, 49.04, 50.61, 56.05, 65.31, 67.09, 68.72, 86.83, 109.59, 151.01; **IR** (DRIFT): ν_{max} = 1071 (C–O); 1101 (C–O); 1638 (C═C) cm^{-1}; **HRMS** (APCI^{+}): *m/z* calcd for $C_{34}H_{59}O_2$ $[M+H]^+$ 499.4510; found 499.4454.

4.1.2. 30-Oxodiethylbetulin **4**

Diethylbetulin **2** (10 g, 20.0 mmol) and SeO_2 (5.563 g, 50.1 mmol) were suspended in 2-methoxyethanol (200 mL) and heated under reflux for 4 h until full conversion was observed. The reaction was monitored by TLC (toluene/Et_2O 20/1). The reaction mixture was filtered over a fritted glass with Celite, diluted with water and extracted with EtOAc 3 times. The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with gradient elution starting at toluene and finishing at toluene/Et_2O 20/1. Collected fractions were evaporated yielding yellow solid residue, which was crystallized from $CHCl_3$/MeOH to give white crystals of **4**. Yield: 7.710 g (75 %), white solid, m. p. 185–187 °C ($CHCl_3$/MeOH); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.75 (s, 3H), 0.81 (s, 3H), 0.94 (s, 6H), 1.01 (s, 3H, 5 × CH_3), 1.16 (t, J = 6.5 Hz, 3H), 1.19 (t, J = 6.4 Hz, 3H, 2 × CH_3 – Et), 1.68 (s, 3H, CH_3–H-30) 2.71 (dd, J_1 = 11.7 Hz, J_2 = 4.2 Hz, 1H, H-3α), 2.78 (td, J_1 = 11.2 Hz, J_2 = 5.4 Hz, 1H, H-19β), 3.11 (d, J = 9.1 Hz, 1H, H-28a), 3.34 (dq, J_1 = 9.5 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.41–3.54 (m, 3H, CH_2 – Et, H-28b), 3.65 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 5.91 (s, 1H, H-29-*pro E*), 6.27 (s, 1H, H-29-*pro Z*), 9.50 (s, 1H, CHO); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.75, 15.31, 15.81, 16.09, 16.20, 16.41, 18.43, 21.05, 23.52, 27.30, 27.89, 28.20, 30.16, 33.01, 34.41, 34.91, 37.27, 38.88, 38.94, 41.08, 42.66, 47.54, 50.41, 52.07, 56.02, 65.29, 67.10, 68.37, 86.79, 133.10, 157.44, 195.09; **IR** (DRIFT): ν_{max} = 1076 (C–O); 1098 (C–O); 1615 (C═C); 1681 (C═O) cm^{-1}; **HRMS** (APCI^{+}): calcd for $C_{34}H_{57}O_3$ $[M+H]^+$ 513.4302, found 513.4247.

4.1.3. 30-(Dibrommethylidene)diethylbetulin **6**

30-Oxodiethylbetulin **4** (100 mg, 0.195 mmol) and CBr_4 (77 mg, 0.234 mmol) were treated with vacuo for 30 min in a reaction vessel to remove all dissolved gasses, moisture etc. The aparature was then flushed with argon and the reagents were dissolved by adding dry dichloromethane (1.5 mL). PPh_3 (107.5 mg, 0.410 mmol) was added in small portions to a stirred solution cooled to 0 °C. The reaction mixture was stirred at the room temperature for 6 h. The reaction was monitored by TLC (toluene) until complete conversion. The solvent was removed under reduced pressure, the residue was dissolved in EtOAc and washed with brine (3 times). The organic phase was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with toluene. Collected fractions were evaporated, which yielded white solid of **6**. Yield: 67 mg (51 %), m. p. 135–137 °C (toluene); **^{1}H NMR** (500 MHz, $CDCl_3$): δ 0.76 (s, 3H), 0.83 (s, 3H), 0.94 (s, 3H), 0.95 (s, 3H); 1.02 (s, 3H, 5 × CH_3), 1.17 (t, J = 7.0 Hz, 3H), 1.19 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 2.47 (td, J_1 = 11.1 Hz, J_2 = 5.7 Hz, 1H, H-19β), 2.73 (dd, J_1 = 11.7 Hz, J_2 = 4.1 Hz, 1H, H-3α), 3.05 (d, J = 9.1 Hz, 1H, H-28a), 3.35 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.41–3.55 (m, 3H, CH_2 – Et and H-28b), 3.66 (dq, J_1 = 9.4 Hz, J_2 = 6.9 Hz, 1H, 1/2 × CH_2 – Et), 5.20 (s, 1H, H-29-*pro E*), 5.23

(s, 1H, H-29-*pro Z*), 6.84 (s, 1H, H-30); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.85, 15.31, 15.81, 16.13, 16.27, 16.41, 18.45, 21.05, 23.53, 26.67, 27.32, 28.21, 30.26, 30.89, 34.42, 34.95, 37.30, 37.39, 38.89, 38.94, 41.12, 42.74, 47.22, 47.27, 50.47, 50.76, 56.03, 65.31, 67.13, 68.79, 86.82, 90.14, 115.88, 136.74, 149.54; **IR** (DRIFT): ν_{max} = 1073 (C–O); 1098 (C–O); 1642 (C═C) cm^{-1}; **HRMS** ($APCI^+$): calcd for $C_{35}H_{57}Br_2O_2$ $[M+H]^+$ 669.2699; found 669.2627.

4.1.4. 30-(Methylidyne)diethylbetulin **8**

30-(Dibromomethylidene)diethylbetulin **6** (100 mg, 0.150 mmol) was treated with vacuo for 30 min in a reaction vessel to remove all dissolved gasses, moisture etc. The flask was then flushed with argon and the reactant was dissolved in dry toluene (4.72 mL). The stirred solution was cooled to −78 °C and then *n*-BuLi was added dropwise (0.2 mL, 2.5 M solution in toluene, 0.500 mmol). The reaction temperature was kept at −78 °C for 3 h and then eventually was brought to the room temperature. The reaction monitoring was done by TLC (toluene) until complete reaction conversion. The reaction mixture was cautiously washed with a saturated solution of NH_4Cl in water (3 times), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with toluene. Collected fractions were evaporated, which yielded a white solid of **5**. Yield: 58 mg (76 %), m. p. 146–148 °C (toluene); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.76 (s, 3H), 0.83 (s, 3H), 0.94 (s, 3H), 0.97 (s, 3H); 1.02 (s, 3H, 5 × CH_3), 1.16 (t, J = 7.0 Hz, 3H), 1.19 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 2.46 (td, J_1 = 10.7 Hz, J_2 = 5.9 Hz, 1H, H-19β), 2.72 (dd, J_1 = 11.7 Hz, J_2 = 4.1 Hz, 1H, H-3α), 2.92 (s, 1H, C≡C–H), 3.05 (d, J = 9.1 Hz, 1H, H-28a), 3.35 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.41–3.56 (m, 3H, H-28b and CH_2 – Et), 3.59–3.71 (m, 1H, 1/2 × CH_2 – Et), 5.26 (d, J = 1.8 Hz, 1H, H-29-*pro E*), 5.28 (d, J = 1.8 Hz, 1H, H-29-*pro Z*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.82, 15.32, 15.82, 16.11, 16.22, 16.43, 18.45, 20.93, 23.55, 25.75, 27.39, 28.21, 30.21, 30.52, 34.42, 34.82, 37.29, 38.87, 38.94, 41.07, 42.90, 47.23, 47.54, 50.26, 50.37, 56.02, 65.30, 67.10, 69.20, 78.39, 83.11, 86.82, 121.52, 137.90; **IR** (DRIFT): ν_{max} = 1073 (C–O); 1103 (C–O); 1607 (C═C); 2343 (C≡C); 3298 (C–H alkyne) cm^{-1}; **HRMS** ($APCI^+$): calcd for $C_{35}H_{57}O_2$ $[M+H]^+$ 509.4353; found 509.4302.

4.1.5. General procedure for preparing Sonogashira coupling conjugates (procedure A)

Aryliodide was added to a stirred suspension of 30-(methylidyne) diethylbetulin **8** (100 mg, 0.197 mmol), PPh_3 (5.2 mg, 0.0198 mmol), CuI (3.8 mg, 0.0200 mmol), Pd/C (10.6 mg, w_{Pd} = 10 %, 9.96 · 10^{-3} mmol) and *N,N*-diisopropylethylamine (103 μL, 76 mg, 0.591 mmol) in dimethylacetamide (1.9 mL) and water (0.1 mL). The reaction mixture was filled with argon and heated to 75 °C.

4.1.5.1. 3β,28-diethoxy-30-(phenylmethylidyne)-lup-20(29)-ene **11a**.

3β,28-Diethoxy-30-(phenylmethylidyne)-lup-20(29)-ene **11a** was prepared according to the general procedure **A** using iodobenzene (31 μL, 56 mg, 0.275 mmol). The reaction was completed after 72 h and monitored by TLC (toluene) until complete conversion. The reaction mixture was filtered, diluted with water and extracted with dichloromethane 3 times. The collected organic phase was washed with brine (3 times), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with toluene/hexane 1/1. Collected fractions were evaporated, which yielded a white solid of **11a**. Yield: 78 mg (68 %), m.p. 70–73 °C (toluene/hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.76 (s, 3H), 0.83 (s, 3H), 0.93 (s, 3H), 1.01 (s, 3H), 1.04 (s, 3H, 5 × CH_3), 1.16 (t, J = 7.0 Hz, 3H), 1.20 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 2.54 (td, J_1 = 10.8 Hz, J_2 = 5.8 Hz, 1H, H-19β), 2.70 (dd, J_1 = 11.7 Hz, J_2 = 4.2 Hz, 1H, H-3α), 3.09 (d, J = 9.1 Hz, 1H, H-28a), 3.34 (dq, J_1 = 9.5 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.49 (q, J = 6.9 Hz, 2H, CH_2 – Et), 3.53 (d, J = 9.0 Hz, 1H, H-28b), 3.65 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 5.26 (d, J = 1.9 Hz, 1H, H-29-*pro E*), 5.28 (d, J = 2.0 Hz, 1H, H-29-*pro Z*), 7.30–7.36 (m, 3H, aryl), 7.43–7.49 (m, 2H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.87, 15.33, 15.81, 16.13, 16.24, 16.41, 18.47, 20.96, 23.54, 25.83, 27.38, 28.21, 30.42, 30.88, 34.46, 35.07, 37.28, 37.30, 38.84, 38.93, 41.09, 42.89, 47.27, 47.90, 50.41, 50.54, 56.03, 65.29, 67.12, 69.23, 86.82, 88.86, 91.01, 119.75, 123.64, 128.23, 128.48, 131.75, 138.82; **IR** (DRIFT): ν_{max} = 1071 (C–O); 1103 (C–O); 1606 (C═C); 2343 (C≡C) cm^{-1}; **HRMS** ($APCI^+$): calcd for $C_{41}H_{61}O_2$ $[M+H]^+$ 585.4666; found 585.4662.

4.1.5.2. 3β,28-diethoxy-30-(4-methoxyphenylmethylidyne)-lup-20(29)-ene **11b**.

3β,28-Diethoxy-30-(4-methoxyphenylmethylidyne)-lup-20 (29)-ene **11b** was prepared according to the general procedure **A** using 4-iodoanisole (65 mg, 0.275 mmol). The reaction was completed after 48 h and monitored by TLC (toluene) until complete conversion. The reaction mixture was filtered, diluted with water and extracted with dichloromethane 3 times. The collected organic phase was washed with brine (3 times), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with gradient elution starting at toluene/hexane 3/1 and finishing at toluene/hexane 5/1. Collected fractions were evaporated, which yielded a white solid of **11b**. Yield: 40 mg (33 %), m. p. 69–72 °C (toluene/hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.76 (s, 3H), 0.83 (s, 3H), 0.93 (s, 3H), 1.01 (s, 3H), 1.04 (s, 3H, 5 × CH_3), 1.16 (t, J = 7 Hz, 3H), 1.20 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 2.53 (td, J_1 = 10.8 Hz, J_2 = 5.8 Hz, 1H, H-19β), 2.70 (dd, J_1 = 11.7 Hz, J_2 = 4.2 Hz, 1H, H-3α), 3.08 (d, J = 9.1 Hz, 1H, H-28a), 3.33 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.45–3.51 (m, 2H, CH_2 – Et), 3.53 (d, J = 8.9 Hz, 1H, H-28b), 3.64 (dq, J_1 = 9.4 Hz, J_2 = 6.9 Hz, 1H, 1/2 × CH_2 – Et), 3.82 (s, 3H, O–CH_3), 5.21 (d, J = 2.0 Hz, 1H, H-29-*pro E*), 5.23 (d, J = 2.0 Hz, 1H, H-29-*pro Z*), 6.79–6.91 (m, 2H, aryl), 7.34–7.44 (m, 2H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.88, 15.33, 15.81, 16.13, 16.23, 16.41, 18.47, 20.95, 23.54, 25.79, 27.39, 28.21, 30.43, 30.89, 34.47, 35.08, 37.28, 37.30, 38.84, 38.93, 41.09, 42.89, 47.26, 47.94, 50.42, 50.50, 55.46, 56.03, 65.29, 67.11, 69.23, 86.83, 87.52, 91.02, 114.15, 115.80, 118.95, 133.17, 139.01, 159.66; **IR** (DRIFT): ν_{max} = 1073 (C–O aliphatic); 1106 (C–O aliphatic); 1247 (C–O aromatic); 1600 (C═C); 2205 (C≡C) cm^{-1}; **HRMS** ($APCI^+$): calcd for $C_{42}H_{63}O_3$ $[M+H]^+$ 615.4772; found 615.4755.

4.1.5.3. 3β,28-diethoxy-30-(4-methylphenylmethylidyne)-lup-20(29)-ene **11c**.

3β,28-Diethoxy-30-(4-methylphenylmethylidyne)-lup-20(29)-ene **11c** was synthesized by the general procedure **A** utilizing 4-iodotoluene (43 mg, 0.275 mmol). The reaction was monitored by TLC (toluene) until the reaction completion – 48 h. The reaction mixture was filtered, diluted with water and extracted with dichloromethane 3 times. The collected organic phase was washed with brine (3 times), dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography on a silica gel with toluene. Collected fractions were evaporated, which yielded a white solid of **11c**. Yield: 44 mg (37 %), m. p. 82–84 °C (toluene); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.76 (s, 3H), 0.83 (s, 3H), 0.94 (s, 3H), 1.01 (s, 3H), 1.04 (s, 3H, 5 × CH_3), 1.16 (t, J = 7.0 Hz, 3H), 1.21 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 2.36 (s, 3H, aryl-CH_3), 2.54 (td, J_1 = 10.8 Hz, J_2 = 5.8 Hz, 1H, H-19β), 2.70 (dd, J_1 = 11.7 Hz, J_2 = 4.2 Hz, 1H, H-3α), 3.08 (d, J = 9.1 Hz, 1H, H-28a), 3.34 (dq, J_1 = 9.5 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.49 (q, J = 6.9 Hz, 2H, CH_2 – Et), 3.53 (d, J = 9.1 Hz, 1H, H-28b), 3.65 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 5.23 (d, J = 2.0 Hz, 1H, H-29-*pro E*), 5.25 (d, J = 2.0 Hz, 1H, H-29-*pro Z*), 7.14 (d, J = 7.8 Hz, 2H, aryl), 7.35 (d, J = 8.1 Hz, 2H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.87, 15.33, 15.81, 16.13, 16.23, 16.41, 18.46, 20.95, 21.64, 23.53, 25.80, 27.38, 28.21, 30.41, 30.88, 34.46, 35.06, 37.28, 37.29, 38.83, 38.93, 41.09, 42.88, 47.26, 47.92, 50.41, 50.51, 56.03, 65.28, 67.11, 69.23, 86.82, 88.18, 91.20, 119.32, 120.56, 129.23, 131.64, 138.33, 138.94; **IR** (DRIFT): ν_{max} = 1072 (C–O); 1107 (C–O); 1603 (C═C); 2205 (C≡C) cm^{-1};**HRMS**

(APCI$^+$): calcd for $C_{42}H_{63}O_2$ $[M+H]^+$ 599.4823; found 599.4786.

*4.1.5.4. 3β,28-diethoxy-30-[4-(trifluoromethyl)phenylmethylidyne]-lup-20 (29)-ene **11d**.* 3β,28-Diethoxy-30-[4-(trifluoromethyl)phenylmethylidyne]-lup-20(29)-ene **11d** was synthesized according to the general procedure **A** using 1-iodo-4-(trifluoromethyl)-benzene (41 μL, 75 mg, 0.275 mmol). The reaction was monitored by TLC (toluene) until complete conversion, which was indicated after 24 h. The reaction mixture was filtered, diluted with water and extracted with dichloromethane 3 times. The collected organic phase was washed with brine (3 times), dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography on a silica with toluene. Collected fractions were concentrated under reduced pressure, which yielded a white solid of **11d**. Yield: 73 mg (57 %), m. p. 73–74 °C (toluene); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.76 (s, 3H), 0.83 (s, 3H), 0.93 (s, 3H), 1.00 (s, 3H), 1.04 (s, 3H, 5 × CH_3), 1.16 (t, J = 7.0 Hz, 3H), 1.20 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 2.56 (td, J_1 = 10.8 Hz, J_2 = 5.9 Hz, 1H, H-19β), 2.70 (dd, J_1 = 11.7 Hz, J_2 = 4.2 Hz, 1H, H-3α), 3.09 (d, J = 9.1 Hz, 1H, H-28a), 3.33 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.48 (q, J = 7.0 Hz, 2H, CH_2 – Et), 3.52 (d, J = 9.3 Hz, 1H, H-28b), 3.64 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 5.32 (d, J = 1.7 Hz, 1H, H-29-*pro E*), 5.33 (d, J = 1.8 Hz, 1H, H-29-*pro Z*), 7.54 (d, J = 8.3 Hz, 2H, aryl), 7.59 (d, J = 8.4 Hz, 2H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.84, 15.33, 15.80, 16.12, 16.24, 16.41, 18.45, 20.95, 23.54, 25.92, 27.35, 28.21, 30.47, 30.92, 34.46, 35.12, 37.25, 37.30, 38.86, 38.94, 41.10, 42.87, 47.29, 47.73, 50.42, 50.69, 56.03, 65.31, 67.14, 69.23, 86.80, 89.59, 91.37, 120.99, 124.46 (q, J = 272.0 Hz, CF_3), 125.45 (q, J = 3.3 Hz, C-C-CF_3) 127.42, 129.95 (q, J = 32.7 Hz, C-CF_3), 131.92, 138.38; **IR** (DRIFT): ν_{max} = 1072 (C–O); 1108 (C–O); 1599 (C═C); 2205 (C≡C) cm^{-1}; **HRMS** (APCI$^+$): calcd for $C_{42}H_{60}F_3O_2$ $[M+H]^+$ 653.4540; found 653.4514.

*4.1.5.5. 3β,28-diethoxy-30-(2-nitrophenylmethylidyne)-lup-20(29)-ene **11e**.* 3β,28-Diethoxy-30-(2-nitrophenylmethylidyne)-lup-20(29)-ene **11e** was prepared according to the general procedure **A** using 1-iodo-2-nitrobenzene (69 mg, 0.275 mmol). The reaction was completed after 72 h and monitored by TLC (toluene) until complete conversion. The reaction mixture was filtered, diluted with water and extracted with dichloromethane 3 times. The collected organic phase was washed with brine (3 times), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with gradient elution starting at toluene/hexane 4/1 and finishing at toluene. Collected fractions were evaporated, which yielded a yellowish solid of **11e**. Yield: 65 mg (53 %), m. p. 84–86 °C (toluene/hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.74 (s, 3H), 0.81 (s, 3H), 0.91 (s, 3H), 0.96 (s, 3H), 1.02 (s, 3H, 5 × CH_3), 1.14 (t, J = 7.0 Hz, 3H), 1.19 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 2.56 (td, J_1 = 10.7 Hz, J_2 = 5.9 Hz, 1H, H-19β), 2.68 (dd, J_1 = 11.7 Hz, J_2 = 4.2 Hz, 1H, H-3α), 3.08 (d, J = 9.1 Hz, 1H, H-28a), 3.32 (dq, J_1 = 9.5 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.43–3.55 (m, 3H, CH_2 – Et and H-28b), 3.63 (dq, J_1 = 9.5 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 5.38 (d, J = 1.9 Hz, 1H, H-29-*pro E*), 5.40 (d, J = 1.9 Hz, 1H, H-29-*pro Z*), 7.40–7.46 (m, 1H, aryl), 7.56 (td, J_1 = 7.6 Hz, J_2 = 1.3 Hz, 1H, aryl), 7.63 (dd, J_1 = 7.8 Hz, J_2 = 1.5 Hz, 1H, aryl), 8.03 (dd, J_1 = 8.2 Hz, J_2 = 1.1 Hz, 1H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.67, 15.30, 15.77, 16.09, 16.17, 16.37, 18.42, 20.90, 23.49, 25.95, 27.30, 28.17, 30.09, 30.80, 34.36, 34.63, 37.24, 37.32, 38.80, 38.88, 41.02, 42.89, 47.31, 47.88, 50.28, 50.40, 55.95, 65.24, 67.08, 69.25, 85.83, 86.79, 96.89, 119.00, 122.39, 127.74, 128.47, 132.78, 135.00, 138.57, 149.66; **IR** (DRIFT): ν_{max} = 1072 (C–O); 1106 (C–O); 1526 (N–O); 1610 (C═C); 2196 (C≡C) cm^{-1}; **HRMS** (APCI$^+$): calcd $C_{41}H_{60}NO_4$ $[M+H]^+$ 630.4517; found 630.4515.

*4.1.5.6. 3β,28-diethoxy-30-(1-methyl-1H-pyrazol-4-ylmethylidyne)-lup-20(29)-ene **11f**.* 3β,28-Diethoxy-30-(1-methyl-1*H*-pyrazol-4-ylmethylidyne)-lup-20(29)-ene **11f** was prepared according to the general procedure **A** using 4-iodo-1-methyl-1*H*-pyrazole (57 mg, 0.275 mmol). The reaction was completed after 144 h and monitored by TLC (toluene) until complete conversion. The reaction mixture was filtered, diluted with water and extracted with dichloromethane 3 times. The collected organic phase was washed with brine (3 times), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with gradient elution starting at toluene and finishing at toluene/Et_2O 10/1. Collected fractions were evaporated, which yielded a yellowish solid of **11f**. Yield: 18 mg (16 %), m. p. 98–101 °C (toluene/hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.75 (s, 3H), 0.83 (s, 3H), 0.94 (s, 3H), 0.99 (s, 3H), 1.03 (s, 3H, 5 × CH_3), 1.16 (t, J = 7.0 Hz, 3H), 1.19 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 2.50 (td, J_1 = 10.8 Hz, J_2 = 5.9 Hz, 1H, H-19β), 2.71 (dd, J_1 = 11.7 Hz, J_2 = 4.2 Hz, 1H, H-3α), 3.07 (d, J = 9.1 Hz, 1H, H-28a), 3.34 (dq, J_1 = 9.5 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.47 (qd, J_1 = 6.9 Hz, J_2 = 0.6 Hz, 2H, CH_2 – Et), 3.51 (d, J = 9.1 Hz, 1H, H-28b), 3.65 (dq, J_1 = 9.5 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.89 (s, 3H, N–CH_3), 5.19 (s, 2H, H-29-*pro E* and H-29-*pro Z*), 7.51 (s, 1H, pyrazole), 7.58 (s, 1H, pyrazole); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.81, 15.32, 15.80, 16.12, 16.23, 16.40, 18.45, 20.95, 23.53, 25.78, 27.38, 28.20, 30.39, 30.91, 34.47, 35.00, 37.23, 37.30, 38.85, 38.93, 39.21, 41.09, 42.88, 47.21, 47.78, 50.44, 50.46, 56.04, 65.29, 67.10, 69.22, 82.16, 86.81, 89.47, 118.81, 125.44, 128.36, 129.17, 138.92; **IR** (DRIFT): ν_{max} = 1072 (C–O); 1106 (C–O); 1606 (C═C); 2208 (C≡C) cm^{-1}; **HRMS** (APCI$^+$): calcd for $C_{39}H_{61}N_2O_2$ $[M+H]^+$ 589.4728; found 589.4700.

*4.1.5.7. 3β,28-diethoxy-30-(thiophene-2-ylmethylidyne)-lup-20(29)-ene **11g**.* 3β,28-Diethoxy-30-(thiophene-2-ylmethylidyne)-lup-20(29)-ene **11g** was prepared according to the general procedure **A** using 2-iodothiophene (31 μL, 58 mg, 0.275 mmol). The reaction was completed after 48 h and monitored by TLC (toluene) until complete conversion. The reaction mixture was filtered, diluted with water and extracted with dichloromethane 3 times. The collected organic phase was washed with brine (3 times), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with toluene. Collected fractions were evaporated, which yielded a yellowish solid of **11g**. Yield: 66 mg (57 %), m. p. 78–81 °C (toluene/hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.76 (s, 3H), 0.83 (s, 3H), 0.94 (s, 3H), 1.02 (s, 3H), 1.04 (s, 3H, 5 × CH_3), 1.16 (t, J = 7.0 Hz, 3H), 1.19 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 2.54 (td, J_1 = 10.8 Hz, J_2 = 5.9 Hz, 1H, H-19β), 2.71 (dd, J_1 = 11.7 Hz, J_2 = 4.2 Hz, 1H, H-3α), 3.08 (d, J = 9.1 Hz, 1H, H-28a), 3.34 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.48 (q, J = 6.9 Hz, 2H, CH_2 – Et), 3.52 (d, J = 8.6 Hz, 1H, H-28b), 3.65 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 5.25–5.27 (m, 2H, H-29-*pro E* and H-29-*pro Z*), 6.99 (dd, J_1 = 5.2 Hz, J_2 = 3.6 Hz, 1H, thiophene), 7.20 (dd, J_1 = 3.6 Hz, J_2 = 1.0 Hz, 1H, thiophene), 7.26 (dd, J_1 = 5.0 Hz, J_2 = 1.1 Hz, 1H, thiophene); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.86, 15.33, 15.81, 16.13, 16.24, 16.41, 18.46, 20.96, 23.54, 25.86, 27.38, 28.21, 30.40, 30.99, 34.46, 35.02, 37.24, 37.30, 38.85, 38.93, 41.09, 42.88, 47.25, 47.78, 50.40, 50.61, 56.02, 65.29, 67.12, 69.23, 84.20, 86.82, 92.85, 119.71, 123.69, 127.18, 127.20, 131.73, 138.59; **IR** (DRIFT): ν_{max} = 1072 (C–O); 1108 (C–O); 1600 (C═C); 2197 (C≡C) cm^{-1}; **HRMS** (APCI$^+$): calcd for $C_{39}H_{59}O_2S$ $[M+H]^+$ 591.4230; found 591.4204.

*4.1.6. 20-[1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl]-3β,28-diethoxy-30-norlup-20(29)-ene **15a***

A solution of copper(II) sulfate pentahydrate (20 mg, 0.0786 mmol) in water (4 mL) was added to a stirred solution of 30-(methylidyne) diethylbetulin **8** (100 mg, 0.197 mmol), 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylazide (147 mg, 0.393 mmol) in *t*-BuOH (8 mL) all at once. The reaction mixture was heated to 55 °C and sodium L-ascorbate (31 mg, 0.157 mmol) was added in small portions. After 48 h copper(II) sulfate pentahydrate (20 mg, 0.0786 mmol) and sodium L-ascorbate (31 mg, 0.157 mmol) were added again and the reaction temperature was raised to 65 °C. The reaction was completed after another 24 h and was monitored by TLC (toluene/MeCN 5/1). The reaction mixture was

diluted with water and extracted with EtOAc 3 times. The collected organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with toluene/Et_2O 5/1. Collected fractions were evaporated, which yielded a white solid of **15a**. Yield: 91 mg (52 %), m. p. 120–121 °C (toluene); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.74 (s, 3H), 0.80 (s, 3H), 0.93 (s, 6H), 1.02 (s, 3H, 5 × CH_3), 1.16 (t, J = 7.0 Hz, 3H), 1.20 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 1.87 (s, 3H), 2.03 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H, 4 × AcO), 2.70 (dd, J_1 = 11.7 Hz, J_2 = 4.3 Hz, 1H, H-3α), 2.77 (td, J_1 = 11.2 Hz, J_2 = 5.5 Hz, 1H, H-19β), 3.15 (d, J = 9.1 Hz, 1H, H-28a), 3.33 (dq, J_1 = 9.5 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.50 (q, J = 7.0 Hz, 2H, CH_2 – Et), 3.55 (d, J = 9.0 Hz, 1H, H-28b), 3.64 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 4.00 (ddd, J_1 = 10,2 Hz, J_2 = 5.1 Hz, J_3 = 2.2 Hz, 1H, H-5′), 4.16 (dd, J_1 = 12.6 Hz, J_2 = 2.1 Hz, 1H, H-6′), 4.31 (dd, J_1 = 12.6 Hz, J_2 = 5.1 Hz, 1H, H-6′), 5.17 (s, 1H, H-29-*pro E*), 5.22–5.29 (m, 1H, H-4′), 5.41 (t, J = 9.3 Hz, 1H, H-3′), 5.47 (t, J = 9.4 Hz, 1H, H-2′), 5.62 (s, 1H, H-29-*pro Z*), 5.86 (d, J = 9.2 Hz, 1H, H-1′), 7.69 (s, 1H, triazole); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.83, 15.35, 15.80, 16.15, 16.23, 16.41, 18.43, 20.32, 20.66, 20.67, 20.83, 21.12, 23.52, 27.29, 28.19, 30.29, 34.44, 34.77, 37.26, 37.46, 38.86, 38.93, 41.12, 42.77, 47.38, 50.47, 56.01, 61.79, 65.29, 67.11, 67.94, 68.68, 70.35, 72.91, 75.29, 85.87, 86.79, 118.08, 144.35, 168.98, 169.50, 170.02, 170.60; **IR** (DRIFT): ν_{max} = 1072 (C–O); 1107 (C–O); 1599 (C═C); 1716 (C═O) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{49}H_{76}N_3O_{11}$ $[M+H]^+$ 882.5474; found 882.5474.

4.1.7. 3β,28-diethoxy-20-[1-(β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene **15b**

A suspension of 20-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]-3β,28-diethoxy-30-norlup-20(29)-ene **15a** (100 mg, 0.113 mmol) in dry EtOH (0.5 mL) was added to a suspension of NaH (18.2 mg, 60 % suspension in mineral oil, 0.454 mmol) in dry EtOH (0.5 mL) and sealed under argon. The reaction completion was confirmed by TLC ($CHCl_3$/MeOH 6/1) after 2 h at the room temperature. pH was decreased by AcOH to 6–7 and the solvent was evaporated. The residue was dissolved in EtOAc, washed with brine (3 times), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with $CHCl_3$/MeOH 5/1. Collected fractions were evaporated, which yielded a white solid of **15b**. Yield: 59 mg (73 %), m. p. 141–143 °C ($CHCl_3$/MeOH); **^{1}H NMR** (500 MHz, $(CD_3)_2SO$): δ = 0.67 (s, 3H), 0.76 (s, 3H), 0.89 (s, 3H), 0.95 (s, 3H), 1.00 (s, 3H, 5 × CH_3), 1.07 (t, J = 7.0 Hz, 3H), 1.13 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 2.71 (dd, J_1 = 11.6 Hz, J_2 = 4.2 Hz, 1H, H-3α), 2.78 (td, J_1 = 11.2 Hz, J_2 = 5.5 Hz, 1H, H-19β), 3.17 (d, J = 9.1 Hz, 1H, H-28a), 3.20–3.28 (m, 2H), 3.37–3.52 (m, 6H), 3.58 (dq, J_1 = 9.5 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.67–3.74 (m, 1H), 3.77–3.85 (m, 1H), 4.60 (t, J = 5.7 Hz, 1H), 5.07 (s, 1H), 5.13 (d, J = 5.5 Hz, 1H), 5.27 (d, J = 4.7 Hz, 1H), 5.33 (d, J = 6.1 Hz, 1H), 5.49 (d, J = 9.3 Hz, 1H), 5.64 (s, 1H), 8.45 (s, 1H, triazole); **^{13}C NMR** (126 MHz, $(CD_3)_2SO$): δ = 14.54, 15.11, 15.58, 15.69, 15.84, 16.27, 17.83, 20.49, 22.61, 26.82, 27.81, 29.56, 33.77, 34.07, 36.59, 36.82, 37.94, 38.25, 40.46, 42.14, 46.71, 49.70, 55.07, 60.73, 63.87, 66.12, 67.54, 69.61, 71.89, 76.96, 79.15, 79.94, 80.15, 85.41, 87.52, 120.53, 144.55; **IR** (DRIFT): ν_{max} = 1072 (C–O); 1107 (C–O); 1599 (C═C); 1716 (C═O), 3358 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{41}H_{68}N_3O_7$ $[M+H]^+$ 714.5052; found 714.5050.

4.1.8. 20-(1-Benzyl-1H-1,2,3-triazol-4-yl)-3β,28-diethoxy-30-norlup-20(29)-ene **15c**

A solution of copper(II) sulfate pentahydrate (20 mg, 0.0786 mmol) in water (4 mL) was added to a stirred solution of 30-(methylidyne) diethylbetulin **8** (100 mg, 0.197 mmol) and benzylazide (49 μL, 52 mg, 0.393 mmol) in *t*-BuOH (8 mL) all at once. The reaction mixture was heated to 55 °C and sodium L-ascorbate (31 mg, 0.157 mmol) was added in small portions. After 3 h, sodium L-ascorbate (15.5 mg, 0.079 mmol) was added again. The reaction was monitored by TLC (toluene/Et_2O 5/1), which indicated reaction completion after 72 h. The reaction mixture was diluted with water, extracted with EtOAc 3 times, washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with toluene/Et_2O 5/1. Collected fractions were evaporated, which yielded a white solid of **15c**. Yield: 50 mg (40 %), m. p. 142–144 °C (toluene); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.75 (s, 3H), 0.80 (s, 3H), 0.90 (s, 3H), 0.94 (s, 3H), 1.01 (s, 3H, 5 × CH_3), 1.16 (t, J = 7.0 Hz, 3H), 1.19 (t, J = 7.0 Hz, 3H, 2 × CH_3 – Et), 2.71 (dd, J_1 = 11.7 Hz, J_2 = 4.3 Hz, 1H, H-3α), 2.82 (td, J_1 = 11.3 Hz, J_2 = 5.6 Hz, 1H, H-19β), 3.14 (d, J = 9.1 Hz, 1H, H-28a), 3.34 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 3.48 (q, J = 7.0 Hz, 2H, CH_2 – Et), 3.53 (d, J = 9.0 Hz, 1H, H-28b), 3.65 (dq, J_1 = 9.4 Hz, J_2 = 7.0 Hz, 1H, 1/2 × CH_2 – Et), 5.10 (s, 1H, H-29-*pro E*), 5.52 (s, 3H, phenyl-CH_2, H-29-*pro Z*), 7.26–7.29 (m, 2H), 7.33–7.40 (m, 4H, 5H – phenyl, 1H – triazole). **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.86, 15.35, 15.81, 16.14, 16.23, 16.41, 18.43, 21.10, 23.53, 27.40, 28.20, 30.32, 34.44, 34.80, 37.27, 37.45, 38.85, 38.93, 41.13, 42.76, 47.36, 50.48, 54.20, 56.02, 65.29, 67.09, 68.71, 86.81, 110.26, 119.90, 128.10, 128.81, 129.25, 135.02, 144.99, 149.62; **IR** (DRIFT): ν_{max} = 1074 (C–O); 1103 (C–O); 1621 (C═C); cm^{-1}; **HRMS** ($APCI^+$): calcd for $C_{42}H_{64}N_3O_2$ $[M+H]^+$ 642.4993; found 642.4916.

4.1.9. 3,28-Bis(TBDMS)betulin **3**

Betulin **1** (10 g, 22.6 mmol), imidazole (9.232 g, 135.6 mmol) and *tert*-butyldimethylsilyl chloride (13.63 g, 90.4 mmol) were dissolved in dry dimethylformamide (270 mL). The reaction vessel was sealed under argon and stirred at r.t. overnight. The reaction was completed after 24 h, which was indicated both by TLC (hexane) and precipitation of the product. The white precipitate of **3** was filtered off and dried under vacuum. The purity was sufficient for subsequent reaction, but the precipitate could be purified by column chromatography on a silica gel with hexane. Yield: 14.707 g (97 %), m. p. 173–175 °C (hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.03 (s, 6H, 2 × Si–CH_3), 0.04 (s, 6H, 2 × Si–CH_3), 0.73 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.89 (s, 12H, Si-*t*Bu, CH_3), 0.90 (s, 9H, Si-*t*Bu), 0.97 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 1.68 (s, 3H, C30–CH_3), 2.39 (td, J_1 = 10.9 Hz, J_2 6.0 Hz, 1H, H-19β), 3.16 (dd, J_1 = 11.1 Hz, J_2 = 4.5 Hz, 1H, H-3α), 3.26 (d, J = 9.6 Hz, 1H, H-28a), 3.67 (d, J = 9.0 Hz, 1H, H-28b), 4.57 (dd, J_1 = 2.3 Hz, J_2 = 1.4 Hz, 1H, H-29-*pro E*), 4.67 (d, J = 2.1 Hz, 1H, H-29-*pro Z*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = −5.29, −4.74, −3.59, 14.90, 16.06, 16.30, 18.29, 18.48, 18.66, 19.30, 21.05, 25.49, 26.10, 26.14, 27.21, 28.02, 28.58, 29.66, 30.15, 34.48, 34.51, 37.24, 37.58, 38.89, 39.61, 41.12, 42.84, 48.24, 48.62, 50.66, 55.58, 60.66, 79.65, 109.48, 151.14; **IR** (DRIFT): ν_{max} = 1068 (C–O); 1088 (C–O); 1644 (C═C) cm^{-1}.

4.1.10. 30-Oxo-3,28-bis(TBDMS)betulin **5**

SeO_2 (4.133 g, 37.24 mmol) was added to a suspension of 3,28-bis (TBDMS)betulin **3** (10 g, 14.90 mmol) in 2-methoxyethanol (155 mL) in one piece. The reaction mixture was heated under reflux for 2 h. The TLC monitoring (hexane/EtOAc 50/1) indicated complete reaction after 2 h. The reaction mixture was filtered over fritted glass with Celite and evaporated under reduced pressure. The residue was dissolved in EtOAc, washed with brine, dried over $MgSO_4$, filtered and sorbed on silica (40 g) under reduced pressure. Purification by column chromatography was done on silica gel with hexane/EtOAc 85/1. Collected fractions were evaporated and crystallized from $CHCl_3$/MeOH, which provided yellowish crystals **5**. Yield: 9.8 g, (96 %), m. p. 188–190 °C ($CHCl_3$/MeOH); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.02 (s, 6H, 2 × Si–CH_3), 0.04 (d, J = 0.9 Hz, 6H, 2 × Si–CH_3), 0.72 (s, 3H, CH_3), 0.80 (s, 3H, CH_3), 0.88 (s, 12H, Si-*t*Bu, CH_3), 0.90 (s, 9H, Si-*t*Bu), 0.94 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 2.77 (td, J_1 = 11.3 Hz, J_2 = 5.5 Hz, 1H, H-19β), 3.14 (dd, J_1 = 11.3 Hz, J_2 = 4.6 Hz, 1H, H-3α), 3.29 (d, J = 9.7 Hz, 1H, H-28a), 3.67 (d, J = 9.6 Hz, 1H, H-28b), 5.91 (s, 1H, H-29-*pro E*), 6.27 (s, 1H, H-29-*pro Z*), 9.51 (s, 1H, CHO); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = −5.31, −5.28, −4.74, −3.61, 14.75, 16.00, 16.04, 16.23, 18.29, 18.45, 18.63, 21.05, 26.08, 26.12, 27.09, 27.90, 27.97, 28.57, 29.56, 32.92, 33.02, 34.43,

34.46, 37.20, 38.87, 39.59, 41.04, 42.68, 48.49, 50.46, 51.65, 51.74, 55.55, 60.33, 79.62, 133.11, 157.56, 195.14; **IR** (DRIFT): ν_{max} = 1070 (C–O); 1088 (C–O); 1619 (C═C); 1686 (C═O) cm^{-1}.

*4.1.11. 30-(Dibromomethylidene)-3,28-bis(TBDMS)betulin **7***

Solution A was prepared by dissolving 30-oxo-3,28-bis(TBDMS)betulin **5** (10 g, 14.59 mmol) in dry dichloromethane (33.4 mL), solution B by dissolving CBr_4 (9.679 g, 29.19 mmol) in dry dichloromethane (16.7 mL) and solution C by dissolving PPh_3 (15.311 g; 58.37 mmol) in its portion of dry dichloromethane (66.9 mL). All solutions were sealed under argon. Solution B was added dropwise over 10 min to a stirred solution C cooled to 0 °C. After another 10 min solution A was added in the same manner. The reaction was monitored by TLC (hexane) until the reaction completion, which was indicated after 2–3 h. The reaction mixture was 3 times washed with brine, dried over $MgSO_4$ and filtered. The crude product was precipitated by adding eightfold volume of MeCN, filtered, washed with MeCN and dried under reduced pressure. The white precipitate of **7** was in sufficient purity for subsequent reaction, however could be purified by column chromatography on silica gel with hexane. Yield: 6.014 g (49 %), m. p. 141–143 °C (hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.03 (s, 6H, 2 × Si–CH_3), 0.04 (s, 6H, 2 × Si–CH_3), 0.72 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.88 (s, 12H, Si-*t*Bu, CH_3), 0.90 (s, 9H, Si-*t*Bu), 0.95 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 2.45 (td, J_1 = 11.1 Hz, J_2 = 5.8 Hz, 1H, H-19β), 3.16 (dd, J_1 = 11.2 Hz, J_2 = 4.5 Hz, 1H, H-3α), 3.23 (d, J = 9.7 Hz, 1H, H-28a), 3.65 (d, J = 9.6 Hz, 1H, H-28b), 5.19 (s, 1H, H-29-*pro E*), 5.23 (s, 1H, H-29-*pro Z*), 6.83 (d, J = 1.0 Hz, 1H, H-30); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = −5.30, −4.75, −3.61, 14.84, 16.03, 16.30, 18.29, 18.47, 18.65, 21.05, 26.10, 26.11, 26.71, 27.12, 27.97, 28.58, 29.68, 30.83, 34.47, 37.22, 37.32, 38.90, 39.59, 41.09, 42.76, 47.33, 48.17, 50.35, 50.54, 55.55, 60.77, 79.63, 90.10, 115.79, 136.76, 149.62; **IR** (DRIFT): ν_{max} = 1069 (C–O); 1089 (C–O); 1617 (C═C); 1647 (C═C) cm^{-1}.

*4.1.12. 30-Methylidyne-3,28-bis(TBDMS)betulin **9***

30-(Dibromomethylidene)-3,28-bis(TBDMS)betulin **7** (10 g, 11.89 mmol) was put in the reaction vessel and dried under vacuum for 30 min. The starting compound was dissolved in hexane (393 mL) under argon and cooled down to −78 °C. Then *n*-BuLi (15 mL, 2.5 M solution in hexane, 37.5 mmol) was added dropwise over 10 min. After that reaction continued at −78 °C for 90 min. The reaction was monitored by TLC (hexane) until complete reaction conversion. The reaction mixture was then brought to the room temperature and cautiously washed with brine (3 times), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica with hexane. Collected fractions were evaporated yielding white solid of **9**. Yield: 7.776 g (96 %), m. p. 152–155 °C (hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.03 (s, 6H, 2 × Si–CH_3), 0.04 (s, 6H, 2 × Si–CH_3), 0.72 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.88 (s, 12H, Si-*t*Bu, CH_3), 0.90 (s, 9H, Si-*t*Bu), 0.97 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 2.46 (td, J_1 = 10.6 Hz, J_2 = 5.8 Hz, 1H, H-19β), 2.92 (s, 1H, C≡C–H), 3.15 (dd, J_1 = 11.2 Hz, J_2 = 4.5 Hz, 1H, H-3α), 3.24 (d, J = 9.6 Hz, 1H, H-28a), 3.66 (d, J = 9.7 Hz, 1H, H-28b), 5.25 (d, J = 1.9 Hz, 1H, H-29-*pro E*), 5.27 (d, J = 1.9 Hz, 1H, H-29-*pro Z*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = −5.30, −4.74, −3.59, 14.82, 16.03, 16.06, 16.25, 18.30, 18.47, 18.66, 20.93, 25.77, 26.10, 26.13, 27.20, 28.02, 28.58, 29.66, 30.50, 34.35, 34.47, 37.23, 37.24, 38.86, 39.60, 41.04, 42.92, 47.60, 48.14, 49.88, 50.43, 55.55, 61.20, 78.38, 79.65, 83.16, 121.47, 138.01; **IR** (DRIFT): ν_{max} = 1069 (C–O); 1089 (C–O); 1647 (C═C); 3312 (C–H, alkyne) cm^{-1}.

*4.1.13. 30-Methylidynebetulin **10***

Tetra-*n*-butylammonium fluoride (14.68 mL, 1 M solution in tetrahydrofuran) was added to 30-methylidyne-3,28-bis(TBDMS)betulin **9** (1 g, 1.47 mmol) under argon and heated to 45 °C. The reaction was monitored by TLC (toluene/Et_2O 1/1), which indicated complete reaction conversion after 48 h. The solvent was then evaporated and the residue dissolved in EtOAc. The solution in EtOAc was then washed with brine 3 times, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with gradient elution starting at toluene/Et_2O 5/1 and finishing at toluene/Et_2O 1/1. Collected fractions were evaporated yielding white solid of **10**. Yield 0.611 g (92 %), m. p. 188–191 °C (toluene); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.76 (s, 3H), 0.82 (s, 3H), 0.97 (s, 3H), 0.99 (s, 3H), 1.02 (s, 3H, 5 × CH_3), 2.43 (td, J_1 = 10.9 Hz, J_2 = 5.9 Hz, 1H, H-19β), 2.92 (s, 1H, C≡C–H), 3.18 (dd, J_1 = 11.4 Hz, J_2 = 4.8 Hz, 1H, H-3α), 3.29 (d, J = 10.8 Hz, 1H, H-28a), 3.80 (dd, J_1 = 10.7 Hz, J_2 = 1.9 Hz, 1H, H-28b), 5.26 (d, J = 1.7 Hz, 1H, H-29-*pro E*), 5.28 (d, J = 1.8 Hz, 1H, H-29-*pro Z*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.83, 15.51, 16.10, 16.21, 18.46, 20.86, 25.66, 27.17, 27.54, 28.14, 29.25, 30.20, 33.90, 34.38, 37.13, 37.30, 38.84, 39.01, 41.01, 42.94, 47.30, 47.84, 50.14, 50.33, 55.43, 61.03, 78.52, 79.12, 82.94, 121.73, 137.55; **IR** (DRIFT): ν_{max} = 1029 (C–O); 1092 (C–O); 1637 (C═C); 2119 (C≡C); 3311 (C–H, alkyne); 3354 (O–H) cm^{-1}.

4.1.14. General procedure for preparing Sonogashira coupling conjugates (procedure B)

Aryliodide was added to a stirred suspension of 30-methylidynebetulin **10** (200 mg, 0.442 mmol), PPh_3 (11.6 mg, 0.0442 mmol), CuI (8.4 mg, 0.0441 mmol), Pd/C (23.6 mg, w_{Pd} = 10 %, 0.0222 mmol) and *N*,*N*-diisopropylethylamine (230 μL, 171 mg, 1.320 mmol) in dimethylacetamide (3.8 mL) and water (0.2 mL). The reaction mixture was filled with argon and heated to 75 °C.

*4.1.14.1. 30-(Phenylmethylidyne)-lup-20(29)-ene-3β,28-diol **12a**.* 30-(Phenylmethylidyne)-lup-20(29)-ene-3β,28-diol **12a** was prepared according to the general procedure **B** using iodobenzene (69 μL, 126 mg, 0.618 mmol). The reaction was completed after 72 h and monitored by TLC (hexane/EtOAc 3/1) until complete conversion. The reaction mixture was filtered, diluted with water and extracted with EtOAc 3 times. The collected organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with hexane/EtOAc 3/1. Collected fractions were evaporated, which yielded a white solid of **12a**. Yield 141 mg (61 %), m. p. 124–126 °C ($CHCl_3$, hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.76 (s, 3H), 0.83 (s, 3H), 0.96 (s, 3H), 1.03 (s, 3H), 1.03 (s, 3H, 5 × CH_3), 2.52 (td, J_1 = 10.8 Hz, J_2 = 5.8 Hz, 1H, H-19β), 3.16 (dd, J_1 = 11.3 Hz, J_2 = 4.9 Hz, 1H, H-3α), 3.33 (d, J = 10.8 Hz, 1H, H-28a), 3.83 (d, J = 10.6 Hz, 1H, H-28b), 5.26 (d, J = 1.9 Hz, 1H, H-29-*pro E*), 5.28 (d, J = 1.9 Hz, 1H, H-29-*pro Z*), 7.29–7.37 (m, 3H, aryl), 7.40–7.49 (m, 2H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.86, 15.50, 16.12, 16.23, 18.47, 20.89, 25.75, 27.17, 27.54, 28.14, 29.47, 30.56, 34.16, 34.42, 37.13, 37.32, 38.81, 39.00, 41.03, 42.94, 47.66, 47.91, 50.37, 50.43, 55.43, 61.09, 79.12, 88.67, 91.10, 119.96, 123.56, 128.29, 128.49, 131.73, 138.48; **IR** (DRIFT): ν_{max} = 1026 (C–O); 1606 (C═C); 3356 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{37}H_{53}O_2$ $[M+H]^+$ 529.4040; found 529.4045.

*4.1.14.2. 30-(4-Methoxyphenylmethylidyne)-lup-20(29)-ene-3β,28-diol **12b**.* 30-(4-Methoxyphenylmethylidyne)-lup-20(29)-ene-3β,28-diol **12b** was synthesized according to the general procedure **B** utilizing 4-iodoanisole (145 mg, 0.620 mmol). Completion of the reaction was indicated by TLC (hexane/EtOAc 3/1) after 72 h. The reaction mixture was filtered, then diluted with water and extracted with EtOAc (3 times). The collected organic phase was washed with brine, dried over $MgSO_4$ followed by filtration and evaporation of the solvent. The residue was purified by column chromatography on a silica gel with gradient elution starting at hexane/EtOAc 3/1 and finishing at hexane/EtOAc 7/3. Fractions were collected and the solvent was evaporated, which provided a white solid of **12b**. Yield 67 mg (27 %), m. p. 120–122 °C ($CHCl_3$, hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.75 (s, 3H), 0.82 (s, 3H), 0.96 (s, 3H), 1.02 (s, 3H), 1.03 (s, 3H, 5 × CH_3), 2.50 (td, J_1 = 10.8 Hz, J_2 = 5.8 Hz, 1H, H-19β), 3.16 (dd, J_1 = 11.3 Hz, J_2 = 4.9 Hz, 1H, H-

3α), 3.33 (d, $J = 10.8$ Hz, 1H, H-28a), 3.80–3.84 (m, 4H, H-28b, O–CH_3), 5.21 (d, $J = 2.0$ Hz, 1H, H-29-*pro E*), 5.23 (d, $J = 2.0$ Hz, 1H, H-29-*pro Z*), 6.86 (d, $J = 8.9$ Hz, 2H, aryl), 7.39 (d, $J = 8.9$ Hz, 2H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): $\delta = 14.88$, 15.50, 16.13, 16.23, 18.48, 20.90, 25.71, 27.18, 27.55, 28.15, 29.48, 30.59, 34.17, 34.44, 37.13, 37.32, 38.82, 39.01, 41.04, 42.94, 47.70, 47.90, 50.39, 55.46, 61.11, 79.14, 87.35, 91.13, 114.17, 115.71, 119.17, 133.16, 138.67, 159.70; **IR** (DRIFT): $\nu_{max} = 1028$ (C–O); 1246 (C–O); 1647 (C═C); 2195 (C≡C); 3392 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{38}H_{55}O_3$ $[M+H]^+$ 559.4146; found 559.4149.

4.1.14.3. 30-(4-Methylphenylmethylidyne)-lup-20(29)-ene-3β,28-diol

12c. 30-(4-Methylphenylmethylidyne)-lup-20(29)-ene-3β,28-diol **12c** was prepared according to the general procedure **B** using 4-iodotoluene (135 mg, 0.619 mmol). The reaction was completed after 72 h and monitored by TLC (hexane/EtOAc 3/1) until complete conversion. The reaction mixture was filtered, diluted with water and extracted with EtOAc (3 times). The collected organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with a mobile phase hexane/EtOAc/$CHCl_3$ 15/4/1. Collected fractions were evaporated, which yielded a white solid of **12c**. Yield 171 mg (71 %), m. p. 145–147 °C ($CHCl_3$, hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): $\delta = 0.75$ (s, 3H), 0.82 (s, 3H), 0.96 (s, 3H), 1.02 (s, 3H), 1.03 (s, 3H, 5 × CH_3), 2.36 (s, 3H, Ar-CH_3), 2.50 (td, $J_1 = 10.8$ Hz, $J_2 = 5.8$ Hz, 1H, H-19β), 3.16 (dd, $J_1 = 11.3$ Hz, $J_2 = 4.7$ Hz, 1H, H-3α), 3.32 (d, $J = 10.8$ Hz, 1H, H-28a), 3.82 (d, $J = 10.7$ Hz, 1H, H-28b), 5.23 (d, $J = 1.9$ Hz, 1H, H-29-*pro E*), 5.25 (d, $J = 1.9$ Hz, 1H, H-29-*pro Z*), 7.14 (d, $J = 8.0$ Hz, 2H, aryl), 7.35 (d, $J = 8.1$ Hz, 2H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): $\delta = 14.85$, 15.49, 16.11, 16.22, 18.46, 20.88, 21.64, 25.71, 27.17, 27.53, 28.14, 29.46, 30.56, 34.15, 34.42, 37.11, 37.30, 38.80, 38.99, 41.02, 42.92, 47.67, 47.89, 50.36, 50.38, 55.43, 61.08, 79.11, 88.00, 91.29, 119.53, 120.47, 129.25, 131.61, 138.41, 138.59; **IR** (DRIFT): $\nu_{max} = 1026$ (C–O); 1683 (C═C); 2200 (C≡C); 3365 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{38}H_{55}O_2$ $[M+H]^+$ 543.4197; found 543.4199.

4.1.14.4. 30-[4-(Trifluoromethyl)phenylmethylidyne]-lup-20(29)-ene-3β,28-diol

12d. 30-[4-(Trifluoromethyl)phenylmethylidyne]-lup-20(29)-ene-3β,28-diol **12d** was prepared according to the general procedure **B** using 1-iodo-4-(trifluoromethyl)-benzene (91 μL, 168 mg, 0.618 mmol). The reaction was monitored by TLC (hexane/EtOAc 3/1) until complete conversion, which was indicated after 72 h. The reaction mixture was filtered, diluted with water and then extracted with EtOAc 3 times. The collected organic phase was washed with brine, dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography on a silica gel with hexane/EtOAc/$CHCl_3$ 15/4/1. Collected fractions were concentrated under reduced pressure, which yielded a white solid of **12d**. Yield 197 mg (75 %), m. p. 188–190 °C ($CHCl_3$, hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): $\delta = 0.75$ (s, 3H), 0.82 (s, 3H), 0.96 (s, 3H), 1.01 (s, 3H), 1.03 (s, 3H, 5 × CH_3), 2.53 (td, $J_1 = 10.8$ Hz, $J_2 = 5.9$ Hz, 1H, H-19β), 3.16 (dd, $J_1 = 11.3$ Hz, $J_2 = 4.9$ Hz, 1H, H-3α), 3.33 (d, $J = 10.8$ Hz, 1H, H-28a), 3.82 (dd, $J_1 = 11.0$ Hz, $J_2 = 1.3$ Hz, 1H, H-28b), 5.32 (d, $J = 1.7$ Hz, 1H, H-29-*pro E*), 5.34 (d, $J = 1.8$ Hz, 1H, H-29-*pro Z*), 7.54 (d, $J = 8.1$ Hz, 2H, aryl), 7.59 (d, $J = 8.3$ Hz, 2H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): $\delta = 14.82$, 15.50, 16.11, 16.22, 18.45, 20.88, 25.84, 27.14, 27.52, 28.14, 29.50, 30.59, 34.20, 34.42, 37.10, 37.31, 38.82, 39.00, 41.03, 42.92, 47.49, 47.92, 50.37, 50.58, 55.43, 61.03, 79.10, 89.65, 91.16, 121.20, 124.08 (q, $J = 272.0$ Hz, CF_3), 125.46 (q, $J = 3.8$ Hz, C-C-CF_3), 127.33, 130.01 (q, $J = 32.7$ Hz, C-CF_3), 131.90, 138.03; **IR** (DRIFT): $\nu_{max} = 1024$ (C–O); 1067 (C–O); 1320 (CF_3); 1616 (C═C); 2213 (C≡C); 3315 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{38}H_{52}F_3O_2$ $[M+H]^+$ 597.3914; found 597.3911 and 579.3809 $[M-H_2O+H]^+$.

4.1.14.5. 30-(2-Nitrophenylmethylidyne)-lup-20(29)-ene-3β,28-diol

12e. 30-(2-Nitrophenylmethylidyne)-lup-20(29)-ene-3β,28-diol **12e** was prepared according to the general procedure **B** using 1-iodo-2-nitrobenzene (154 mg, 0.618 mmol). The reaction was completed after 72 h and monitored by TLC (hexane/EtOAc 7/3) until complete conversion. The reaction mixture was filtered, diluted with water and extracted with EtOAc 3 times. The collected organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with hexane/EtOAc/$CHCl_3$ 13/6/1. Collected fractions were evaporated, which yielded a yellowish solid of **12e**. Yield 165 mg (65 %), m. p. 130–134 °C ($CHCl_3$, hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): $\delta = 0.75$ (s, 3H), 0.82 (s, 3H), 0.94 (s, 3H), 0.98 (s, 3H), 1.02 (s, 3H, 5 × CH_3), 2.54 (td, $J_1 = 10.9$ Hz, $J_2 = 5.9$ Hz, 1H, H-19β), 3.15 (dd, $J_1 = 11.3$ Hz, $J_2 = 5.0$ Hz, 1H, H-3α), 3.32 (d, $J = 10.8$ Hz, 1H, H-28a), 3.82 (dd, $J_1 = 10.8$ Hz, $J_2 = 1.4$ Hz, 1H, H-28b), 5.39 (d, $J = 1.8$ Hz, 1H, H-29-*pro E*), 5.41 (d, $J = 1.8$ Hz, 1H, H-29-*pro Z*), 7.44 (ddd, $J_1 = 8.4$ Hz, $J_2 = 7.5$ Hz, $J_3 = 1.5$ Hz, 1H, aryl), 7.57 (td, $J_1 = 7.6$ Hz, $J_2 = 1.3$ Hz, 1H, aryl), 7.64 (dd, $J_1 = 7.8$ Hz, $J_2 = 1.4$ Hz, 1H, aryl), 8.04 (dd, $J_1 = 8.3$ Hz, $J_2 = 1.1$ Hz, 1H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): $\delta = 14.69$, 15.48, 16.12, 16.20, 18.46, 20.87, 25.91, 27.12, 27.53, 28.13, 29.09, 30.49, 33.67, 34.36, 37.21, 37.29, 38.81, 38.99, 40.99, 42.99, 47.72, 47.99, 50.25, 50.28, 55.40, 61.11, 79.13, 85.96, 96.70, 118.97, 122.65, 124.79, 128.55, 132.84, 135.05, 138.32, 149.70; **IR** (DRIFT): $\nu_{max} = 1026$ (C–O); 1525 (N–O); 1609 (C═C); 2195 (C≡C); 3384 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{37}H_{52}NO_4$ $[M+H]^+$ 574.3891; found 574.3888.

4.1.14.6. 30-(1-Methyl-1H-pyrazol-4-ylmethylidyne)-lup-20(29)-ene-3β,28-diol

12f. 30-(1-Methyl-1*H*-pyrazol-4-ylmethylidyne)-lup-20(29)-ene-3β,28-diol **12f** was prepared according to the general procedure **B** using 4-iodo-1-methyl-1*H*-pyrazole (129 mg, 0.208 mmol). The reaction was completed after 72 h and monitored by TLC (hexane/EtOAc 1/1) until complete conversion. The reaction mixture was filtered, diluted with water and extracted with EtOAc 3 times. The collected organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with hexane/EtOAc 1/1. Collected fractions were evaporated, which yielded a white solid of **12f**. Yield 46 mg (20 %), m. p. 208–211 °C ($CHCl_3$, hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): $\delta = 0.75$ (s, 3H), 0.82 (s, 3H), 0.96 (s, 3H), 1.00 (s, 3H), 1.02 (s, 3H, 5 × CH_3), 2.48 (td, $J_1 = 10.8$ Hz, $J_2 = 5.9$ Hz, 1H, H-19β), 3.17 (dd, $J_1 = 11.3$ Hz, $J_2 = 4.9$ Hz, 1H, H-3α), 3.31 (d, $J = 10.7$ Hz, 1H, H-28a), 3.81 (dd, $J_1 = 10.7$ Hz, $J_2 = 1.3$ Hz, 1H, H-28b), 3.90 (s, 3H, N–CH_3), 5.18–5.21 (m, 2H, H-29), 7.47 (s, 1H, pyrazole), 7.56 (s, 1H, pyrazole); **^{13}C NMR** (126 MHz, $CDCl_3$): $\delta = 14.81$, 15.50, 16.11, 16.23, 18.46, 20.89, 25.70, 27.18, 27.54, 28.15, 29.45, 30.60, 34.10, 34.43, 37.08, 37.31, 38.82, 39.01, 39.24, 41.03, 42.92, 47.55, 47.85, 50.35, 50.40, 55.45, 61.05, 79.11, 82.20, 89.30, 103.62, 119.02, 132.53, 138.59, 142.11; **IR** (DRIFT): $\nu_{max} = 1026$ (C–O); 1653 (C═C); 2208 (C≡C); 3398 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{35}H_{53}N_2O_2$ $[M+H]^+$ 533.4102; found 533.4105.

4.1.14.7. 30-(Thiophene-2-ylmethylidyne)-lup-20(29)-ene-3β,28-diol

12g. 30-(Thiophene-2-ylmethylidyne)-lup-20(29)-ene-3β,28-diol **12g** was prepared according to the general procedure **B** using 2-iodothiophene (68 μL, 130 mg, 0.618 mmol). The reaction was completed after 24 h and monitored by TLC (hexane/EtOAc 3/1) until complete conversion. The reaction mixture was filtered, diluted with water and extracted with EtOAc (3 times). The collected organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with gradient mobile phase – starting at hexane/EtOAc 3/1 and finishing at hexane/EtOAc 2/1. Collected fractions were evaporated, which yielded a white solid of **12g**. Yield 134 mg (57 %), m. p. 126–129 °C ($CHCl_3$, hexane); **^{1}H NMR** (500 MHz, $CDCl_3$): $\delta = 0.76$ (s,

3H), 0.83 (s, 3H), 0.96 (s, 3H), 1.03 (s, 6H, 5 × CH_3), 2.51 (td, $J_1 = 10.8$ Hz, $J_2 = 5.9$ Hz, 1H, H-19β), 3.17 (dd, $J_1 = 11.4$ Hz, $J_2 = 4.9$ Hz, 1H, H-3α), 3.32 (d, $J = 10.8$ Hz, 1H, H-28a), 3.82 (dd, $J_1 = 10.8$ Hz, $J_2 = 1.5$ Hz, 1H, H-28b), 5.26 (d, $J = 1.8$ Hz, 1H, H-29-*pro E*), 5.27 (d, $J = 1.8$ Hz, 1H, H-29-*pro Z*), 6.99 (dd, $J_1 = 5.2$ Hz, $J_2 = 3.6$ Hz, 1H, thiophene), 7.20 (dd, $J_1 = 3.6$ Hz, $J_2 = 1.1$ Hz, 1H, thiophene), 7.26 (dd, $J_1 = 5.2$ Hz, $J_2 =$ 1.2 Hz, 1H, thiophene); **^{13}C NMR** (126 MHz, $CDCl_3$): $\delta =$ 14.86, 15.50, 16.12, 16.23, 18.47, 20.90, 25.78, 27.16, 27.55, 28.15, 29.44, 30.67, 34.11, 34.42, 37.09, 37.31, 38.81, 39.01, 41.03, 42.93, 47.54, 47.89, 50.36, 50.49, 55.43, 61.07, 79.12, 84.30, 92.66, 119.91, 123.60, 127.24, 131.76, 138.24; **IR** (DRIFT): $\nu_{max} = 1026$ (C–O); 1604 (C═C); 2194 (C≡C); 3379 (O–H) cm^{-1}; **HRMS** (ESI$^+$): calcd for $C_{35}H_{51}O_2S$ $[M+H]^+$ 535.3604; found 535.3610.

4.1.15. General procedure for acetylation of conjugates 12a–12g (procedure C)

4-Dimethylaminopyridine (92.5 mg, 0.757 mmol) in dry dichloromethane (5 mL) and Ac_2O (125 μL, 135 mg, 1.322 mmol) was added to a stirred solution of triterpene **12a–12g** (0.189 mmol). The reaction flask was then sealed under argon and the reaction continued at r.t. The reaction was monitored by TLC (hexane/EtOAc 3/1), which indicated a complete reaction after an hour. The reaction was diluted with dichloromethane and the pH of the reaction was neutralized by water solution of $NaHCO_3$. The mixture was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure.

4.1.15.1. 3β,28-diacetoxy-30-(phenylmethylidyne)-lup-20(29)-ene 13a.

3β,28-Diacetoxy-30-(phenylmethylidyne)-lup-20(29)-ene **13a** was obtained by acetylation of **12a** according to the general procedure **C**. White solid of **13a** was obtained directly from extraction without any further purification. Yield 70 mg (61 %), m. p. 90–94 °C (MeOH, H_2O); **^{1}H NMR** (500 MHz, $CDCl_3$): $\delta = 0.83$ (s, 6H), 0.85 (s, 3H), 1.01 (s, 3H), 1.04 (s, 3H, 5 × CH_3), 2.03 (s, 3H), 2.08 (s, 3H, 2 × Ac), 2.57 (td, $J_1 =$ 10.9 Hz, $J_2 = 5.8$ Hz, 1H, H-19β), 3.85 (d, $J = 11.0$ Hz, 1H, H-28a), 4.27 (dd, $J_1 = 11.0$ Hz, $J_2 = 1.3$ Hz, 1H, H-28b), 4.45 (dd, $J_1 = 10.6$ Hz, $J_2 =$ 5.8 Hz, 1H, H-3α), 5.26 (d, $J = 1.9$ Hz, 1H, H-29-*pro E*), 5.29 (d, $J = 1.8$ Hz, 1H, H-29-*pro Z*), 7.31–7.36 (m, 3H, aryl), 7.43–7.47 (m, 2H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): $\delta =$ 14.83, 16.17, 16.27, 16.63, 18.34, 20.87, 21.19, 21.44, 23.84, 25.68, 27.17, 28.10, 30.03, 30.37, 34.32, 34.73, 37.22, 37.38, 37.94, 38.50, 41.01, 42.91, 46.40, 47.60, 50.25, 50.40, 55.52, 63.24, 81.06, 88.50, 91.18, 120.15, 123.47, 128.34, 128.51, 131.71, 138.16, 171.10, 171.74; **IR** (DRIFT): $\nu_{max} =$ 1028 (C–O); 1239 (C–O); 1606 (C═C); 1732 (C═O) cm^{-1}.

4.1.15.2. 3β,28-diacetoxy-30-(4-methoxyphenylmethylidyne)-lup-20(29)-ene 13b.

3β,28-Diacetoxy-30-(4-methoxyphenylmethylidyne)-lup-20(29)-ene **13b** was synthesized according to the general procedure **C** from conjugate **12b**. The organic phase was purified by column chromatography on a silica gel with hexane/EtOAc 8/1. Collected fractions were evaporated yielding **13b** as a white solid. Yield 113 mg (93 %), m. p. 98–101 °C (MeOH, H_2O); **^{1}H NMR** (500 MHz, $CDCl_3$): $\delta = 0.83$ (s, 6H), 0.85 (s, 3H), 1.01 (s, 3H), 1.04 (s, 3H, 5 × CH_3), 2.03 (s, 3H), 2.07 (s, 3H, 2 × Ac), 2.55 (td, $J_1 = 10.9$ Hz, $J_2 = 5.8$ Hz, 1H, H-19β), 3.82 (s, 3H, O–CH_3), 3.84 (d, $J = 11.4$ Hz, 1H, H-28a), 4.27 (dd, $J = 10.9$ Hz, 1.3 Hz, 1H, H-28b), 4.45 (dd, $J_1 = 10.4$ Hz, $J_2 = 6.0$ Hz, 1H, H-3α), 5.22 (d, $J =$ 1.9 Hz, 1H, H-29-*pro E*), 5.24 (d, $J = 1.9$ Hz, 1H, H-29-*pro Z*), 6.84–6.89 (m, 2H, aryl), 7.36–7.41 (m, 2H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): $\delta =$ 14.84, 16.17, 16.27, 16.62, 18.33, 20.86, 21.19, 21.44, 23.84, 25.63, 27.18, 28.09, 30.04, 30.39, 34.33, 34.73, 37.22, 37.37, 37.94, 38.50, 41.01, 42.91, 46.38, 47.63, 50.27, 50.35, 55.46, 55.52, 63.27, 81.07, 87.17, 91.21, 114.19, 115.60, 119.36, 133.13, 138.34, 159.73, 171.11, 171.75; **IR** (DRIFT): $\nu_{max} = 1030$ (C–O); 1242 (C–O); 1601 (C═C); 1732 (C═O); 2203 (C≡C) cm^{-1}.

4.1.15.3. 3β,28-diacetoxy-30-(4-methylphenylmethylidyne)-lup-20(29)-ene 13c.

3β,28-Diacetoxy-30-(4-methylphenylmethylidyne)-lup-20(29)-ene **13c** was prepared from conjugate **12c** according to the general procedure **C**. The residue after extraction was purified by column chromatography on silica gel with hexane/EtOAc/$CHCl_3$ 17/2/1. A white solid of **13c** was obtained after evaporation of all solvents. Yield 102 mg (86 %), m. p. 87–90 °C (MeOH, H_2O); **^{1}H NMR** (500 MHz, $CDCl_3$): $\delta = 0.83$ (s, 6H), 0.85 (s, 3H), 1.01 (s, 3H), 1.04 (s, 3H, 5 × CH_3), 2.03 (s, 3H), 2.08 (s, 3H, 2 × Ac), 2.36 (s, 3H, aryl-CH_3), 2.56 (td, $J_1 =$ 10.9 Hz, $J_2 = 5.8$ Hz, 1H, H-19β), 3.84 (d, $J = 11.0$ Hz, 1H, H-28a), 4.27 (dd, $J_1 = 10.8$ Hz, $J_2 = 1.0$ Hz, 1H, H-28b), 4.45 (dd, $J_1 = 10.5$ Hz, $J_2 =$ 5.8 Hz, 1H, H-3α), 5.24 (d, $J = 1.9$ Hz, 1H, H-29-*pro E*), 5.26 (d, $J = 1.9$ Hz, 1H, H-29-*pro Z*), 7.14 (d, $J = 7.9$ Hz, 2H, aryl), 7.34 (d, $J = 8.1$ Hz, 2H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): $\delta =$ 14.83, 16.17, 16.27, 16.62, 18.33, 20.86, 21.19, 21.44, 21.64, 23.84, 25.65, 27.18, 28.10, 30.03, 30.37, 34.32, 34.72, 37.22, 37.38, 37.94, 38.50, 41.00, 42.91, 46.38, 47.62, 50.26, 50.36, 55.52, 63.26, 81.07, 87.83, 91.38, 119.72, 120.38, 129.28, 131.60, 138.29, 138.47, 171.11, 171.76; **IR** (DRIFT): $\nu_{max} =$ 1029 (C–O); 1239 (C–O); 1603 (C═C); 1732 (C═O) cm^{-1}.

4.1.15.4. 3β,28-diacetoxy-30-[4-(trifluoromethyl)phenylmethylidyne]-lup-20(29)-ene 13d.

3β,28-Diacetoxy-30-[4-(trifluoromethyl)phenylmethylidyne]-lup-20(29)-ene **13d** was prepared according to the general procedure **C** from conjugate **12d**. The organic phase was purified by column chromatography on silica gel with hexane/EtOAc 8/1 yielding **13d** as a white solid. Yield 109 mg (85 %), m. p. 98–101 °C (MeOH, H_2O); **^{1}H NMR** (500 MHz, $CDCl_3$): $\delta = 0.83$ (s, 6H), 0.85 (s, 3H), 1.00 (s, 3H), 1.04 (s, 3H, 5 × CH_3), 2.03 (s, 3H), 2.08 (s, 3H, 2 × Ac), 2.58 (td, J_1 = 10.9 Hz, $J_2 = 5.8$ Hz, 1H, H-19β), 3.85 (d, $J = 11.0$ Hz, 1H, H-28a), 4.26 (dd, $J_1 = 11.0$ Hz, $J_2 = 0.9$ Hz, 1H, H-28b), 4.45 (dd, $J_1 = 10.1$ Hz, $J_2 = 6.2$ Hz, 1H, H-3α), 5.33 (d, $J = 1.7$ Hz, 1H, H-29-*pro E*), 5.34 (d, $J =$ 1.7 Hz, 1H, H-29-*pro Z*), 7.54 (d, $J = 8.1$ Hz, 2H, aryl), 7.60 (d, $J = 8.3$ Hz, 2H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): $\delta =$ 14.74, 16.10, 16.21, 16.55, 18.26, 20.79, 21.11, 21.36, 23.75, 25.72, 27.07, 28.02, 29.99, 30.33, 34.26, 34.69, 37.15, 37.28, 37.86, 38.45, 40.95, 42.83, 46.35, 47.36, 50.21, 50.49, 55.46, 63.07, 80.97, 89.66, 90.92, 121.30, 124.00 (q, $J = 272.1$ Hz, CF_3), 125.42 (q, $J = 3.0$ Hz, C-C-CF_3), 127.17, 130.00 (q, $J = 32.6$ Hz, C-CF_3), 131.83, 137.65, 171.04, 171.66; IR (DRIFT): $\nu_{max} = 1031$ (C–O); 1240 (C–O); 1616 (C═C); 1732 (C═O) cm^{-1}.

4.1.15.5. 3β,28-diacetoxy-30-(2-nitrophenylmethylidyne)-lup-20(29)-ene 13e.

3β,28-Diacetoxy-30-(2-nitrophenylmethylidyne)-lup-20(29)-ene **13e** was prepared from conjugate **12e** by following general procedure **C**. The residue after extraction was purified by column chromatography on silica gel with hexane/EtOAc 8/1. Collected fractions were evaporated, which provided a yellow solid of **13e**. Yield 68 mg (55 %), m. p. 202–205 °C (MeOH, H_2O); **^{1}H NMR** (500 MHz, $CDCl_3$): $\delta = 0.82$ (s, 6H), 0.84 (s, 3H), 0.96 (s, 3H), 1.03 (s, 3H, 5 × CH_3), 2.02 (s, 3H), 2.08 (s, 3H, 2 × Ac), 2.58 (td, $J_1 = 10.$ Hz 9, $J_2 = 5.8$ Hz, 1H, H-19β), 3.85 (d, $J =$ 11.0 Hz, 1H, H-28a), 4.26 (dd, $J_1 = 10.7$ Hz, $J_2 = 1.1$ Hz, 1H, H-28b), 4.43 (dd, $J_1 = 10.4$ Hz, $J_2 = 5.9$ Hz, 1H, H-3α), 5.39 (d, $J = 1.7$ Hz, 1H, H-29-*pro E*), 5.41 (d, $J = 1.7$ Hz, 1H, H-29-*pro Z*), 7.42–7.48 (m, 1H, aryl), 7.58 (td, $J_1 = 7.6$ Hz, $J_2 = 1.2$ Hz, 1H, aryl), 7.65 (dd, $J_1 = 7.8$ Hz, $J_2 = 1.4$ Hz, 1H, aryl), 8.05 (dd, $J_1 = 8.3$ Hz, $J_2 = 1.0$ Hz, 1H, aryl); **^{13}C NMR** (126 MHz, $CDCl_3$): $\delta =$ 14.64, 16.17, 16.24, 16.61, 18.32, 20.85, 21.20, 21.43, 23.82, 25.84, 27.12, 28.09, 29.63, 29.83, 30.27, 34.19, 34.25, 37.19, 37.47, 37.92, 38.49, 40.97, 42.97, 46.48, 47.68, 50.17, 55.49, 63.26, 81.09, 86.09, 96.51, 118.90, 122.81, 124.81, 128.60, 132.88, 135.06, 138.06, 149.68, 171.11, 171.79; **IR** (DRIFT): $\nu_{max} =$ 1028 (C–O); 1246 (C–O); 1601 (C═C); 1720 (C═O); 1732 (C═O); 1508 (N–O); 2198 (C≡C) cm^{-1}. **HRMS** (ESI$^+$): calcd for $C_{41}H_{56}N_1O_6$ $[M+H]^+$ 658.4102; found 658.4105.

*4.1.15.6. 3β,28-diacetoxy-30-(1-methyl-1H-pyrazol-4-ylmethylidyne)-lup-20(29)-ene **13f**.* Following general procedure **C**, 3β,28-diacetoxy-30-(1-methyl-1*H*-pyrazol-4-ylmethylidyne)-lup-20(29)-ene **13f** was prepared by acetylation of conjugate **12f**. The organic phase was purified by column chromatography on a silica gel with hexane/EtOAc/dichloromethane 14/5/1. Evaporation of the solvents provided **13f** as a white solid. Yield 84 mg (72 %), m. p. 100–103 °C (MeOH, H_2O); **^{1}H NMR** (500 MHz, CD_2Cl_2): δ = 0.84 (s, 6H), 0.86 (s, 3H), 1.01 (s, 3H), 1.05 (s, 3H, 5 × CH_3), 2.00 (s, 3H), 2.04 (s, 3H, 2 × Ac), 2.57 (td, J_1 = 10.9 Hz, J_2 = 5.8 Hz, 1H, H-19β), 3.82 (d, J = 11.0 Hz 1H, H-28a), 3.87 (s, 3H, N–CH_3), 4.25 (dd, J_1 = 11.1 Hz, J_2 = 1.4 Hz, 1H, H-28b), 4.42 (dd, J_1 = 9.5 Hz, J_2 = 6.9 Hz, 1H, H-3α), 5.18 (d, J = 1.9 Hz, 1H, H-29-*pro E*), 5.21 (d, J = 1.9 Hz, 1H, H-29-*pro Z*), 7.50 (s, 1H, pyrazole), 7.52 (s, 1H, pyrazole); **^{13}C NMR** (126 MHz, CD_2Cl_2): δ = 15.01, 16.36, 16.49, 16.80, 18.75, 21.29, 21.34, 21.60, 24.26, 26.15, 27.61, 28.27, 30.42, 30.88, 34.74, 35.07, 37.64, 37.79, 38.33, 38.93, 39.59, 41.45, 43.33, 46.84, 47.95, 50.70, 50.79, 55.89, 63.41, 81.31, 82.84, 89.43, 103.67, 119.24, 132.99, 139.08, 142.18, 171.20, 171.82; **IR** (DRIFT): ν_{max} = 1027 (C–O); 1240 (C–O); 1607 (C═C); 1732 (C═O); 2207 (C≡C) cm^{-1}. **HRMS** (ESI^+): calcd for $C_{39}H_{57}N_2O_4$ $[M+H]^+$ 617.4313; found 617.4312.

*4.1.15.7. 3β,28-diacetoxy-30-(thiophene-2-ylmethylidyne)-lup-20(29)-ene **13g**.* 3β,28-Diacetoxy-30-(thiophene-2-ylmethylidyne)-lup-20(29)-ene **13g** was synthesized according to the general procedure **C** from the conjugate **12g**. The organic phase was purified by column chromatography on a silica gel with gradient elution starting at hexane/EtOAc 8/1 and finishing at hexane/EtOAc 3/1. Solvents from collected fractions were removed under reduced pressure, which yielded **13g** as a white solid. Yield 110 mg (94 %), m. p. 187–189 °C (MeOH, H_2O); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.83 (s, 3H), 0.84 (s, 3H), 0.85 (s, 3H), 1.02 (s, 3H), 1.04 (s, 3H, 5 × CH_3), 2.03 (s, 3H), 2.08 (s, 3H, 2 × Ac), 2.56 (td, J_1 = 10.9 Hz, J_2 = 5.8 Hz, 1H, H-19β), 3.84 (d, J = 11.0 Hz, 1H, H-28a), 4.26 (dd, J_1 = 11.0 Hz, J_2 = 0.9 Hz, 1H, H-28b), 4.45 (dd, J_1 = 10.6 Hz, J_2 = 5.8 Hz, 1H, H-3α), 5.26 (d, J = 1.7 Hz, 1H, H-29-*pro E*), 5.27 (d, J = 1.8 Hz, 1H, H-29-*pro Z*), 7.00 (dd, J_1 = 5.2 Hz, J_2 = 3.6 Hz, 1H, thiophene), 7.20 (dd, J_1 = 3.6 Hz, J_2 = 1.1 Hz, 1H, thiophene), 7.27 (dd, J_1 = 5.2 Hz, J_2 = 1.1 Hz, 1H, thiophene); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.84, 16.18, 16.28, 16.63, 18.34, 20.88, 21.20, 21.44, 23.84, 25.72, 27.17, 28.11, 30.01, 30.48, 34.32, 34.67, 37.22, 37.34, 37.95, 38.51, 41.01, 42.92, 46.39, 47.48, 50.26, 50.47, 55.52, 63.21, 81.07, 84.41, 92.48, 120.13, 123.50, 127.25, 127.31, 131.81, 137.94, 171.10, 171.74; **IR** (DRIFT): ν_{max} = 1028 (C–O); 1239 (C–O); 1605 (C═C); 1732 (C═O); 2195 (C≡C) cm^{-1}. **HRMS** (ESI^+): calcd for $C_{39}H_{55}O_4S$ $[M+H]^+$ 619.3816; found 619.3819.

*4.1.16. General procedure for synthesis of triazoles **16a**–**16j** (procedure D)*

Appropriate azides needed for the synthesis of triazoles **16a** and **16c–16h** were prepared firstly. NaN_3 (57.5 mg, 0.884 mmol) and appropriate bromide (0.442 mmol) were dissolved in dimethylformamide (20 mL) at r.t. The reaction was monitored by TLC and was completed after 24 h. The reaction mixture was diluted with dichloromethane, washed with brine 3 times, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Freshly prepared azides were directly utilized in the synthesis of triazoles **16a** and **16c–16h**. Azides for the synthesis of triazoles **16i–16j** were prepared as follows. 1,8-Diazabicyklo[5.4.0]undec-7-en (79 μL, 81 mg, 0.530 mmol) was added to a solution of appropriate alcohol (0.442 mmol) and diphenylphosphoryl azide (114 μL, 146 mg, 0.530 mmol) in dry toluene (1 mL) cooled to 0 °C. The reaction was being kept at 0 °C and under argon for 2 more hours and then the temperature was elevated to r.t. The reaction was diluted with water and 3 times extracted with toluene. The collected organic phase was washed with brine, dried over $MgSO_4$, filtered and evaporated. Freshly prepared azides were used directly in the synthesis of the triazoles **16i–16j**. Synthesis of the triazole **16b** will be discussed individually. 30-methylidynebetulin **12** (100 mg, 0.221 mmol) and appropriate azide (0.442 mmol) were dissolved in dry tetrahydrofuran (27 mL), after which copper(I) 3-methylsalicylate (4.7 mg, 0.022 mmol) was added. The reaction continued under argon at 60 °C.

*4.1.16.1. 20-[1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol **16a**.* 20-[1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol **16a** was prepared according to the general procedure **D** using 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylazide. The reaction conversion was monitored by TLC (toluene/Et_2O 1/1) and indicated a complete reaction after 32 h. The solvent was evaporated and the residue was purified by column chromatography on silica gel with gradient elution starting at hexane/EtOAc 1/1 and finishing at 1/2. Collected fraction were evaporated yielding white solid of **16a**. Yield: 150 mg (82 %), m. p. 142–144 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.74 (s, 3H), 0.80 (s, 3H), 0.94 (s, 3H), 0.95 (s, 3H), 1.01 (s, 3H, 5 × CH_3), 1.87 (s, 3H), 2.03 (s, 3H), 2.07 (s, 3H), 2.08 (s, 3H, 4 × AcO), 2.76 (td, J_1 = 11.3 Hz, J_2 = 5.6 Hz, 1H, H-19β), 3.16 (dd, J_1 = 11.3 Hz, J_2 = 4.7 Hz, 1H, H-3α), 3.41 (d, J = 10.8 Hz, 1H, H-28a), 3.83 (d, J = 10.6 Hz, 1H, H-28b), 4.00 (ddd, J_1 = 10.1 Hz, J_2 = 5.0 Hz, J_3 = 2.1 Hz, 1H, H-5′), 4.15 (dd, J_1 = 12.6 Hz, J_2 = 2.1 Hz, 1H, H-6′), 4.31 (dd, J_1 = 12.6 Hz, J_2 = 5.1 Hz, 1H, H-6′), 5.17 (s, 1H, H-29-*pro E*), 5.24 (dd, J_1 = 10.2 Hz, J_2 = 9.1 Hz, 1H, H-4′), 5.41 (t, J = 9.2 Hz, 1H, H-3′), 5.46 (t, J = 9.1 Hz, 1H, H-2′), 5.59 (s, 1H, H-29-*pro Z*), 5.86 (d, J = 9.0 Hz, 1H, H-1′), 7.70 (s, 1H, H-triazole); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.84, 15.50, 16.14, 16.22, 18.43, 20.32, 20.66, 20.66, 20.83, 21.05, 27.05, 27.19, 27.51, 28.13, 29.41, 29.49, 29.79, 29.83, 32.06, 33.85, 34.41, 37.27, 37.31, 38.84, 39.00, 41.05, 42.82, 48.00, 50.44, 55.43, 60.66, 61.77, 67.92, 70.38, 72.87, 75.30, 79.09, 85.88, 111.72, 118.20, 144.13, 149.73, 168.96, 169.50, 170.02, 170.59; **IR** (DRIFT): ν_{max} = 1033 (C–O); 1062 (C–O); 1647 (C═C); 1749 (C═O); 3422 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{45}H_{67}N_3O_{11}$ $[M+H]^+$ 826.4848; found 826.4852.

*4.1.16.2. 20-[1-(β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol **16b**.* Instead of following general procedure **D**, 20-[1-(β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol **16b** was prepared from 20-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol **16a**. Compound Triazole **16a** (100 mg, 0.121 mmol) was dissolved in dry EtOH (0.33 mL) as well as NaH (24.2 mg, 60 % suspension in mineral oil. 0.605 mmol) was dissolved in dry EtOH (0.33 mL). Both solutions were slowly combined under argon. The reaction continued at r.t. and was monitored by TLC ($CHCl_3$/MeOH 4/1), which indicated a complete conversion in 2 h pH of the reaction was brought down to 6–7 with AcOH and the reaction mixture was sorbed on a silica gel (400 mg) under reduced pressure. Purification by column chromatography was done on silica gel with $CHCl_3$/MeOH 4/1. Collected fractions were collected and evaporated yielding white solid of **16b**. Yield: 53 mg (67 %), m. p. 210–212 °C ($CHCl_3$/MeOH); **^{1}H NMR** (500 MHz, CD_3OD): δ = 0.79 (s, 3H), 0.88 (s, 3H), 0.99 (s, 3H), 1.05 (s, 3H), 1.12 (s, 3H, 5 × CH_3), 2.84 (td, J_1 = 11.3 Hz, J_2 = 5.5 Hz, 1H, H-19β), 3.16 (dd, J_1 = 11.4 Hz, J_2 = 4.8 Hz, 1H, H-3α), 3.43 (d, J = 11.1 Hz, 1H, H-28a), 3.53–3.65 (m, 3H, H-5′, 2 × H-6′), 3.77 (dd, J_1 = 12.2 Hz, J_2 = 5.5 Hz, 1H, H-4′), 3.84 (d, J = 11.0 Hz, 1H, H-28b), 3.93 (dd, J_1 = 12.2 Hz, J_2 = 2.0 Hz, 1H, H-3′), 3.98 (t, J = 9.1 Hz, 1H, H-2′), 5.22 (s, 1H, H-29-*pro E*), 5.63 (d, J = 9.2 Hz, 1H, H-1′), 5.68 (s, 1H, H-29-*pro Z*), 8.28 (s, 1H, triazole); **^{13}C NMR** (126 MHz, CD_3OD): δ = 15.24, 16.11, 16.58, 16.68, 19.43, 22.11, 28.01, 28.21, 28.62, 28.72, 30.42, 30.68, 33.63, 34.76, 35.50, 38.25, 38.56, 39.93, 40.02, 42.15, 43.78, 44.34, 44.37, 51.56, 51.75, 56.78, 60.34, 62.37, 70.88, 73.88, 78.55, 79.62, 81.12, 89.56, 110.66, 121.79, 145.99, 150.12; **IR** (DRIFT): ν_{max} = 1029 (C–O); 1094 (C–O); 1637 (C═C); 3354 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{37}H_{59}N_3O_7$ $[M+H]^+$ 658.4426; found 658.4430.

4.1.16.3. 20-(1-Benzyl-1H-1,2,3-triazol-4-yl)-30-norlup-20(29)-ene-3β,28-diol ***16c.*** 20-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-30-norlup-20(29)-ene-3β,28-diol **16c** was prepared according to the general procedure **D** using benzylazide. The reaction was monitored by TLC (hexane/EtOAc 1/1), which indicated a complete reaction after 96 h. The solvent was evaporated, the residue was dissolved in EtOAc, washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography on a silica gel with gradient elution starting at hexane/EtOAc 1/1 and finishing at hexane/EtOAc 2/3. Collected fractions were evaporated yielding a white solid of **16c**. Yield: 76 mg (59 %), m. p. 124–126 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.75 (s, 3H), 0.79 (s, 3H), 0.92 (s, 3H), 0.96 (s, 3H), 1.00 (s, 3H, 5 × CH_3), 2.80 (td, J_1 = 11.3 Hz, J_2 = 5.6 Hz, 1H, H-19β), 3.17 (dd, J_1 = 11.3 Hz, J_2 = 4.7 Hz, 1H, H-3α), 3.39 (d, J = 10.8 Hz, 1H, H-28a), 3.81 (d, J = 10.1 Hz, 1H, H-28b), 5.10 (s, 1H, H-29-*pro E*), 5.50 (s, 1H, H-29-*pro Z*), 5.51 (s, 2H, phenyl-CH_2), 7.26 (s, 1H, triazole), 7.27 (d, J = 2.1 Hz, 1H), 7.34–7.41 (m, 4H, 5H-phenyl); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.87, 15.50, 16.14, 16.23, 18.44, 21.04, 22.83, 27.21, 27.53, 28.14, 29.43, 29.50, 29.84, 32.43, 33.89, 34.42, 37.28, 37.30, 38.83, 39.00, 41.06, 42.82, 47.98, 50.33, 50.45, 54.22, 55.44, 60.65, 79.12, 110.58, 119.97, 128.13, 128.85, 129.26, 134.97, 144.76, 149.55; **IR** (DRIFT): ν_{max} = 1030 (C–O); 1079 (C–O); 1635 (C═C); 1954 (C–H, Ar); 3386 (O–H) cm^{-1}; **HRMS** (ESI$^+$): calcd for $C_{38}H_{55}N_3O_2$ $[M+H]^+$ 586.4367; found 586.4371.

4.1.16.4. 20-[1-(4-Carboxyphenylmethyl)-1H-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol ***16d.*** 20-[1-(4-Carboxyphenylmethyl)-1*H*-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol **16d** was synthesized according to the general procedure **D** using 4-(azidomethyl)benzoic acid. The reaction was monitored by TLC (hexane/EtOAc 5/1 + a drop of AcOH) and was discontinued after 7 days with incomplete conversion. The solvent was removed under reduce pressure and the residue was purified by column chromatography on a silica with hexane/EtOAc 1/2 and 0.1 % of AcOH. Collected fractions were evaporated yielding a white solid of **16d**. Yield: 82 mg (59 %), m. p. 170–173 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $(CD_3)_2SO$): δ = 0.64 (s, 3H), 0.73 (s, 3H), 0.84 (s, 3H), 0.87 (s, 3H), 0.96 (s, 3H, 5 × CH_3), 2.74 (td, J_1 = 11.1 Hz, J_2 = 5.7 Hz, 1H, H-19β), 2.92–3.01 (m, 1H, H-3α), 3.16 (d, J = 9.9 Hz, 1H, H-28a), 3.55 (d, J = 9.9 Hz, 1H, H-28b), 4.20–4.27 (m, 2H, 2 × OH), 5.06 (s, 1H, H-29-*pro E*), 5.53 (s, 1H, H-29-*pro Z*), 5.66 (s, 2H, phenyl-CH_2), 7.36 (d, J = 8.1 Hz, 2H, phenyl-*ortho*), 7.93 (d, J = 8.1 Hz, 2H, phenyl-*meta*), 8.29 (s, 1H, triazole), 12.97 (s, 1H, COOH); **^{13}C NMR** (126 MHz, $(CD_3)_2SO$): δ = 14.41, 15.67, 15.77, 15.83, 17.93, 20.40, 26.67, 27.12, 28.06, 29.00, 30.36, 30.64, 33.43, 33.82, 36.58, 36.61, 38.20, 38.45, 40.41, 40.43, 42.12, 47.41, 49.55, 49.73, 52.32, 54.78, 57.91, 76.71, 121.96, 127.68, 128.10, 129.68, 129.78, 130.48, 140.93, 144.73, 166.84.; **IR** (DRIFT): ν_{max} = 1070 (C–O); 1088 (C–O); 1615 (C═C); 1685 (C═O); 3362 (O–H) cm^{-1}; **HRMS** (ESI$^+$): calcd for $C_{39}H_{55}N_3O_4$ $[M+H]^+$ 630.4265; found 630.4267.

4.1.16.5. 20-{1-[3-(Trifluoromethyl)phenylmethyl]-1H-1,2,3-triazol-4-yl}-30-norlup-20(29)-ene-3β,28-diol ***16e.*** 20-{1-[3-(Trifluoromethyl) phenylmethyl]-1*H*-1,2,3-triazol-4-yl}-30-norlup-20(29)-ene-3β,28-diol **16e** was prepared according to the general procedure **D** utilizing 1-(azidomethyl)-3-(trifluoromethyl)benzene. The reaction conversion was monitored by TLC (hexane/EtOAc 2/1) and the completion of the reaction was indicated after 96 h. The solvent was removed under reduced pressure, the residue was dissolved in dichloromethane, washed with brine (3 times), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue purified was purified by column chromatography on a silica gel with hexane/EtOAc 1/1. Collected fractions were evaporated yielding a white solid of **16e**. Yield: 104 mg (72 %), m. p. 115–118 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.74 (s, 3H), 0.79 (s, 3H), 0.91 (s, 3H), 0.96 (s, 3H), 1.00 (s, 3H, 5 × CH_3), 2.80 (td, J_1 = 11.3 Hz, J_2 = 5.6 Hz, 1H, H-19β), 3.16 (dd, J_1 = 11.3 Hz, J_2 = 4.7 Hz, 1H, H-3α), 3.40 (d, J = 10.8 Hz, 1H, H-28a), 3.82 (d, J = 10.1 Hz, 1H, H-28b), 5.13 (s, 1H, H-29-*pro E*), 5.52 (s, 1H, H-29-*pro Z*), 5.57 (s, 2H, phenyl-CH_2), 7.42–7.46 (m, 2H, 1H-triazole, 1H, phenyl-*para*), 7.51 (t, J = 7.8 Hz, 1H, phenyl-*meta*), 7.55 (s, 1H, phenyl-*ortho*), 7.63 (d, J = 7.7 Hz, 1H, phenyl-*ortho*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.82, 15.50, 16.14, 16.21, 18.43, 21.03, 22.83, 27.20, 27.52, 28.13, 29.44, 29.50, 29.84, 31.05, 32.42, 33.90, 34.41, 37.27, 37.31, 38.81, 39.00, 41.06, 42.81, 47.99, 50.43, 53.60, 55.42, 60.66, 79.12, 110.94, 123.84 (q, J = 272.3 Hz, CF_3), 124.79 (q, J = 3.4 Hz, phenyl-*ortho*), 125.78 (q, J = 3.3 Hz, phenyl-*ortho*), 129.91, 131.36, 131.73 (q, J = 32.7 Hz, C-CF_3), 136.01, 144.58, 149.88; **IR** (DRIFT): ν_{max} = 1030 (C–O); 1076 (C–O); 1328 (C–F); 1629 (C═C); 3385 (O–H) cm^{-1}; **HRMS** (ESI$^+$): calcd for $C_{39}H_{54}F_3N_3O_2$ $[M+H]^+$ 654.4241; found 654.4243.

4.1.16.6. 20-[1-(4-Fluorophenylmethyl)-1H-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol ***16f.*** 20-[1-(4-Fluorophenylmethyl)-1*H*-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol **16f** was prepared according to the general procedure **D** using 1-(azidomethyl)-4-fluorobenzene. The reaction conversion was monitored by TLC (hexane/EtOAc 1/1), which indicated a complete reaction after 6 h. The solvent was removed under reduced pressure and was the residue was dissolved in dichloromethane. The solution was washed with brine (3 times), dried over $MgSO_4$, filtered and evaporated in vacuo. The residue purified was purified by column chromatography on a silica gel with gradient elution starting at hexane/EtOAc 1/1 and finishing at hexane/EtOAc 1/2. Collected fractions were evaporated yielding a white solid of **16f**. Yiled: 92 mg (69 %), m. p. 113–114 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.74 (s, 3H), 0.79 (s, 3H), 0.91 (s, 3H), 0.96 (s, 3H), 1.00 (s, 3H, 5 × CH_3), 2.79 (td, J_1 = 11.3 Hz, J_2 = 5.6 Hz, 1H, H-19β), 3.17 (dd, J_1 = 11.3 Hz, J_2 = 4.7 Hz, 1H, H-3α), 3.39 (d, J = 10.8 Hz, 1H, H-28a), 3.81 (d, J = 10.7 Hz, 1H, H-28b), 5.10 (s, 1H, H-29-*pro E*), 5.48 (s, 2H, phenyl-CH_2), 5.50 (s, 1H, H-29-*pro Z*), 7.03–7.09 (m, 2H), 7.24–7.29 (m, 2H, 4H-phenyl), 7.37 (s, 1H, triazole); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.85, 15.50, 16.13, 16.22, 18.43, 21.04, 27.19, 27.51, 28.12, 29.44, 29.83, 32.41, 33.88, 34.40, 37.26, 37.29, 38.83, 38.99, 41.05, 42.81, 43.45, 47.97, 50.31, 50.45, 53.47, 55.42, 60.62, 79.09, 110.63, 116.26 (d, J = 21.8 Hz, C–C–F), 119.85, 129.99 (d, J = 8.4 Hz, C–C–C–F), 130.81 (d, J = 3.2 Hz, C–C–C–C–F), 144.66, 149.68, 162.98 (d, J = 248.0 Hz, C–F); **IR** (DRIFT): ν_{max} = 1030 (C–O); 1107 (C–O); 1226 (C–F); 1608 (C═C); 3365 (O–H) cm^{-1}; **HRMS** (ESI$^+$): calcd for $C_{38}H_{54}FN_3O_2$ $[M+H]^+$ 604.4273; found 604.4274.

4.1.16.7. 20-[1-(4-Formylphenylmethyl)-1H-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol ***16g.*** 20-[1-(4-Formylphenylmethyl)-1*H*-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol **16g** was synthesized according to the general procedure **D** using 4-(azidomethyl) benzaldehyde. The reaction was monitored by TLC (hexane/EtOAc 2/1) and completed after 2 h. The solvent was removed under reduced pressure, dissolved in dichloromethane, washed with brine 3 times, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue purified was purified by column chromatography on a silica gel with gradient elution starting at hexane/EtOAc 1/2 and finishing at hexane/EtOAc 1/3. Collected fractions were evaporated yielding a white solid of **16g**. Yiled: 111 mg (82 %), m. p. 100–104 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.74 (s, 3H), 0.79 (s, 3H), 0.92 (s, 3H), 0.96 (s, 3H), 1.00 (s, 3H, 5 × CH_3), 2.80 (td, J_1 = 11.3 Hz, J_2 = 5.6 Hz, 1H, H-19β), 3.17 (dd, J_1 = 11.3 Hz, J_2 = 4.6 Hz, 1H, H-3α), 3.39 (d, J = 10.8 Hz, 1H, H-28a), 3.82 (d, J = 10.8 Hz, 1H, H-28b), 5.13 (s, 1H, H-29-*pro E*), 5.52 (s, 1H, H-29-*pro Z*), 5.60 (s, 2H, phenyl-CH_2), 7.40 (d, J = 8.1 Hz, 2H, phenyl-*meta*), 7.44 (s, 1H, triazole), 7.89 (d, J = 8.2 Hz, 2H, phenyl-*ortho*), 10.02 (s, 1H, CHO); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.85, 15.50, 16.13, 16.22, 18.42, 21.05, 27.19, 27.24, 27.51, 28.12, 29.44, 29.49, 29.82, 33.89, 34.40, 37.27, 37.30, 38.85, 38.99, 41.06, 42.82, 47.99, 50.36, 50.44, 53.70, 55.42, 60.63, 79.06, 110.92, 120.16, 128.42, 130.55, 136.65, 141.44, 144.54, 149.91, 191.50; **IR**

(DRIFT): ν_{max} = 1030 (C–O); 1107 (C–O); 1610 (C═C); 1694 (C═O); 3385 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{39}H_{55}N_3O_3$ $[M+H]^+$ 614.4316; found 614.4319.

*4.1.16.8. 20-[1-(Pyridin-3-ylmethyl)-1H-1,2,3-triazol-4-yl]-30-norlup-20 (29)-ene-3β,28-diol **16h**.* 20-[1-(Pyridin-3-ylmethyl)-1*H*-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol **16h** was synthesized according to the general procedure **D** utilizing β-(azidomethyl)pyridine. The reaction conversion was monitored by TLC (hexane/EtOAc 2/1) and the reaction completion was indicated after 72 h. The reaction solvent was removed and the residue was dissolved again in EtOAc. The solution was 3 times washed with brine, dried over $MgSO_4$, filtered and evaporated under reduce pressure. The residue purified was purified by column chromatography on a silica gel with gradient elution starting at dichloromethane/MeOH 25/1 and finishing at dichloromethane/MeOH 15/1. Collected fractions were evaporated yielding a white solid of **16h**. Yiled: 54 mg (42 %), m. p. 167–171 °C (dichloromethane/hexane); **^{1}H NMR** (500 MHz, CD_3OD): δ = 0.75 (s, 3H), 0.83 (s, 3H), 0.89 (s, 3H), 0.95 (s, 3H), 1.05 (s, 3H, 5 × CH_3), 2.78 (td, J_1 = 11.3 Hz, J_2 = 5.6 Hz, 1H, H-19β), 3.11 (dd, J_1 = 11.1 Hz, J_2 = 4.8 Hz, 1H, H-3α), 3.35 (d, J = 11.1 Hz, 1H, H-28a), 3.77 (d, J = 10.9 Hz, 1H, H-28b), 5.16 (s, 1H, H-29-*pro E*), 5.56 (s, 1H, H-29-*pro Z*), 5.68 (s, 2H, Aryl-CH_2), 7.41–7.50 (m, 1H, pyridine), 7.81 (dt, J_1 = 7.9 Hz, J_2 = 1.8 Hz, 1H, pyridine), 8.09 (s, 1H, triazole), 8.53 (d, J = 4.0 Hz, 1H, pyridine), 8.59 (s, 1H, pyridine). **^{13}C NMR** (126 MHz, CD_3OD): δ = 15.15, 16.11, 16.54, 16.66, 19.42, 22.01, 28.02, 28.15, 28.36, 28.61, 30.42, 33.20, 34.79, 35.48, 38.25, 38.51, 39.93, 40.02, 42.12, 43.76, 45.07, 49.63, 51.61, 51.72, 52.08, 56.78, 60.35, 79.63, 111.56, 122.72, 125.56, 133.68, 137.89, 146.00, 149.74, 150.24, 150.43; **IR** (DRIFT): ν_{max} = 1030 (C–O); 1107 (C–O); 1636 (C═C); 3366 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{37}H_{54}N_4O_2$ $[M+H]^+$ 587.4320; found 587.4322.

*4.1.16.9. 20-[1-(Thiophen-2-ylmethyl)-1H-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol **16i**.* 20-[1-(Thiophen-2-ylmethyl)-1*H*-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol **16i** was prepared according to the general procedure **D** using 2-(azidomethyl)thiophene. The reaction was monitored until complete conversion, which was indicated by TLC (hexane/EtOAc 3/1) after 24 h. The solvent was then removed under reduced pressure, and the residue was dissolved in EtOAc. The solution was 3 times washed with brine, dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography on a silica gel with hexane/EtOAc/$CHCl_3$ 10/10/1. Removal of the solvents provided a white solid of **16i**. Yield 88 mg (67 %), m. p. 140–143 °C ($CHCl_3$/hexane); **^{1}H NMR** (500 MHz, ($CDCl_3$): δ = 0.75 (s, 3H), 0.79 (s, 3H), 0.92 (s, 3H), 0.96 (s, 3H), 1.00 (s, 3H, 5 × CH_3), 2.80 (td, J_1 = 11.3 Hz, J_2 = 5.6 Hz, 1H, H-19β), 3.16 (dd, J_1 = 11.3 Hz, J_2 = 4.7 Hz, 1H, H-3α), 3.40 (d, J = 10.8 Hz, 1H, H-28a), 3.82 (dd, J_1 = 10.9 Hz, J_2 = 1.1 Hz, 1H, H-28b), 5.11 (s, 1H, H-29-*pro E*), 5.51 (s, 1H, H-29-*pro Z*), 5.69 (s, 2H, aryl-CH_2), 7.02 (dd, J_1 = 5.1 Hz, J_2 = 3.5 Hz, 1H, thiophene), 7.11 (dd, J_1 = 3.6 Hz, J_2 = 0.9 Hz, 1H, thiophene), 7.34 (dd, J_1 = 5.1 Hz, J_2 = 1.2 Hz, 1H, thiophene), 7.44 (s, 1H, triazole); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.87, 15.50, 16.14, 16.22, 18.43, 21.04, 27.21, 27.52, 28.13, 29.44, 29.83, 32.39, 33.89, 34.41, 37.27, 37.30, 38.83, 38.99, 41.06, 42.82, 43.44, 47.98, 48.62, 50.28, 50.44, 55.42, 60.64, 79.11, 110.67, 119.64, 127.17, 127.48, 128.24, 136.36, 144.62, 149.45; **IR** (DRIFT): ν_{max} = 1014 (C–O); 1074 (C–O); 1631 (C═C); 3374 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{36}H_{54}N_3O_2S$ $[M+H]^+$ 592.3931; found 592.3930.

*4.1.16.10. 20-[1-(Furan-2-ylmethyl)-1H-1,2,3-triazol-4-yl]-30-norlup-20 (29)-ene-3β,28-diol **16j**.* 20-[1-(Furan-2-ylmethyl)-1*H*-1,2,3-triazol-4-yl]-30-norlup-20(29)-ene-3β,28-diol **16j** was obtained by following general procedure **D** with 2-(azidomethyl)furan. The reaction was monitored by TLC (hexane/EtOAc 3/1) until complete conversion was spotted (24 h). The reaction solvent was removed and the residue was dissolved again in EtOAc. The solution was washed with brine 3 times, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography on a silica gel with hexane/EtOAc 1/1. Collected fractions were evaporated yielding **16j** as a white solid. Yield 107 mg (84 %), m. p. 121–124 °C ($CHCl_3$/hexane); **^{1}H NMR** (500 MHz, ($CDCl_3$): δ = 0.74 (s, 3H), 0.79 (s, 3H), 0.93 (s, 3H), 0.96 (s, 3H), 1.00 (s, 3H, 5 × CH_3), 2.81 (td, J_1 = 11.3 Hz, J_2 = 5.6 Hz, 1H, H-19β), 3.16 (dd, J_1 = 11.3 Hz, J_2 = 4.7 Hz, 1H, H-3α), 3.41 (d, J = 10.8 Hz, 1H, H-28a), 3.83 (d, J = 10.5 Hz, 1H, H-28b), 5.11 (s, 1H, H-29-*pro E*), 5.51 (s, 2H, aryl-CH_2), 5.52 (s, 1H, H-29-*pro Z*), 6.39 (dd, J_1 = 3.2 Hz, J_2 = 1.9 Hz, 1H, furan), 6.45 (d, J = 3.2 Hz, 1H, furan), 7.44 (d, J = 1.3 Hz, 1H, furan), 7.48 (s, 1H, triazole); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.86, 15.50, 16.14, 16.22, 18.44, 21.05, 27.22, 27.52, 28.13, 29.29, 29.46, 32.41, 33.89, 34.41, 37.27, 37.30, 38.82, 38.99, 41.07, 42.82, 43.43, 46.81, 47.98, 50.25, 50.44, 55.43, 60.64, 79.11, 110.37, 111.00, 119.85, 143.75, 144.62, 147.57, 149.40; **IR** (DRIFT): ν_{max} = 1013 (C–O); 1074 (C–O); 1631 (C═C); 3377 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{36}H_{54}N_3O_3$ $[M+H]^+$ 576.4160; found 576.4161.

*4.1.17. 30-Methylidynebetulonic acid **14***

Suspension of 30-methylidynebetulin **10** (1 g, 2.21 mmol) in acetone (30 mL) was cooled to 0 °C. Solution of CrO_3 (3.4 g, 34 mmol) in H_2SO_4 (3.36 mL, 95 %) was cooled to 0 °C too and cautiously diluted with distilled water (8.10 mL). The solution of Jones reagent was added to the stirred suspension of the starting compound dropwise over 10 min and the reaction then continued at 0 °C. The reaction was monitored by TLC (toluene/Et_2O 5/1), which indicated a complete reaction after 30 min. Then EtOAc was added and the mixture was washed 3 times with brine, which contained 0.1 % AcOH (v/v). The collected organic phase was dried over $MgSO_4$, filtered and evaporated under reduce pressure. The residue purified was purified by column chromatography on a silica gel with hexane/EtOAc 2/1 + 0.1 % of AcOH. Collected fractions were evaporated yielding a white solid of **14**. Yiled: 493 mg (48 %), m. p. 178–180 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.93 (s, 3H), 0.98 (s, 3H), 1.00 (s, 3H), 1.02 (s, 3H), 1.07 (s, 3H, 5 × CH_3), 2.92 (s, 1H, C≡C–H), 3.10 (td, J_1 = 10.7 Hz, J_2 = 4.7 Hz, 1H, H-19β), 5.32 (d, J = 1.8 Hz, 1H, H-29 *pro-E*), 5.34 (d, J = 1.8 Hz, 1H, H-29 *pro-Z*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.73, 15.93, 16.07, 19.79, 21.16, 21.40, 25.76, 26.81, 29.84, 30.77, 32.04, 33.74, 34.27, 36.97, 37.06, 38.62, 39.75, 40.74, 42.73, 46.78, 47.48, 49.80, 50.54, 55.08, 56.34, 78.52, 82.88, 122.30, 137.31, 181.53, 218.31; **IR** (DRIFT): ν_{max} = 1028 (C–O); 1105 (C–O); 1686 (C═C); 1705 (C═O); 2116 (C≡C); 3293 (C–H alkyne); 3376 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{31}H_{44}O_3$ $[M+H]^+$ 465.3363; found 465.3365.

*4.1.18. General procedure for the synthesis of triazoles **17a–17j** (procedure E)*

Appropriate azides needed for the synthesis of triazoles **17a–17h** were prepared firstly. NaN_3 (56 mg, 0.860 mmol) and appropriate bromide (0.430 mmol) were dissolved in dimethylformamide (20 mL) at r.t. The reaction was monitored by TLC and was completed after 24 h. The reaction mixture was diluted with dichloromethane, washed with brine 3 times, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Freshly prepared azides were directly utilized in the synthesis of triazoles **17a–17h**. Azides for the synthesis of triazoles **17i–17j** were prepared the following way. 1,8-Diazabicyklo[5.4.0] undec-7-en (79 μL, 81 mg, 0.530 mmol) was added to a stirred solution of appropriate alcohol (0.442 mmol) and diphenylphosphoryl azide (114 μL, 146 mg, 0.530 mmol) in dry toluene (1 mL) cooled to 0 °C. The reaction was cooled down to 0 °C and under argon for 2 h and then the temperature was raised to r.t. The reaction was diluted with water and extracted with toluene 3 times. The collected organic phase was washed with brine, dried over $MgSO_4$, and after filtration the solvent was evaporated. Freshly prepared azides were used directly in the synthesis of the triazoles **17i–17j**. 30-methylidynebetulonic acid **14** (100 mg,

0.215 mmol) and appropriate azide (0.430 mmol) were dissolved in dry tetrahydrofuran (26 mL), after which copper(I) 3-methylsalicylate (4.7 mg, 0.022 mmol) was added. The reaction continued under argon at 60 °C.

4.1.18.1. 20-[1-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid ***17a.*** 20-[1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17a** was synthesized according to the general procedure **E** using 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylazide. The reaction conversion was monitored by TLC (hexane/EtOAc 2/1 + a drop of AcOH) and indicated a completion of the reaction after 4 h. The solvent was evaporated and the residue was dissolved again in dichloromethane. The mixture was washed with brine 3 times, which contained 0.1 % (v/v) of AcOH. The collected organic phase was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue purified was purified by column chromatography on a silica gel with gradient elution starting at hexane/EtOAc 3/2 + 0.1 % of AcOH and finishing at hexane/EtOAc 2/3 + 0.1 % of AcOH. Collected fractions were evaporated yielding a white solid of **17a**. Yiled: 147 mg (97 %), m. p. 153–155 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$): $\delta = 0.91$ (s, 3H), 0.93 (s, 3H), 0.97 (s, 3H), 1.00 (s, 3H), 1.05 (s, 3H, 5 × CH_3), 1.87 (s, 3H), 2.03 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H, 4 × AcO), 3.36 (td, J_1 = 11.1 Hz, J_2 = 4.6 Hz, 1H, H-19β), 4.01 (ddd, J_1 = 10.1 Hz, J_2 = 5.0 Hz, J_3 = 2.1 Hz, 1H, H-5′), 4.16 (dd, J_1 = 12.6 Hz, J_2 = 2.0 Hz, 1H, H-6′), 4.31 (dd, J_1 = 12.6 Hz, J_2 = 5.1 Hz, 1H, H-6′), 5.19 (s, 1H, H-29-*pro E*), 5.25 (t, J = 10.0 Hz, 1H, H-4′), 5.42 (t, J = 9.8 Hz, 1H, H-3′), 5.46 (t, J = 9.3 Hz, 1H, H-2′), 5.62 (s, 1H, H-29-*pro Z*), 5.86 (d, J = 8.9 Hz, 1H, H-1′), 7.74 (s, 1H, triazole); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.74, 16.03, 16.06, 18.89, 19.75, 20.35, 20.67, 20.85, 21.13, 21.57, 22.78, 26.74, 29.20, 29.87, 31.72, 32.12, 32.45, 33.77, 34.26, 36.82, 37.04, 38.68, 39.75, 40.76, 42.60, 47.47, 49.92, 50.45, 55.12, 56.56, 61.79, 67.93, 70.51, 72.86, 75.32, 85.92, 118.58, 143.99, 149.62, 168.89, 169.51, 170.06, 170.63, 181.38, 218.25; **IR** (DRIFT): ν_{max} = 1214 (C–O esters); 1642 (C═C); 1697 (C═O); 1751 (C═O) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{45}H_{63}N_3O_{12}$ $[M+H]^+$ 838.4485; found 838.4488.

4.1.18.2. 20-[1-(β-D-Glucopyranosyl)-1H-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid ***17b.*** 20-[1-(β-D-Glucopyranosyl)-1*H*-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17b** was prepared by the general procedure **E** using β-D-gluckopyranosylazide. The reaction monitoring was processed by TLC ($CHCl_3$/MeOH 5/1 + a drop of AcOH) and indicated a complete reaction after 72 h. The reaction mixture concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel with $CHCl_3$/MeOH 5/1 + 0.1 % of AcOH. Collected fractions were evaporated yielding a white solid of **17b**. Yiled: 26 mg (18 %), m. p. 160–161 °C ($CHCl_3$/MeOH); **^{1}H NMR** (500 MHz, $(CD_3)_2SO$): δ = 0.83 (s, 3H), 0.92 (s, 6H), 0.95 (s, 3H), 0.98 (s, 3H, 5 × CH_3), 3.43–3.48 (m, 2H, H-6′ and H-5′), 3.70 (d, J = 10.1 Hz, 1H, H-6′), 3.81 (t, J = 9.0 Hz, 1H, H-4′), 4.60 (s, 1H, H-3′), 5.11 (s, 1H, H-29-*pro E*), 5.38 (s, 1H, H-2′), 5.50 (d, J = 9.3 Hz, 1H, H-1′), 5.58 (s, 1H, H-29-*pro Z*), 8.35 (s, 1H, triazole); **^{13}C NMR** (126 MHz, $(CD_3)_2SO$): δ = 13.46, 14.35, 15.51, 15.64, 19.15, 20.67, 21.11, 23.04, 26.39, 26.63, 26.72, 29.34, 31.61, 31.68, 33.12, 33.58, 36.09, 36.34, 37.63, 38.78, 42.05, 46.48, 49.02, 49.63, 53.80, 55.51, 60.76, 64.95, 69.57, 71.89, 76.55, 76.98, 79.94, 87.48, 120.62, 144.95, 147.79, 216.53; **IR** (DRIFT): ν_{max} = 1698 (C═O); 3327 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{37}H_{55}N_3O_8$ $[M+H]^+$ 670.4062; found 670.4073.

4.1.18.3. 20-(1-Benzyl-1H-1,2,3-triazol-4-yl)-3-oxo-30-norlup-20(29)-en-28-oic acid ***17c.*** 20-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-3-oxo-30-norlup-20(29)-en-28-oic acid **17c** was synthesized according to the general procedure **E** utilizing benzylazide. The reaction was monitored by TLC (hexane/EtOAc 2/1 + a drop of AcOH), which indicated a complete reaction conversion after 24 h. The reaction solvent was removed under reduced pressure and the residue was dissolved in dichloromethane. The solution was washed with brine 3 times, which contained 0.1 % (v/v) of AcOH, dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by column chromatography on a silica gel with gradient elution starting at hexane/EtOAc 2/1 + 0.1 % of AcOH and finishing at hexane/EtOAc 1/1 + 0.1 % of AcOH. Collected fractions were evaporated yielding a white solid of **17c**. Yiled: 76 mg (59 %), m. p. 106–108 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.89 (s, 3H), 0.93 (s, 3H), 0.95 (s, 3H), 1.00 (s, 3H), 1.06 (s, 3H, 5 × CH_3), 3.32 (td, J_1 = 11.1 Hz, J_2 = 4.6 Hz, 1H, H-19β), 5.13 (s, 1H, H-29-*pro E*), 5.52 (s, 2H, phenyl-CH_2), 5.59 (s, 1H, H-29-*pro Z*), 7.25–7.28 (m, 2H), 7.33–7.40 (m, 3H, 5H-phenyl), 7.43 (s, 1H, triazole); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.25, 14.76, 16.01, 16.08, 19.77, 21.13, 21.57, 26.81, 26.91, 29.84, 29.88, 32.16, 32.59, 33.77, 34.24, 36.87, 37.03, 38.68, 39.72, 40.76, 42.61, 47.46, 49.91, 50.32, 54.20, 55.06, 56.57, 120.33, 128.04, 128.82, 129.24, 135.04, 144.41, 181.63, 218.29; **IR** (DRIFT): ν_{max} = 1638 (C═C); 1695 (C═O) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{38}H_{51}N_3O_3$ $[M+H]^+$ 598.4003; found 598.4013.

4.1.18.4. 20-[1-(4-Carboxyphenylmethyl)-1H-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid ***17d.*** 20-[1-(4-Carboxyphenylmethyl)-1*H*-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17d** was prepared by general procedure **E** using 4-(azidomethyl)benzoic acid. The reaction was monitored by TLC (hexane/EtOAc 2/1 + a drop of AcOH) and after 76 h the reaction was discontinued with incomplete reaction conversion. The reaction solvent was removed under reduced pressure and the residue was dissolved in dichloromethane. The solution was washed with brine (3 times), which contained 0.1 % of AcOH, dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by column chromatography on a silica gel with hexane/EtOAc 1/1 + 0.1 % of AcOH. Collected fractions were evaporated yielding a white solid of **17d**. Yiled: 58 mg (42 %), m. p. 145–148 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.87 (s, 3H), 0.94 (s, 6H), 0.99 (s, 3H), 1.06 (s, 3H, 5 × CH_3), 3.24 (td, J_1 = 11.0 Hz, J_2 = 3.9 Hz, 1H, H-19β), 5.15 (s, 1H, H-29-*pro E*), 5.56–5.65 (m, 2H, phenyl-CH_2), 5.70 (s, 1H, H-29-*pro Z*), 7.34 (d, J = 8.2 Hz, 2H, phenyl-*meta*), 7.44 (s, 1H, triazole), 8.09 (d, J = 8.1 Hz, 2H, phenyl-*ortho*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.76, 15.97, 16.09, 19.78, 21.14, 21.62, 26.82, 27.11, 29.84, 32.12, 32.82, 33.76, 34.22, 36.91, 37.02, 38.60, 39.73, 40.78, 42.62, 47.48, 49.95, 50.51, 53.77, 55.03, 56.63, 110.81, 120.40, 128.01, 128.07, 130.04, 131.07, 140.65, 144.06, 149.95, 170.64, 182.49, 218.76; **IR** (DRIFT): ν_{max} = 1616 (C═C); 1691 (C═O) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{39}H_{51}N_3O_5$ $[M+H]^+$ 642.3901; found 642.3908.

4.1.18.5. 20-{1-[3-(Trifluoromethyl)phenylmethyl]-1H-1,2,3-triazol-4-yl}-3-oxo-30-norlup-20(29)-en-28-oic acid ***17e.*** 20-{1-[3-(Trifluoromethyl)phenylmethyl]-1*H*-1,2,3-triazol-4-yl}-3-oxo-30-norlup-20(29)-en-28-oic acid **17e** was prepared according to the general procedure **E** using 1-(azidomethyl)-3-(trifluoromethyl)benzene. The reaction conversion was monitored by TLC (hexane/EtOAc 2/1 + a drop of AcOH), which indicated a complete reaction after 48 h. The reaction solvent was removed under vacuum and the residue was dissolved again in dichloromethane. The solution was washed with brine 3 times, which contained 0.1 % of AcOH, dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by column chromatography on a silica gel with gradient elution starting at hexane/EtOAc 2/1 + 0.1 % of AcOH and finishing at hexane/EtOAc 1/1 + 0.1 % of AcOH. Collected fractions were evaporated yielding a white solid of **17e**. Yiled: 70 mg (49 %), m. p. 118–120 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.88 (s, 3H), 0.92 (s, 3H), 0.95 (s, 3H), 1.00 (s, 3H), 1.06 (s, 3H, 5 × CH_3), 3.33 (td, J_1 = 11.1 Hz, J_2 = 4.7 Hz, 1H, H-19β), 5.15 (s, 1H, H-29-*pro E*), 5.58 (s, 2H, phenyl-CH_2), 5.62 (s, 1H, H-29-*pro Z*), 7.45 (d, J = 7.8 Hz, 1H, phenyl-*para*), 7.48 (s, 1H, triazole), 7.51 (t, J = 7.8 Hz, 1H,

phenyl-*meta*), 7.54 (s, 1H, phenyl-*ortho*), 7.62 (d, $J = 7.7$ Hz, 1H, phenyl-*ortho*); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.71, 15.99, 16.07, 19.76, 21.11, 21.55, 26.82, 26.97, 29.88, 32.14, 32.66, 33.75, 34.22, 36.87, 37.02, 38.69, 39.68, 40.75, 42.60, 43.51, 47.44, 49.88, 50.41, 53.57, 55.03, 56.59, 111.11, 120.36, 123.85 (q, $J = 272.5$ Hz, CF_3), 124.72 (q, $J = 3.2$ Hz, phenyl-*ortho*), 125.75 (q, $J = 3.4$ Hz, phenyl-*ortho*), 129.88, 131.31, 131.62 (q, $J = 33.0$ Hz, C-CF_3), 136.07, 144.26, 149.82, 181.87, 218.28; **IR** (DRIFT): ν_{max} = 1328 (C–F); 1696 (C═O) cm^{-1}; **HRMS** (ESI$^+$): calcd for $C_{39}H_{50}F_3N_3O_3$ $[M+H]^+$ 666.3877; found 666.3884.

*4.1.18.6. 20-[1-(4-Fluorophenylmethyl)-1H-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17f**.* 20-[1-(4-Fluorophenylmethyl)-1*H*-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17f** was synthesized according to the general procedure **E** utilizing 1-(azidomethyl)-4-fluorobenzene. The reaction was monitored by TLC (hexane/EtOAc 2/1 + a drop of AcOH), which indicated a complete reaction after 24 h. The reaction solvent was removed under reduced pressure and the residue was dissolved again in dichloromethane. The solution was washed with brine (3 times), which contained 0.1 % of AcOH, dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by column chromatography on a silica gel with gradient elution starting at hexane/EtOAc 2/1 + 0.1 % of AcOH and finishing at hexane/EtOAc 1/1 + 0.1 % of AcOH. Collected fractions were removed under vacuum yielding a white solid of **17f**. Yiled: 110 mg (83 %), m. p. 155–158 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$): δ = 0.89 (s, 3H), 0.93 (s, 3H), 0.95 (s, 3H), 1.00 (s, 3H), 1.06 (s, 3H, 5 × CH_3), 3.32 (td, $J_1 = 11.1$ Hz, $J_2 = 4.7$ Hz, 1H, H-19β), 5.13 (s, 1H, H-29-*pro E*), 5.49 (s, 2H, phenyl-CH_2), 5.60 (s, 1H, H-29-*pro Z*), 7.03–7.08 (m, 2H, phenyl-*meta*), 7.24–7.29 (m, 2H, phenyl-*ortho*), 7.42 (s, 1H, triazole); **^{13}C NMR** (126 MHz, $CDCl_3$): δ = 14.76, 16.01, 16.09, 19.77, 21.12, 21.59, 22.83, 26.83, 26.98, 29.50, 29.84, 29.88, 32.07, 32.15, 32.66, 33.77, 34.22, 36.86, 37.03, 38.68, 39.72, 40.76, 42.62, 43.50, 47.44, 49.91, 50.33, 53.47, 55.04, 56.57, 110.87, 116.25 (d, $J = 22.0$ Hz, C–C–F), 120.17, 129.96 (d, $J = 8.4$ Hz, C–C–C–F), 130.88 (d, $J = 3.1$ Hz, C–C–C–C–F), 144.33, 149.65, 162.99 (d, $J = 247.9$ Hz, C–F), 181.46, 218.25; **IR** (DRIFT): ν_{max} = 1225 (C–F); 1636 (C═C); 1698 (C═O); 1870 (C–H, Ar) cm^{-1}; **HRMS** (ESI$^+$): calcd for $C_{38}H_{50}FN_3O_3$ $[M+H]^+$ 616.3909; found 616.3918.

*4.1.18.7. 20-[1-(4-Formylphenylmethyl)-1H-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17g**.* 20-[1-(4-Formylphenylmethyl)-1*H*-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17g** was synthesized according to the general procedure **E** using 4-(azidomethyl) benzaldehyde. The reaction was monitored by TLC (hexane/EtOAc 2/1 + a drop of AcOH), which indicated a complete reaction conversion after 72 h. The reaction solvent was removed under vacuum and the residue was dissolved again in dichloromethane. The solution was washed with brine (3 times), which contained 0.1 % of AcOH, dried over $MgSO_4$, filtered and concentrated under reduce pressure. The residue was purified by column chromatography on a silica gel with gradient elution starting at hexane/EtOAc 2/1 + 0.1 % of AcOH and finishing at hexane/EtOAc 1/1 + 0.1 % of AcOH. Collected fractions were removed under vacuum yielding a white solid of **17g**. Yiled: 53 mg (39 %), m. p. 84–87 °C (hexane/EtOAc); **^{1}H NMR** (500 MHz, $(CD_3)_2SO$): δ = 0.82 (s, 3H), 0.85 (s, 3H), 0.89 (s, 3H), 0.92 (s, 3H), 0.98 (s, 3H, 5 × CH_3), 3.36 (td, $J_1 = 11.2$ Hz, $J_2 = 5.1$ Hz, 1H, H-19β), 5.11 (s, 1H, H-29-*pro E*), 5.52 (s, 1H, H-29-*pro Z*), 5.71 (s, 2H, phenyl-CH_2), 7.48 (d, $J = 8.0$ Hz, 2H, phenyl-*meta*), 7.91 (d, $J = 8.1$ Hz, 2H, phenyl-*ortho*), 8.28 (s, 1H, triazole), 10.00 (s, 1H, CHO), 12.09 (s, 1H, COOH); **^{13}C NMR** (126 MHz, $(CD_3)_2SO$): δ = 14.20, 15.42, 15.59, 19.12, 20.67, 21.02, 22.05, 26.38, 28.97, 29.25, 31.58, 32.19, 33.06, 33.56, 36.00, 36.30, 37.66, 38.77, 41.99, 46.46, 48.96, 49.64, 52.32, 53.76, 55.46, 109.91, 122.04, 128.28, 129.88, 135.76, 142.70, 144.71, 147.86, 177.22, 192.62, 216.47; **IR** (DRIFT): ν_{max} = 1610 (C═C); 1697 (C═O); 3400 (O–H) cm^{-1}; **HRMS** (ESI$^+$): calcd for $C_{39}H_{51}N_3O_4$ $[M+H]^+$ 626.3952; found 626.3949.

*4.1.18.8. 20-[1-(Pyridin-3-ylmethyl)-1H-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17h**.* 20-[1-(Pyridin-3-ylmethyl)-1*H*-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17h** was synthesized by the general procedure **E** using β-(azidomethyl)pyridine. The reaction was monitored by TLC (dichloromethane/MeOH 20/1), which indicated a completion of the reaction after 72 h. The solvent was removed from the reaction under reduced pressure. The residue was purified by column chromatography on a silica gel with gradient elution starting at dichloromethane/MeOH 40/1 and finishing at dichloromethane/MeOH 20/1. Collected fractions were removed under vacuum yielding a white solid of **17h**. Yiled: 50 mg (39 %), m. p. 146–150 °C (dichloromethane/hexane); **^{1}H NMR** (500 MHz, $(CD_3)_2SO$): δ = 0.82 (s, 3H), 0.86 (s, 3H), 0.89 (s, 3H), 0.91 (s, 3H), 0.97 (s, 3H, 5 × CH_3), 5.10 (s, 1H, H-29-*pro E*), 5.52 (s, 1H, H-29-*pro Z*), 5.73 (s, 2H, phenyl-CH_2), 6.66–7.31 (m, 1H, pyridine), 7.31–8.22 (m, 3H, pyridine), 8.28 (s, 1H, triazole), 12.13 (s, 1H, COOH); **^{13}C NMR** (126 MHz, $(CD_3)_2SO$): δ = 13.90, 14.20, 15.41, 15.59, 19.11, 20.65, 21.00, 26.35, 28.97, 29.25, 30.11, 31.57, 32.19, 33.05, 33.56, 35.99, 36.29, 37.65, 38.76, 41.98, 46.46, 48.94, 49.53, 53.77, 55.46, 69.75, 109.69, 117.72, 121.76, 129.99, 130.09, 133.22, 135.17, 144.65, 147.84, 177.20, 216.46.; **IR** (DRIFT): ν_{max} = 1700 (C═O; 1931 (C–H aromatic); 3400 (O–H) cm^{-1}; **HRMS** (ESI$^+$): calcd for $C_{37}H_{50}N_4O_3$ $[M+H]^+$ 599.3956; found 599.3954.

*4.1.18.9. 20-[1-(Thiophen-2-ylmethyl)-1H-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17i**.* 20-[1-(Thiophen-2-ylmethyl)-1*H*-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17i** was prepared by following general procedure **E** using 2-(azidomethyl)thiophene. The reaction was monitored by TLC (hexane/EtOAc 2/1 + a drop of AcOH) until complete conversion (6 h). Then the reaction was diluted with water, acidified with HCl and extracted with EtOAc 3 times. The collected organic phase was dried over MgSO4, filtered and evaporated. The residue was purified by column chromatography on a silica gel with hexane/EtOAc/$CHCl_3$ 12/7/1 + 0.1 % AcOH. Solvents from the collected fractions were removed under vacuum providing a white solid of **17i**. Yield 98 mg (75 %), m. p. 142–145 °C ($CHCl_3$/hexane); **^{1}H NMR** (500 MHz, ($CDCl_3$): δ = 0.89 (s, 3H), 0.92 (s, 3H), 0.95 (s, 3H), 1.00 (s, 3H), 1.06 (s, 3H, 5 × CH_3), 3.33 (td, $J_1 = 10.9$ Hz, $J_2 = 4.3$ Hz, 1H, H-19β), 5.13 (s, 1H, H-29-*pro E*), 5.59 (s, 1H, H-29-*pro Z*), 5.70 (s, 2H, aryl-CH_2), 7.01 (dd, $J_1 = 5.1$ Hz, $J_2 = 3.5$ Hz, 1H, thiophene), 7.11 (d, $J = 3.0$ Hz, 1H, thiophene), 7.32 (dd, $J_1 = 5.1$ Hz, $J_2 = 1.1$ Hz, 1H, thiophene), 7.49 (s, 1H, triazole); **^{13}C NMR** (126 MHz, ($CDCl_3$): δ = 14.77, 16.01, 16.08, 19.76, 21.13, 21.57, 26.81, 26.91, 29.83, 29.88, 32.16, 32.57, 33.76, 34.24, 36.87, 37.03, 38.68, 39.72, 40.76, 42.62, 47.45, 48.63, 49.89, 50.27, 55.05, 56.58, 111.03, 119.99, 127.11, 127.46, 128.14, 136.50, 144.33, 149.43, 181.78, 218.29; **IR** (DRIFT): ν_{max} = 1695 (C═O); 2895 (O–H) cm^{-1}; **HRMS** (ESI$^+$): calcd for $C_{36}H_{50}N_3O_3S$ $[M+H]^+$ 604.3567; found 604.3556.

*4.1.18.10. 20-[1-(Furan-2-ylmethyl)-1H-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17j**.* 20-[1-(Furan-2-ylmethyl)-1*H*-1,2,3-triazol-4-yl]-3-oxo-30-norlup-20(29)-en-28-oic acid **17j** was synthesized using 2-(azidomethyl)furan according to the general procedure **E**. The complete reaction conversion was indicated by TLC (hexane/EtOAc 2/1 + a drop of AcOH) after 6 h. The solvent from the reaction was removed under reduced pressure and the residue was purified by column chromatography on a silica gel with hexane/EtOAc/$CHCl_3$ 14/5/1 + 0.1 % of AcOH. Collected fractions were evaporated under vacuum providing a white yield of **17j**. Yield 113 mg (90 %), m. p. 138–142 °C ($CHCl_3$/hexane); **^{1}H NMR** (500 MHz, ($CDCl_3$): δ = 0.89 (s, 3H), 0.94 (s, 3H), 0.96 (s, 3H), 1.00 (s, 3H), 1.06 (s, 3H, 5 × CH_3), 3.34 (td, $J_1 = 11.1$ Hz, $J_2 = 4.7$ Hz, 1H, H-19β), 5.13 (s, 1H, H-29-*pro E*), 5.51 (s, 2H, aryl-CH_2), 5.60 (s, 1H, H-29-*pro Z*), 6.38 (dd, $J_1 = 3.2$ Hz, $J_2 = 1.9$ Hz, 1H,

furan), 6.44 (d, $J = 3.1$ Hz, 1H, furan), 7.43 (dd, $J_1 = 1.8$ Hz, $J_2 = 0.6$ Hz, 1H, furan), 7.52 (s, 1H, triazole); **^{13}C NMR** (126 MHz, ($CDCl_3$): δ = 14.75, 16.01, 16.08, 19.76, 21.12, 21.58, 26.81, 26.95, 29.89, 32.17, 32.59, 33.76, 34.23, 36.87, 37.03, 38.69, 39.71, 40.76, 42.62, 43.52, 46.80, 47.44, 49.90, 50.24, 55.04, 56.57, 110.30, 110.98, 120.20, 143.71, 144.29, 147.62, 149.33, 181.75, 218.31; **IR** (DRIFT): ν_{max} = 1693 (C═O); 2923 (O–H) cm^{-1}; **HRMS** (ESI^+): calcd for $C_{36}H_{50}N_3O_4$ $[M+H]^+$ 588.3796; found 588.3798.

4.2. Biological evaluation

4.2.1. Cell culture and MTS cytotoxicity assay

Cytotoxicity screening was done according to the routine protocol, which was developed at our department in the past [69,70]. All cells (if not indicated otherwise) were obtained from the American Tissue Culture Collection (ATCC). The CCRF-CEM cell-line with high chemoselectivity is derived from T lymphoblastic leukemia, K562 represent cells from an chronic myeloid leukemia patient sample with bcr-abl translocation, U2OS cells are derived from osteosarcoma, HCT116 are cells from colorectal tumor and its p53 gene knock-down equivalent (HCT116p53−/−, Horizon Discovery Ltd, UK) is a model of human cancers with p53 mutation, which is frequently associated with poor prognosis and A549 cell-line is lung adenocarcinoma. BJ and MRC-5 cells represent human fibroblasts and were used as a non-tumor control. The cells were maintained in nunc/corning 75 cm^2 plastic tissue culture flasks and cultured in appropriate cell culture medium according to the ATCC or Horizon recommendations (DMEM/RPMI 1640 with 5 g/L glucose, 2 mM glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin, 10 % fetal calf serum, and $NaHCO_3$). The cytotoxicity MTS assays were performed according to the standard procedure used at our department and they were repeated 3 independent times.

4.2.2. Annexin V binding assay

We employed an apoptosis detection assay based on Annexin V-FITC and propidium iodide staining, utilizing a commercial kit from Exbio. The procedure followed the manufacturer's protocol with minor modifications. Briefly, cells were adjusted to the appropriate concentration, washed with Annexin V binding buffer, and subsequently stained with Annexin V-FITC and propidium iodide. To reduce cytotoxic effects observed in CCRF-CEM cells, we used only half of the recommended volume of propidium iodide, as the standard concentration caused acute toxicity. Stained cells were incubated for 15 min at room temperature in the dark, centrifuged, and resuspended in 100 µl of Annexin V binding buffer. Samples were immediately subjected to flow cytometric analysis using a FACSAria II cytometer (Becton Dickinson), with a minimum acquisition of 10,000 cells per sample.

4.2.3. JC-1-based mitochondrial membrane potential assay

Changes in mitochondrial membrane potential were evaluated using a fluorescent cationic dye sensitive to membrane polarization. CCRF-CEM cells were exposed to test compounds at concentrations corresponding to 1 × and 5 × their respective IC_{50} values for 24 h. Following treatment, cells were adjusted to a density of 0.5 × 10^6 cells/mL and incubated with the potential-sensitive dye at a final concentration of 1 µM for 10 min at room temperature. As a depolarization control, CCCP was applied at 50 µM 5 min prior to dye addition. After labeling, cells were collected by centrifugation (1500 rpm, 5 min, room temperature), resuspended in 0.5 mL of phosphate-buffered saline, and promptly analyzed by flow cytometry (FACSAria II, Becton Dickinson) using a 488 nm excitation laser. Emission from the dye's monomeric and aggregated forms, indicative of depolarized and polarized mitochondria respectively, was recorded using appropriate filters for detection at 529 nm and 590 nm. A minimum of 10,000 events was acquired for each condition.

4.2.4. Cell cycle analysis

Assessment of apoptosis and cell cycle distribution was conducted via flow cytometry. CCRF-CEM cells were seeded at a density of 5 × 10^5 cells/mL in 6-well plates and treated with either 1 × or 5 × IC_{50} concentrations of the tested compound. Cells were cultured for 24 h in RPMI 1640 medium supplemented with 10 % fetal calf serum, 100 U/mL penicillin, and 100 µg/mL streptomycin under standard conditions (37 °C, 5 % CO_2, humidified atmosphere). A vehicle-only control was harvested in parallel. After incubation, cells were collected, washed with ice-cold PBS, and fixed in 70 % ethanol at −20 °C overnight. The following day, fixed cells were washed with hypotonic citrate buffer, treated with RNase A (50 µg/mL), and stained with propidium iodide for total DNA content analysis. Flow cytometric measurements were performed using a cytometer equipped with a 488 nm excitation laser (FACSCalibur, Becton Dickinson). Cell cycle phase distribution was quantified using Kaluza software (Beckman Coulter).

4.2.5. Assessment of DNA synthesis via BrdU incorporation

Cells were cultured and treated under the same conditions as those used for cell cycle analysis. Prior to collection, they were pulse-labeled with 10 µM 5-bromo-2′-deoxyuridine (BrdU) for 30 min to mark newly synthesized DNA. Following labeling, cells were harvested by trypsinization, fixed in ice-cold 70 % ethanol, and incubated on ice for 30 min. After washing with PBS, DNA was denatured by resuspension in 2 M HCl for 30 min at room temperature. The reaction was neutralized with 0.1 M sodium tetraborate ($Na_2B_4O_7$), after which cells were washed in PBS containing 0.5 % Tween-20 and 1 % BSA. BrdU incorporation was detected by staining with a primary anti-BrdU antibody (Exbio) for 30 min at room temperature in the dark. Following another PBS wash, cells were incubated with a FITC-conjugated secondary anti-mouse antibody (Sigma). Finally, cells were stained with propidium iodide (0.1 mg/mL) and treated with RNase A (0.5 mg/mL) for 1 h at room temperature in the dark. Samples were analyzed by flow cytometry using a FACSCalibur cytometer (Becton Dickinson) equipped with a 488 nm excitation laser.

4.2.6. Assessment of RNA synthesis via BrU incorporation

Cells were cultured and treated under the same experimental conditions as described for cell cycle analysis. To monitor RNA synthesis, cells were pulse-labeled with 1 mM 5-bromouridine (BrU) for 30 min prior to harvesting. Following labeling, cells were fixed in 1 % paraformaldehyde (buffered in PBS) containing 0.05 % NP-40 for 15 min at room temperature and subsequently stored at 4 °C overnight to enhance fixation. The next day, cells were washed with 1 % glycine in PBS to quench residual aldehyde groups and then rinsed again in PBS. For detection of BrU incorporation, cells were incubated with a primary anti-BrdU antibody (Exbio), which cross-reacts with BrU, for 30 min at room temperature in the dark. After washing with PBS, cells were stained with a FITC-conjugated secondary anti-mouse antibody (Sigma) under identical conditions. Following antibody staining, a secondary fixation step was performed using 1 % paraformaldehyde (PBS-buffered) with 0.05 % NP-40 to stabilize fluorescence labeling. After a final PBS wash, cells were incubated with a staining solution containing propidium iodide (0.1 mg/mL) and RNase A (0.5 mg/mL) for 1 h at room temperature in the dark. Samples were analyzed by flow cytometry using a FACSCalibur (Becton Dickinson) equipped with a 488 nm excitation laser.

4.2.7. Western blot

CCRF-CEM cells were treated with tested compounds at concentrations corresponding to 1 × IC_{50} and 5 × IC_{50} for 24 h. Following treatment, the cells were thoroughly washed with ice-cold 1 × PBS, and total cellular proteins 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). Protein concentrations were determined using the Pierce™ BCA Protein Assay Kit (Thermo

Scientific) according to the manufacturer's instructions. Aliquots containing 20 μg of total protein were denatured in Laemmli sample buffer (50 mM DTT, 0.06 % bromophenol blue, 47 % glycerol, 12 % SDS, 0.5 M Tris-HCl, pH 6.8) and separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Following electrophoresis, proteins were transferred onto nitrocellulose membranes using the Trans-Blot® Turbo™ Transfer System (Bio-Rad). Membranes were blocked in 5 % bovine serum albumin (BSA) in TBS containing 0.1 % Tween 20 (TBS-T) for 1 h at room temperature and then incubated overnight at 4 °C with primary antibodies specific for β-actin, Cyclin A, and p21 (all from Sigma-Aldrich); Chk1 (Santa Cruz Biotechnology); and phospho-Chk1 (Ser345), γH2AX, Bcl-2, caspase-8, caspase-3, and PARP (all from Cell Signaling Technology). After incubation with primary antibodies, membranes were washed with TBS-T and subsequently incubated with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (Sigma-Aldrich) for 1 h at room temperature. Chemiluminescent signals were developed using the ECL Prime reagent (Amersham) and visualized with the Li-COR Odyssey imaging system (LI-COR Biotechnology). β-actin was used as a loading control to confirm equal protein loading.

4.2.8. Pharmacokinetic parameters

The detailed experimental procedure for the measurement of the pharmacological parameters is described in our earlier work [7,13].

CRediT authorship contribution statement

Šimon Orság: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Ivo Frydrych:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Barbora Lišková:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Soňa Gurská:** Validation, Investigation, Formal analysis. **Adam Přibylka:** Validation, Methodology, Investigation, Data curation. **Jiří Hodoň:** Visualization, Validation, Methodology, Investigation. **Petr Džubák:** Writing – review & editing, Validation, Supervision. **Marián Hajdúch:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Milan Urban:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Data curation, 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

This work was supported by the SALVAGE project, registration number: CZ.02.01.01/00/22_008/0004644, supported by OP JAK, with co-financing from the EU and the State Budget. The infrastructure was supported in parts by the infrastructural projects (CZ-OPENSCREEN – LM2023052; EATRIS-CZ – LM2023053). Pharmacology studies and stipendia for students were supported by internal Palacky University grants IGA_LF_2025_021 and IGA_PrF_2025_014.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2025.118268.

Data availability

Data will be made available on request.

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